(12) 按照专利合作条约所公布的国际申请

(19) 世界知识产权组织
国 际 局

(43) 国际公布日
2022 年 10 月 20 日 (20.10.2022)

WIPO | PCT

(10) 国际公布号
WO 2022/218384 A1

(51) 国际专利分类号:
C07K 16/28 (2006.01) *C07K 19/00* (2006.01)
C12N 15/13 (2006.01)

(21) 国际申请号: PCT/CN2022/086890

(22) 国际申请日: 2022 年 4 月 14 日 (14.04.2022)

(25) 申请语言: 中文

(26) 公布语言: 中文

(30) 优先权:
202110404033.2 2021年4月14日 (14.04.2021) CN

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本国际公布:
— 包括国际检索报告 (条约第21条(3))。
— 包括按细则13之二规定在说明书以外提交的关于生物材料保藏的说明 (细则13之二.4(d)(i)和48.2(a)(viii))。

WO 2022/218384 A1

(54) **Title:** ANTI-CD47 MONOCLONAL ANTIBODY AND USE THEREOF

(54) 发明名称: 抗CD47的单克隆抗体及其用途

(57) **Abstract:** An anti-CD47 monoclonal antibody and the use thereof in combination with a monoclonal antibody, a bispecific antibody and/or a tumor therapeutic agent, wherein the anti-CD47 monoclonal antibody is secreted by a hybridoma cell line with a deposit number of CCTCC NO: C2018135.

(57) 摘要: 一种抗CD47的单克隆抗体及其联合单克隆抗体、双特异性抗体和/或肿瘤治疗剂的用途，所述抗CD47的单克隆抗体由保藏号为CCTCC NO:C2018135的杂交瘤细胞株分泌。

ANTI-CD47 MONOCLONAL ANTIBODY AND USE THEREOF

TECHNICAL FIELD

The present invention relates to the fields of autoimmune diseases treatment and molecular immunology, and particularly to an anti-CD47 antibody, a pharmaceutical composition comprising the same, and use thereof. More particularly, the present invention relates to an anti-CD47 monoclonal antibody.

BACKGROUND

CD47 is also referred to as integrin associated protein (IAP). CD47 is a five-span transmembrane protein with a molecular weight of about 50 kDa and belongs to the immunoglobulin superfamily. Its extracellular N terminus is an IgV domain and is connected to αvβ3 (CD51/CD61) and αIIbβ3 (CD41/CD61) integrins. CD47 is involved in a variety of physiological functions, such as cell transfer, T cell and dendritic cell (DC) activation, and axonal development.

CD47 is expressed on all types of cells including erythrocytes, and is highly expressed on tumor cells. CD47 has two ligands, namely signal regulatory protein-α (SIRPα) and thrombospondin-1 (TSP1). SIRPα, a receptor transmembrane glycoprotein comprises an immunoglobulin domain, belongs to the SIRP family, and is mainly expressed on macrophages and nerve cells. In the CD47-SIRPα pathway, CD47 protein binds to SIRPα and phosphorylates its immunoreceptor tyrosine-based inhibitory motif (ITIM), and intracellularly recruits SHP-1 protein to produce a series of cascade reactions to inhibit macrophage phagocytosis (Matozaki T, Murata Y, Okazawa H, et al., Functions and molecular mechanisms of the CD47–SIRPα signaling pathway. *Trends in cell biology*, 2009, 19(2): 72-80.). However, normal red blood cells are not phagocytosed due to the inhibitory signal generated by the binding of CD47 on the surface of the cell membrane to SIPRα of macrophages. (Oldenborg P A, Zheleznyak A, Fang Y F, et al., Role of CD47 as a marker of self on red blood cells. *Science,* 2000, 288(5473): 2051-2054.). TSP1, a homotrimer composed of 3 peptide chains, is involved in cell proliferation,

apoptosis, adhesion, migration, angiogenesis and other processes through interaction with other cell surface receptors, matrix components and growth factors (Jiang P, Lagenaur CF, Narayanan V. Integrin-associated Protein Is a Ligand for the P84 Neural Adhesion Molecule. *Journal of Biological Chemistry* 1999. 274:559-62).

Macrophages are derived from monocytes, which in turn are derived from precursor cells in the bone marrow. Their main functions are to phagocytose cell debris and pathogens and to activate lymphocytes or other immune cells to respond to the pathogens in the form of fixed cells or free cells. At present, researches suggest that tumor cells have a mechanism of escaping macrophage phagocytosis. During the growth of tumor cells, specific proteins such as calreticulin are formed on the surface, exposing the identity of the tumor cells, such that the tumor cells are phagocytosed by the attracted macrophages. However, tumor cells with highly expressed CD47 are mistakenly recognized as normal cells by macrophages with SIRPα and thus escape macrophage phagocytosis, since the CD47-SIRPα pathway activates the inhibition of the macrophage phagocytosis (CD47 is upregulated on circulating hematopoietic stem cells and leukemia cells to avoid phagocytosis. Jaiswal S, Jamieson C H M, Pang W W, et al., *Cell*, 2009, 138(3) 271-285).

At present, researches suggest that anti-CD47 antibodies kill tumor cells primarily through two mechanisms. 1. Binding of anti-CD47 antibodies to CD47 blocks the CD47-SIRPα pathway, allowing macrophages' phagocytosis. 2. Anti-CD47 antibodies exert a tumor-killing effect through DC cells and CD8+ T cells. DC cells phagocytose tumor cells through the synergism between anti-CD47 antibodies and pro-phagocytic molecules such as calreticulin, and present tumor-associated antigens to CD8+ T cells, thereby exerting the specific killing effect of CD8+ T cells on tumors (CD47 blockade as another immune checkpoint therapy for cancer. Vonderheide R H. *Nature Medicine*, 2015, 21(10):1122). With the two mechanisms, it is suggested that anti-CD47 antibodies are very likely to have the ability to activate both non-specific immunity and specific immunity.

At present, anti-CD47 monoclonal antibody drugs have promising utility in a variety of applications and good efficacy in treating tumors, and can be used for

treating various tumors. The anti-CD47 monoclonal antibody drug Hu5F9-G4 effectively inhibits the growth and metastasis of hematological malignancies and solid tumors in preclinical studies (Abstract PR13: The anti-CD47 antibody Hu5F9-G4 is a novel immune checkpoint inhibitor with synergistic efficacy in combination with clinically active cancer targeting antibodies [J] Chao M P, McKenna K M, Cha A, et al., 2016), wherein the amino acid sequence of the heavy chain is set forth in SEQ ID NO: 85, and the amino acid sequence of the light chain is set forth in SEQ ID NO: 86.

Therefore, the development of antibody drugs with high affinity for CD47, higher efficacy and fewer toxic side effects for treating tumors is of great significance.

SUMMARY

Mammalian cell expression systems were used to express recombinant human CD47 as an antigen to immunize mice, and hybridoma cells were obtained by fusion of mouse spleen cells and myeloma cells. The following hybridoma cell lines are obtained by screening a large number of the samples.

The inventors found that:

The hybridoma cell line LT012 can secrete a monoclonal antibody (named 6F7) capable of specifically binding to CD47, and the monoclonal antibody can compete with the receptor SIRPα ECD-hFc-Biotin for binding to CD47, effectively blocking the binding of SIRPα to CD47 and further promoting the phagocytosis of tumor cells by macrophages.

Furthermore, the inventors have prepared humanized versions of the monoclonal antibody 6F7 (named 6F7H1L1, 6F7H2L2 and 6F7H3L3).

The present invention is detailed below.

The amino acid sequences of the CDR regions of the antibodies are analyzed by technical means well known to those skilled in the art, for example, by VBASE2 database.

The antibodies 6F7, 6F7 H1L1, 6F7 H2L2 and 6F7 H3L3 disclosed herein share the same HCDR1-3 and LCDR1-3.

The 3 CDRs of the heavy chain variable region have the following amino acid sequences:

HCDR1: GYTFTSYW (SEQ ID NO: 5),

HCDR2: IDPSDSET (SEQ ID NO: 6),

HCDR3: ARLYRWYFDV (SEQ ID NO: 7);

the 3 CDRs of the light chain variable region have the following amino acid sequences:

LCDR1: EIVGTY (SEQ ID NO: 8),

LCDR2: GAS (SEQ ID NO: 9),

LCDR3: GQSYNFPYT (SEQ ID NO: 10).

In some embodiments, the antibodies disclosed herein are selected from the group consisting of:

an amino acid sequence of a heavy chain of 6F7 H1L1 (G1M) (SEQ ID NO: 59)

an amino acid sequence of a light chain of 6F7 H1L1 (G1M) (SEQ ID NO: 60)

an amino acid sequence of a heavy chain of 6F7 H2L2 (G1M) (SEQ ID NO: 61)

an amino acid sequence of a light chain of 6F7 H2L2 (G1M) (SEQ ID NO: 62)

an amino acid sequence of a heavy chain of 6F7 H3L3 (G1M) (SEQ ID NO: 63)

an amino acid sequence of a light chain of 6F7 H3L3 (G1M) (SEQ ID NO: 64)

an amino acid sequence of a heavy chain of 6F7 H1L1 (hG4) (SEQ ID NO: 65)

an amino acid sequence of a light chain of 6F7 H1L1 (hG4) (SEQ ID NO: 66)

an amino acid sequence of a heavy chain of 6F7 H2L2 (hG4) (SEQ ID NO: 67)

an amino acid sequence of a light chain of 6F7 H2L2 (hG4) (SEQ ID NO: 68)

an amino acid sequence of a heavy chain of 6F7 H3L3 (hG4) (SEQ ID NO: 69)

an amino acid sequence of a light chain of 6F7 H3L3 (hG4) (SEQ ID NO: 70)

One aspect of the present invention relates to an isolated polypeptide comprising sequences set forth in SEQ ID NOs: 5, 6 and 7, wherein the polypeptide, as part of an anti-human CD47 antibody, specifically binds to human CD47, the antibody further comprising sequences set forth in SEQ ID NOs: 8, 9 and 10.

One aspect of the present invention relates to an isolated polypeptide comprising sequences set forth in SEQ ID NOs: 8, 9 and 10, wherein the polypeptide, as part of

an anti-human CD47 antibody, specifically binds to human CD47, the antibody further comprising sequences set forth in SEQ ID NOs: 5, 6 and 7.

One aspect of the present invention relates to an isolated polypeptide comprising a sequence selected from sequences set forth in SEQ ID NOs: 2, 12, 16 and 20 or a variant thereof, wherein the polypeptide, as part of an anti-human CD47 antibody, specifically binds to human CD47, the antibody further comprising a sequence selected from sequences set forth in SEQ ID NOs: 4, 14, 18 and 22 or a variant thereof, wherein the variant has at least 85%, preferably 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98% or 99% or higher sequence identity to the corresponding sequence, or has one or more (preferably 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 21, 22, 23, 24, 25, 26, 27, 28, 29 or 30) conservative amino acid mutations (preferably substitutions, insertions or deletions) compared to the corresponding sequence; or

one aspect of the present invention relates to an isolated polypeptide comprising a sequence selected from sequences set forth in SEQ ID NOs: 4, 14, 18 and 22 or a variant thereof, wherein the polypeptide, as part of an anti-human CD47 antibody, specifically binds to human CD47, the monoclonal antibody further comprising a sequence selected from sequences set forth in SEQ ID NOs: 2, 12, 16 and 20 or a variant thereof, wherein the variant has at least 85%, preferably 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98% or 99% or higher sequence identity to the corresponding sequence, or has one or more (preferably 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 21, 22, 23, 24, 25, 26, 27, 28, 29 or 30) conservative amino acid mutations (preferably substitutions, insertions or deletions) compared to the corresponding sequence.

In one embodiment of the present invention, the antigen-binding fragment is selected from Fab, Fab', $F(ab')_2$, Fd, Fv, dAb, Fab/c, complementarity determining region (CDR) fragment, single-chain antibody (e.g., scFv), bivalent antibody and domain antibody.

In one embodiment of the present invention, the antibody that specifically binds to CD47 is a humanized antibody, a chimeric antibody or a multispecific antibody

(e.g., a bispecific antibody).

In one embodiment of the present invention, the antibody that specifically binds to CD47 binds to human CD47 protein with a KD less than about 10^{-5} M, e.g., less than about 10^{-6} M, 10^{-7} M, 10^{-8} M, 10^{-9} M or 10^{-10} M or less. Preferably, the KD is measured by a Fortebio system.

In one embodiment of the present invention, the antibody that specifically binds to CD47 binds to human CD47 protein with an EC_{50} less than about 100 nM, e.g., less than about 10 nM, 1 nM, 0.9 nM, 0.8 nM, 0.7 nM, 0.6 nM, 0.5 nM, 0.4 nM, 0.3 nM, 0.2 nM or 0.1 nM or less. Specifically, the EC_{50} is measured by indirect ELISA.

In one embodiment of the present invention, the antibody that specifically binds to CD47 comprises constant regions, and the constant regions are derived from species other than murine, e.g., from a human antibody, preferably from a human IgG, more preferably from IgG1 or IgG4.

In one embodiment of the present invention, the constant regions of the antibody that specifically binds to CD47 are humanized, e.g., the heavy chain constant region is Ig gamma-1 chain C region, more preferably Ig gamma-1 chain C region under GenBank ACCESSION No. P01857 (SEQ ID NO: 58), or is Ig gamma-4 chain C region, more preferably Ig gamma-4 chain C region under GenBank ACCESSION No. P01861.1 (SEQ ID NO: 56); the light chain constant region is Ig kappa chain C region, more preferably Ig kappa chain C region under GenBank ACCESSION No. P01834 (SEQ ID NO: 57). The antibodies disclosed herein use the following constant regions on the basis of the variable regions of 6F7 H1L1, 6F7 H2L2 and 6F7 H3L3: the heavy chain constant region is Ig gamma-1 chain C region under ACCESSION No. P01857 (SEQ ID NO: 58) or the heavy chain constant region is Ig gamma-4 chain C region under ACCESSION No. P01861.1 (SEQ ID NO: 56), and on this basis, S228P mutation is introduced to improve the stability; the light chain constant region is Ig kappa chain C region, ACCESSION No. P01834 (SEQ ID NO: 57). Another aspect of the present invention relates to an isolated polynucleotide encoding a polypeptide comprising sequences set forth in SEQ ID NOs: 5, 6 and 7, wherein the polypeptide, as part of an anti-human CD47 antibody,

specifically binds to human CD47, the antibody further comprising sequences set forth in SEQ ID NOs: 8, 9 and 10.

One aspect of the present invention relates to an isolated polynucleotide encoding a polypeptide comprising sequences set forth in SEQ ID NOs: 8, 9 and 10, wherein the polypeptide, as part of an anti-human CD47 antibody, specifically binds to human CD47, the antibody further comprising sequences set forth in SEQ ID NOs: 5, 6 and 7.

One aspect of the present invention relates to an isolated polynucleotide encoding a polypeptide comprising a sequence selected from sequences set forth in SEQ ID NOs: 2, 12, 16 and 20 or a variant thereof, wherein the polypeptide, as part of an anti-human CD47 antibody, specifically binds to human CD47, the antibody further comprising a sequence selected from sequences set forth in SEQ ID NOs: 4, 14, 18 and 22 or a variant thereof,

wherein the variant comprises a sequence having at least 85%, preferably 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98% or 99% or higher sequence identity to the corresponding sequence or having one or more (preferably 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 21, 22, 23, 24, 25, 26, 27, 28, 29 or 30) conservative amino acid mutations (preferably substitutions, insertions or deletions) compared to the corresponding sequence; or

one aspect of the present invention relates to an isolated polynucleotide encoding a polypeptide comprising a sequence selected from sequences set forth in SEQ ID NOs: 4, 14, 18 and 22 or a variant thereof, wherein the polypeptide, as part of an anti-human CD47 antibody, specifically binds to human CD47, the antibody further comprising a sequence selected from sequences set forth in SEQ ID NOs: 2, 12, 16 and 20 or a variant thereof,

wherein the variant has at least 85%, preferably 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98% or 99% or higher sequence identity to the corresponding sequence, or has one or more (preferably 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 21, 22, 23, 24, 25, 26, 27, 28, 29 or 30) conservative amino acid mutations (preferably substitutions, insertions or deletions)

compared to the corresponding sequence;

specifically, the polynucleotide molecule comprises or consists of a nucleotide sequence set forth in SEQ ID NO: 1, SEQ ID NO: 11, SEQ ID NO: 15 or SEQ ID NO: 19, or a sequence having at least 85%, preferably 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98% or 99% or higher sequence identity to the sequence.

Specifically, the polynucleotide molecule comprises or consists of a nucleotide sequence set forth in SEQ ID NO: 3, SEQ ID NO: 13, SEQ ID NO: 17 or SEQ ID NO: 21, or a sequence having at least 85%, preferably 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98% or 99% or higher sequence identity to the sequence.

One aspect of the present invention further provides a hybridoma cell line selected from the hybridoma cell line LT012 under CCTCC NO. 2018135, and a monoclonal antibody produced by the hybridoma cell line.

The present invention further relates to the following aspects:

1. A pharmaceutical composition, preferably for treating a tumor, comprising a component A and a component B, wherein the component A is an antibody that specifically binds to CD47 or an antigen-binding fragment thereof;

the component B is selected from one or more of the following: a component B1, a component B2 and a component B3, wherein the component B1 is a bispecific antibody or an antigen-binding fragment thereof, the component B2 is an anti-tumor chemotherapeutic, and the component B3 is an anti-PD-1 monoclonal antibody or an antigen-binding fragment thereof;

preferably, the composition further comprises a component C, wherein the component C is an anti-tumor therapeutic agent, and the component C is different from component B;

for example, according to the Kabat, IMGT, Chothia or AbM numbering system,

the antibody that specifically binds to CD47 comprises CDR sequences selected from those comprised in the following heavy chain variable regions and light chain variable regions:

(1) an HCDR1, an HCDR2 and an HCDR3 comprised in a heavy chain variable region set forth in SEQ ID NO: 2, and
an LCDR1, an LCDR2 and an LCDR3 comprised in a light chain variable region set forth in SEQ ID NO: 4; or
(2) an HCDR1, an HCDR2 and an HCDR3 comprised in a heavy chain variable region set forth in SEQ ID NO: 12, and
an LCDR1, an LCDR2 and an LCDR3 comprised in a light chain variable region set forth in SEQ ID NO: 14; or
(3) an HCDR1, an HCDR2 and an HCDR3 comprised in a heavy chain variable region set forth in SEQ ID NO: 16, and
an LCDR1, an LCDR2 and an LCDR3 comprised in a light chain variable region set forth in SEQ ID NO: 18; or
(4) an HCDR1, an HCDR2 and an HCDR3 comprised in a heavy chain variable region set forth in SEQ ID NO: 20, and
an LCDR1, an LCDR2 and an LCDR3 comprised in a light chain variable region set forth in SEQ ID NO: 22
(preferably, according to the IMGT numbering system, the antibody comprises:
an HCDR1 comprising or consisting of a sequence set forth in SEQ ID NO: 5 or a variant thereof,
an HCDR2 comprising or consisting of a sequence set forth in SEQ ID NO: 6 or a variant thereof, and
an HCDR3 comprising or consisting of a sequence set forth in SEQ ID NO: 7 or a variant thereof; and the antibody further comprises:
an LCDR1 comprising or consisting of amino acids set forth in SEQ ID NO: 8 or a variant thereof,
an LCDR2 comprising or consisting of an amino acid sequence set forth in SEQ ID NO: 9 or a variant thereof, and
an LCDR3 comprising or consisting of a sequence set forth in SEQ ID NO: 10 or a variant thereof);
the bispecific antibody is an anti-PD-1/anti-CTLA4 antibody or an

anti-PD-1/anti-VEGFA antibody or a combination of the two; the bispecific antibody comprises a first protein functional region and a second protein functional region, wherein the first protein functional region targets PD-1, and the second protein functional region targets CTLA4 or VEGFA; the first protein functional region is an immunoglobulin, and the second protein functional region is a single-chain antibody; or the first protein functional region is a single-chain antibody, and the second protein functional region is an immunoglobulin;

preferably, two single-chain antibody molecules (preferably, two identical single-chain antibody molecules) are linked to one immunoglobulin molecule; preferably, the immunoglobulin is of IgG1 subtype (preferably, human IgG1 subtype); preferably, the single-chain antibody is linked to the N terminus or C terminus of the heavy chain of the immunoglobulin, wherein the numbers of the first protein functional region and the second protein functional region are each independently 1, 2 or more; preferably, the first protein functional region is linked to the second protein functional region either directly or via a first linker fragment; and/or the heavy chain variable region of the single-chain antibody is linked to the light chain variable region of the single-chain antibody either directly or via a second linker fragment; the first linker fragment and the second linker fragment are identical or different; preferably, the linker fragment is (GGGGS)n, wherein n is a positive integer, or more preferably, n is 1, 2, 3, 4, 5 or 6; preferably, the first linker fragment and the second linker fragment comprise amino acid sequences independently selected from SEQ ID NO: 119 and SEQ ID NO: 120; more preferably, the first linker fragment and the second linker fragment comprise an amino acid sequence set forth in SEQ ID NO: 120;

when the bispecific antibody is an anti-PD-1/anti-CTLA4 antibody,

the first protein functional region comprises HCDR1–HCDR3 comprised in a heavy chain variable region set forth in SEQ ID NO: 87, and LCDR1–LCDR3 comprised in a light chain variable region set forth in SEQ ID NO: 88 (preferably, according to the IMGT numbering system, the first protein functional region comprises an HCDR1 comprising or consisting of a sequence set forth in SEQ ID NO: 89 or a

variant thereof,
an HCDR2 comprising or consisting of a sequence set forth in SEQ ID NO: 90 or a variant thereof,
an HCDR3 comprising or consisting of a sequence set forth in SEQ ID NO: 91 or a variant thereof,
an LCDR1 comprising or consisting of amino acids set forth in SEQ ID NO: 92 or a variant thereof,
an LCDR2 comprising or consisting of an amino acid sequence set forth in SEQ ID NO: 93 or a variant thereof, and
an LCDR3 comprising or consisting of a sequence set forth in SEQ ID NO: 94 or a variant thereof);
the second protein functional region comprises HCDR1–HCDR3 in a heavy chain variable region set forth in SEQ ID NO: 95, and LCDR1–LCDR3 in a light chain variable region set forth in SEQ ID NO: 96 (preferably, according to the IMGT numbering system, the second protein functional region comprises an HCDR1 comprising or consisting of a sequence set forth in SEQ ID NO: 97 or a variant thereof,
an HCDR2 comprising or consisting of a sequence set forth in SEQ ID NO: 98 or a variant thereof,
an HCDR3 comprising or consisting of a sequence set forth in SEQ ID NO: 99 or a variant thereof,
an LCDR1 comprising or consisting of amino acids set forth in SEQ ID NO: 100 or a variant thereof,
an LCDR2 comprising or consisting of an amino acid sequence set forth in SEQ ID NO: 101 or a variant thereof, and
an LCDR3 comprising or consisting of a sequence set forth in SEQ ID NO: 102 or a variant thereof);
or
when the bispecific antibody is an anti-PD-1/anti-VEGFA antibody,
the first protein functional region comprises HCDR1–HCDR3 comprised in a heavy

chain variable region set forth in SEQ ID NO: 103, and LCDR1–LCDR3 comprised in a light chain variable region set forth in SEQ ID NO: 104 (preferably, according to the IMGT numbering system, the first protein functional region comprises an HCDR1 comprising or consisting of a sequence set forth in SEQ ID NO: 105 or a variant thereof,

an HCDR2 comprising or consisting of a sequence set forth in SEQ ID NO: 106 or a variant thereof,

an HCDR3 comprising or consisting of a sequence set forth in SEQ ID NO: 107 or a variant thereof,

an LCDR1 comprising or consisting of amino acids set forth in SEQ ID NO: 108 or a variant thereof,

an LCDR2 comprising or consisting of an amino acid sequence set forth in SEQ ID NO: 109 or a variant thereof, and

an LCDR3 comprising or consisting of a sequence set forth in SEQ ID NO: 110 or a variant thereof);

the second protein functional region comprises HCDR1–HCDR3 in a heavy chain variable region set forth in SEQ ID NO: 111, and LCDR1–LCDR3 in a light chain variable region set forth in SEQ ID NO: 112 (preferably, according to the IMGT numbering system, the second protein functional region comprises an HCDR1 comprising or consisting of a sequence set forth in SEQ ID NO: 113 or a variant thereof,

an HCDR2 comprising or consisting of a sequence set forth in SEQ ID NO: 114 or a variant thereof,

an HCDR3 comprising or consisting of a sequence set forth in SEQ ID NO: 115 or a variant thereof,

an LCDR1 comprising or consisting of amino acids set forth in SEQ ID NO: 116 or a variant thereof,

an LCDR2 comprising or consisting of an amino acid sequence set forth in SEQ ID NO: 117 or a variant thereof, and

an LCDR3 comprising or consisting of a sequence set forth in SEQ ID NO: 118 or a

variant thereof);

wherein,

the anti-PD1 monoclonal antibody comprises HCDR1–HCDR3 comprised in a heavy chain variable region set forth in SEQ ID NO: 121, and LCDR1–LCDR3 comprised in a light chain variable region set forth in SEQ ID NO: 122 (preferably, according to the IMGT numbering system, the anti-PD1 monoclonal antibody comprises an HCDR1 comprising or consisting of a sequence set forth in SEQ ID NO: 123 or a variant thereof,

an HCDR2 comprising or consisting of a sequence set forth in SEQ ID NO: 124 or a variant thereof,

an HCDR3 comprising or consisting of a sequence set forth in SEQ ID NO: 125 or a variant thereof,

an LCDR1 comprising or consisting of amino acids set forth in SEQ ID NO: 126 or a variant thereof,

an LCDR2 comprising or consisting of an amino acid sequence set forth in SEQ ID NO: 127 or a variant thereof, and

an LCDR3 comprising or consisting of a sequence set forth in SEQ ID NO: 128 or a variant thereof);

wherein the variant comprises a sequence having at least 80%, 81%, 82%, 83%, 84%, 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99% homology to the corresponding sequence, or the variant comprises a sequence having one or more (preferably 1, 2 or 3) conservative amino acid mutations (preferably substitutions, insertions or deletions) compared to the corresponding sequence.

2. The pharmaceutical composition according to item 1, wherein the antibody that specifically binds to CD47 further comprises framework regions (FRs) in the heavy chain variable region and framework regions (FRs) in the light chain variable region selected from the group consisting of the following:

(1) the framework regions (FRs) in the heavy chain variable region including an FR-H1, an FR-H2, an FR-H3 and an FR-H4, wherein the FR-H1 comprises or

consists of an amino acid sequence set forth in SEQ ID NO: 23 or a variant thereof; the FR-H2 comprises or consists of an amino acid sequence set forth in SEQ ID NO: 24 or a variant thereof; the FR-H3 comprises or consists of an amino acid sequence set forth in SEQ ID NO: 25 or a variant thereof; the FR-H4 comprises or consists of an amino acid sequence set forth in SEQ ID NO: 26 or a variant thereof; the framework regions (FRs) in the light chain variable region including an FR-L1, an FR-L2, an FR-L3 and an FR-L4, wherein the FR-L1 comprises or consists of an amino acid sequence set forth in SEQ ID NO: 27 or a variant thereof; the FR-L2 comprises or consists of an amino acid sequence set forth in SEQ ID NO: 28 or a variant thereof; the FR-L3 comprises or consists of an amino acid sequence set forth in SEQ ID NO: 29 or a variant thereof; the FR-L4 comprises or consists of an amino acid sequence set forth in SEQ ID NO: 30 or a variant thereof;

(2) the framework regions (FRs) in the heavy chain variable region including an FR-H1, an FR-H2, an FR-H3 and an FR-H4, wherein the FR-H1 comprises or consists of an amino acid sequence set forth in SEQ ID NO: 31 or a variant thereof; the FR-H2 comprises or consists of an amino acid sequence set forth in SEQ ID NO: 32 or a variant thereof; the FR-H3 comprises or consists of an amino acid sequence set forth in SEQ ID NO: 33 or a variant thereof; the FR-H4 comprises or consists of an amino acid sequence set forth in SEQ ID NO: 34 or a variant thereof; the framework regions (FRs) in the light chain variable region including an FR-L1, an FR-L2, an FR-L3 and an FR-L4, wherein the FR-L1 comprises or consists of an amino acid sequence set forth in SEQ ID NO: 35 or a variant thereof; the FR-L2 comprises or consists of an amino acid sequence set forth in SEQ ID NO: 36 or a variant thereof; the FR-L3 comprises or consists of an amino acid sequence set forth in SEQ ID NO: 37 or a variant thereof; the FR-L4 comprises or consists of an amino acid sequence set forth in SEQ ID NO: 38 or a variant thereof;

(3) the framework regions (FRs) in the heavy chain variable region including an FR-H1, an FR-H2, an FR-H3 and an FR-H4, wherein the FR-H1 comprises or consists of an amino acid sequence set forth in SEQ ID NO: 39 or a variant thereof; the FR-H2 comprises or consists of an amino acid sequence set forth in SEQ ID

NO: 40 or a variant thereof; the FR-H3 comprises or consists of an amino acid sequence set forth in SEQ ID NO: 41 or a variant thereof; the FR-H4 comprises or consists of an amino acid sequence set forth in SEQ ID NO: 42 or a variant thereof; the framework regions (FRs) in the light chain variable region including an FR-L1, an FR-L2, an FR-L3 and an FR-L4, wherein the FR-L1 comprises or consists of an amino acid sequence set forth in SEQ ID NO: 43 or a variant thereof; the FR-L2 comprises or consists of an amino acid sequence set forth in SEQ ID NO: 44 or a variant thereof; the FR-L3 comprises or consists of an amino acid sequence set forth in SEQ ID NO: 45 or a variant thereof; the FR-L4 comprises or consists of an amino acid sequence set forth in SEQ ID NO: 46 or a variant thereof;

(4) the framework regions (FRs) in the heavy chain variable region including an FR-H1, an FR-H2, an FR-H3 and an FR-H4, wherein the FR-H1 comprises or consists of an amino acid sequence set forth in SEQ ID NO: 47 or a variant thereof; the FR-H2 comprises or consists of an amino acid sequence set forth in SEQ ID NO: 48 or a variant thereof; the FR-H3 comprises or consists of an amino acid sequence set forth in SEQ ID NO: 49 or a variant thereof; the FR-H4 comprises or consists of an amino acid sequence set forth in SEQ ID NO: 50 or a variant thereof; the framework regions (FRs) in the light chain variable region including an FR-L1, an FR-L2, an FR-L3 and an FR-L4, wherein the FR-L1 comprises or consists of an amino acid sequence set forth in SEQ ID NO: 51 or a variant thereof; the FR-L2 comprises or consists of an amino acid sequence set forth in SEQ ID NO: 52 or a variant thereof; the FR-L3 comprises or consists of an amino acid sequence set forth in SEQ ID NO: 53 or a variant thereof; the FR-L4 comprises or consists of an amino acid sequence set forth in SEQ ID NO: 54 or a variant thereof,

wherein the variant comprises a sequence having at least 80%, preferably at least 81%, 82%, 83%, 84%, 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98% or 99% homology to the corresponding sequence or having one or more (preferably 1, 2, 3, 4, 5, 6, 7, 8, 9 or 10) conservative amino acid mutations (preferably substitutions, insertions or deletions) compared to the corresponding sequence.

3. The pharmaceutical composition according to item 1 or 2, wherein the antibody that specifically binds to CD47 comprises:

(1) a heavy chain variable region comprising or consisting of:

an amino acid sequence set forth in SEQ ID NO: 2 or a variant thereof, and

a light chain variable region comprising or consisting of:

an amino acid sequence set forth in SEQ ID NO: 4 or a variant thereof;

(2) a heavy chain variable region comprising or consisting of:

an amino acid sequence set forth in SEQ ID NO: 12 or a variant thereof, and

a light chain variable region comprising or consisting of:

an amino acid sequence set forth in SEQ ID NO: 14 or a variant thereof;

(3) a heavy chain variable region comprising or consisting of:

an amino acid sequence set forth in SEQ ID NO: 16 or a variant thereof, and

a light chain variable region comprising or consisting of:

an amino acid sequence set forth in SEQ ID NO: 18 or a variant thereof; and

(4) a heavy chain variable region comprising or consisting of:

an amino acid sequence set forth in SEQ ID NO: 20 or a variant thereof, and

a light chain variable region comprising or consisting of:

an amino acid sequence set forth in SEQ ID NO: 22 or a variant thereof,

the first protein functional region of the anti-PD-1/anti-CTLA4 antibody comprises:

(1) a heavy chain variable region comprising or consisting of:

a sequence set forth in SEQ ID NO: 87 or a variant thereof, and

a light chain variable region comprising or consisting of:

a sequence set forth in SEQ ID NO: 88 or a variant thereof; and

(2) a heavy chain variable region comprising or consisting of:

a sequence set forth in SEQ ID NO: 129 or a variant thereof, and

a light chain variable region comprising or consisting of:

a sequence set forth in SEQ ID NO: 130 or a variant thereof;

the second protein functional region of the anti-PD-1/anti-CTLA4 antibody comprises:

(1) a heavy chain variable region comprising or consisting of:

a sequence set forth in SEQ ID NO: 95 or a variant thereof, and
a light chain variable region comprising or consisting of:
a sequence set forth in SEQ ID NO: 96 or a variant thereof;
(2) a heavy chain variable region comprising or consisting of:
a sequence set forth in SEQ ID NO: 133 or a variant thereof, and
a light chain variable region comprising or consisting of:
a sequence set forth in SEQ ID NO: 134 or a variant thereof;
(3) a heavy chain variable region comprising or consisting of:
a sequence set forth in SEQ ID NO: 135 or a variant thereof, and
a light chain variable region comprising or consisting of:
a sequence set forth in SEQ ID NO: 136 or a variant thereof;
(4) a heavy chain variable region comprising or consisting of:
a sequence set forth in SEQ ID NO: 137 or a variant thereof, and
a light chain variable region comprising or consisting of:
a sequence set forth in SEQ ID NO: 138 or a variant thereof; and
(5) a heavy chain variable region comprising or consisting of:
a sequence set forth in SEQ ID NO: 131 or a variant thereof, and
a light chain variable region comprising or consisting of:
a sequence set forth in SEQ ID NO: 132 or a variant thereof;
preferably, the immunoglobulin of the anti-PD-1/anti-CTLA4 antibody comprises a heavy chain having an amino acid sequence set forth in SEQ ID NO: 139, and targets PD-1;
the first protein functional region of the anti-PD-1/anti-VEGFA antibody comprises:
(1) a heavy chain variable region comprising or consisting of:
a sequence set forth in SEQ ID NO: 103 or a variant thereof, and
a light chain variable region comprising or consisting of:
a sequence set forth in SEQ ID NO: 104 or a variant thereof;
the second protein functional region of the anti-PD-1/anti-VEGFA antibody comprises:
(1) a heavy chain variable region comprising or consisting of:

a sequence set forth in SEQ ID NO: 111 (preferably encoded by a nucleotide sequence set forth in SEQ ID NO: 145) or a variant thereof, and

a light chain variable region comprising or consisting of:

a sequence set forth in SEQ ID NO: 112 (preferably encoded by a nucleotide sequence set forth in SEQ ID NO: 146) or a variant thereof;

preferably, the immunoglobulin of the anti-PD-1/anti-VEGFA antibody comprises a heavy chain having an amino acid sequence set forth in SEQ ID NO: 140, and targets VEGFA;

the anti-PD-1 monoclonal antibody comprises or consists of a heavy chain variable region set forth in SEQ ID NO: 121 and a light chain variable region set forth in SEQ ID NO: 122;

wherein the variant has at least 85%, preferably 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98% or 99% or higher sequence identity to the corresponding sequence, or comprises an amino acid sequence having one or more (preferably 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 21, 22, 23, 24, 25, 26, 27, 28, 29 or 30) conservative amino acid mutations (preferably substitutions, insertions or deletions) compared to the corresponding sequence.

4. The pharmaceutical composition according to any one of items 1–3, wherein the antibody that specifically binds to CD47 further comprises a heavy chain constant region and a light chain constant region, and the constant regions are derived from species other than murine, e.g., from a human antibody, preferably from a human IgG or IgM, and more preferably from IgG1; preferably, the heavy chain constant region is Ig gamma-1 chain C region, ACCESSION No. P01857 (SEQ ID NO: 58) or Ig gamma-4 chain C region, ACCESSION No. P01861.1 (SEQ ID NO: 56); the light chain constant region is Ig kappa chain C region, ACCESSION No. P01834 (SEQ ID NO: 57); preferably, the antibody that specifically binds to CD47 is secreted by a hybridoma cell line LT012 under CCTCC NO. 2018135.

5. The pharmaceutical composition according to any one of items 1–4, wherein, according to the EU numbering system, the antibody that specifically binds to CD47 and the immunoglobulin comprise a heavy chain constant region having mutations

at any 1, 2 or 3 of positions 234, 235 and 237, and the affinity constant of the bispecific antibody for FcγRIIIa and/or C1q is reduced after the mutation as compared to that before the mutation; preferably, the affinity constant is measured by a Fortebio Octet system;

preferably, the antibody that specifically binds to CD47 comprises an L234A and/or L235A mutation according to the EU numbering system;

more preferably, the heavy chain constant region of the immunoglobulin has one of the following combinations of mutations:

L234A and L235A; or

L234A and G237A; or

L235A and G237A; or

L234A, L235A and G237A;

still more preferably,

according to the EU numbering system, the heavy chain constant region of the immunoglobulin further has one of the combinations of the following mutations:

N297A, D265A, D270A, P238D, L328E, E233D, H268D, P271G, A330R, C226S, C229S, E233P, P331S, S267E, L328F, A330L, M252Y, S254T, T256E, N297Q, P238S, P238A, A327Q, A327G, P329A, K322A, T394D, G236R, G236A, L328R, A330S, P331S, H268A, E318A and K320A.

6. The pharmaceutical composition according to any one of items 1–5, wherein the antibody that specifically binds to CD47 comprises or consists of a heavy chain and a light chain selected from the group consisting of the following:

(1) a heavy chain set forth in SEQ ID NO: 59 and a light chain set forth in SEQ ID NO: 60;

(2) a heavy chain set forth in SEQ ID NO: 61 and a light chain set forth in SEQ ID NO: 62;

(3) a heavy chain set forth in SEQ ID NO: 63 and a light chain set forth in SEQ ID NO: 64;

(4) a heavy chain set forth in SEQ ID NO: 65 and a light chain set forth in SEQ ID NO: 66;

(5) a heavy chain set forth in SEQ ID NO: 67 and a light chain set forth in SEQ ID NO: 68; and

(6) a heavy chain set forth in SEQ ID NO: 69 and a light chain set forth in SEQ ID NO: 70;

the anti-PD-1/anti-CTLA4 antibody comprises or consists of a heavy chain and a light chain selected from the group consisting of the following:

a heavy chain set forth in SEQ ID NO: 78 (preferably encoded by a nucleotide sequence set forth in SEQ ID NO: 77) and a light chain set forth in SEQ ID NO: 80 (preferably encoded by a nucleotide sequence set forth in SEQ ID NO: 79);

the anti-PD-1/anti-VEGFA antibody comprises or consists of a heavy chain and a light chain selected from the group consisting of the following:

a heavy chain set forth in SEQ ID NO: 141 (preferably encoded by a nucleotide sequence set forth in SEQ ID NO: 142) and a light chain set forth in SEQ ID NO: 143 (preferably encoded by a nucleotide sequence set forth in SEQ ID NO: 144);

the anti-PD-1 monoclonal antibody comprises or consists of a heavy chain and a light chain selected from the group consisting of the following: a heavy chain set forth in SEQ ID NO: 82 (preferably encoded by a nucleotide sequence set forth in SEQ ID NO: 81) and a light chain set forth in SEQ ID NO: 84 (preferably encoded by a nucleotide sequence set forth in SEQ ID NO: 83).

7. The pharmaceutical composition according to any one of items 1–6, wherein the antigen-binding fragment is selected from Fab, Fab', $F(ab')_2$, Fd, Fv, dAb, Fab/c, complementarity determining region (CDR) fragment, single-chain antibody (e.g., scFv), bivalent antibody and domain antibody.

8. The pharmaceutical composition according to any one of items 1–7, wherein the antibody that specifically binds to CD47 is a humanized antibody, a chimeric antibody or a multispecific antibody (e.g., a bispecific antibody).

9. The pharmaceutical composition according to any one of items 1–8, wherein the antibody that specifically binds to CD47 binds to human CD47 protein with a KD less than about 10^{-5} M, e.g., less than about 10^{-6} M, 10^{-7} M, 10^{-8} M, 10^{-9} M, or 10^{-10} M or less, or the antibody that specifically binds to CD47 binds to human CD47

protein with an EC_{50} less than about 100 nM, e.g., less than about 10 nM, 1 nM, 0.9 nM, 0.8 nM, 0.7 nM, 0.6 nM, 0.5 nM, 0.4 nM, 0.3 nM, 0.2 nM, 0.1 nM or less.

10. The pharmaceutical composition according to any one of items 1–9, wherein the antibody that specifically binds to CD47 is in the form of an antibody conjugate comprising the antibody that specifically binds to CD47 or the antigen-binding fragment thereof according to any one of items 1–9 and a conjugated moiety conjugated thereto, wherein the conjugated moiety is a purification tag (e.g., a His tag), a cytotoxic agent or a detectable label; preferably, the conjugated moiety is a radioisotope, a luminescent substance, a colored substance, an enzyme or polyethylene glycol; or

the antibody that specifically binds to CD47 is in the form of a multispecific antibody, preferably a bispecific antibody, comprising the antibody that specifically binds to CD47 or the antigen-binding fragment thereof according to any one of items 1–9, and an antibody against another antigen and/or another antigenic epitope or an antigen-binding fragment thereof; or the antibody that specifically binds to CD47 is in the form of a fusion protein comprising the antibody that specifically binds to CD47 or the antigen-binding fragment thereof according to any one of items 1–9.

11. The pharmaceutical composition according to any one of items 1–10, wherein the anti-tumor therapeutic agent is selected from one or more of the following: a tyrosine kinase inhibitor, a DNA polymerase inhibitor, an anti-human CD20 antibody, an anti-human PDL-1 antibody, an anti-human PD-1 antibody, an anti-human CTLA-4 antibody, a BCL-2 inhibitor, an anti-human EGFR antibody, an anti-human HER2 antibody, an anti-human HER3 antibody, a cyclin-dependent kinase inhibitor, an anti-human VEGFR2 antibody, an anti-human VEGF antibody, a proteasome inhibitor, an angiogenesis inhibitor, a rapidly accelerated fibrosarcoma (RAF) inhibitor, acalabrutinib, a bispecific antibody and a fusion protein drug; preferably, the therapeutic agent is cetuximab, obinutuzumab or rituximab;

preferably, the anti-tumor chemotherapeutic is selected from one or more of the following: an alkylating agent, an anthracycline, an antimetabolite, an antibiotic, a

plant-based and/or hormonal drug, a platinum-based drug (e.g., cisplatin, carboplatin and oxaliplatin), adriamycin, cyclophosphamide, a taxane (e.g., albumin-bound paclitaxel, liposome paclitaxel and docetaxel), etoposide, gemcitabine, pemetrexed, capecitabine, olaparib, rucaparib, niraparib, talazoparib, fluzoparib, vinca alkaloid, tamoxifen, megestrol, goserelin, asparaginase, a fluorouracil antineoplastic drug, azacitidine, cytarabine and cyclocytidine.

12. The pharmaceutical composition according to any one of items 1–11, wherein the pharmaceutical composition further comprises a pharmaceutically acceptable carrier and/or excipient.

13. The pharmaceutical composition according to any one of items 1–12, wherein, based on the mass of the antibody, the component A and the component B1 are in a mass ratio of (1:5)–(5:1), e.g., 1:5, 1:4, 1:3, 1:2, 1:1, 2:1, 3:1, 4:1 or 5:1;

the component A and the component B3 are in a mass ratio of (1:5)–(5:1), e.g., 1:5, 1:4, 1:3, 1:2, 1:1, 2:1, 3:1, 4:1 or 5:1;

or

the component B2 and the component A are in a mass ratio of 1:(1–1000), preferably 1:(5–500), and more preferably 1:(10–100);

preferably, the component A and the component B in the pharmaceutical composition are in a form suitable for administration by intravenous drip infusion, subcutaneous injection, intradermal injection, intravenous injection, intramuscular injection or intralesional injection.

14. A kit, preferably for treating a tumor, comprising a product A and a product B, wherein the product A comprises the antibody that specifically binds to CD47 or the antigen-binding fragment thereof as defined in any one of items 1–13;

the product B is selected from one or more of the following: a product B1, a product B2 and a product B3, wherein the product B1 is the bispecific antibody or the antigen-binding fragment thereof as defined in any one of items 1–13 (preferably, when the bispecific antibody is a combination of an anti-PD-1/anti-CTLA4 antibody and an anti-PD-1/anti-VEGFA antibody, the anti-PD-1/anti-CTLA4 antibody and the anti-PD-1/anti-VEGFA antibody are packaged either separately or together), the

product B2 is the anti-tumor chemotherapeutic as defined in any one of items 1–13, and the product B3 is the anti-PD-1 monoclonal antibody or the antigen-binding fragment thereof as defined in any one of items 1–13, wherein the product B1, the product B2 and the product B3 are packaged either separately or together;

preferably, the kit further comprises a product C, wherein the product C is the anti-tumor therapeutic agent as defined in any one of items 1–13, and the product C is different from the product B;

preferably, the kit further comprises a package insert.

15. The kit according to item 14, wherein, based on the mass of the antibody, the product A and the product B1 are in a mass ratio of (1:5)–(5:1), e.g., 1:5, 1:4, 1:3, 1:2, 1:1, 2:1, 3:1, 4:1 or 5:1;

the product A and the product B3 are in a mass ratio of (1:5)–(5:1), e.g., 1:5, 1:4, 1:3, 1:2, 1:1, 2:1, 3:1, 4:1 or 5:1;

or

the anti-tumor chemotherapeutic is in a unit dose of 0.1–100 mg, 0.5–50 mg, 1–20 mg, 2–15 mg, 4–12 mg or 8–12 mg;

preferably, the product A, the product B or the product C in the kit is in a form suitable for administration by intravenous drip infusion, subcutaneous injection, intradermal injection, intravenous injection, intramuscular injection or intralesional injection.

16. A method for treating or preventing a tumor, comprising administering to a subject in need an effective amount of the component A and the component B as defined in any one of items 1–13, wherein preferably, the method further comprises administering to the subject in need an effective amount of the component C as defined in any one of items 1–13;

preferably, the component A, the component B and the component C are administered simultaneously or sequentially; more preferably, the component A, the component B and the component C are administered before or after a surgical treatment and/or before or after a radiotherapy;

preferably, when the component B is the component B1 and/or the component B3,

the component A or the component B is administered at a unit dose of 0.1–100 mg, preferably 1–10 mg per kg body weight of the subject; or the component A or the component B is administered at a unit dose of 10–1000 mg, preferably 50–500 mg, 100–400 mg, 150–300 mg, 150–250 mg or 200 mg in each subject;

preferably, the component B2 is in a unit dose of 0.1–100 mg, 0.5–50 mg, 1–20 mg, 2–15 mg, 4–12 mg or 8–12 mg;

preferably, the dose is administered from twice daily to about once every other day, or once every 3 days, 4 days, 5 days, 6 days, 10 days, 1 week, 2 weeks, 3 weeks, 4 weeks, 5 weeks or 6 weeks;

preferably, the route of administration is intravenous drip infusion, subcutaneous injection, intradermal injection, intravenous injection, intramuscular injection or intralesional injection.

17. The pharmaceutical composition according to any one of items 1–13, the kit according to item 14 or 15, or the method according to item 16, wherein the tumor is preferably a tumor expressing CD47, and preferably a cancer; the cancer includes a solid tumor, a hematological tumor, lymphoma, blastoma, sarcoma, leukemia or lymphoid malignancy, and more preferably includes squamous cell carcinoma, myeloma, lung cancer, small cell lung cancer, non-small cell lung cancer, head and neck squamous cell carcinoma, glioma (e.g., neuroglioma and recurrent glioma), acute myelocytic leukemia, Hodgkin's lymphoma, non-Hodgkin's lymphoma, diffuse large B-cell lymphoma, follicular lymphoma, acute lymphocytic leukemia, acute myeloblastic leukemia, chronic lymphocytic leukemia, chronic myeloblastic leukemia, primary mediastinal large B-cell lymphoma, mantle cell lymphoma, small lymphocytic lymphoma, T cell/histiocyte-rich large B-cell lymphoma, multiple myeloma, myeloid leukemia-1 protein, relapsed and refractory peripheral T-cell lymphoma, myelodysplastic syndrome, anaplastic large cell lymphoma, mantle cell lymphoma, marginal zone lymphoma, myelofibrosis, polycythemia vera, bone marrow cancer, myeloproliferative disease, aggressive systemic mastocytosis, eosinophilia, dermatofibrosarcoma protuberans, chronic eosinophilic leukemia, gastrointestinal cancer, gastric adenocarcinoma or gastroesophageal junction

adenocarcinoma, ovarian cancer, liver cancer, lymphocytic leukemia, large intestine cancer, endometrial cancer, prostate cancer, thyroid cancer, melanoma, chondrosarcoma, neuroblastoma, adenocarcinoma, pancreatic cancer, pancreatic ductal adenocarcinoma, glioblastoma multiforme, bone cancer, Ewing's sarcoma, cervical cancer, nasopharyngeal cancer, brain cancer, bladder cancer, breast cancer, triple negative breast cancer, intestinal cancer, rectal cancer, colorectal cancer, colon cancer, hepatocellular carcinoma, renal cell carcinoma, clear cell renal cell carcinoma, head and neck cancer, throat cancer, hepatobiliary cancer, central nervous system cancer, esophageal carcinoma, esophageal squamous cell carcinoma, malignant pleural mesothelioma, systemic light chain amyloidosis, lymphocytic lymphoma, myeloproliferative neoplasm, neuroendocrine tumor, Merkel cell carcinoma, testicular cancer and skin cancer, peritoneal cancer, fallopian tube cancer, urothelial cancer, microsatellite instability-high (MSI-H) or mismatch repair deficient (dMMR) cancer and mesothelioma.

18. A unit formulation, preferably for treating a tumor, comprising 1–10000 mg (preferably 10–1000 mg, and preferably 50–500 mg, 100–400 mg, 150–300 mg, 150–250 mg or 200 mg) of the component A and the component B as defined in any one of items 1–13,

wherein, when the component B is the component B1 and/or the component B3, the unit formulation comprises 1–10000 mg (preferably 1–1000 mg, and preferably 50–500 mg, 100–400 mg, 150–300 mg, 150–250 mg, 200 mg or 100 mg) of the component B as defined in any one of items 1–13;

when the component B is the component B2, the unit formulation comprises 0.1–100 mg, 0.5–50 mg, 1–20 mg, 2–15 mg, 4–12 mg, or 8–12 mg of the component B;

preferably, the unit formulation further comprises one or more of the components C as defined in any one of items 1–13;

wherein the component A, the component B and the component C are packaged separately.

19. A single dose unit, preferably for treating a tumor, comprising 0.1–10000 mg (preferably 1–1000 mg, and preferably 50–500 mg, 100–400 mg, 150–300 mg, 150–

250 mg, 200 mg or 100 mg) of the component A and the component B as defined in any one of items 1–13,

wherein, when the component B is the component B1 and/or the component B3, the single dose unit comprises 0.1–10000 mg (preferably 1–1000 mg, and preferably 50–500 mg, 100–400 mg, 150–300 mg, 150–250 mg, 200 mg or 100 mg) of the component B as defined in any one of items 1–13;

when the component B is the component B2, the single dose unit comprises 0.1–100 mg, 0.5–50 mg, 1–20 mg, 2–15 mg, 4–12 mg, or 8–12 mg of the component B.

In a specific embodiment, the anti-tumor chemotherapeutic is selected from azacitidine, an anthracycline, cytarabine, gemcitabine and cyclocytidine.

The anti-human PD-1 antibody is selected from pembrolizumab, nivolumab, cemiplimab, toripalimab, sintilimab, camrelizumab and tislelizumab.

The anti-human CD20 antibody is selected from rituximab, obinutuzumab, BCD-132, H-02, B-001, IMM-0306, ACE-1755, IMM-03, JMT-601, TXB-4BC1 and GB-4542.

The anti-human EGFR antibody is selected from cetuximab, panitumumab, necitumumab, nepidermin, nimotuzumab, amivantamab, HS-627, amelimumab, depatuxizumab, FmAb-2, GC-1118A, imgatuzumab, MVC-101, SCT-200, QL-1203, tomuzotuximab, JMT-101, MCLA-158, QL-1105, SYN-004, MCLA-129, WBP-297, AM-105, BH-2922, BMX-002, CMAB-017, DF-203, GB-263, JZB-29, SAH-EJ1, SFR-9X0122, UBP-1215, ABX-901, MCLA-125, TXB-4BC2, 111-In-ch806, depatuxizumab mafodotin, DR-50201, DXL-1218, ENLS-1, FS-101 and GI-3000.

The BCL-2 inhibitor is selected from venetoclax.

The anti-HER2 or anti-HER3 antibody is selected from trastuzumab, trastuzumab emtansine, trastuzumab deruxtecan, margetuximab, pertuzumab, disitamab vedotin, U-31402, ISU-104, SIB-001, 9F7-F11, EV-20Sap, U-31402, ARX-788, BAT-8001, HL-02, TAA-013, trastuzumab duocarmazine, A-166, AU-101, AU-105, BPX-603, ISB-1302, KN-026, MB-103, MRG-002, zanidatamab, zenocutuzumab, ACE-1702, ALTP-7, B-002, BAT-8001, BAT-1006, BAY-2701439, BTRC-4017A, CAMH-2,

cinrebafusp alfa, CT-0508, DP-303c, DX-126262, FS-102, FS-1502, GQ-1001, HS-630, LCB-14, M-802, MBS-301, MT-5111, NJH-395, PF-06804103, SBT-6050, SENL-006, SHRA-1201, SHRA-1811, TT-16, ZW-49 and LZM-006.

The anti-human VERFR2 antibody is selected from ramucirumab, AK109, VXM-01, AVI-3207, gentuximab, KD-035 and JY-025.

The taxane is selected from paclitaxel, albumin-bound paclitaxel, liposome paclitaxel and docetaxel.

The cyclin-dependent kinase inhibitor is selected from palbociclib, seliciclib, milciclib, lerociclib, abemaciclib, ebvaciclib, ribociclib succinate, trilaciclib, SHR-6390, alvocidib hydrochloride, AT-7519, AZD-4573, BEY-1107, BPI-1178, CT-7001, FCN-437c, FIT-039, NUV-422, PF-07104091, XZP-3287, zotiraciclib citrate, AGM-130, AUR-102, fadraciclib, LY-3405105, HS-10342, ON-123300, QHRD-107, TQ-05510, voruciclib, JS-101, XH-30002, AZ-5576, ETH-155008, JS-104 and RMC-4550.

The proteasome inhibitor is selected from bortezomib, carfilzomib and ixazomib.

The tyrosine kinase inhibitor is selected from anlotinib, acalabrutinib, brigatinib, alectinib hydrochloride, ibrutinib, radotinib dihydrochloride, bosutinib monohydrate, ponatinib hydrochloride, crizotinib, ruxolitinib phosphate, nilotinib hydrochloride hydrate, dasatinib hydrate and imatinib mesylate.

The angiogenesis inhibitor antibody drug is selected from bevacizumab, ramucirumab and ranibizumab.

The rapidly accelerated fibrosarcoma (RAF) inhibitor antibody drug is selected from LY3009120, dabrafenib, vemurafenib and sorafenib.

The hormonal inhibitor is selected from dexamethasone, tamoxifen, toremifen, flutamide, nilutamidt, leuprolide, goserelin, buserelin, aminoglutethimide and formesfane.

The anti-human PD-1 antibody is selected from pembrolizumab, nivolumab, cemiplimab, toripalimab, sintilimab, camrelizumab, tislelizumab and penpulimab.

The anti-human PDL-1 antibody is selected from atezolizumab, avelumab, durvalumab and AK105.

The anti-human CTLA-4 antibody is selected from ZW25, ipilimumab and tremelimumab.

The anti-human PD-1/CTLA-4 bispecific antibody is selected from cadonilimab, SI-B003, QL1706, XmAb-20717, KN046, MGD019 and MEDI5752.

The anti-human PD-1/VEGFA bispecific antibody is selected from AK112 (i.e., VP101(hG1DM)).

The platinum-based drug is selected from cisplatin (DDP), carboplatin (CBP) and oxaliplatin (L-OHP).

The anti-human PDL-1 antibody is selected from avelumab, atezolizumab, durvalumab, JS-003, CS-100L, LY-3300054›KD-033, CK-301, CCX-4503, CX-072, KN-035, HRP00052, HRP00049, FAZ-053, GR-1405, KD-005, HLX-20, KL-Al67, CBT-502, STI-A1014, REMD-290, BGB-A333, BCD-135 and MCLA-145O.

The fusion protein drug is selected from aflibercept, AVID200, trebananib and M7824.

In one embodiment of the present invention, the drug is in a form suitable for injection, preferably in a form suitable for administration by subcutaneous injection, intradermal injection, intravenous injection, intramuscular injection or intralesional injection.

In the present invention, unless otherwise defined, the scientific and technical terms used herein have the meanings generally understood by those skilled in the art. In addition, the laboratory operations of cell culture, molecular genetics, nucleic acid chemistry and immunology used in the present invention are the routine operations widely used in the corresponding fields. Meanwhile, in order to better understand the present invention, the definitions and explanations of the relevant terms are provided below.

As used herein, the term "antigen-binding region" means a protein or a portion of a protein that specifically binds to a given antigen. For example, a portion of an antibody comprising amino acid residues that interact with an antigen and confer the antibody the specificity and affinity for the antigen is referred to as an

"antigen-binding region". The antigen-binding region generally comprises one or more "complementarity determining regions" (CDRs). Certain antigen-binding regions further comprise one or more "framework" regions (FRs). CDRs are amino acid sequences that contribute to antigen-binding specificity and affinity.

As used herein, the term "antibody" refers to an intact immunoglobulin of any isotype or an antigen-binding fragment thereof that can compete with an intact antibody for specifically binding to a target antigen, and includes, for example, chimeric, humanized, fully humanized and bispecific antibodies or antigen-binding fragments thereof. Such "antibodies" are antigen-binding proteins. An intact antibody generally comprises at least two full-length heavy chains and two full-length light chains, but, in some cases, may comprise fewer chains, such as an antibody naturally existing in camelids that may comprise only a heavy chain. An antibody or an antigen-binding fragment thereof may be derived from a single source only, or may be "chimeric", i.e., different portions of the antibody may be derived from two different sources as further described below. An antibody or an antigen-binding fragment thereof may be produced in hybridomas by recombinant DNA technology, or by enzymatic or chemical cleavage of intact antibodies. Unless otherwise indicated, the term "antibody", in addition to antibodies comprising two full-length heavy chains and two full-length light chains, also includes derivatives, variants and fragments thereof.

As used herein, the term "antigen-binding fragment" (or abbreviated as "fragment") of an "antibody" or an "immunoglobulin chain" (heavy or light chain) comprises part of an antibody (whether obtained or synthesized) that lacks at least some of the amino acids present in the full length of the antibody but is capable of specifically binding to the antigen. Such fragments are biologically active as they specifically bind to a target antigen and can compete with other antibodies or antigen-binding fragments thereof for specifically binding to a given epitope. In one aspect, such fragments will retain at least one CDR present in the full-length light or heavy chain of the antibody, and in some embodiments, will comprise a single heavy and/or light chain or a portion thereof. Such biologically active fragments can be produced by

recombinant DNA technology, or, for example, by enzymatic or chemical cleavage of intact antibodies. Immunologically functional immunoglobulin fragments include, but are not limited to, Fab, Fab', $F(ab')_2$, Fab/c, dAb, Fv, domain antibodies and single-chain antibodies, and can be derived from any mammalian source, including, but not limited to, human, mouse, rat, camelid and rabbit. It is further contemplated that a functional portion of an antibody disclosed herein, such as one or more CDRs, can be covalently bound to a second protein or a micromolecule to generate a therapeutic agent directed to a particular target in the body, thereby having bifunctional therapeutic properties or having an extended serum half-life, such as a fusion protein.

As used herein, the terms "antibody full-length chain", "full-length antibody", "intact antibody" and "whole antibody" are used interchangeably herein to refer to an antibody having a substantially similar structure to a natural antibody structure or having heavy chains in the Fc region as defined herein.

The term "light chain" includes full-length light chains and fragments thereof with sufficient variable region sequences to confer the binding specificity. The full-length light chain comprises a variable region domain V_L and a constant region domain C_L. The variable region domain of the light chain is at the amino terminus of the polypeptide. Light chains include kappa (κ) and lambda (λ) chains.

The term "heavy chain" includes full-length heavy chains and fragments thereof with sufficient variable region sequences to confer the binding specificity. The full-length heavy chain includes a variable region domain V_H and 3 constant region domains C_{H1}, C_{H2} and C_{H3}. The V_H domain is at the amino terminus of the polypeptide, and the C_H domains are at the carboxyl terminus, the C_{H3} being closest to the carboxyl terminus of the polypeptide. The heavy chain may be of any isotype, including IgG (including IgG1, IgG2, IgG3 and IgG4 subtypes), IgA (including IgA1 and IgA2 subtypes), IgM and IgE.

As used herein, the term "Fab fragment" consists of one light chain, C_{H1} and the variable region of one heavy chain. The heavy chain of an Fab molecule cannot form disulfide bonds with another heavy chain molecule.

As used herein, the term "Fc" region comprises two heavy chain fragments comprising the C_{H1} and C_{H2} domains of an antibody. The two heavy chain fragments are held together by two or more disulfide bonds and by the hydrophobic interaction of the C_{H3} domains.

As used herein, the term "Fab' fragment" comprises one light chain and part of one heavy chain (containing the V_H domain, the C_{H1} domain, and part of the region between the C_{H1} and C_{H2} domains), such that interchain disulfide bonds can be formed between the two heavy chains of two Fab' fragments to form an $F(ab')_2$ molecule.

As used herein, the term "$F(ab')_2$ fragment" comprises two light chains and two heavy chains containing part of the constant region between the C_{H1} and C_{H2} domains, such that interchain disulfide bonds are formed between the two heavy chains. Thus, the $F(ab')_2$ fragment consists of two Fab' fragments held together by disulfide bonds between the two heavy chains.

As used herein, the term "Fv region" comprises the variable regions from the heavy and light chains, but lacks the constant regions.

As used herein, the term "Fd" fragment refers to an antibody fragment consisting of V_H and C_{H1} domains (Ward et al., *Nature*, 341:544-546 (1989)).

As used herein, the term "dAb" fragment consists of V_H domains (Ward et al., *Nature* 341:544-546 (1989)).

As used herein, the term "Fab'-SH" is the designation herein for Fab', wherein one or more cysteine residues of the constant domain carry a free thiol group.

As used herein, the term "Fab/c" fragment is an intermediate formed by pepsin digestion of an immunoglobulin, and combines the advantages of Fab and Fc regions, i.e., strong diffusibility and low metabolic clearance *in vivo*, while retaining high affinity (Liu Jianjun, *Chinese Journal of Cellular and Molecular Immunology*, 1989(4):29-29).

As used herein, the term "single-chain antibody" is an Fv molecule in which the heavy and light chain variable regions are connected by a flexible linker to form a single polypeptide chain (which forms an antigen-binding region) (see, e.g., Bird et

al., *Science*, 242:423-426 (1988), and Huston et al., *Proc. Natl. Acad. Sci. USA.*, 90:5879-5883 (1988)). Single-chain antibodies are described in detail in International Patent Publication No. WO 88/01649 and U.S. Patent Nos. 4,946,778 and 5,260,203, the disclosures of which are incorporated herein by reference.

As used herein, the term "domain antibody" is an immunofunctional immunoglobulin fragment that comprises only the variable region of the heavy chain or the light chain, including multivalent domain antibodies or bivalent domain antibodies. In some cases, two or more V_H regions are covalently linked by a peptide linker to generate a multivalent domain antibody (particularly a bivalent domain antibody). The two V_H regions of the bivalent domain antibody may target the same or different antigens.

As used herein, the term "bivalent antigen-binding protein" or "bivalent antibody" comprises two antigen-binding sites. In some cases, the two binding sites have the same antigen specificity. The bivalent antibody may be bispecific.

As used herein, the term "multispecific antigen-binding protein" or "multispecific antibody" is an antigen-binding protein or antibody that targets more than one antigen or epitope.

As used herein, the term "bispecific", "dual-specificity" or "bifunctional" antigen-binding protein or antibody is a hybrid antigen-binding protein or an antibody having two different antigen-binding sites. A bispecific antibody is a multispecific antigen-binding protein or a multispecific antibody, and can be produced by a variety of methods, including but not limited to, fusion of hybridomas or linkage of Fab' fragments. See, e.g., Songsivilai and Lachmann, 1990, *Clin. Exp. Immunol.*, 79:315-321; Kostelny et al., 1992, *J. Immunol.*, 148:1547-1553. The two binding sites of a bispecific antigen-binding protein or antibody will bind to two different epitopes present in the same or different protein targets.

As used herein, the terms "mAb" and "monoclonal antibody" refer to an antibody or a fragment of an antibody that is derived from a group of highly homologous antibodies, i.e., from a group of identical antibody molecules except for natural

mutations that may occur spontaneously. The monoclonal antibody is highly specific for a single epitope on an antigen. The polyclonal antibody, relative to the monoclonal antibody, generally comprises at least 2 or more different antibodies which generally recognize different epitopes on an antigen. Monoclonal antibodies can generally be obtained using the hybridoma technique first reported by Kohler et al. (*Nature*, 256:495, 1975), but can also be obtained using recombinant DNA technology (see, e.g., U.S. Patent No. 4,816,567).

As used herein, the term "humanized antibody" refers to an antibody or an antibody fragment obtained when all or part of the CDR regions of a human immunoglobulin (receptor antibody) are replaced by the CDR regions of a non-human antibody (donor antibody), wherein the donor antibody may be a non-human (e.g., mouse, rat or rabbit) antibody having expected specificity, affinity or reactivity. In addition, some amino acid residues in the framework regions (FRs) of the receptor antibody can also be replaced by the amino acid residues of corresponding non-human antibodies or by the amino acid residues of other antibodies to further improve or optimize the performance of the antibody. For more details on humanized antibodies, see, for example, Jones et al., *Nature*, 321:522–525 (1986); Reichmann et al., *Nature*, 332:323–329 (1988); Presta, *Curr. Op. Struct. Biol.*, 2:593–596 (1992); and Clark, *Immunol. Today*, 21: 397–402 (2000).

As used herein, the term "epitope" refers to a site on an antigen to which an immunoglobulin or an antibody specifically binds. "Epitope" is also referred to in the art as an "antigenic determinant". The epitope or antigenic determinant generally consists of chemically active surface groups of molecules such as amino acids, carbohydrates or sugar side chains, and usually has specific three-dimensional structural characteristics and specific charge characteristics. For example, the epitope generally comprises at least 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14 or 15 consecutive or non-consecutive amino acids in a unique spatial conformation, which can be "linear" or "conformational". See, e.g., *Epitope Mapping Protocols* in *Methods in Molecular Biology*, Vol. 66, G. E. Morris, Ed. (1996). In a linear epitope, all interaction sites between a protein and an interaction molecule (e.g., an

antibody) are located linearly along the primary amino acid sequence of the protein. In a conformational epitope, the interaction sites are located across amino acid residues of a protein that are separated from each other.

The terms "polypeptide" or "protein" are used interchangeably herein to refer to a polymer of amino acid residues. The term is also used to refer to an amino acid polymer in which one or more amino acid residues are analogs or mimetics of naturally existing amino acids, and to naturally existing amino acid polymers. The term may also include, for example, amino acid polymers that have been modified by addition of saccharide residues to form glycoproteins, or have been phosphorylated. Polypeptides and proteins can be produced by naturally existing cells and non-recombinant cells, or they may be produced by genetically engineered or recombinant cells, and comprise a molecule having the amino acid sequence of a native protein or a molecule having deletions, insertions and/or substitutions in one or more amino acids of the native sequence.

In some embodiments, the terms "polypeptide" and "protein" particularly include antibodies, such as anti-human CD47 antibodies (also referred to as CD47 antibodies), CD47-binding proteins, or variants thereof, e.g., antibodies or sequences having deletions, insertions, and/or substitutions of one or more amino acids.

The term "polypeptide fragment" refers to a polypeptide having an amino-terminal deletion, a carboxyl-terminal deletion, and/or an internal deletion as compared to a full-length protein. Such fragments may also comprise modified amino acids as compared to the full-length protein. In certain embodiments, such fragments are about 5 to 500 amino acids in length. For example, the fragment can be at least 5, 6, 8, 10, 14, 20, 50, 70, 100, 110, 150, 200, 250, 300, 350, 400 or 450 amino acids in length. Useful polypeptide fragments include immunologically functional fragments of antibodies, including binding domains. In the case of human CD47 antibodies, useful fragments include but are not limited to CDR regions, variable domains of heavy or light chains, parts of antibody chains, variable domains exactly comprising 2 CDRs, or the like.

A "derivative" of a polypeptide is a polypeptide (e.g., an antigen-binding protein or an antibody) that is chemically modified in other manners than insertion, deletion or substitution, e.g., by conjugation with another chemical moiety, e.g., a PEG-conjugated polypeptide.

In the present invention, letters before the position number represent amino acids before mutation, and letters after the position number represent amino acids after mutation, unless otherwise specified.

In some embodiments of the present invention, a disulfide bond is present between the VH and VL of the single-chain antibody. Methods for introducing a disulfide bond between the VH and VL of an antibody are well known in the art, see, for example, US 5,747,654; Rajagopal et al., *Prot. Engin.* 10(1997)1453-1459; Reiter et al., *Nat. Biotechnol.* 14(1996)1239-1245; Reiter et al., *Protein Engineering* 8(1995)1323-1331; Webber et al., *Molecular Immunology* 32(1995)249-258; Reiter et al., *Immunity* 2(1995)281-287; Reiter et al., *JBC* 269(1994)18327-18331; Reiter et al., *Inter. J. of Cancer* 58(1994)142-149; or Reiter et al., *Cancer Res.* 54(1994)2714-2718, which are incorporated herein by reference.

As used herein, the term "specifically bind" refers to a non-random binding reaction between two molecules, such as a reaction between an antibody and an antigen it targets. In some embodiments, an antibody that specifically binds to an antigen (or an antibody that is specific for an antigen) means that the antibody binds to the antigen with an affinity (K_D) less than about 10^{-5} M, such as less than about 10^{-6} M, 10^{-7} M, 10^{-8} M, 10^{-9} M or 10^{-10} M or less.

As used herein, the term "K_D" refers to a dissociation equilibrium constant for a specific antibody-antigen interaction, which is used to describe the binding affinity between the antibody and the antigen. Among several parameters measured by molecular binding kinetics, the K_D value is the dissociation equilibrium constant. In antibody drug research, it is the parameter characterizing the intensity of the affinity between an antibody of interest and the target antigen molecule, and is calculated by the formula: $KD = k_{dis}/k_{on}$. A smaller equilibrium dissociation constant indicates a more intensive antibody-antigen binding and a higher affinity between the antibody

and the antigen. k_{on} (association rate constant) is the rate of antigen-antibody complex formation, and a smaller k_{on} suggests a faster binding of an antibody to an antigen. k_{dis} (dissociation rate constant) is the rate at which an antibody dissociates from an antigen-antibody complex, and a smaller k_{dis} suggests a slower rate for the antibody dissociating from the antigen and a firmer binding between the antibody and the antigen. Generally, an antibody binds to an antigen (e.g., L1 protein) with a dissociation equilibrium constant (K_D) of less than about 10^{-5} M, e.g., less than about 10^{-6} M, 10^{-7} M, 10^{-8} M, 10^{-9} M or 10^{-10} M or less, for example, as measured by surface plasmon resonance (SPR) on a BIACORE system or by a Fortebio system.

As used herein, the terms "monoclonal antibody" and "McAb" have the same meaning and can be used interchangeably; the terms "polyclonal antibody" and "PcAb" have the same meaning and can be used interchangeably; the terms "polypeptide" and "protein" have the same meaning and can be used interchangeably. Besides, as used herein, amino acids are generally represented by single-letter and three-letter abbreviations known in the art. For example, alanine can be represented by A or Ala.

As used herein, the terms "hybridoma" and "hybridoma cell line" can be used interchangeably, and when referring to the terms "hybridoma" and "hybridoma cell line", subclones and progeny cells of the hybridoma are also included.

As used herein, the terms "percent sequence identity" and "percent sequence homology" are used interchangeably.

As used herein, the terms "similarity", "sequence similarity" and "identity" refer to the correlation between the sequences of two or more protein or polypeptide molecules, as determined by aligning and comparing the sequences. "Percent identity" refers to the percentage of identical amino acid residues in the molecules compared, and can be calculated based on the size of the smallest molecule to be compared. For such calculations, gaps in the alignment (if any) must be addressed by a particular mathematical model or computer program (i.e., an "algorithm"). The term "substantial identity", when used for polypeptides, means that two peptide sequences, when optimally aligned, for example using the programs GAP or

BESTFIT, using default gap weights provided by the programs, have at least 70%, 75% or 80% sequence identity, at least 90% or 95% sequence identity, or at least 97%, 98% or 99% sequence identity. In some cases, residue positions that are not identical differ in conservative amino acid substitutions. "Conservative amino acid substitution" is one in which the amino acid residue is substituted with another amino acid residue having a side chain R group that possesses similar chemical properties (e.g., charge or hydrophilicity). Generally, conservative amino acid substitutions will substantially retain the functions and properties of the protein. In cases where two or more amino acid sequences differ from each other by conservative substitutions, the percent sequence identity may be elevated to correct for the conservative nature of the substitution. Methods for making this adjustment are well known to those skilled in the art. See, e.g., Pearson, *Methods Mol. Biol.*, 243:307-31 (1994). Examples of groups of amino acids having side chains with similar chemical properties include: 1) aliphatic hydroxyl side chain: glycine, alanine, valine, leucine and isoleucine, 2) aliphatic hydroxyl side chain: serine and threonine, 3) amide-containing side chain: asparagine and glutamine, 4) aromatic side chain: phenylalanine, tyrosine and tryptophan, 5) basic side chain: lysine, arginine and histidine, 6) acidic side chain: aspartic acid and glutamic acid, and 7) sulfur-containing side chain: cysteine and methionine. For example, the conservative amino acid substitution groups are valine-leucine-isoleucine-glycine-alanine, phenylalanine-tyrosine, threonine-serine, lysine-arginine, glutamic acid-aspartic acid and asparagine-glutamine.

Optionally, a conservative substitution is any change with a positive value in the PAM250 log-likelihood matrix disclosed in Gonnet et al., *Science*, 256:1443-45 (1992), which is incorporated herein by reference. A "moderately conservative" substitution is any change with a non-negative value in the PAM250 log-likelihood matrix.

The sequence identity of polypeptides is usually measured by a sequence analysis software. Protein analysis software aligns sequences using a measure of similarity assigned to different substitutions, deletions and other modifications (including

conservative amino acid substitutions). For example, GCG, including programs such as "Gap" and "Bestfit", (using default parameters specified by the program) can be used to determine sequence homology or sequence identity between closely related polypeptides (e.g., homologous polypeptides from different biological species) or between a wild-type protein and its mutant protein. See, e.g., GCG Version 6.1 (University of Wisconsin, WI). Polypeptide sequences can also be compared using FASTA with default or recommended parameters. See GCG Version 6.10 FASTA (e.g., FASTA2 and FASTA3), which provides alignments for regions of optimal overlap between the challenge and query sequences and percent sequence identities (Pearson, *Methods Enzymol.*, 183:63-98 (1990); Pearson, *Methods Mol. Biol.*, 132:185-219 (2000)). Another preferred algorithm when comparing sequences with a database containing massive sequences from different organisms is the computer program BLAST, in particular, blastp or tblastn (using default parameters provided by the program). See, e.g., Altschul et al., *Mol. Biol.*, 215:403-410 (1990); Al tschul et al., *Nucleic Acids Res.*, 25:3389-402 (1997).

As used herein, the term "pharmaceutically acceptable carrier and/or excipient" refers to a carrier and/or excipient that is pharmacologically and/or physiologically compatible with the subject and the active ingredient. Such carriers and/or excipients are well known in the art (see, e.g., *Remington's Pharmaceutical Sciences*, edited by Gennaro AR, 19th Ed., Pennsylvania, Mack Publishing Company, 1995), including but not limited to: pH regulators, surfactants, adjuvants and ionic strength enhancers. For example, the pH regulators include, but are not limited to, phosphate buffer; the surfactants include, but are not limited to, cationic, anionic or non-ionic surfactants, such as Tween-80; the ionic strength enhancers include, but are not limited to, sodium chloride.

As used herein, the term "effective amount" refers to an amount sufficient to obtain or at least partially obtain a desired effect. For example, a prophylactically effective amount against a disease (e.g., a tumor) refers to an amount sufficient to prevent, stop or delay the onset of the disease (e.g., a tumor); a therapeutically effective amount refers to an amount sufficient to cure or at least partially stop a disease and

complications thereof in patients suffering from the disease.

The term "single dose unit" means a single pharmaceutical dosage form, such as an injection, e.g., packaged in an ampoule, comprising the anti-CD47 antibody and the combined therapeutic agents (e.g., the bispecific antibody, the anti-PD-1 monoclonal antibody and/or the anti-tumor chemotherapeutic) according to the present invention to be administered to a subject at time points of a regimen, preferably per kg body weight of the subject. In specific embodiments of the present invention, the regimen comprises, for example, administrations of the single dose unit according to a treatment cycle of from twice daily to about once every other day, or once every 3 days, 4 days, 5 days, 6 days, 10 days, 1 week, 2 weeks, 3 weeks, 4 weeks, 5 weeks or 6 weeks.

In the present invention, the terms "first" (e.g., first protein functional region or first linker fragment) and "second" (e.g., second protein functional region or second linker fragment) are used for distinguishing or clarity in expression and do not carry typical sequential meanings, unless otherwise specified.

A "therapeutically effective amount" or "therapeutically effective dose" of a drug or therapeutic agent is any amount of a drug that, when used alone or in combination with another therapeutic agent, protects a subject from the onset of a disease or promotes disease regression as evidenced by the reduction in the severity of disease symptoms, increase in the frequency and duration of disease symptom-free periods, or the prevention of damage or disability caused by the affliction of the disease. The ability of a therapeutic agent to promote disease regression can be evaluated using a variety of methods known to skilled practitioners, such as in a human subject in clinical trials, in an animal model system that predicts the efficacy in humans, or by determining the activity of the drug in an *in vitro* assay.

The "prophylactically effective amount" of a drug refers to any amount of a drug that inhibits the occurrence or recurrence of cancer when administered, alone or in combination with an antineoplastic agent, to a subject at risk of developing cancer (e.g., a subject having a premalignant condition) or a subject at risk of recurrence of cancer. In some embodiments, the prophylactically effective amount completely

prevents the occurrence or recurrence of cancer. "Inhibiting" the occurrence or recurrence of cancer means reducing the possibility of the occurrence or recurrence of cancer or completely preventing the occurrence or recurrence of cancer.

Compared with the prior art, the present invention has the following advantages:

The anti-CD47 monoclonal antibody involved in the present invention can effectively block the binding of SIRPα to CD47 by specifically binding to CD47, thereby promoting the phagocytosis of tumor cells by macrophages, and can be used in combination with immune checkpoint inhibitors or other drugs, showing more excellent anti-tumor activity.

BRIEF DESCRIPTION OF THE DRAWINGS

FIG. 1 shows the phagocytic index of HPMMs on Raji cells mediated by 6F7H1L1(hG4) in combination with rituximab.

FIG. 2 shows the phagocytic index of HPMMs on HL-60 cells mediated by 6F7H1L1(hG4) in combination with azacitidine.

FIG. 3 shows the activation of immune cells promoted by 6F7H1L1(hG4) in combination with PD-1/CTLA-4 bispecific antibody.

FIG. 4 shows the immune response of immune cells against human lung adenocarcinoma A549 cells promoted by 6F7H1L1(hG4) in combination with a PD-1 antibody.

FIG. 5 shows the immune response of immune cells against human ovarian cancer SK-OV-3 cells promoted by 6F7H1L1(hG4) in combination with a PD-1 antibody.

FIG. 6 shows the immune response of immune cells against human prostate cancer LNCAP cells promoted by 6F7H1L1(hG4) in combination with a PD-1 antibody.

FIG. 7 shows the phagocytic index of HPMMs on HT-29 cells mediated by 6F7H1L1(hG4) in combination with cetuximab.

FIG. 8 shows the phagocytic index of HPMMs on Raji cells mediated by 6F7H1L1(hG4) in combination with obinutuzumab.

FIG. 9 shows the efficacy of 6F7H1L1(hG4) in combination with anti-PD-1/anti-CTLA-4 bispecific antibody cadonilimab on a

BALB/c-hPD1/hSIRPα mouse CT26-hCD47 tumor model.

FIG. 10 shows the body weight changes in the BALB/c-hPD1/hSIRPα mouse CT26-hCD47 tumor model receiving 6F7H1L1(hG4) in combination with anti-PD-1/anti-CTLA-4 bispecific antibody cadonilimab.

FIG. 11 shows the efficacy of 6F7H1L1(hG4) in combination with anti-PD-1/anti-VEGFA bispecific antibody VP101(hG1DM) on an SCID Beige mouse MDA-MB-231 tumor model.

FIG. 12 shows the body weight changes in the SCID Beige mouse MDA-MB-231 tumor model receiving 6F7H1L1(hG4) in combination with anti-PD-1/anti-VEGFA bispecific antibody VP101(hG1DM).

Notes on the deposit of biological materials:

Hybridoma cell line LT012 was deposited at China Center for Type Culture Collection (CCTCC) on Jun. 21, 2018 under CCTCC NO. C2018135, the depository address being Wuhan University, Wuhan, China, postal code: 430072.

DETAILED DESCRIPTION

The embodiments of the present invention will be described in detail below with reference to the examples. Those skilled in the art will appreciate that the following examples are only for illustrating the present invention, and should not be construed as limitations to the scope of the present invention. Examples where the specific technologies or conditions are not specified are performed according to the technologies or conditions described in the publications of the art (e.g., see, *Guide to Molecular Cloning Experiments*, authored by J. Sambrook et al., and translated by Huang Peitang et al., third edition, Science Press) or according to the product instruction. Reagents or instruments used are commercially available conventional products if the manufacturers thereof are not specified.

In the following examples of the present invention, BALB/C mice were purchased from Guangdong Medical Laboratory Animal Center.

In the following examples of the present invention, the isotype control antibodies

used, i.e., hIgG1 and hIgG4, were antibodies targeting human anti-hen egg lysozyme (HEL), and the variable region sequences of these antibodies were from the study reported by Acierno et al., entitled "Affinity maturation increases the stability and plasticity of the Fv domain of anti-protein antibodies" (Acierno et al., *J Mol biol.*, 2007; 374(1): 130–46, wherein the sequence of the heavy chain variable region is set forth in SEQ ID NO: 75, and the sequence of the light chain variable region is set forth in SEQ ID NO: 76). In the constant region fragment of hIgG1, the heavy chain constant region is Ig gamma-1 chain C region, ACCESSION No. P01857, and the light chain constant region is Ig kappa chain C region, ACCESSION No. P01834. In hIgG4, the heavy chain constant region is Ig gamma-4 chain C region, ACCESSION No. P01861.1 with S228P mutation introduced to improve the stability, and the light chain constant region is Ig kappa chain C region, ACCESSION No. P01834. Both hIgG1 and hIgG4 were prepared by Akeso Biopharma, Inc.

In the following examples of the present invention, the peripheral blood mononuclear cells (PBMCs) used were isolated and prepared by Akeso Biopharma, Inc. PBMCs were isolated from healthy human peripheral blood according to the manual of Ficoll-PaqueTM Plus reagent, and the isolated PBMCs were counted and frozen. The healthy human peripheral blood and PBMCs were isolated and extracted with the informed consent of the donors.

In the following examples of the present invention, the human peripheral monocyte-derived macrophages (HPMMs) used were derived from the PBMCs.

In the following examples of the present invention, the light and heavy chain sequences of anti-PD-1/CTLA-4 bispecific antibody cadonilimab, i.e., CP004(hG1TM) antibody, were derived from the sequences listed in *WHO Drug Information*, Proposed INN: List 124 (*WHO Drug Information*, 2020; 34(4):947–949, wherein the heavy chain sequence is set forth in SEQ ID NO: 78, and its coding nucleotide sequence is set forth in SEQ ID NO: 77; the light chain sequence is set forth in SEQ ID NO: 80, and its coding nucleotide sequence is set forth in SEQ ID NO: 79). The light and heavy chain sequences of anti-PD-1 antibody

penpulimab were derived from the sequences listed in *WHO Drug Information*, Proposed INN: List 123 (*WHO Drug Information*, 2020; 34(2): 375–376, wherein the heavy chain sequence is set forth in SEQ ID NO: 82, and its coding nucleotide sequence is set forth in SEQ ID NO: 81; the light chain sequence is set forth in SEQ ID NO: 84, and its coding nucleotide sequence is set forth in SEQ ID NO: 83). The heavy chain sequence and the light chain sequence of the anti-PD-1/anti-VEGFA antibody are set forth in SEQ ID NO: 141 (encoded by a nucleotide sequence set forth in SEQ ID NO: 142) and SEQ ID NO: 143 (encoded by a nucleotide sequence set forth in SEQ ID NO: 144), respectively.

The method for preparing cadonilimab, anti-PD-1/anti-VEGFA antibody and penpulimab are as follows. The DNA sequence of the heavy chain-coding gene (including the constant region and the variable region) and the DNA sequence of the light chain-coding gene (including the constant region and the variable region) of the candidate antibodies were separately cloned into pUC57simple (supplied by GenScript) vectors to obtain plasmids pUC57simple-H and pUC57simple-L, respectively. The above plasmids were enzymatically digested (HindIII&EcoRI), and the heavy and light chains isolated by electrophoresis were separately subcloned into expression vector pcDNA3.1 (purchased from Invitrogen). The recombinant plasmids were extracted, and a combination of pcDNA3.1-H + pcDNA3.1-L was co-transfected into 293F cells. After 7 days of cell culture, the culture was centrifuged at high speed. The supernatant was concentrated, loaded onto a HiTrap MabSelect SuRe column, and purified using a protein purification liquid chromatography system (AKTA Purifier 10, GE). The purified sample, i.e., antibody cadonilimab, was added to both a reduced protein electrophoresis loading buffer and a non-reduced protein electrophoresis loading buffer, and then boiled for SDS-PAGE electrophoresis. By screening and confirming whether the protein conforms to the theoretical size, a purified antibody was obtained.

Example 1: Preparation of anti-human CD47 antibody 6F7

1. Preparation of hybridoma cell line 6F7

The antigen used for preparing the anti-CD47 antibody with the hybridoma cell line 6F7 was CD47 IgV TEV-His (including human CD47 mature peptide of positions 19–141 of GenbankID: NP 942088.1 and TEV (amino acid sequence: ENLYFQG, SEQ ID NO: 74)-his tag fusion protein, synthesized by Akeso Biopharma, Inc.) and 3T3-CD47 cells (NIH/3T3, manufacturer: ATCC, Cat. No: CRL-1658; the human CD47 mature peptide was transfected into the cells on the basis of NIH/3T3 to construct a cell line stably expressing 3T3-CD47). Spleen cells of immunized mice were fused with myeloma cells of the mice to prepare hybridoma cells. With CD47 IgV TEV-His and 3T3-CD47 cells separately taken as antigens, the hybridoma cells were screened by indirect ELISA to obtain hybridoma cells capable of secreting antibodies capable of specifically binding to CD47. The hybridoma cells obtained by ELISA screening were screened by competitive ELISA to obtain a hybridoma cell line capable of secreting a monoclonal antibody capable of competing for binding to CD47 IgV TEV-His with the receptor human SIRPαECD-hFc-Biotin (SIRPαECD refers to the extracellular region of SIRPα, positions 31–373 of protein GenBank ACCESSION No. NP_542970.1; hFc refers to a human IgG Fc purification label, specifically to Ig gamma-1 chain C region, positions 114–330 of GenbankID: P01857), which was then subjected to dilution cloning to obtain a stable hybridoma cell line. The hybridoma cell line was designated hybridoma cell line LT012, and the monoclonal antibody secreted by the cell line was designated 6F7.

Hybridoma cell line LT012 (CD47-6F7) was deposited at China Center for Type Culture Collection (CCTCC) on Jun. 21, 2018 under CCTCC NO. C2018135, the depository address being Wuhan University, Wuhan, China, postal code: 430072.

2. Preparation of anti-CD47 antibody 6F7

The cell lines LT011, LT012 and LT015 prepared above were separately cultured with a chemical defined medium (CD medium; containing 1% penicillin-streptomycin) in a 5% CO_2, 37 °C incubator. After 7 days, the supernatants were collected and purified by high-speed centrifugation and vacuum filtration through a microfiltration membrane and through a HiTrap protein A HP

column to obtain antibody 6F7.

Example 2: Sequence analysis of anti-CD47 antibody 6F7

mRNA was extracted from the cell line LT012 cultured in Example 1 according to the method described in the manual of RNAprep pure Cell/Bacteria Kit (Tiangen, Cat. No. DP430).

cDNA was synthesized according to the manual of Invitrogen SuperScript® III First-Strand Synthesis System for RT-PCR and amplified by PCR.

The PCR-amplified products were directly subjected to TA cloning according to the manual of the pEASY-T1 Cloning Kit (Transgen CT101).

The TA-cloned products were directly sequenced, and the sequencing results are as follows:

The nucleic acid sequence of the heavy chain variable region is set forth in SEQ ID NO: 1 with a length of 351 bp.

The encoded amino acid sequence is set forth in SEQ ID NO: 2 with a length of 117 aa, and the sequences of heavy chain CDR1, CDR2 and CDR3 are set forth in SEQ ID NOs: 5, 6 and 7, respectively.

The nucleic acid sequence of the light chain variable region is set forth in SEQ ID NO: 3 with a length of 321 bp.

The encoded amino acid sequence is set forth in SEQ ID NO: 4 with a length of 107 aa, and the sequences of light chain CDR1, CDR2 and CDR3 are set forth in SEQ ID NOs: 8, 9 and 10, respectively.

Example 3: Design and preparation of light and heavy chains of humanized anti-human CD47 antibodies 6F7 H1L1(hG4), 6F7 H2L2(hG4) and 6F7 H3L3(hG4)

1. Design of light and heavy chains of humanized anti-human CD47 antibodies 6F7 H1L1(hG4), 6F7 H2L2(hG4) and 6F7 H3L3(hG4)

Based on the three-dimensional crystal structure of human CD47 protein (Hage T, Reinemer P, Sebald W., Crystals of a 1:1 Complex Between Human Interleukin-4

and the Extracellular Domain of Its Receptor Alpha Chain, *Eur. J. Biochem.*, 1998; 258(2):831-6.) and the sequence of antibody 6F7 obtained in Example 2, the variable region sequences of antibodies 6F7 H1L1, 6F7 H2L2 and 6F7 H3L3 were obtained by computer modeling and mutation design (antibody constant region sequences from NCBI database: the heavy chain constant region is Ig gamma-4 chain C region, ACCESSION No. P01861.1 with S228P mutation introduced to improve the stability on this basis; the light chain constant region is Ig kappa chain C region, ACCESSION No. P01834). The intact antibodies containing the constant regions were denoted as 6F7 H1L1(hG4), 6F7 H2L2(hG4) and 6F7 H3L3(hG4), respectively.

The designed variable region sequences are as follows:

(1) Heavy and light chain variable region sequences of humanized monoclonal antibody 6F7 H1L1

The nucleic acid sequence of the heavy chain variable region is set forth in SEQ ID NO: 11 with a length of 351 bp.

The encoded amino acid sequence is set forth in SEQ ID NO: 12 with a length of 117 aa, and the sequences of heavy chain CDR1, CDR2 and CDR3 are set forth in SEQ ID NOs: 5, 6 and 7, respectively.

The nucleic acid sequence of the light chain variable region is set forth in SEQ ID NO: 13 with a length of 321 bp.

The encoded amino acid sequence is set forth in SEQ ID NO: 14 with a length of 107 aa, and the sequences of light chain CDR1, CDR2 and CDR3 are set forth in SEQ ID NOs: 8, 9 and 10, respectively.

(2) Heavy and light chain variable region sequences of humanized monoclonal antibody 6F7 H2L2

The nucleic acid sequence of the heavy chain variable region is set forth in SEQ ID NO: 15 with a length of 351 bp.

The encoded amino acid sequence is set forth in SEQ ID NO: 16 with a length of 117 aa, and the sequences of heavy chain CDR1, CDR2 and CDR3 are set forth in SEQ ID NOs: 5, 6 and 7, respectively.

The nucleic acid sequence of the light chain variable region is set forth in SEQ ID NO: 17 with a length of 321 bp.

The encoded amino acid sequence is set forth in SEQ ID NO: 18 with a length of 107 aa, and the sequences of light chain CDR1, CDR2 and CDR3 are set forth in SEQ ID NOs: 8, 9 and 10, respectively.

(3) Heavy and light chain variable region sequences of humanized monoclonal antibody 6F7 H3L3

The nucleic acid sequence of the heavy chain variable region is set forth in SEQ ID NO: 19 with a length of 351 bp.

The encoded amino acid sequence is set forth in SEQ ID NO: 20 with a length of 117 aa, and the sequences of heavy chain CDR1, CDR2 and CDR3 are set forth in SEQ ID NOs: 5, 6 and 7, respectively.

The nucleic acid sequence of the light chain variable region is set forth in SEQ ID NO: 21 with a length of 321 bp.

The encoded amino acid sequence is set forth in SEQ ID NO: 22 with a length of 107 aa, and the sequences of light chain CDR1, CDR2 and CDR3 are set forth in SEQ ID NOs: 8, 9 and 10, respectively.

2. Preparation of humanized antibodies 6F7 H1L1(hG4), 6F7 H2L2(hG4) and 6F7 H3L3(hG4)

The heavy chain cDNA and light chain cDNA of 6F7H1L1(hG4), the heavy chain cDNA and light chain cDNA of 6F7H2L2(hG4), and the heavy chain cDNA and light chain cDNA of 6F7H3L3(hG4) were separately cloned into pUC57simple (provided by Genscript) vectors to obtain pUC57simple-6F7H1**(hG4)** and pUC57simple-6F7L1, pUC57simple-6F7H2**(hG4)** and pUC57simple-6F7L2, and pUC57simple-6F7H3**(hG4)** and pUC57simple-6F7L3, respectively. With reference to the standard techniques described in *Molecular Cloning: A Laboratory Manual* (Second Edition), the heavy and light chain full-length genes synthesized by EcoRI&HindIII digestion of genes were subcloned into expression vector pcDNA3.1 by restriction enzyme EcoRI&HindIII to obtain expression plasmids pcDNA3.1-6F7H1**(hG4)** and pcDNA3.1-6F7L1, pcDNA3.1-6F7H2**(hG4)** and

pcDNA3.1-6F7L2, and pcDNA3.1-6F7H3**(hG4)** and pcDNA3.1-6F7L3, and the heavy and light chain genes of the recombinant expression plasmids were further sequenced. Then the designed gene combinations comprising corresponding light and heavy chain recombinant plasmids (pcDNA3.1-6F7H1**(hG4)**/pcDNA3.1-6F7L1, pcDNA3.1-6F7H2**(hG4)**/pcDNA3.1-6F7L2, and pcDNA3.1-6F7H3**(hG4)**/pcDNA3.1-6F7L3) were separately co-transfected into 293F cells, and the cultures were collected and purified. After the sequences were verified, endotoxin-free expression plasmids were prepared and were transiently transfected into HEK293 cells for antibody expression. The cultures were collected after 7 days, and subjected to affinity purification on a Protein A column (MabSelect SURE (GE)) to obtain humanized antibodies.

Example 4: Design and preparation of light and heavy chains of humanized anti-human CD47 antibodies 6F7 H1L1(G1M), 6F7 H2L2(G1M) and 6F7 H3L3(G1M)

1. Design of light and heavy chains of humanized anti-human CD47 antibodies 6F7 H1L1(G1), 6F7 H2L2(G1) and 6F7 H3L3(G1)

Based on the three-dimensional crystal structure of human CD47 protein (Hage T, Reinemer P, Sebald W., Crystals of a 1:1 Complex Between Human Interleukin-4 and the Extracellular Domain of Its Receptor Alpha Chain, *Eur. J. Biochem.*, 1998; 258(2):831-6.) and the sequence of antibody 6F7 obtained in Example 2, the variable region sequences of antibodies 6F7 H1L1, 6F7 H2L2 and 6F7 H3L3 were obtained by computer modeling and mutation design (antibody constant region sequences from NCBI database: the heavy chain constant region is Ig gamma-1 chain C region, ACCESSION No. P01857; the light chain constant region is Ig kappa chain C region, ACCESSION No. P01834). For distinction from the humanized antibodies in Example 3, the humanized antibodies above were designated 6F7 H1L1(G1), 6F7 H2L2(G1) and 6F7 H3L3(G1), respectively.

The variable region sequences of the humanized antibodies 6F7 H1L1(G1), 6F7

H2L2(G1) and 6F7 H3L3(G1) designed in this example were identical to those of 6F7 H1L1(hG4), 6F7 H2L2(hG4) and 6F7 H3L3(hG4) in Example 3.

2. Preparation of humanized antibodies 6F7 H1L1(G1), 6F7 H2L2(G1) and 6F7 H3L3(G1)

On the basis of 6F7 H1L1(G1), 6F7 H2L2(G1) and 6F7 H3L3(G1), new humanized antibodies were obtained by introducing a leucine-to-alanine point mutation at position 234 (L234A, according to the EU numbering system) and a leucine-to-alanine point mutation at position 235 (L235A, according to the EU numbering system) in the hinge region of the heavy chain, and were designated 6F7 H1L1(G1M), 6F7 H2L2(G1M) and 6F7 H3L3(G1M), respectively.

The heavy chain cDNA and light chain cDNA of 6F7H1L1(G1M), the heavy chain cDNA and light chain cDNA of 6F7H2L2(G1M), and the heavy chain cDNA and light chain cDNA of 6F7H3L3(G1M) were separately cloned into pUC57simple (provided by Genscript) vectors to obtain pUC57simple-6F7H1(G1M) and pUC57simple-6F7L1, pUC57simple-6F7H2(G1M) and pUC57simple-6F7L2, and pUC57simple-6F7H3(G1M) and pUC57simple-6F7L3, respectively. With reference to the standard techniques described in *Molecular Cloning: A Laboratory Manual* (Second Edition), the heavy and light chain full-length genes synthesized by EcoRI&HindIII digestion of genes were subcloned into expression vector pcDNA3.1 by restriction enzyme EcoRI&HindIII to obtain expression plasmids pcDNA3.1-6F7H1(G1M) and pcDNA3.1-6F7L1, pcDNA3.1-6F7H2(G1M) and pcDNA3.1-6F7L2, and pcDNA3.1-6F7H3(G1M) and pcDNA3.1-6F7L3, and the heavy and light chain genes of the recombinant expression plasmids were further sequenced. Then the designed gene combinations comprising corresponding light and heavy chain recombinant plasmids (pcDNA3.1-6F7H1(G1M)/pcDNA3.1-6F7L1, pcDNA3.1-6F7H2(G1M)/pcDNA3.1-6F7L2, and pcDNA3.1-6F7H3(G1M)/pcDNA3.1-6F7L3) were separately co-transfected into 293F cells, and the cultures were collected and purified. After the sequences were verified, endotoxin-free expression plasmids were prepared and were transiently

transfected into HEK293 cells for antibody expression. The cultures were collected after 7 days, and subjected to affinity purification on a Protein A column (MabSelect SURE (GE)) to obtain humanized antibodies.

Example 5: Phagocytosis of tumor cells by macrophages promoted by 6F7H1L1(hG4) in combination with rituximab or azacitidine

Materials: Azacitidine (purchased from Baxter Oncology GmbH); Hu5F9-G4 (supplied by Akeso Biopharma, Inc.); Raji cells (Chinese Academy of Sciences Shanghai Branch); HL-60 cells (ATCC); RPMI 1640 (Gibco, Cat. No: 22400-105); pancreatin (0.25% Typsin-EDTA (1×)) (Gibco, Cat. No: 25200072); fetal bovine serum (FBS; Excell bio, Cat. No: FSP500); fluorescent dye 56-carboxyfluorescein diacetatesuccinimidyl ester (CFSE; Biolegend, Cat. No: 423801); recombinant human macrophage colony-stimulating factor (human M-CSF; PeproTech, Cat. No: 300-25); APC anti-human CD11c antibody (Biolegend, Cat. No: 301614); isotype control (human IgG4; supplied by Akeso Biopharma, Inc.); rituximab (Roche); BSA (Sigma, Cat. No: V900933-1KG); Ficoll paque plus (GE, Cat. No: 17-1440-02).

Human peripheral monocyte-derived macrophages (HPMMs) were prepared by inducing PBMCs. Frozen PBMCs were conventionally thawed and incubated in an incubator overnight. The next day, PBMCs were collected, centrifuged at 170× g for 5 min, resuspended in a 1640 medium containing 2% FBS, and incubated in an incubator for 2 h. The supernatant was removed, and the cells were washed twice with PBS. 1640 complete medium (containing 10% FBS) and 100 ng/mL human M-CSF were added and the cells were inducted for 7 days. The medium was exchanged and 100 ng/mL human M-CSF was added on days 3 and 5. After the induction was completed on day 7, HPMMs were collected and centrifuged at 170× g for 5 min. The supernatant was discarded, and the cells were resuspended in 1640 complete medium (containing 10% FBS), with the cell density adjusted to 1×10^{10} cells/mL. Then the cells were aliquoted into 1.5 mL EP tubes for later use.

1. Phagocytosis of tumor cells by macrophages promoted by 6F7H1L1(hG4) in combination with rituximab

Objective: In this study, the effects of 6F7H1L1(hG4) in combination with

rituximab and 6F7H1L1(hG4) alone on the antibody-dependent cellular phagocytosis (ADCP) mediated by human Burkitt's lymphoma Raji cells were compared at the cytological level *in vitro*. In the assay system, HPMMs were used as the effector cells and Burkitt's lymphoma Raji cells were used as the target cells.

Procedures: Raji cells in the logarithmic phase were conventionally collected, centrifuged at 170× g for 5 min, resuspended and then washed once with PBS. Raji cells were resuspended in CFSE (2.5 μM) to a staining density of 1×10^7 cells/mL and incubated in an incubator for 20 min. 6 mL of 1640 complete medium was added to stop staining. The cells were centrifuged at 170× g for 5 min, and the supernatant was discarded. Then 1 mL of 1640 complete medium was added for resuspension, and the cells were incubated in an incubator for 10 min. The antibodies were diluted with 1640 complete medium to the working concentrations shown in the figure, and an isotype control antibody (an anti-HEL antibody, human IgG4) group was set. The antibodies and the target cells were added into EP tubes containing HPMMs at a volume ratio of 1:1 (the final volume was 100 μL, the effector-to-target ratio was 5×10^4:1.5×10^5), and the mixtures were well mixed for resuspension and incubated in an incubator at 37 °C for 2 h. 800 μL of 1% PBSA (PBS containing 1% of BSA) at room temperature was added to each tube, and the system was centrifuged at 1200× g for 5 min, and the supernatant was discarded. The cells were washed once with 800 μL of 1% PBSA. APC anti-human CD11c antibody (Biolegend, Lot No: 371506; diluted 400-fold with 1% PBSA) was added to the corresponding samples at 100 μL/sample for labeling, and the samples were incubated on ice for 40 min. 800 μL of 1% PBSA was added to each tube. The mixture was centrifuged at 1200× g for 5 min and the supernatant was discarded. The cells were washed once with 800 μL of 1% PBSA. 200 μL of 1% PBSA was added to each tube for resuspension, and the cells were detected on an FACS Calibur flow cytometer. Macrophages in the system were APC-$CD11b^+$ positive, and macrophages involved in phagocytosis were APC-$CD11b^+$ and $CFSE^+$ dual positive. The phagocytic index was determined as the ratio of the number of dual positive cells to the number of APC positive cells, and the antibody-mediated ADCP

activity was evaluated. The ADCP activity of each group, represented by P%, was calculated according to the following formula:

$$P\% = \frac{Number\ of\ macrophages\ involved\ in\ phagocytosis}{Total\ number\ of\ macrophages} \times 100\%$$

Results: In this study, the phagocytic activity (ADCP) mediated by 6F7H1L1(hG4) in combination with rituximab was determined using HPMMs as the effector cells and Raji cells as the target cells. The results, as shown in **Error! Citation source not found.**, demonstrated that the phagocytic indexes of rituximab and 6F7H1L1(hG4) were significantly higher than those of the blank control and the isotype control (human IgG4), indicating that rituximab and 6F7H1L1(hG4) have ADCP activity. The phagocytic indexes of 6F7H1L1(hG4) in combination with rituximab were significantly higher than those of 6F7H1L1(hG4), indicating that the ADCP activity of 6F7H1L1(hG4) in combination with rituximab is stronger than that of the monotherapy.

2. Phagocytosis of tumor cells by macrophages promoted by 6F7H1L1(hG4) in combination with azacitidine

Objective: In this study, the effects of 6F7H1L1(hG4) in combination with azacitidine and 6F7H1L1(hG4) alone on the antibody-dependent cellular phagocytosis (ADCP) mediated by acute myelocytic leukemia (AML) HL-60 cells were compared at the cytological level *in vitro*. In the assay system, HPMMs were used as the effector cells, and AML HL-60 cells treated with different concentrations of azacitidine were used as the target cells.

Procedures: HL-60 cells were conventionally collected and plated in a 6-well plate at 4×10^5 cells/2 mL of 1640 complete medium per well. Different concentrations of azacitidine (at working concentrations of 3 μM or 1 μM) were added. A blank HL-60 group was set. The cells were incubated in an incubator for 24 h. The next day, the treated and blank HL-60 cells were collected, centrifuged at 170× g for 5 min, resuspended and counted. Then the cells were analyzed for viability and washed once with PBS. CFSE was diluted to 2.5 μM with PBS. HL-60 cells were

resuspended in the diluted CFSE at a staining density of 1×10^7 cells/mL and incubated in an incubator for 20 min. 6 mL of 1640 complete medium (containing 10% FBS) was added to stop staining. The cells were centrifuged at 170× g for 5 min, and the supernatant was discarded. Then 1 mL of 1640 complete medium was added for resuspension, and the cells were incubated in an incubator for 10 min. The antibodies were diluted with 1640 complete medium to 20 μg/mL and 2 μg/mL (the working concentrations were 10 μg/mL and 1 μg/mL), and an isotype control antibody (an anti-HEL antibody, human IgG4) group was set. The antibodies and the target cells were added into V-bottom plates containing HPMMs (the final volume was 100 μL, the effector-to-target ratio was 5×10^4:1.5×10^5), and the mixtures were well mixed for resuspension and incubated in an incubator at 37 °C for 2 h. 150 μL of 1% PBSA (PBS containing 1% of BSA) at room temperature was added to each well, and the system was centrifuged at 1000× g for 5 min, and the supernatant was discarded. The cells were washed once with 200 μL of 1% PBSA. APC anti-human CD11c antibody (diluted 400-fold with 1% PBSA) was added to the corresponding samples at 100 μL/sample for labeling, and the samples were incubated on ice for 40 min. 150 μL of 1% PBSA was added to each well. The mixture was centrifuged at 1000× g for 5 min and the supernatant was discarded. The cells were washed once with 200 μL of 1% PBSA. 200 μL of 1% PBSA was added to each well for resuspension, and the cells were detected on an FACS Calibur flow cytometer. Macrophages in the system were APC-$CD11b^+$ positive, and macrophages involved in phagocytosis were APC-$CD11b^+$ and $CFSE^+$ dual positive. The phagocytic index was determined as the ratio of the number of dual positive cells to the number of APC positive cells, and the antibody-mediated ADCP activity was evaluated. The ADCP activity of each group, represented by P%, was calculated according to the formula shown in (1) of this example.

Results: In this study, the ADCP activity assay of 6F7H1L1(hG4) in combination with azacitidine was conducted by using Hu5F9-G4 targeting CD47 as the positive control, HPMMs as the effector cells and HL-60 cells as the target cells. The results, as shown in **Error! Citation source not found.**, demonstrated that the phagocytic

indexes of azacitidine, 6F7H1L1(hG4) and Hu5F9-G4 were significantly higher than those of the blank control and the isotype control (human IgG4), indicating that azacitidine, 6F7H1L1(hG4) and Hu5F9-G4 have pro-phagocytic activity. The phagocytic indexes of 6F7H1L1(hG4) and Hu5F9-G4 in combination with azacitidine were significantly higher than those of antibody monotherapies, indicating that the ADCP activities of 6F7H1L1(hG4) and Hu5F9-G4 in combination with azacitidine are stronger than those of antibody monotherapies. Compared with the control antibody Hu5F9-G4 for the same target, the ADCP activity of 6F7H1L1(hG4) in combination with azacitidine was comparable to that of Hu5F9-G4 in combination with azacitidine.

Example 6: Activation of immune cells promoted by 6F7H1L1(hG4) in combination with PD-1/CTLA-4 bispecific antibody or PD-1 antibody

Materials: PBMCs (isolated and prepared by Akeso Biopharma, Inc.); A549 cells (the Cell Center of the Chinese Academy of Sciences, Cat. No: KCB-200434YJ); SK-VO-3 cells (ATCC, Cat. No: HTB-77); HL-60 cells (ATCC, Cat. No: CCL-240); LNCAP (Chinese Academy of Sciences, Shanghai Institutes for Biological Sciences Cell Center); Raji-PDL1 cells (constructed by Akeso Biopharma, Inc., through constructing a PDL-1 lentiviral vector, infecting Raji cells after virus encapsulation and screening with blasticidin with conventional procedures); DMEM medium (Gibco, Cat. No: 11995-081); Trypsin-EDTA (0.25%); phenol red (Gibco, Cat. No: 25200072); RPMI 1640 medium (Gibco, Cat. No: 22400-105); Blasticidin (Gibco, Cat. No: R210-01); FBS (Excell bio, Cat. No: FSP500); SEB (*Staphylococcus aureus* enterotoxin; Dianotech, Cat. No: S010201); MMC (mitomycin C; Stressmarq, Cat. No: SIH-246); Ficoll-PaqueTM PLUS (GE Healthcare, Cat. No: 17-1440-02); human IL-2 ELISA KIT (Dakewe, Cat. No: 1110202), human IFN-γ ELISA KIT (Dakewe, Cat. No: 1110002); isotype control (human IgG1, or anti-HEL-isotype control).

1. Activation of immune cells promoted by 6F7H1L1(hG4) in combination with PD-1/CTLA-4 bispecific antibody

Objective: In this study, the activities of 6F7H1L1(hG4) in combination with a PD-1/CTLA-4 bispecific antibody (cadonilimab) and 6F7H1L1(hG4) alone in inducing the immune response of immune cells against human Burkitt's lymphoma Raji-PDL1 cells overexpressing human PDL-1 were compared at the cytological level *in vitro*.

Procedures: Raji-PDL1 cells were conventionally cultured in 1640 + 10% FBS complete medium. PBMCs were thawed and activated with 0.5 μg/mL SEB for two days. On the day of the experiment, Raji-PDL1 cells were treated with 2 μg/mL MMC for 1 h. SEB-activated PBMCs and MMC-treated Raji-PDL1 cells were collected, washed twice with PBS, resuspended in 1640 + 10% FBS complete medium and counted. Raji-PDL1 and PBMC cells were seeded on 96-well plates at 1×10^5 cells/well. The diluted antibody was added according to the study design. The mixture was mixed evenly and incubated in a 5% CO_2 incubator at 37 °C for 3 days. After 3 days, the cell culture supernatant was collected and analyzed for IL-2 and IFN-γ according to the manual of the ELISA KIT (Dakewe Biotech Co., Ltd.). The media in this study were all 10% FBS + 1640.

Results: The results, as shown in FIG. 3, demonstrated that 6F7H1L1(hG4) or PD-1/CTLA-4 bispecific antibody monotherapy can significantly stimulate the secretion of IFN-γ in PBMCs, while 6F7H1L1(hG4) in combination with the PD-1/CTLA-4 bispecific antibody showed superior activity to the monotherapies, i.e., enhances more significant immune response in immune cells against human Burkitt's lymphoma Raji-PDL1 cells.

2. Activation of immune cells promoted by 6F7H1L1(hG4) in combination with PD-1 antibody

(1) Immune response of immune cells against human lung adenocarcinoma A549 cells promoted by 6F7H1L1(hG4) in combination with PD-1 antibody

Objective: In this study, the activities of 6F7H1L1(hG4) in combination with a PD-1 antibody (penpulimab) and 6F7H1L1(hG4) alone in inducing the immune responses of immune cells against Raji-PDL1 cells and human lung adenocarcinoma A549 cells were compared at the cytological level *in vitro*.

Procedures: Raji-PDL1 cells were conventionally cultured in 1640 + 10% FBS complete medium, and A549 cells were conventionally cultured in DMEM + 10% FBS complete medium. PBMCs were thawed and activated with 0.5 μg/mL SEB for two days. On the day of the experiment, Raji-PDL1 cells were treated with 2 μg/mL MMC for 1 h. SEB-activated PBMCs and MMC-treated Raji-PDL1 cells were collected, washed twice with PBS, resuspended in 1640 + 10% FBS complete medium and counted. Raji-PDL1 and PBMC cells were seeded on 96-well plates at 1×10^5 cells/well. A549 cells in the logarithmic phase were collected and seeded on the 96-well plate at 5×10^4 cells/well. The diluted antibody was added according to the study design. The mixture was mixed evenly and incubated in a 5% CO_2 incubator at 37 °C for 3 days. After 3 days, the cell culture supernatant was collected and analyzed for IL-2 and IFN-γ according to the manual of the ELISA KIT. The media in this study were all 10% FBS + 1640.

Results: The results, as shown in FIG. 4, demonstrated that 6F7H1L1(hG4) or PD-1 antibody monotherapy can significantly stimulate the secretion of IFN-γ by PBMC cells, while 6F7H1L1(hG4) in combination with the PD-1 antibody showed superior activity to the monotherapies, i.e., enhances more significant immune response in immune cells against human lung adenocarcinoma A549 cells.

(2) Immune response of immune cells against human ovarian cancer SK-OV-3 **cells promoted by 6F7H1L1(hG4) in combination with PD-1 antibody**

Objective: In this study, the activities of 6F7H1L1(hG4) in combination with a PD-1 antibody (penpulimab) and 6F7H1L1(hG4) alone in inducing the immune responses of immune cells against Raji-PDL1 cells and human ovarian cancer SK-OV-3 cells were compared at the cytological level *in vitro*.

Procedures: Raji-PDL1 cells were conventionally cultured in 1640 + 10% FBS complete medium, and SK-OV-3 cells were conventionally cultured in McCoy's 5A (Modified) Medium + 10% FBS complete medium. PBMCs were thawed and activated with 0.5 μg/mL SEB for two days. On the day of the experiment, Raji-PDL1 cells were treated with 2 μg/mL MMC for 1 h. SEB-activated PBMCs and MMC-treated Raji-PDL1 cells were collected, washed twice with PBS,

resuspended in 1640 + 10% FBS complete medium and counted. Raji-PDL1 and PBMC cells were seeded on 96-well plates at 1×10^5 cells/well. SK-OV-3 cells in the logarithmic phase were collected and seeded on the 96-well plate at 5×10^4 cells/well. The diluted antibody was added according to the study design. The mixture was mixed evenly and incubated in a 5% CO_2 incubator at 37 °C for 3 days. After 3 days, the cell culture supernatant was collected and analyzed for IL-2 and IFN-γ according to the manual of the ELISA KIT. The media in this study were all 10% FBS + 1640.

Results: The results, as shown in FIG. 5, demonstrated that 6F7H1L1(hG4) or PD-1 antibody monotherapy can significantly stimulate the secretion of IL-2 by PBMC cells, while 6F7H1L1(hG4) in combination with the PD-1 antibody showed superior activity to the monotherapies, i.e., enhances more significant immune response in immune cells against human ovarian cancer SK-OV-3 cells.

(3) Immune response of immune cells against human prostate cancer LNCAP cells promoted by 6F7H1L1(hG4) in combination with PD-1 antibody

Objective: In this study, the activities of 6F7H1L1(hG4) in combination with a PD-1 antibody (penpulimab) and 6F7H1L1(hG4) alone in inducing the immune responses of immune cells against Raji-PDL1 cells and human prostate cancer LNCAP cells were compared at the cytological level *in vitro*.

Methodology: Raji-PDL1 cells were conventionally cultured in 1640 + 10% FBS complete medium, and LNCAP cells were conventionally cultured in RPMI-1640 + 10% FBS complete medium. PBMCs were thawed and activated with 0.5 μg/mL SEB for two days. On the day of the experiment, Raji-PDL1 cells were treated with 2 μg/mL MMC for 1 h. SEB-activated PBMCs and MMC-treated Raji-PDL1 cells were collected, washed twice with PBS, resuspended in 1640 + 10% FBS complete medium and counted. Raji-PDL1 and PBMC cells were seeded on 96-well plates at 1×10^5 cells/well. LNCAP cells in the logarithmic phase were collected and seeded on the 96-well plate at 5×10^4 cells/well. The diluted antibody was added according to the study design. The mixture was mixed evenly and incubated in a 5% CO_2 incubator at 37 °C for 3 days. After 3 days, the cell culture supernatant was

collected and analyzed for IL-2 and IFN-γ according to the manual of the ELISA KIT. The media in this study were all 10% FBS + 1640.

Results: The results, as shown in FIG. 6, demonstrated that 6F7H1L1(hG4) or PD-1 antibody monotherapy can significantly stimulate the secretion of IL-2 by PBMC cells, while 6F7H1L1(hG4) in combination with the PD-1 antibody showed superior activity to the monotherapies, i.e., enhances more significant immune response in immune cells against human prostate cancer LNCAP cells.

Example 7: Phagocytosis of tumor cells by macrophages promoted by 6F7H1L1(hG4) in combination with cetuximab

PBMCs (prepared by Akeso Biopharma, Inc., Lot No: 20200521-E) were thawed and incubated with 1640 complete medium + 10% FBS (Excell bio, Cat. No: FSP500, Lot No: 11I025) overnight. The next day, the medium was changed to 1640 complete medium + 2% FBS, and the cells were incubated with the medium for 2 h. The supernatant was removed, and the cells were washed twice with PBS and incubated in an incubator (90% 1640 + 10% FBS + M-CSF-100 ng/mL) to induce adult human macrophages (HPMMs, prepared by Akeso Biopharma, Inc.). The medium was exchanged and the treatment was given on days 3 and 5. The experiment was performed on day 7 of induction.

HT-29 cells (Chinese Academy of Sciences, Shanghai Institutes for Biological Sciences Cell Center, Cat. No: TCHu103) were conventionally collected, centrifuged at 170× g for 5 min, resuspended and then counted. Then the cells were analyzed for viability and washed once with PBS. CFSE (Biolegend, Cat. No: 423801) was diluted to 2.5 μM with PBS. The cells were resuspended in a proper amount of the diluted CFSE to a staining density of 1×10^7 cells/mL and incubated in an incubator for 20 min. 6 mL of 1640 complete medium (containing 10% FBS) was added to stop staining. The cells were centrifuged at 170× g for 5 min, and the supernatant was discarded. 1 mL of 1640 complete medium was added, and the cells were incubated in an incubator for 10 min, resuspended and counted. The number of cells was adjusted.

6F7H1L1(hG4) and cetuximab (prepared by Akeso Biopharma, Inc., Lot No: 20200917) were diluted to the desired concentrations with 1640 complete medium, and an isotype control antibody group was set. Human macrophages were collected and centrifuged at 7170× g for 5 min, and the supernatant was discarded. The cells were counted, transferred to 1.5 mL EP tubes and centrifuged at 1200× g for 5 min, and the supernatant was discarded. The tumor cells + antibody suspension (50 μL each) were added into the 1.5 mL EP tubes containing macrophages, and the mixtures were well mixed for resuspension and incubated in an incubator at 37 °C for 2 h. 800 μL of 1% PBSA at room temperature was added to each tube. The tubes were centrifuged at 1200× g for 5 min, and the supernatant was discarded. The cells were washed once with 800 μL of PBSA. APC anti-mouse/human CD11b antibody (Biolegend, Cat. No: 101212, concentration: 0.2 mg/mL; diluted 500-fold with PBSA) was added to the corresponding samples at 100 μL/sample, and the mixtures were well mixed and incubated on ice for 40 min. 800 μL of 1% PBSA was added to each tube. The tubes were centrifuged at 1200× g for 5 min, and the supernatant was discarded. The cells in each tube were washed once with 800 μL of PBSA. 200 μL of 1% PBSA was added to each tube for resuspension, and the cells were transferred to a flow cytometry tube and detected.

Results: In this study, the phagocytic activity (ADCP) mediated by 6F7H1L1(hG4) in combination with cetuximab was determined using HPMMs as the effector cells and HT29 cells as the target cells. The results, as shown in FIG. 7, demonstrated that the phagocytic indexes of cetuximab and 6F7H1L1(hG4) were significantly higher than those of the blank control and the isotype control (human IgG4), indicating that cetuximab and 6F7H1L1(hG4) have ADCP activity. The phagocytic indexes of 6F7H1L1(hG4) in combination with cetuximab were significantly higher than those of 6F7H1L1(hG4), indicating that the ADCP activity of 6F7H1L1(hG4) in combination with cetuximab is stronger than that of the monotherapy.

Example 8: Phagocytosis of tumor cells by macrophages promoted by 6F7H1L1(hG4) in combination with obinutuzumab

PBMCs (prepared by Akeso Biopharma, Inc., Lot No: 20201126-G) were thawed and incubated with 1640 complete medium + 10% FBS (Excell bio, Cat. No: FSP500, Lot No: 11I025) overnight. The next day, the medium was changed to 1640 complete medium + 2% FBS, and the cells were incubated with the medium for 2 h. The supernatant was removed, and the cells were washed twice with PBS and incubated in an incubator (90% 1640 + 10% FBS + M-CSF-100 ng/mL) to induce adult human macrophages (HPMMs). The medium was exchanged and the treatment was given on days 3 and 5. The experiment was performed on day 7 of induction.

Raji cells (purchased from ATCC) were conventionally collected and washed once with PBS. CFSE was diluted to 2.5 μM with PBS. The cells were resuspended in a proper amount of the diluted CFSE to a staining density of 1×10^7 cells/mL and incubated in an incubator for 20 min. 6 mL of 1640 complete medium (containing 10% FBS) was added to stop staining. The cells were centrifuged at 170× g for 5 min, and the supernatant was discarded. 1 mL of 1640 complete medium was added, and the cells were incubated in an incubator for 10 min with the cell density adjusted to 3×10^6 cells/mL. The antibodies were diluted to the desired concentrations with 1640 complete medium, and isotype control antibody groups were set. Macrophages were collected and centrifuged at 170× g for 5 min. The cells were adjusted to a density of 1×10^6 cells/mL, transferred to 96-well V-bottom plates and centrifuged at 750× g for 5 min, and the supernatant was discarded. The tumor cell + antibody suspension was added to the 96-well V-bottom plates containing macrophages, and the mixtures were well mixed for resuspension and incubated in an incubator at 37 °C for 2 h. 150 μL of 1% PBSA at room temperature was added to each well. The mixture was centrifuged at 750× g for 5 min, and the supernatant was discarded. The cells were washed once with 200 μL of PBSA. APC anti-mouse/human CD11b antibody (diluted 500-fold with PBSA) was added to the corresponding samples at 100 μL/sample, and the mixtures were well mixed and incubated on ice for 40 min. 150 μL of 1% PBSA was added to each well. The cells were centrifuged at 750× g for 5 min, and the supernatant was discarded. The cells

in each well were washed once with 200 μL of PBSA. 200 μL of 1% PBSA was added to each tube for resuspension, and the cells were transferred to a flow cytometry tube and detected.

Results: In this study, the phagocytic activity (ADCP) mediated by 6F7H1L1(hG4) in combination with obinutuzumab was determined using HPMMs as the effector cells and Raji cells as the target cells. The results, as shown in FIG. 8, demonstrated that the phagocytic indexes of obinutuzumab and 6F7H1L1(hG4) were significantly higher than those of the blank control and the isotype controls (human IgG4 and human IgG1), indicating that obinutuzumab and 6F7H1L1(hG4) have ADCP activity. The phagocytic indexes of 6F7H1L1(hG4) in combination with obinutuzumab were higher than those of 6F7H1L1(hG4), indicating that the ADCP activity of 6F7H1L1(hG4) in combination with obinutuzumab is stronger than that of the monotherapy.

Example 9: Effective anti-tumor treatment with 6F7H1L1(hG4) in combination with anti-PD-1/anti-CTLA-4 bispecific antibody

In order to determine the tumor inhibitory activity *in vivo* of 6F7H1L1(hG4) antibody in combination with anti-PD-1/anti-CTLA-4 bispecific antibody cadonilimab, female BALB/c-hPD1/hSIRPα mice aged 6.43–8.43 weeks (purchased from GemPharmatech Co., Ltd.) were grafted subcutaneously with CT26-hCD47 cells (mouse colon cancer cells, purchased from GemPharmatech Co., Ltd.). When the mean tumor volume reached 80–120 mm^3, the mice were randomized into 4 groups of 6 by tumor volume. The day of grouping was defined as D0, and dosing was started on the day of grouping D0. The regimen of the combination therapy group is as follows: the drugs were formulated separately and administered sequentially (no certain order or time interval was required, and one treatment should be given after the completed administration of the other). The modeling and specific regimen are shown in Table 1. After the administration, the length and width of tumors in each group were measured, and the tumor volume was

calculated. The data were subjected to an independent sample T-test, SPSS 17.0 *P <0.05, **P<0.01.

Table 1: Regimen for treating CT26-hCD47 xenograft tumor BALB/c-hPD1/hSIRPα mouse model with 6F7H1L1(hG4) antibody in combination with anti-PD-1/anti-CTLA-4 bispecific antibody cadonilimab

Group	Number	Grafting	Administration
Isotype control 5 mg/kg	6	CT26-hCD47, 5×10^5 cells, BALB/c-hPD1/hSIRPα mice, SC	Isotype control antibody hIgG4, intraperitoneally administered twice per week for 4 weeks, 7 doses
6F7H1L1(hG4) 5mg/kg	6		6F7H1L1(hG4), 5 mg/kg; intraperitoneally administered twice per week for 4 weeks, 7 doses.
Cadonilimab 0.5mg/kg	6		Cadonilimab (prepared by Akeso Biopharma, Inc., Lot No: B104C20191206), 0.5 mg/kg; intraperitoneally administered twice per week for 4 weeks, 7 doses.
6F7H1L1(hG4) 5mg/kg, Cadonilimab 0.5mg/kg	6		6F7H1L1(hG4), 5 mg/kg; cadonilimab, 0.5 mg/kg; intraperitoneally administered twice per week for 4 weeks, 7 doses.

The results are shown in FIG. 9. The results showed that compared with the isotype control antibody hIgG4, both 6F7H1L1(hG4) and PD-1/CTLA-4 bispecific antibody cadonilimab can effectively inhibit the growth of tumors in mice, and the

6F7H1L1(hG4) + cadonilimab combination therapy group showed synergic anti-tumor efficacy in the model and was superior to the monotherapy groups in inhibiting tumors.

In addition, as shown in FIG. 10, both 6F7H1L1(hG4) and cadonilimab were well tolerated by the tumor-bearing mice, either alone or in combination, and no effect on the body weight of the tumor-bearing mice was found in the groups.

Example 10: Effective anti-tumor treatment with 6F7H1L1(hG4) in combination with anti-PD-1/anti-VEGFA bispecific antibody

In order to determine the tumor inhibitory activity *in vivo* of 6F7H1L1(hG4) antibody in combination with anti-PD-1/anti-VEGFA bispecific antibody VP101(hG1DM), SCID Beige mice aged 5-7 weeks (purchased from Charles River, Beijing) were grafted subcutaneously with MDA-MB-231 cells (purchased from ATCC). When the mean tumor volume reached 120-150 mm^3, the mice were randomized into 4 groups of 6 by tumor volume. The day of grouping was defined as D0. Dosing was started on the day of grouping D0, and the mice were injected intraperitoneally with activated PBMCs. The regimen of the combination therapy group is as follows: the drugs were formulated separately and administered sequentially (no certain order or time interval was required, and one treatment should be given after the completed administration of the other). The modeling and specific regimen are shown in Table 2. After the administration, the length and width of tumors in each group were measured, and the tumor volume was calculated. The data were subjected to a one-way analysis of variance (Bonferroni post hoc test), $*P<0.05$, $**P<0.01$.

Table 2: Regimen for treating MDA-MB-231 xenograft tumor SCID Beige mouse model with 6F7H1L1(hG4) antibody in combination with anti-PD-1/anti-VEGFA bispecific antibody VP101(hG1DM)

Grouping	Number	Grafting	Administration
Isotype control	6	MDA-MB-231,	Isotype control antibody hIgG4, 0.05

		8×10^6 cells, SCID Beige mice, SC; on the day of grouping, the mice were grafted with 4×10^6 CD3 antibody-activated human PBMCs via intraperitoneal injection	mg/kg; intraperitoneally administered twice per week for 4 weeks; Isotype control antibody hIgG1DM, 3.75 mg/kg; intraperitoneally administered once per week for 4 weeks
6F7H1L1(hG4) 0.05mg/kg	6		6F7H1L1(hG4), 0.05 mg/kg; intraperitoneally administered twice per week for 4 weeks
VP101(hG1DM) 2mg/kg	6		VP101(hG1DM) (prepared by Akeso Biopharma, Inc., Lot No: B112C20201205), 2 mg/kg; intraperitoneally administered once per week for 4 weeks
6F7H1L1(hG4) 0.05mg/kg, VP101(hG1DM) 2mg/kg	6		6F7H1L1(hG4), 0.05 mg/kg; intraperitoneally administered twice per week for 4 weeks; VP101(hG1DM), 2 mg/kg; intraperitoneally administered once per week for 4 weeks

The results are shown in FIG. 11. The results showed that compared with the isotype control group, both 6F7H1L1(hG4) and VP101(hG1DM) can effectively inhibit the growth of tumors in mice, and the 6F7H1L1(hG4) + VP101(hG1DM) combination therapy group showed synergistic anti-tumor efficacy on the model and was superior to the monotherapy groups in inhibiting tumors.

In addition, as shown in FIG. 12, both 6F7H1L1(hG4) and VP101(hG1DM) were well tolerated by the tumor-bearing mice, either alone or in combination, and no effect on the body weight of the tumor-bearing mice was found in the groups.

The embodiments of the present invention have been described above in detail, but

the present invention is not limited to the embodiments. Those skilled in the art can make various equivalent modifications or replacements without departing from the spirit of the present invention. These equivalent modifications or replacements shall fall within the scope defined by the claims of the present application.

CLAIMS

1. A pharmaceutical composition, preferably for treating a tumor, comprising a component A and a component B, wherein the component A is an antibody that specifically binds to CD47 or an antigen-binding fragment thereof;

the component B is selected from one or more of the following: a component B1, a component B2 and a component B3, wherein the component B1 is a bispecific antibody or an antigen-binding fragment thereof, the component B2 is an anti-tumor chemotherapeutic, and the component B3 is an anti-PD-1 monoclonal antibody or an antigen-binding fragment thereof;

preferably, the composition further comprises a component C, wherein the component C is an anti-tumor therapeutic agent, and the component C is different from component B;

for example, according to the Kabat, IMGT, Chothia or AbM numbering system,

the antibody that specifically binds to CD47 comprises CDR sequences selected from those comprised in the following heavy chain variable regions and light chain variable regions:

(1) an HCDR1, an HCDR2 and an HCDR3 comprised in a heavy chain variable region set forth in SEQ ID NO: 2, and

an LCDR1, an LCDR2 and an LCDR3 comprised in a light chain variable region set forth in SEQ ID NO: 4; or

(2) an HCDR1, an HCDR2 and an HCDR3 comprised in a heavy chain variable region set forth in SEQ ID NO: 12, and

an LCDR1, an LCDR2 and an LCDR3 comprised in a light chain variable region set forth in SEQ ID NO: 14; or

(3) an HCDR1, an HCDR2 and an HCDR3 comprised in a heavy chain variable region set forth in SEQ ID NO: 16, and

an LCDR1, an LCDR2 and an LCDR3 comprised in a light chain variable region set forth in SEQ ID NO: 18; or

(4) an HCDR1, an HCDR2 and an HCDR3 comprised in a heavy chain variable

region set forth in SEQ ID NO: 20, and
an LCDR1, an LCDR2 and an LCDR3 comprised in a light chain variable region set forth in SEQ ID NO: 22
(preferably, according to the IMGT numbering system, the antibody comprises:
an HCDR1 comprising or consisting of a sequence set forth in SEQ ID NO: 5 or a variant thereof,
an HCDR2 comprising or consisting of a sequence set forth in SEQ ID NO: 6 or a variant thereof, and
an HCDR3 comprising or consisting of a sequence set forth in SEQ ID NO: 7 or a variant thereof; and the antibody further comprises:
an LCDR1 comprising or consisting of amino acids set forth in SEQ ID NO: 8 or a variant thereof,
an LCDR2 comprising or consisting of an amino acid sequence set forth in SEQ ID NO: 9 or a variant thereof, and
an LCDR3 comprising or consisting of a sequence set forth in SEQ ID NO: 10 or a variant thereof);
the bispecific antibody is an anti-PD-1/anti-CTLA4 antibody or an anti-PD-1/anti-VEGFA antibody or a combination of the two; the bispecific antibody comprises a first protein functional region and a second protein functional region, wherein the first protein functional region targets PD-1, and the second protein functional region targets CTLA4 or VEGFA; the first protein functional region is an immunoglobulin, and the second protein functional region is a single-chain antibody; or the first protein functional region is a single-chain antibody, and the second protein functional region is an immunoglobulin;
preferably, two single-chain antibody molecules (preferably, two identical single-chain antibody molecules) are linked to one immunoglobulin molecule; preferably, the immunoglobulin is of IgG1 subtype (preferably, human IgG1 subtype); preferably, the single-chain antibody is linked to the N terminus or C terminus of the heavy chain of the immunoglobulin, wherein the numbers of the first protein functional region and the second protein functional region are each

independently 1, 2 or more; preferably, the first protein functional region is linked to the second protein functional region either directly or via a first linker fragment; and/or the heavy chain variable region of the single-chain antibody is linked to the light chain variable region of the single-chain antibody either directly or via a second linker fragment; the first linker fragment and the second linker fragment are identical or different; preferably, the linker fragment is (GGGGS)n, wherein n is a positive integer, or more preferably, n is 1, 2, 3, 4, 5 or 6; preferably, the first linker fragment and the second linker fragment comprise amino acid sequences independently selected from SEQ ID NO: 119 and SEQ ID NO: 120; more preferably, the first linker fragment and the second linker fragment comprise an amino acid sequence set forth in SEQ ID NO: 120;

when the bispecific antibody is an anti-PD-1/anti-CTLA4 antibody,

the first protein functional region comprises HCDR1–HCDR3 comprised in a heavy chain variable region set forth in SEQ ID NO: 87, and LCDR1–LCDR3 comprised in a light chain variable region set forth in SEQ ID NO: 88 (preferably, according to the IMGT numbering system, the first protein functional region comprises an HCDR1 comprising or consisting of a sequence set forth in SEQ ID NO: 89 or a variant thereof,

an HCDR2 comprising or consisting of a sequence set forth in SEQ ID NO: 90 or a variant thereof,

an HCDR3 comprising or consisting of a sequence set forth in SEQ ID NO: 91 or a variant thereof,

an LCDR1 comprising or consisting of amino acids set forth in SEQ ID NO: 92 or a variant thereof,

an LCDR2 comprising or consisting of an amino acid sequence set forth in SEQ ID NO: 93 or a variant thereof, and

an LCDR3 comprising or consisting of a sequence set forth in SEQ ID NO: 94 or a variant thereof);

the second protein functional region comprises HCDR1–HCDR3 in a heavy chain variable region set forth in SEQ ID NO: 95, and LCDR1–LCDR3 in a light chain

variable region set forth in SEQ ID NO: 96 (preferably, according to the IMGT numbering system, the second protein functional region comprises an HCDR1 comprising or consisting of a sequence set forth in SEQ ID NO: 97 or a variant thereof,

an HCDR2 comprising or consisting of a sequence set forth in SEQ ID NO: 98 or a variant thereof,

an HCDR3 comprising or consisting of a sequence set forth in SEQ ID NO: 99 or a variant thereof,

an LCDR1 comprising or consisting of amino acids set forth in SEQ ID NO: 100 or a variant thereof,

an LCDR2 comprising or consisting of an amino acid sequence set forth in SEQ ID NO: 101 or a variant thereof, and

an LCDR3 comprising or consisting of a sequence set forth in SEQ ID NO: 102 or a variant thereof);

or

when the bispecific antibody is an anti-PD-1/anti-VEGFA antibody,

the first protein functional region comprises HCDR1–HCDR3 comprised in a heavy chain variable region set forth in SEQ ID NO: 103, and LCDR1–LCDR3 comprised in a light chain variable region set forth in SEQ ID NO: 104 (preferably, according to the IMGT numbering system, the first protein functional region comprises an HCDR1 comprising or consisting of a sequence set forth in SEQ ID NO: 105 or a variant thereof,

an HCDR2 comprising or consisting of a sequence set forth in SEQ ID NO: 106 or a variant thereof,

an HCDR3 comprising or consisting of a sequence set forth in SEQ ID NO: 107 or a variant thereof,

an LCDR1 comprising or consisting of amino acids set forth in SEQ ID NO: 108 or a variant thereof,

an LCDR2 comprising or consisting of an amino acid sequence set forth in SEQ ID NO: 109 or a variant thereof, and

an LCDR3 comprising or consisting of a sequence set forth in SEQ ID NO: 110 or a variant thereof);

the second protein functional region comprises HCDR1–HCDR3 in a heavy chain variable region set forth in SEQ ID NO: 111, and LCDR1–LCDR3 in a light chain variable region set forth in SEQ ID NO: 112 (preferably, according to the IMGT numbering system, the second protein functional region comprises an HCDR1 comprising or consisting of a sequence set forth in SEQ ID NO: 113 or a variant thereof,

an HCDR2 comprising or consisting of a sequence set forth in SEQ ID NO: 114 or a variant thereof,

an HCDR3 comprising or consisting of a sequence set forth in SEQ ID NO: 115 or a variant thereof,

an LCDR1 comprising or consisting of amino acids set forth in SEQ ID NO: 116 or a variant thereof,

an LCDR2 comprising or consisting of an amino acid sequence set forth in SEQ ID NO: 117 or a variant thereof, and

an LCDR3 comprising or consisting of a sequence set forth in SEQ ID NO: 118 or a variant thereof);

wherein,

the anti-PD1 monoclonal antibody comprises HCDR1–HCDR3 comprised in a heavy chain variable region set forth in SEQ ID NO: 121, and LCDR1–LCDR3 comprised in a light chain variable region set forth in SEQ ID NO: 122 (preferably, according to the IMGT numbering system, the anti-PD1 monoclonal antibody comprises an HCDR1 comprising or consisting of a sequence set forth in SEQ ID NO: 123 or a variant thereof,

an HCDR2 comprising or consisting of a sequence set forth in SEQ ID NO: 124 or a variant thereof,

an HCDR3 comprising or consisting of a sequence set forth in SEQ ID NO: 125 or a variant thereof,

an LCDR1 comprising or consisting of amino acids set forth in SEQ ID NO: 126 or

a variant thereof,

an LCDR2 comprising or consisting of an amino acid sequence set forth in SEQ ID NO: 127 or a variant thereof, and

an LCDR3 comprising or consisting of a sequence set forth in SEQ ID NO: 128 or a variant thereof);

wherein the variant comprises a sequence having at least 80%, 81%, 82%, 83%, 84%, 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99% homology to the corresponding sequence, or the variant comprises a sequence having one or more (preferably 1, 2 or 3) conservative amino acid mutations (preferably substitutions, insertions or deletions) compared to the corresponding sequence.

2. The pharmaceutical composition according to claim 1, wherein the antibody that specifically binds to CD47 further comprises framework regions (FRs) in the heavy chain variable region and framework regions (FRs) in the light chain variable region selected from the group consisting of the following:

(1) the framework regions (FRs) in the heavy chain variable region including an FR-H1, an FR-H2, an FR-H3 and an FR-H4, wherein the FR-H1 comprises or consists of an amino acid sequence set forth in SEQ ID NO: 23 or a variant thereof; the FR-H2 comprises or consists of an amino acid sequence set forth in SEQ ID NO: 24 or a variant thereof; the FR-H3 comprises or consists of an amino acid sequence set forth in SEQ ID NO: 25 or a variant thereof; the FR-H4 comprises or consists of an amino acid sequence set forth in SEQ ID NO: 26 or a variant thereof; the framework regions (FRs) in the light chain variable region including an FR-L1, an FR-L2, an FR-L3 and an FR-L4, wherein the FR-L1 comprises or consists of an amino acid sequence set forth in SEQ ID NO: 27 or a variant thereof; the FR-L2 comprises or consists of an amino acid sequence set forth in SEQ ID NO: 28 or a variant thereof; the FR-L3 comprises or consists of an amino acid sequence set forth in SEQ ID NO: 29 or a variant thereof; the FR-L4 comprises or consists of an amino acid sequence set forth in SEQ ID NO: 30 or a variant thereof;

(2) the framework regions (FRs) in the heavy chain variable region including an

FR-H1, an FR-H2, an FR-H3 and an FR-H4, wherein the FR-H1 comprises or consists of an amino acid sequence set forth in SEQ ID NO: 31 or a variant thereof; the FR-H2 comprises or consists of an amino acid sequence set forth in SEQ ID NO: 32 or a variant thereof; the FR-H3 comprises or consists of an amino acid sequence set forth in SEQ ID NO: 33 or a variant thereof; the FR-H4 comprises or consists of an amino acid sequence set forth in SEQ ID NO: 34 or a variant thereof; the framework regions (FRs) in the light chain variable region including an FR-L1, an FR-L2, an FR-L3 and an FR-L4, wherein the FR-L1 comprises or consists of an amino acid sequence set forth in SEQ ID NO: 35 or a variant thereof; the FR-L2 comprises or consists of an amino acid sequence set forth in SEQ ID NO: 36 or a variant thereof; the FR-L3 comprises or consists of an amino acid sequence set forth in SEQ ID NO: 37 or a variant thereof; the FR-L4 comprises or consists of an amino acid sequence set forth in SEQ ID NO: 38 or a variant thereof;

(3) the framework regions (FRs) in the heavy chain variable region including an FR-H1, an FR-H2, an FR-H3 and an FR-H4, wherein the FR-H1 comprises or consists of an amino acid sequence set forth in SEQ ID NO: 39 or a variant thereof; the FR-H2 comprises or consists of an amino acid sequence set forth in SEQ ID NO: 40 or a variant thereof; the FR-H3 comprises or consists of an amino acid sequence set forth in SEQ ID NO: 41 or a variant thereof; the FR-H4 comprises or consists of an amino acid sequence set forth in SEQ ID NO: 42 or a variant thereof; the framework regions (FRs) in the light chain variable region including an FR-L1, an FR-L2, an FR-L3 and an FR-L4, wherein the FR-L1 comprises or consists of an amino acid sequence set forth in SEQ ID NO: 43 or a variant thereof; the FR-L2 comprises or consists of an amino acid sequence set forth in SEQ ID NO: 44 or a variant thereof; the FR-L3 comprises or consists of an amino acid sequence set forth in SEQ ID NO: 45 or a variant thereof; the FR-L4 comprises or consists of an amino acid sequence set forth in SEQ ID NO: 46 or a variant thereof;

(4) the framework regions (FRs) in the heavy chain variable region including an FR-H1, an FR-H2, an FR-H3 and an FR-H4, wherein the FR-H1 comprises or consists of an amino acid sequence set forth in SEQ ID NO: 47 or a variant thereof;

the FR-H2 comprises or consists of an amino acid sequence set forth in SEQ ID NO: 48 or a variant thereof; the FR-H3 comprises or consists of an amino acid sequence set forth in SEQ ID NO: 49 or a variant thereof; the FR-H4 comprises or consists of an amino acid sequence set forth in SEQ ID NO: 50 or a variant thereof;
the framework regions (FRs) in the light chain variable region including an FR-L1, an FR-L2, an FR-L3 and an FR-L4, wherein the FR-L1 comprises or consists of an amino acid sequence set forth in SEQ ID NO: 51 or a variant thereof; the FR-L2 comprises or consists of an amino acid sequence set forth in SEQ ID NO: 52 or a variant thereof; the FR-L3 comprises or consists of an amino acid sequence set forth in SEQ ID NO: 53 or a variant thereof; the FR-L4 comprises or consists of an amino acid sequence set forth in SEQ ID NO: 54 or a variant thereof,
wherein the variant comprises a sequence having at least 80%, preferably at least 81%, 82%, 83%, 84%, 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98% or 99% homology to the corresponding sequence or having one or more (preferably 1, 2, 3, 4, 5, 6, 7, 8, 9 or 10) conservative amino acid mutations (preferably substitutions, insertions or deletions) compared to the corresponding sequence.

3. The pharmaceutical composition according to claim 1 or 2, wherein the antibody that specifically binds to CD47 comprises:

(1) a heavy chain variable region comprising or consisting of:
an amino acid sequence set forth in SEQ ID NO: 2 or a variant thereof, and
a light chain variable region comprising or consisting of:
an amino acid sequence set forth in SEQ ID NO: 4 or a variant thereof;

(2) a heavy chain variable region comprising or consisting of:
an amino acid sequence set forth in SEQ ID NO: 12 or a variant thereof, and
a light chain variable region comprising or consisting of:
an amino acid sequence set forth in SEQ ID NO: 14 or a variant thereof;

(3) a heavy chain variable region comprising or consisting of:
an amino acid sequence set forth in SEQ ID NO: 16 or a variant thereof, and
a light chain variable region comprising or consisting of:

an amino acid sequence set forth in SEQ ID NO: 18 or a variant thereof; and
(4) a heavy chain variable region comprising or consisting of:
an amino acid sequence set forth in SEQ ID NO: 20 or a variant thereof, and
a light chain variable region comprising or consisting of:
an amino acid sequence set forth in SEQ ID NO: 22 or a variant thereof,
the first protein functional region of the anti-PD-1/anti-CTLA4 antibody comprises:
(1) a heavy chain variable region comprising or consisting of:
a sequence set forth in SEQ ID NO: 87 or a variant thereof, and
a light chain variable region comprising or consisting of:
a sequence set forth in SEQ ID NO: 88 or a variant thereof; and
(2) a heavy chain variable region comprising or consisting of:
a sequence set forth in SEQ ID NO: 129 or a variant thereof, and
a light chain variable region comprising or consisting of:
a sequence set forth in SEQ ID NO: 130 or a variant thereof;
the second protein functional region of the anti-PD-1/anti-CTLA4 antibody comprises:
(1) a heavy chain variable region comprising or consisting of:
a sequence set forth in SEQ ID NO: 95 or a variant thereof, and
a light chain variable region comprising or consisting of:
a sequence set forth in SEQ ID NO: 96 or a variant thereof;
(2) a heavy chain variable region comprising or consisting of:
a sequence set forth in SEQ ID NO: 133 or a variant thereof, and
a light chain variable region comprising or consisting of:
a sequence set forth in SEQ ID NO: 134 or a variant thereof;
(3) a heavy chain variable region comprising or consisting of:
a sequence set forth in SEQ ID NO: 135 or a variant thereof, and
a light chain variable region comprising or consisting of:
a sequence set forth in SEQ ID NO: 136 or a variant thereof;
(4) a heavy chain variable region comprising or consisting of:
a sequence set forth in SEQ ID NO: 137 or a variant thereof, and

a light chain variable region comprising or consisting of:
a sequence set forth in SEQ ID NO: 138 or a variant thereof; and
(5) a heavy chain variable region comprising or consisting of:
a sequence set forth in SEQ ID NO: 131 or a variant thereof, and
a light chain variable region comprising or consisting of:
a sequence set forth in SEQ ID NO: 132 or a variant thereof;
preferably, the immunoglobulin of the anti-PD-1/anti-CTLA4 antibody comprises a heavy chain having an amino acid sequence set forth in SEQ ID NO: 139, and targets PD-1;
the first protein functional region of the anti-PD-1/anti-VEGFA antibody comprises:
(1) a heavy chain variable region comprising or consisting of:
a sequence set forth in SEQ ID NO: 103 or a variant thereof, and
a light chain variable region comprising or consisting of:
a sequence set forth in SEQ ID NO: 104 or a variant thereof;
the second protein functional region of the anti-PD-1/anti-VEGFA antibody comprises:
(1) a heavy chain variable region comprising or consisting of:
a sequence set forth in SEQ ID NO: 111 (preferably encoded by a nucleotide sequence set forth in SEQ ID NO: 145) or a variant thereof, and
a light chain variable region comprising or consisting of:
a sequence set forth in SEQ ID NO: 112 (preferably encoded by a nucleotide sequence set forth in SEQ ID NO: 146) or a variant thereof;
preferably, the immunoglobulin of the anti-PD-1/anti-VEGFA antibody comprises a heavy chain having an amino acid sequence set forth in SEQ ID NO: 140, and targets VEGFA;
the anti-PD-1 monoclonal antibody comprises or consists of a heavy chain variable region set forth in SEQ ID NO: 121 and a light chain variable region set forth in SEQ ID NO: 122;
wherein the variant has at least 85%, preferably 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98% or 99% or higher sequence identity to the

corresponding sequence, or comprises an amino acid sequence having one or more (preferably 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 21, 22, 23, 24, 25, 26, 27, 28, 29 or 30) conservative amino acid mutations (preferably substitutions, insertions or deletions) compared to the corresponding sequence.

4. The pharmaceutical composition according to any one of claims 1–3, wherein the antibody that specifically binds to CD47 further comprises a heavy chain constant region and a light chain constant region, and the constant regions are derived from species other than murine, e.g., from a human antibody, preferably from a human IgG or IgM, and more preferably from IgG1; preferably, the heavy chain constant region is Ig gamma-1 chain C region, ACCESSION No. P01857 (SEQ ID NO: 58) or Ig gamma-4 chain C region, ACCESSION No. P01861.1 (SEQ ID NO: 56); the light chain constant region is Ig kappa chain C region, ACCESSION No. P01834 (SEQ ID NO: 57); preferably, the antibody that specifically binds to CD47 is secreted by a hybridoma cell line LT012 under CCTCC NO. 2018135.

5. The pharmaceutical composition according to any one of claims 1–4, wherein, according to the EU numbering system, the antibody that specifically binds to CD47 and the immunoglobulin comprise a heavy chain constant region having mutations at any 1, 2 or 3 of positions 234, 235 and 237, and the affinity constant of the bispecific antibody for FcγRIIIa and/or C1q is reduced after the mutation as compared to that before the mutation; preferably, the affinity constant is measured by a Fortebio Octet system;

preferably, the antibody that specifically binds to CD47 comprises an L234A and/or L235A mutation according to the EU numbering system;

more preferably, the heavy chain constant region of the immunoglobulin has one of the following combinations of mutations:

L234A and L235A; or

L234A and G237A; or

L235A and G237A; or

L234A, L235A and G237A;

still more preferably,

according to the EU numbering system, the heavy chain constant region of the immunoglobulin further has one of the combinations of the following mutations: N297A, D265A, D270A, P238D, L328E, E233D, H268D, P271G, A330R, C226S, C229S, E233P, P331S, S267E, L328F, A330L, M252Y, S254T, T256E, N297Q, P238S, P238A, A327Q, A327G, P329A, K322A, T394D, G236R, G236A, L328R, A330S, P331S, H268A, E318A and K320A.

6. The pharmaceutical composition according to any one of claims 1–5, wherein the antibody that specifically binds to CD47 comprises or consists of a heavy chain and a light chain selected from the group consisting of the following:

(1) a heavy chain set forth in SEQ ID NO: 59 and a light chain set forth in SEQ ID NO: 60;

(2) a heavy chain set forth in SEQ ID NO: 61 and a light chain set forth in SEQ ID NO: 62;

(3) a heavy chain set forth in SEQ ID NO: 63 and a light chain set forth in SEQ ID NO: 64;

(4) a heavy chain set forth in SEQ ID NO: 65 and a light chain set forth in SEQ ID NO: 66;

(5) a heavy chain set forth in SEQ ID NO: 67 and a light chain set forth in SEQ ID NO: 68; and

(6) a heavy chain set forth in SEQ ID NO: 69 and a light chain set forth in SEQ ID NO: 70;

the anti-PD-1/anti-CTLA4 antibody comprises or consists of a heavy chain and a light chain selected from the group consisting of the following:

a heavy chain set forth in SEQ ID NO: 78 (preferably encoded by a nucleotide sequence set forth in SEQ ID NO: 77) and a light chain set forth in SEQ ID NO: 80 (preferably encoded by a nucleotide sequence set forth in SEQ ID NO: 79);

the anti-PD-1/anti-VEGFA antibody comprises or consists of a heavy chain and a light chain selected from the group consisting of the following:

a heavy chain set forth in SEQ ID NO: 141 (preferably encoded by a nucleotide sequence set forth in SEQ ID NO: 142) and a light chain set forth in SEQ ID NO:

143 (preferably encoded by a nucleotide sequence set forth in SEQ ID NO: 144);
the anti-PD-1 monoclonal antibody comprises or consists of a heavy chain and a light chain selected from the group consisting of the following: a heavy chain set forth in SEQ ID NO: 82 (preferably encoded by a nucleotide sequence set forth in SEQ ID NO: 81) and a light chain set forth in SEQ ID NO: 84 (preferably encoded by a nucleotide sequence set forth in SEQ ID NO: 83).

7. The pharmaceutical composition according to any one of claims 1–6, wherein the antigen-binding fragment is selected from Fab, Fab', $F(ab')_2$, Fd, Fv, dAb, Fab/c, complementarity determining region (CDR) fragment, single-chain antibody (e.g., scFv), bivalent antibody and domain antibody.

8. The pharmaceutical composition according to any one of claims 1–7, wherein the antibody that specifically binds to CD47 is a humanized antibody, a chimeric antibody or a multispecific antibody (e.g., a bispecific antibody).

9. The pharmaceutical composition according to any one of claims 1–8, wherein the antibody that specifically binds to CD47 binds to human CD47 protein with a KD less than about 10^{-5} M, e.g., less than about 10^{-6} M, 10^{-7} M, 10^{-8} M, 10^{-9} M, or 10^{-10} M or less, or the antibody that specifically binds to CD47 binds to human CD47 protein with an EC_{50} less than about 100 nM, e.g., less than about 10 nM, 1 nM, 0.9 nM, 0.8 nM, 0.7 nM, 0.6 nM, 0.5 nM, 0.4 nM, 0.3 nM, 0.2 nM, 0.1 nM or less.

10. The pharmaceutical composition according to any one of claims 1–9, wherein the antibody that specifically binds to CD47 is in the form of an antibody conjugate comprising the antibody that specifically binds to CD47 or the antigen-binding fragment thereof according to any one of claims 1–9 and a conjugated moiety conjugated thereto, wherein the conjugated moiety is a purification tag (e.g., a His tag), a cytotoxic agent or a detectable label; preferably, the conjugated moiety is a radioisotope, a luminescent substance, a colored substance, an enzyme or polyethylene glycol; or
the antibody that specifically binds to CD47 is in the form of a multispecific antibody, preferably a bispecific antibody, comprising the antibody that specifically binds to CD47 or the antigen-binding fragment thereof according to any one of

claims 1–9, and an antibody against another antigen and/or another antigenic epitope or an antigen-binding fragment thereof; or the antibody that specifically binds to CD47 is in the form of a fusion protein comprising the antibody that specifically binds to CD47 or the antigen-binding fragment thereof according to any one of claims 1–9.

11. The pharmaceutical composition according to any one of claims 1–10, wherein the anti-tumor therapeutic agent is selected from one or more of the following: a tyrosine kinase inhibitor, a DNA polymerase inhibitor, an anti-human CD20 antibody, an anti-human PDL-1 antibody, an anti-human PD-1 antibody, an anti-human CTLA-4 antibody, a BCL-2 inhibitor, an anti-human EGFR antibody, an anti-human HER2 antibody, an anti-human HER3 antibody, acalabrutinib, a cyclin-dependent kinase inhibitor, an anti-human VEGFR2 antibody, an anti-human VEGF antibody, a proteasome inhibitor, an angiogenesis inhibitor, a rapidly accelerated fibrosarcoma (RAF) inhibitor, a bispecific antibody and a fusion protein drug; preferably, the therapeutic agent is cetuximab, obinutuzumab or rituximab;

preferably, the anti-tumor chemotherapeutic is selected from one or more of the following: an alkylating agent, an anthracycline, an antimetabolite, an antibiotic, a plant-based and/or hormonal drug, a platinum-based drug (e.g., cisplatin, carboplatin and oxaliplatin), adriamycin, cyclophosphamide, a taxane (e.g., albumin-bound paclitaxel, liposome paclitaxel and docetaxel), etoposide, gemcitabine, pemetrexed, capecitabine, olaparib, rucaparib, niraparib, talazoparib, fluzoparib, vinca alkaloid, tamoxifen, megestrol, goserelin, asparaginase, a fluorouracil antineoplastic drug (e.g., 5-fluorouracil), azacitidine, cytarabine and cyclocytidine.

12. The pharmaceutical composition according to any one of claims 1–11, wherein the pharmaceutical composition further comprises a pharmaceutically acceptable carrier and/or excipient.

13. The pharmaceutical composition according to any one of claims 1–12, wherein, based on the mass of the antibody, the component A and the component B1 are in a mass ratio of (1:5)–(5:1), e.g., 1:5, 1:4, 1:3, 1:2, 1:1, 2:1, 3:1, 4:1 or 5:1;

the component A and the component B3 are in a mass ratio of (1:5)–(5:1), e.g., 1:5, 1:4, 1:3, 1:2, 1:1, 2:1, 3:1, 4:1 or 5:1;

or

the component B2 and the component A are in a mass ratio of 1:(1–1000), preferably 1:(5–500), and more preferably 1:(10–100);

preferably, the component A and the component B in the pharmaceutical composition are in a form suitable for administration by intravenous drip infusion, subcutaneous injection, intradermal injection, intravenous injection, intramuscular injection or intralesional injection.

14. A kit, preferably for treating a tumor, comprising a product A and a product B, wherein the product A comprises the antibody that specifically binds to CD47 or the antigen-binding fragment thereof as defined in any one of claims 1–13;

the product B is selected from one or more of the following: a product B1, a product B2 and a product B3, wherein the product B1 is the bispecific antibody or the antigen-binding fragment thereof as defined in any one of claims 1–13 (preferably, when the bispecific antibody is a combination of an anti-PD-1/anti-CTLA4 antibody and an anti-PD-1/anti-VEGFA antibody, the anti-PD-1/anti-CTLA4 antibody and the anti-PD-1/anti-VEGFA antibody are packaged either separately or together), the product B2 is the anti-tumor chemotherapeutic as defined in any one of claims 1–13, and the product B3 is the anti-PD-1 monoclonal antibody or the antigen-binding fragment thereof as defined in any one of claims 1–13, wherein the product B1, the product B2 and the product B3 are packaged either separately or together;

preferably, the kit further comprises a product C, wherein the product C is the anti-tumor therapeutic agent as defined in any one of claims 1–13, and the product C is different from the product B;

preferably, the kit further comprises a package insert.

15. The kit according to claim 14, wherein, based on the mass of the antibody, the product A and the product B1 are in a mass ratio of (1:5)–(5:1), e.g., 1:5, 1:4, 1:3, 1:2, 1:1, 2:1, 3:1, 4:1 or 5:1;

the product A and the product B3 are in a mass ratio of (1:5)–(5:1), e.g., 1:5, 1:4,

1:3, 1:2, 1:1, 2:1, 3:1, 4:1 or 5:1;

or

the anti-tumor chemotherapeutic is in a unit dose of 0.1–100 mg, 0.5–50 mg, 1–20 mg, 2–15 mg, 4–12 mg or 8–12 mg;

preferably, the product A, the product B or the product C in the kit is in a form suitable for administration by intravenous drip infusion, subcutaneous injection, intradermal injection, intravenous injection, intramuscular injection or intralesional injection.

16. A method for treating or preventing a tumor, comprising administering to a subject in need an effective amount of the component A and the component B as defined in any one of claims 1–13, wherein preferably, the method further comprises administering to the subject in need an effective amount of the component C as defined in any one of claims 1–13;

preferably, the component A, the component B and the component C are administered simultaneously or sequentially; more preferably, the component A, the component B and the component C are administered before or after a surgical treatment and/or before or after a radiotherapy;

preferably, when the component B is the component B1 and/or the component B3, the component A or the component B is administered at a unit dose of 0.1–100 mg, preferably 1–10 mg per kg body weight of the subject; or the component A or the component B is administered at a unit dose of 10–1000 mg, preferably 50–500 mg, 100–400 mg, 150–300 mg, 150–250 mg or 200 mg in each subject;

preferably, the component B2 is in a unit dose of 0.1–100 mg, 0.5–50 mg, 1–20 mg, 2–15 mg, 4–12 mg or 8–12 mg;

preferably, the dose is administered from twice daily to about once every other day, or once every 3 days, 4 days, 5 days, 6 days, 10 days, 1 week, 2 weeks, 3 weeks, 4 weeks, 5 weeks or 6 weeks;

preferably, the route of administration is intravenous drip infusion, subcutaneous injection, intradermal injection, intravenous injection, intramuscular injection or intralesional injection.

17. The pharmaceutical composition according to any one of claims 1–13, the kit according to claim 14 or 15, or the method according to claim 16, wherein the tumor is preferably a tumor expressing CD47, and preferably a cancer; the cancer includes a solid tumor, a hematological tumor, lymphoma, blastoma, sarcoma, leukemia or lymphoid malignancy, and more preferably includes squamous cell carcinoma, myeloma, lung cancer, small cell lung cancer, non-small cell lung cancer, head and neck squamous cell carcinoma, glioma (e.g., neuroglioma and recurrent glioma), acute myelocytic leukemia, Hodgkin's lymphoma, non-Hodgkin's lymphoma, diffuse large B-cell lymphoma, follicular lymphoma, acute lymphocytic leukemia, acute myeloblastic leukemia, chronic lymphocytic leukemia, chronic myeloblastic leukemia, primary mediastinal large B-cell lymphoma, mantle cell lymphoma, small lymphocytic lymphoma, T cell/histiocyte-rich large B-cell lymphoma, multiple myeloma, myeloid leukemia-1 protein, relapsed and refractory peripheral T-cell lymphoma, myelodysplastic syndrome, anaplastic large cell lymphoma, mantle cell lymphoma, marginal zone lymphoma, myelofibrosis, polycythemia vera, bone marrow cancer, myeloproliferative disease, aggressive systemic mastocytosis, eosinophilia, dermatofibrosarcoma protuberans, chronic eosinophilic leukemia, gastrointestinal cancer, gastric adenocarcinoma or gastroesophageal junction adenocarcinoma, ovarian cancer, liver cancer, lymphocytic leukemia, large intestine cancer, endometrial cancer, prostate cancer, thyroid cancer, melanoma, chondrosarcoma, neuroblastoma, adenocarcinoma, pancreatic cancer, pancreatic ductal adenocarcinoma, glioblastoma multiforme, bone cancer, Ewing's sarcoma, cervical cancer, nasopharyngeal cancer, brain cancer, bladder cancer, breast cancer, triple negative breast cancer, intestinal cancer, rectal cancer, colorectal cancer, colon cancer, hepatocellular carcinoma, renal cell carcinoma, clear cell renal cell carcinoma, head and neck cancer, throat cancer, hepatobiliary cancer, central nervous system cancer, esophageal carcinoma, esophageal squamous cell carcinoma, malignant pleural mesothelioma, systemic light chain amyloidosis, lymphocytic lymphoma, myeloproliferative neoplasm, neuroendocrine tumor, Merkel cell carcinoma, testicular cancer and skin cancer, peritoneal cancer, fallopian

tube cancer, urothelial cancer, microsatellite instability-high (MSI-H) or mismatch repair deficient (dMMR) cancer and mesothelioma.

18. A unit formulation, preferably for treating a tumor, comprising 1–10000 mg (preferably 10–1000 mg, and preferably 50–500 mg, 100–400 mg, 150–300 mg, 150–250 mg or 200 mg) of the component A and the component B as defined in any one of claims 1–13,

wherein, when the component B is the component B1 and/or the component B3, the unit formulation comprises 1–10000 mg (preferably 1–1000 mg, and preferably 50–500 mg, 100–400 mg, 150–300 mg, 150–250 mg, 200 mg or 100 mg) of the component B as defined in any one of claims 1–13;

when the component B is the component B2, the unit formulation comprises 0.1–100 mg, 0.5–50 mg, 1–20 mg, 2–15 mg, 4–12 mg, or 8–12 mg of the component B;

preferably, the unit formulation further comprises one or more of the components C as defined in any one of claims 1–13;

wherein the component A, the component B and the component C are packaged separately.

19. A single dose unit, preferably for treating a tumor, comprising 0.1–10000 mg (preferably 1–1000 mg, and preferably 50–500 mg, 100–400 mg, 150–300 mg, 150–250 mg, 200 mg or 100 mg) of the component A and the component B as defined in any one of claims 1–13,

wherein, when the component B is the component B1 and/or the component B3, the single dose unit comprises 0.1–10000 mg (preferably 1–1000 mg, and preferably 50–500 mg, 100–400 mg, 150–300 mg, 150–250 mg, 200 mg or 100 mg) of the component B as defined in any one of claims 1–13;

when the component B is the component B2, the single dose unit comprises 0.1–100 mg, 0.5–50 mg, 1–20 mg, 2–15 mg, 4–12 mg, or 8–12 mg of the component B.

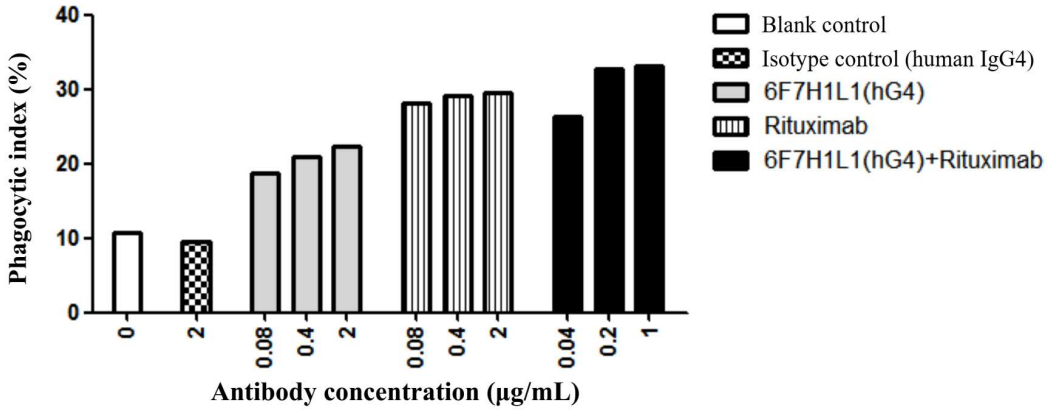
Phagocytic index (%)
40
30
20
10
0
0
2
0.08
0.4
2
0.08
0.4
2
0.04
0.2
1
Antibody concentration (μg/mL)
Blank control
Isotype control (human IgG4)
6F7H1L1(hG4)
Rituximab
6F7H1L1(hG4)+Rituximab

FIG. 1

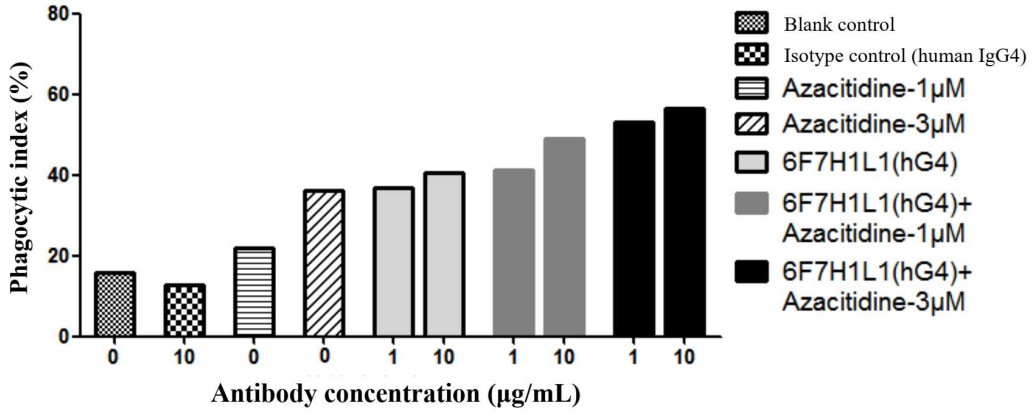
Phagocytic index (%)
80
60
40
20
0
0
10
0
0
1
10
1
10
1
10
Antibody concentration (μg/mL)
Blank control
Isotype control (human IgG4)
Azacitidine-1μM
Azacitidine-3μM
6F7H1L1(hG4)
6F7H1L1(hG4)+
Azacitidine-1μM
6F7H1L1(hG4)+
Azacitidine-3μM

FIG. 2

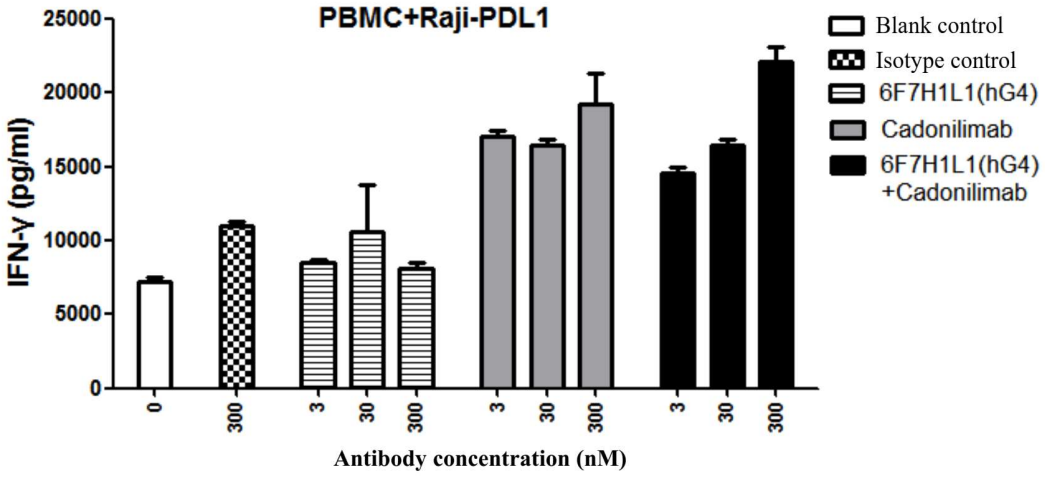

FIG. 3

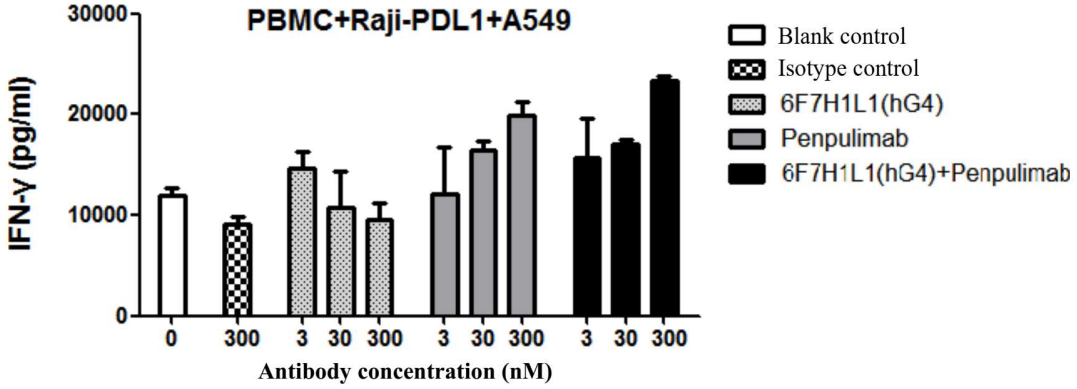

FIG. 4

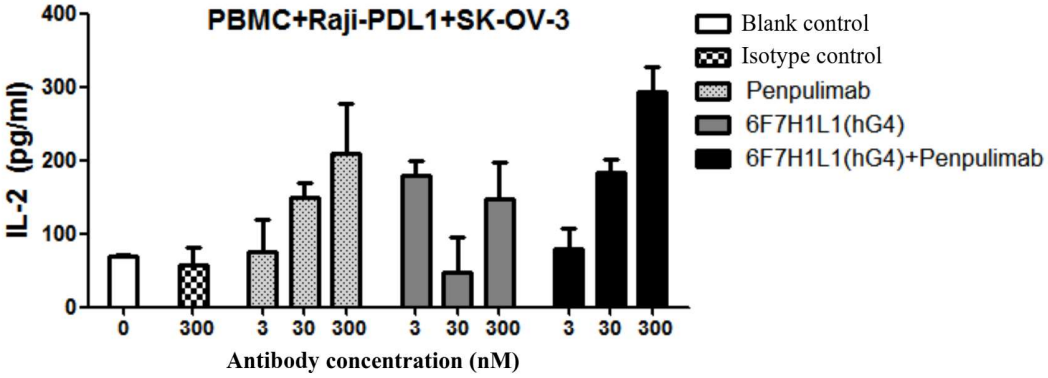

FIG. 5

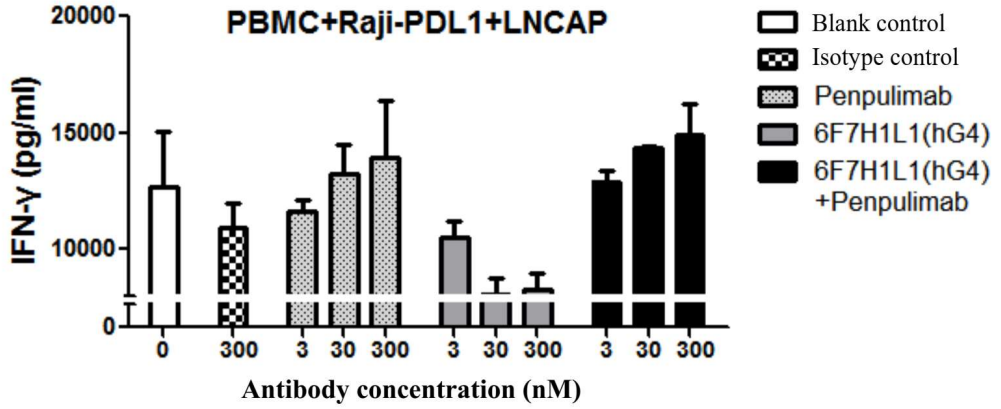

FIG. 6

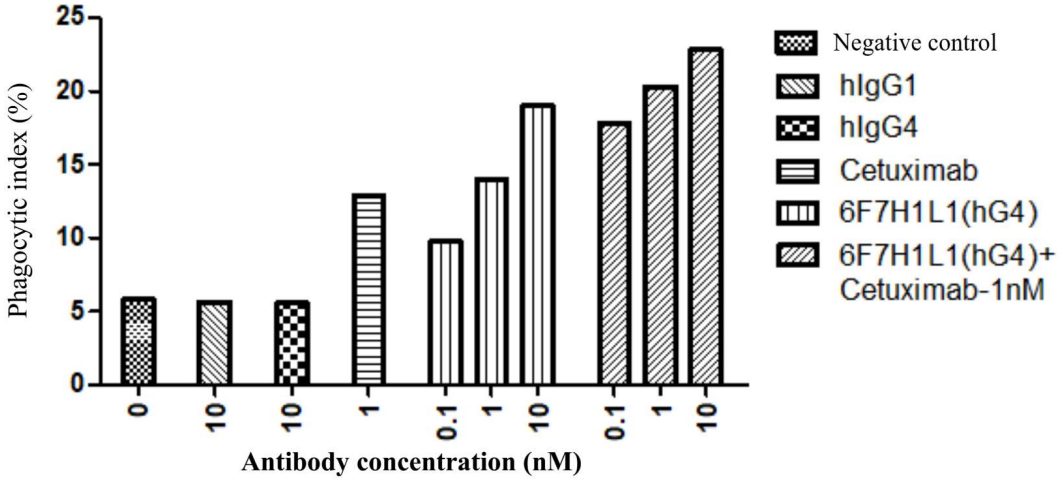

FIG. 7

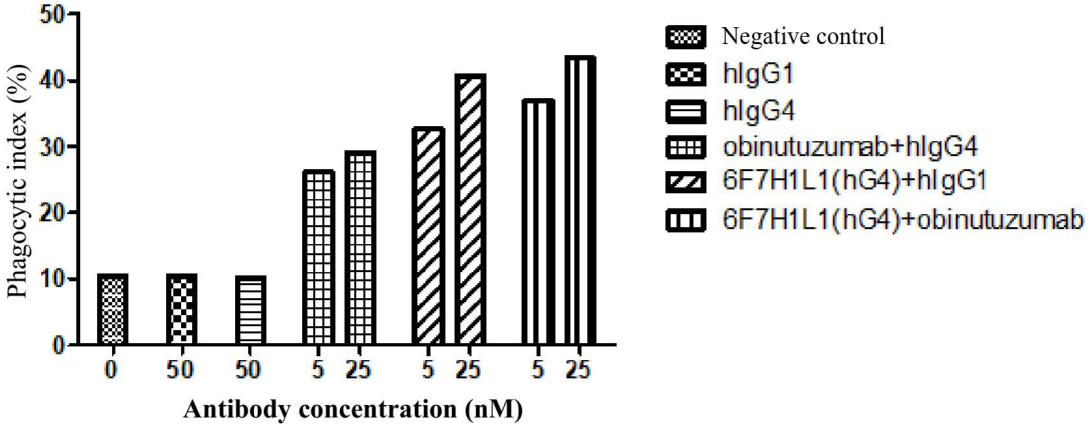

FIG. 8

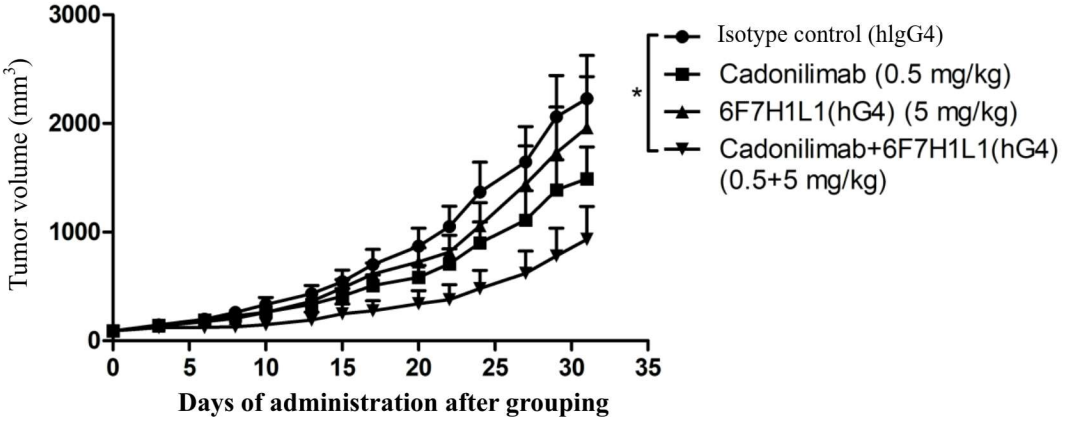

FIG. 9

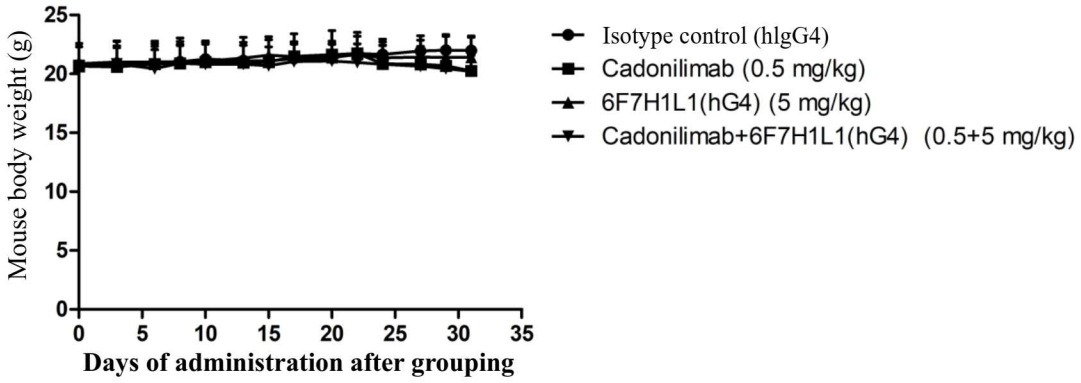

FIG. 10

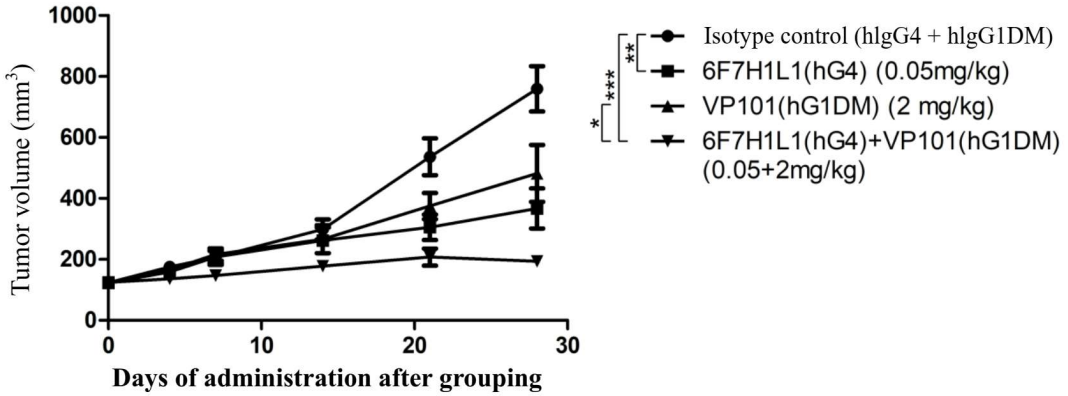

FIG. 11

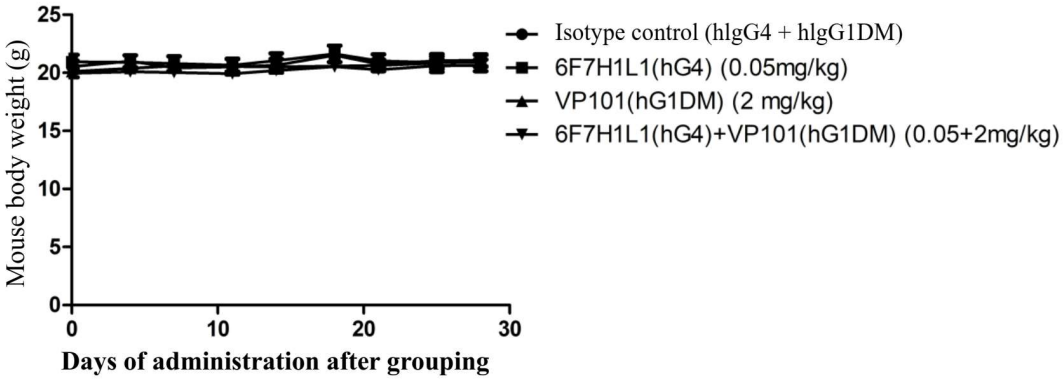
Mouse body weight (g)
25
20
15
10
5
0
0
10
20
30
Days of administration after grouping
Isotype control (hlgG4 + hlgG1DM)
6F7H1L1(hG4) (0.05mg/kg)
VP101(hG1DM) (2 mg/kg)
6F7H1L1(hG4)+VP101(hG1DM) (0.05+2mg/kg)

FIG. 12

SEQUENCE LISTING

SEQ ID NO: 1: nucleotide sequence of heavy chain variable region of 6F7

CAGGTGCAGCTGCAGCAGCCAGGAGCAGAGCTGGTGAGGCCAGGAGCATCCGTG
AAGCTGTCTTGTAAGGCCAGCGGCTACACCTTCACATCCTATTGGATGAACTGGGT
GAAGCAGAGGCCTGGACAGGGACTGGAGTGGATCGGCATGATCGACCCAAGCGA
TTCCGAGACCCACAACAATCAGATGTTTAAGGACAAGGCCACCCTGACAGTGGAT
AAGAGCTCCAATACCGCCTACATGCACCTGTCTAGCCTGACATCTGAGGACAGCG
CCGTGTATCACTGCGCCCGGCTGTACAGATGGTATTTTGACGTGTGGGGAGCAGG
AACCACAGTGACCGTGTCCTCT

SEQ ID NO: 2: amino acid sequence of heavy chain variable region of 6F7

QVQLQQPGAELVRPGASVKLSCKASGYTFTSYWMNWVKQRPGQGLEWIGMIDPSD
SETHNNQMFKDKATLTVDKSSNTAYMHLSSLTSEDSAVYHCARLYRWYFDVWGAGT
TVTVSS

SEQ ID NO: 3: nucleotide sequence of light chain variable region of 6F7

AACATCGTGATGACCCAGTCCCCCAAGTCTATGAGCATGTCCCTGGGCGAGAGGG
TGACCCTGTCCTGTAAGGCCTCTGAGATCGTGGGCACATACGTGTCTTGGTTTCAG
CAGAAGCCACACCAGAGCCCCAAGCTGCTGATCTACGGCGCCTCCAATCGGTATA
CAGGCGTGCCTGACAGATTCACCGGCTCTGGCAGCGCCACAGACTTCACCCTGAC
AATCTCTAACGTGCAGGCCGAGGACCTGGCCGATTATCACTGCGGCCAGAGCTAC
AATTTCCCTTATACCTTTGGCGGCGGCACAAAGCTGGAGATCAAG

SEQ ID NO: 4: amino acid sequence of light chain variable region of 6F7

NIVMTQSPKSMSMSLGERVTLSCKASEIVGTYVSWFQQKPHQSPKLLIYGASNRYTG
VPDRFTGSGSATDFTLTISNVQAEDLADYHCGQSYNFPYTFGGGTKLEIK

SEQ ID NO: 5 HCDR1 of 6F7CDR

GYTFTSYW

SEQ ID NO: 6 HCDR2 of 6F7CDR

IDPSDSET

SEQ ID NO: 7 HCDR3 of F7CDR

ARLYRWYFDV

SEQ ID NO: 8 LCDR1 of 6F7CDR

EIVGTY

SEQ ID NO: 9 LCDR2 of 6F7CDR

GAS

SEQ ID NO: 10 LCDR3 of 6F7CDR

GQSYNFPYT

SEQ ID NO: 11 nucleotide sequence of heavy chain variable region of 6F7H1

CAGGTGCAGCTGGTGCAGAGCGGAGCAGAGGTGGTGAAGCCAGGAGCCTCTGTG
AAGCTGAGCTGTAAGGCCTCCGGCTACACCTTCACAAGCTATTGGATGAACTGGG
TGCGGCAGAGACCAGGACAGGGACTGGAGTGGATCGGAATGATCGACCCTTCCG
ATTCTGAGACCCACAATGCCCAGAAGTTTCAGGGCAAGGCCACCCTGACAGTGG
ACAAGAGCACCTCCACAGCCTACATGCACCTGAGCTCCCTGCGGTCCGAGGACAC
AGCCGTGTACTATTGCGCCAGGCTGTACCGCTGGTATTTTGACGTGTGGGGAGCAG
GAACCACAGTGACCGTGTCTAGC

SEQ ID NO: 12 amino acid sequence of heavy chain variable region of 6F7H1

QVQLVQSGAEVVKPGASVKLSCKASGYTFTSYWMNWVRQRPGQGLEWIGMIDPSD
SETHNAQKFQGKATLTVDKSTSTAYMHLSSLRSEDTAVYYCARLYRWYFDVWGAGT
TVTVSS

SEQ ID NO: 13 nucleotide sequence of light chain variable region of 6F7L1

AACATCGTGATGACCCAGTCCCCAGCCACAATGTCTATGAGCCCAGGAGAGAGGG
TGACCCTGTCCTGTAGAGCCTCTGAGATCGTGGGCACATACGTGTCTTGGTTTCAG

CAGAAGCCAGGACAGGCACCTAGGCTGCTGATCTACGGAGCAAGCAACAGGTAT
ACCGGAGTGCCAGCACGCTTCTCCGGCTCTGGCAGCGGCACAGACTTTACCCTGA
CAATCAGCTCCGTGCAGCCTGAGGACCTGGCCGATTATCACTGCGGCCAGTCTTAC
AATTTCCCATATACCTTTGGCGGCGGCACAAAGCTGGAGATCAAG

SEQ ID NO: 14 amino acid sequence of light chain variable region of 6F7L1
NIVMTQSPATMSMSPGERVTLSCRASEIVGTYVSWFQQKPGQAPRLLIYGASNRYTG
VPARFSGSGSGTDFTLTISSVQPEDLADYHCGQSYNFPYTFGGGTKLEIK

SEQ ID NO: 15 nucleotide sequence of heavy chain variable region of 6F7H2
CAGGTGCAGCTGGTGCAGAGCGGAGCAGAGGTGGTGAAGCCAGGAGCCTCTGTG
AAGGTGAGCTGTAAGGCCTCCGGCTACACCTTCACATCCTATTGGATGAACTGGGT
GCGGCAGAGACCAGGACAGGGACTGGAGTGGATCGGAATCATCGACCCTTCCGA
TTCTGAGACCTCTAATGCCCAGAAGTTTCAGGGCCGGGTGACCCTGACAGTGGAC
AAGAGCACCTCCACAGCCTACATGCACCTGAGCTCCCTGAGGAGCGAGGACACA
GCCGTGTACTATTGCGCCAGGCTGTACCGCTGGTATTTTGACGTGTGGGGAGCAGG
AACCACAGTGACCGTGTCTAGC

SEQ ID NO: 16 amino acid sequence of heavy chain variable region of 6F7H2
QVQLVQSGAEVVKPGASVKVSCKASGYTFTSYWMNWVRQRPGQGLEWIGIIDPSDS
ETSNAQKFQGRVTLTVDKSTSTAYMHLSSLRSEDTAVYYCARLYRWYFDVWGAGTT
VTVSS

SEQ ID NO: 17 nucleotide sequence of light chain variable region of 6F7L2
AACATCGTGATGACCCAGTCCCCAGCCACACTGTCTCTGAGCCCAGGAGAGAGG
GTGACCCTGTCCTGTAGAGCCTCTGAGATCGTGGGCACATACGTGTCTTGGTTTCA
GCAGAAGCCAGGACAGGCACCTAGGCTGCTGATCTATGGCGCCAGCAACAGGGC
AACCGGCATCCCCGCACGCTTCTCCGGCTCTGGCAGCGGCACAGACTTTACCCTG

ACAATCAGCTCCCTGCAGCCTGAGGACCTGGCCGATTACTATTGCGGCCAGTCTTA
CAATTTCCCATATACCTTTGGCGGCGGCACAAAGCTGGAGATCAAG

SEQ ID NO: 18 amino acid sequence of light chain variable region of 6F7L2
NIVMTQSPATLSLSPGERVTLSCRASEIVGTYVSWFQQKPGQAPRLLIYGASNRATGIP
ARFSGSGSGTDFTLTISSLQPEDLADYYCGQSYNFPYTFGGGTKLEIK

SEQ ID NO: 19 nucleotide sequence of heavy chain variable region of 6F7H3
CAGGTGCAGCTGGTGCAGAGCGGAGCAGAGGTGGTGAAGCCAGGAGCCTCTGTG
AAGGTGAGCTGTAAGGCCTCCGGCTACACCTTCACATCCTATTGGATGAACTGGGT
GCGGCAGGCACCAGGACAGGGACTGGAGTGGATCGGCATCATCGACCCTTCCGAT
TCTGAGACCTCTTACGCCCAGAAGTTTCAGGGCAGGGTGACCCTGACAGTGGACA
AGAGCACCTCCACAGCCTATATGGAGCTGAGCTCCCTGCGCAGCGAGGACACAGC
CGTGTACTATTGCGCCCGGCTGTACAGATGGTATTTTGACGTGTGGGGAGCAGGAA
CCACAGTGACCGTGTCTAGC

SEQ ID NO: 20 amino acid sequence of heavy chain variable region of 6F7H3
QVQLVQSGAEVVKPGASVKVSCKASGYTFTSYWMNWVRQAPGQGLEWIGIIDPSDS
ETSYAQKFQGRVTLTVDKSTSTAYMELSSLRSEDTAVYYCARLYRWYFDVWGAGTT
VTVSS

SEQ ID NO: 21 nucleotide sequence of light chain variable region of 6F7L3
AACATCGTGATGACCCAGTCCCCAGCCACACTGTCTCTGAGCCCAGGAGAGAGG
GTGACCCTGTCCTGTAGAGCCTCTGAGATCGTGGGCACATACCTGTCTTGGTATCA
GCAGAAGCCAGGACAGGCACCTAGGCTGCTGATCTACGGAGCCAGCACCAGGGC
AACAGGCATCCCCGCACGCTTCTCCGGCTCTGGCAGCGGCACCGACTTTACCCTG
ACAATCAGCTCCCTGCAGCCTGAGGATTTTGCCGTGTACTATTGCGGCCAGTCTTA
CAATTTCCCATATACCTTTGGCGGCGGCACAAAGCTGGAGATCAAG

SEQ ID NO: 22 amino acid sequence of light chain variable region of 6F7L3

NIVMTQSPATLSLSPGERVTLSCRASEIVGTYLSWYQQKPGQAPRLLIYGASTRATGIP
ARFSGSGSGTDFTLTISSLQPEDFAVYYCGQSYNFPYTFGGGTKLEIK

SEQ ID NO: 23 amino acid sequence of heavy chain framework region FR-H1 of 6F7

QVQLQQPGAELVRPGASVKLSCKAS

SEQ ID NO: 24 amino acid sequence of heavy chain framework region FR-H2 of 6F7

MNWVKQRPGQGLEWIGM

SEQ ID NO: 25 amino acid sequence of heavy chain framework region FR-H3 of 6F7

HNNQMFKDKATLTVDKSSNTAYMHLSSLTSEDSAVYHC

SEQ ID NO: 26 amino acid sequence of heavy chain framework region FR-H4 of 6F7

WGAGTTVTVSS

SEQ ID NO: 27 amino acid sequence of light chain framework region FR-L1 of 6F7

NIVMTQSPKSMSMSLGERVTLSCKAS

SEQ ID NO: 28 amino acid sequence of light chain framework region FR-L2 of 6F7

VSWFQQKPHQSPKLLIY

SEQ ID NO: 29 amino acid sequence of light chain framework region FR-L3 of 6F7

NRYTGVPDRFTGSGSATDFTLTISNVQAEDLADYHC

SEQ ID NO: 30 amino acid sequence of light chain framework region FR-L4 of 6F7

FGGGTKLEIK

SEQ ID NO: 31 amino acid sequence of framework region FR-H1 of 6F7H1

QVQLVQSGAEVVKPGASVKLSCKAS

SEQ ID NO: 32 amino acid sequence of framework region FR-H2 of 6F7H1

MNWVRQRPGQGLEWIGM

SEQ ID NO: 33 amino acid sequence of framework region FR-H3 of 6F7H1

HNAQKFQGKATLTVDKSTSTAYMHLSSLRSEDTAVYYC

SEQ ID NO: 34 amino acid sequence of framework region FR-H4 of 6F7H1

WGAGTTVTVSS

SEQ ID NO: 35 amino acid sequence of framework region FR-L1 of 6F7L1

NIVMTQSPATMSMSPGERVTLSCRAS

SEQ ID NO: 36 amino acid sequence of framework region FR-L2 of 6F7L1

VSWFQQKPGQAPRLLIY

SEQ ID NO: 37 amino acid sequence of framework region FR-L3 of 6F7L1

NRYTGVPARFSGSGSGTDFTLTISSVQPEDLADYHC

SEQ ID NO: 38 amino acid sequence of framework region FR-L4 of 6F7L1

FGGGTKLEIK

SEQ ID NO: 39 amino acid sequence of framework region FR-H1 of 6F7H2

QVQLVQSGAEVVKPGASVKVSCKAS

SEQ ID NO: 40 amino acid sequence of framework region FR-H2 of 6F7H2

MNWVRQRPGQGLEWIGI

SEQ ID NO: 41 amino acid sequence of framework region FR-H3 of 6F7H2

SNAQKFQGRVTLTVDKSTSTAYMHLSSLRSEDTAVYYC

SEQ ID NO: 42 amino acid sequence of framework region FR-H4 of 6F7H2

WGAGTTVTVSS

SEQ ID NO: 43 amino acid sequence of framework region FR-L1 of 6F7L2

NIVMTQSPATLSLSPGERVTLSCRAS

SEQ ID NO: 44 amino acid sequence of framework region FR-L2 of 6F7L2

VSWFQQKPGQAPRLLIY

SEQ ID NO: 45 amino acid sequence of framework region FR-L3 of 6F7L2

NRATGIPARFSGSGSGTDFTLTISSLQPEDLADYYC

SEQ ID NO: 46 amino acid sequence of framework region FR-L4 of 6F7L2

FGGGTKLEIK

SEQ ID NO: 47 amino acid sequence of framework region FR-H1 of 6F7H3

QVQLVQSGAEVVKPGASVKVSCKAS

SEQ ID NO: 48 amino acid sequence of framework region FR-H2 of 6F7H3

MNWVRQAPGQGLEWIGI

SEQ ID NO: 49 amino acid sequence of framework region FR-H3 of 6F7H3

SYAQKFQGRVTLTVDKSTSTAYMELSSLRSEDTAVYYC

SEQ ID NO: 50 amino acid sequence of framework region FR-H4 of 6F7H3

WGAGTTVTVSS

SEQ ID NO: 51 amino acid sequence of framework region FR-L1 of 6F7L3

NIVMTQSPATLSLSPGERVTLSCRAS

SEQ ID NO: 52 amino acid sequence of framework region FR-L2 of 6F7L3

LSWYQQKPGQAPRLLIY

SEQ ID NO: 53 amino acid sequence of framework region FR-L3 of 6F7L3

TRATGIPARFSGSGSGTDFTLTISSLQPEDFAVYYC

SEQ ID NO: 54 amino acid sequence of framework region FR-L4 of 6F7L3

FGGGTKLEIK

SEQ ID NO: 55 amino acid sequence of heavy chain constant region of IgG1M

ASTKGPSVFPLAPSSKSTSGGTAALGCLVKDYFPEPVTVSWNSGALTSGVHTFPAVLQ
SSGLYSLSSVVTVPSSSLGTQTYICNVNHKPSNTKVDKKVEPKSCDKTHTCPPCPAPE
AAGGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSHEDPEVKFNWYVDGVEVHNAKT
KPREEQYNSTYRVVSVLTVLHQDWLNGKEYKCKVSNKALPAPIEKTISKAKGQPREP
QVYTLPPSRDELTKNQVSLTCLVKGFYPSDIAVEWESNGQPENNYKTTPPVLDSDGSF
FLYSKLTVDKSRWQQGNVFSCSVMHEALHNHYTQKSLSLSPGK

SEQ ID NO: 56 amino acid sequence of heavy chain constant region Ig gamma-4 chain C region

ASTKGPSVFPLAPCSRSTSESTAALGCLVKDYFPEPVTVSWNSGALTSGVHTFPAVLQS
SGLYSLSSVVTVPSSSLGTKTYTCNVDHKPSNTKVDKRVESKYGPPCPSCPAPEFLGG
PSVFLFPPKPKDTLMISRTPEVTCVVVDVSQEDPEVQFNWYVDGVEVHNAKTKPREE
QFNSTYRVVSVLTVLHQDWLNGKEYKCKVSNKGLPSSIEKTISKAKGQPREPQVYTL
PPSQEEMTKNQVSLTCLVKGFYPSDIAVEWESNGQPENNYKTTPPVLDSDGSFFLYSR
LTVDKSRWQEGNVFSCSVMHEALHNHYTQKSLSLSLGK

SEQ ID NO: 57 amino acid sequence of light chain constant region Ig kappa chain C region

RTVAAPSVFIFPPSDEQLKSGTASVVCLLNNFYPREAKVQWKVDNALQSGNSQESVT
EQDSKDSTYSLSSTLTLSKADYEKHKVYACEVTHQGLSSPVTKSFNRGEC

SEQ ID NO: 58 amino acid sequence of heavy chain constant region Ig gamma-1 chain C region

ASTKGPSVFPLAPSSKSTSGGTAALGCLVKDYFPEPVTVSWNSGALTSGVHTFPAVLQ
SSGLYSLSSVVTVPSSSLGTQTYICNVNHKPSNTKVDKKVEPKSCDKTHTCPPCPAPE
LLGGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSHEDPEVKFNWYVDGVEVHNAKT
KPREEQYNSTYRVVSVLTVLHQDWLNGKEYKCKVSNKALPAPIEKTISKAKGQPREP
QVYTLPPSRDELTKNQVSLTCLVKGFYPSDIAVEWESNGQPENNYKTTPPVLDSDGSF
FLYSKLTVDKSRWQQGNVFSCSVMHEALHNHYTQKSLSLSPGK

SEQ ID NO: 59 amino acid sequence of heavy chain of 6F7H1L1(G1M)

QVQLVQSGAEVVKPGASVKLSCKASGYTFTSYWMNWVRQRPGQGLEWIGMIDPSD
SETHNAQKFQGKATLTVDKSTSTAYMHLSSLRSEDTAVYYCARLYRWYFDVWGAGT
TVTVSSASTKGPSVFPLAPSSKSTSGGTAALGCLVKDYFPEPVTVSWNSGALTSGVHT
FPAVLQSSGLYSLSSVVTVPSSSLGTQTYICNVNHKPSNTKVDKKVEPKSCDKTHTCP
PCPAPEAAGGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSHEDPEVKFNWYVDGVEV
HNAKTKPREEQYNSTYRVVSVLTVLHQDWLNGKEYKCKVSNKALPAPIEKTISKAK
GQPREPQVYTLPPSRDELTKNQVSLTCLVKGFYPSDIAVEWESNGQPENNYKTTPPVL
DSDGSFFLYSKLTVDKSRWQQGNVFSCSVMHEALHNHYTQKSLSLSPGK

SEQ ID NO: 60 amino acid sequence of light chain of 6F7H1L1(G1M)

NIVMTQSPATMSMSPGERVTLSCRASEIVGTYVSWFQQKPGQAPRLLIYGASNRYTG
VPARFSGSGSGTDFTLTISSVQPEDLADYHCGQSYNFPYTFGGGTKLEIKRTVAAPSVF
IFPPSDEQLKSGTASVVCLLNNFYPREAKVQWKVDNALQSGNSQESVTEQDSKDSTY
SLSSTLTLSKADYEKHKVYACEVTHQGLSSPVTKSFNRGEC

SEQ ID NO: 61 amino acid sequence of heavy chain of 6F7H2L2(G1M)

QVQLVQSGAEVVKPGASVKVSCKASGYTFTSYWMNWVRQRPGQGLEWIGIIDPSDS
ETSNAQKFQGRVTLTVDKSTSTAYMHLSSLRSEDTAVYYCARLYRWYFDVWGAGTT
VTVSSASTKGPSVFPLAPSSKSTSGGTAALGCLVKDYFPEPVTVSWNSGALTSGVHTF
PAVLQSSGLYSLSSVVTVPSSSLGTQTYICNVNHKPSNTKVDKKVEPKSCDKTHTCPP
CPAPEAAGGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSHEDPEVKFNWYVDGVEVH
NAKTKPREEQYNSTYRVVSVLTVLHQDWLNGKEYKCKVSNKALPAPIEKTISKAKG
QPREPQVYTLPPSRDELTKNQVSLTCLVKGFYPSDIAVEWESNGQPENNYKTTPPVLD
SDGSFFLYSKLTVDKSRWQQGNVFSCSVMHEALHNHYTQKSLSLSPGK

SEQ ID NO: 62 amino acid sequence of light chain of 6F7H2L2(G1M)

NIVMTQSPATLSLSPGERVTLSCRASEIVGTYVSWFQQKPGQAPRLLIYGASNRATGIP
ARFSGSGSGTDFTLTISSLQPEDLADYYCGQSYNFPYTFGGGTKLEIKRTVAAPSVFIFP
PSDEQLKSGTASVVCLLNNFYPREAKVQWKVDNALQSGNSQESVTEQDSKDSTYSL
SSTLTLSKADYEKHKVYACEVTHQGLSSPVTKSFNRGEC

SEQ ID NO: 63 amino acid sequence of heavy chain of 6F7H3L3(G1M)

QVQLVQSGAEVVKPGASVKVSCKASGYTFTSYWMNWVRQAPGQGLEWIGIIDPSDS
ETSYAQKFQGRVTLTVDKSTSTAYMELSSLRSEDTAVYYCARLYRWYFDVWGAGTT
VTVSSASTKGPSVFPLAPSSKSTSGGTAALGCLVKDYFPEPVTVSWNSGALTSGVHTF
PAVLQSSGLYSLSSVVTVPSSSLGTQTYICNVNHKPSNTKVDKKVEPKSCDKTHTCPP
CPAPEAAGGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSHEDPEVKFNWYVDGVEVH
NAKTKPREEQYNSTYRVVSVLTVLHQDWLNGKEYKCKVSNKALPAPIEKTISKAKG
QPREPQVYTLPPSRDELTKNQVSLTCLVKGFYPSDIAVEWESNGQPENNYKTTPPVLD
SDGSFFLYSKLTVDKSRWQQGNVFSCSVMHEALHNHYTQKSLSLSPGK

SEQ ID NO: 6F764 amino acid sequence of light chain of H3L3(G1M)

NIVMTQSPATLSLSPGERVTLSCRASEIVGTYLSWYQQKPGQAPRLLIYGASTRATGIP

ARFSGSGSGTDFTLTISSLQPEDFAVYYCGQSYNFPYTFGGGTKLEIKRTVAAPSVFIFP
PSDEQLKSGTASVVCLLNNFYPREAKVQWKVDNALQSGNSQESVTEQDSKDSTYSL
SSTLTLSKADYEKHKVYACEVTHQGLSSPVTKSFNRGEC

SEQ ID NO: 65 amino acid sequence of heavy chain of 6F7H1L1(hG4)

QVQLVQSGAEVVKPGASVKLSCKASGYTFTSYWMNWVRQRPGQGLEWIGMIDPSD
SETHNAQKFQGKATLTVDKSTSTAYMHLSSLRSEDTAVYYCARLYRWYFDVWGAGT
TVTVSSASTKGPSVFPLAPCSRSTSESTAALGCLVKDYFPEPVTVSWNSGALTSGVHT
FPAVLQSSGLYSLSSVVTVPSSSLGTKTYTCNVDHKPSNTKVDKRVESKYGPPCPSCP
APEFLGGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSQEDPEVQFNWYVDGVEVHNA
KTKPREEQFNSTYRVVSVLTVLHQDWLNGKEYKCKVSNKGLPSSIEKTISKAKGQPR
EPQVYTLPPSQEEMTKNQVSLTCLVKGFYPSDIAVEWESNGQPENNYKTTPPVLDSD
GSFFLYSRLTVDKSRWQEGNVFSCSVMHEALHNHYTQKSLSLSLGK

SEQ ID NO: 66 amino acid sequence of light chain of 6F7H1L1(hG4)

NIVMTQSPATMSMSPGERVTLSCRASEIVGTYVSWFQQKPGQAPRLLIYGASNRYTG
VPARFSGSGSGTDFTLTISSVQPEDLADYHCGQSYNFPYTFGGGTKLEIKRTVAAPSVF
IFPPSDEQLKSGTASVVCLLNNFYPREAKVQWKVDNALQSGNSQESVTEQDSKDSTY
SLSSTLTLSKADYEKHKVYACEVTHQGLSSPVTKSFNRGEC

SEQ ID NO: 67 amino acid sequence of heavy chain of 6F7H2L2(hG4)

QVQLVQSGAEVVKPGASVKVSCKASGYTFTSYWMNWVRQRPGQGLEWIGIIDPSDS
ETSNAQKFQGRVTLTVDKSTSTAYMHLSSLRSEDTAVYYCARLYRWYFDVWGAGTT
VTVSSASTKGPSVFPLAPCSRSTSESTAALGCLVKDYFPEPVTVSWNSGALTSGVHTF
PAVLQSSGLYSLSSVVTVPSSSLGTKTYTCNVDHKPSNTKVDKRVESKYGPPCPSCPA
PEFLGGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSQEDPEVQFNWYVDGVEVHNAK
TKPREEQFNSTYRVVSVLTVLHQDWLNGKEYKCKVSNKGLPSSIEKTISKAKGQPRE
PQVYTLPPSQEEMTKNQVSLTCLVKGFYPSDIAVEWESNGQPENNYKTTPPVLDSDG
SFFLYSRLTVDKSRWQEGNVFSCSVMHEALHNHYTQKSLSLSLGK

SEQ ID NO: 68 amino acid sequence of light chain of 6F7H2L2(hG4)

NIVMTQSPATLSLSPGERVTLSCRASEIVGTYVSWFQQKPGQAPRLLIYGASNRATGIP
ARFSGSGSGTDFTLTISSLQPEDLADYYCGQSYNFPYTFGGGTKLEIKRTVAAPSVFIFP
PSDEQLKSGTASVVCLLNNFYPREAKVQWKVDNALQSGNSQESVTEQDSKDSTYSL
SSTLTLSKADYEKHKVYACEVTHQGLSSPVTKSFNRGEC

SEQ ID NO: 69 amino acid sequence of heavy chain of 6F7H3L3(hG4)

QVQLVQSGAEVVKPGASVKVSCKASGYTFTSYWMNWVRQAPGQGLEWIGIIDPSDS
ETSYAQKFQGRVTLTVDKSTSTAYMELSSLRSEDTAVYYCARLYRWYFDVWGAGTT
VTVSSASTKGPSVFPLAPCSRSTSESTAALGCLVKDYFPEPVTVSWNSGALTSGVHTF
PAVLQSSGLYSLSSVVTVPSSSLGTKTYTCNVDHKPSNTKVDKRVESKYGPPCPSCPA
PEFLGGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSQEDPEVQFNWYVDGVEVHNAK
TKPREEQFNSTYRVVSVLTVLHQDWLNGKEYKCKVSNKGLPSSIEKTISKAKGQPRE
PQVYTLPPSQEEMTKNQVSLTCLVKGFYPSDIAVEWESNGQPENNYKTTPPVLDSDG
SFFLYSRLTVDKSRWQEGNVFSCSVMHEALHNHYTQKSLSLSLGK

SEQ ID NO: 70 amino acid sequence of light chain of 6F7H3L3(hG4)

NIVMTQSPATLSLSPGERVTLSCRASEIVGTYLSWYQQKPGQAPRLLIYGASTRATGIP
ARFSGSGSGTDFTLTISSLQPEDFAVYYCGQSYNFPYTFGGGTKLEIKRTVAAPSVFIFP
PSDEQLKSGTASVVCLLNNFYPREAKVQWKVDNALQSGNSQESVTEQDSKDSTYSL
SSTLTLSKADYEKHKVYACEVTHQGLSSPVTKSFNRGEC

SEQ ID NO: 71 sequence of mFc tag

PRGPTIKPCPPCKCPAPNLLGGPSVFIFPPKIKDVLMISLSPIVTCVVVDVSEDDPDVQIS
WFVNNVEVHTAQTQTHREDYNSTLRVVSALPIQHQDWMSGKEFKCKVNNKDLPAPI
ERTISKPKGSVRAPQVYVLPPPEEEMTKKQVTLTCMVTDFMPEDIYVEWTNNGKTEL
NYKNTEPVLDSDGSYFMYSKLRVEKKNWVERNSYSCSVVHEGLHNHHTTKSFSRTP
GK

SEQ ID NO: 72 sequence of heavy chain of hIgG

EVQLEQSGAELMKPGASVKISCKATGYTFTTYWIEWIKQRPGHSLEWIGEILPGSDST
YYNEKVKGKVTFTADASSNTAYMQLSSLTSEDSAVYYCARGDGFYVYWGQGTTLTV
SSASTKGPSVFPLAPCSRSTSESTAALGCLVKDYFPEPVTVSWNSGALTSGVHTFPAVL
QSSGLYSLSSVVTVPSSSLGTKTYTCNVDHKPSNTKVDKRVESKYGPPCPPCPAPEFL
GGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSQEDPEVQFNWYVDGVEVHNAKTKP
REEQFNSTYRVVSVLTVLHQDWLNGKEYKCKVSNKGLPSSIEKTISKAKGQPREPQV
YTLPPSQEEMTKNQVSLTCLVKGFYPSDIAVEWESNGQPENNYKTTPPVLDSDGSFFL
YSRLTVDKSRWQEGNVFSCSVMHEALHNHYTQKSLSLSLGK

SEQ ID NO: 73 sequence of light chain of hIgG

DIELTQSPATLSVTPGDSVSLSCRASQSISNNLHWYQQKSHESPRLLIKYTSQSMSGIPS
RFSGSGSGTDFTLSINSVETEDFGVYFCQQSGSWPRTFGGGTKLDIKRTVAAPSVFIFP
PSDEQLKSGTASVVCLLNNFYPREAKVQWKVDNALQSGNSQESVTEQDSKDSTYSL
SSTLTLSKADYEKHKVYACEVTHQGLSSPVTKSFNRGEC

SEQ ID NO: 74 amino acid sequence of TEV

ENLYFQG

SEQ ID NO: 75 sequence of heavy chain variable region of hIgG

EVQLEQSGAELMKPGASVKISCKATGYTFTTYWIEWIKQRPGHSLEWIGEILPGSDST
YYNEKVKGKVTFTADASSNTAYMQLSSLTSEDSAVYYCARGDGFYVYWGQGTTLTV
SS

SEQ ID NO: 76 sequence of light chain variable region of hIgG

DIELTQSPATLSVTPGDSVSLSCRASQSISNNLHWYQQKSHESPRLLIKYTSQSMSGIPS
RFSGSGSGTDFTLSINSVETEDFGVYFCQQSGSWPRTFGGGTKLDIK

SEQ ID NO: 77 nucleic acid sequence of heavy chain of CP004(hG1TM)

GAAGTGCAGCTGGTCGAGTCTGGGGGAGGGCTGGTGCAGCCCGGCGGGTCACTG
CGACTGAGCTGCGCAGCTTCCGGATTCGCCTTTAGCTCCTACGACATGTCCTGGGT
GCGACAGGCACCAGGAAAGGGACTGGATTGGGTCGCTACTATCTCAGGAGGCGG
GAGATACACCTACTATCCTGACAGCGTCAAGGGCCGGTTCACAATCTCTAGAGATA
ACAGTAAGAACAATCTGTATCTGCAGATGAACAGCCTGAGGGCTGAGGACACCGC
ACTGTACTATTGTGCCAACCGCTACGGGGAAGCATGGTTTGCCTATTGGGGGCAGG
GAACCCTGGTGACAGTCTCTAGTgccagcaccaaagggcccagcgtgtttcctctcgccccctcctccaaaagc
accagcggaggaaccgctgctctcggatgtctggtgaaggactacttccctgaacccgtcaccgtgagctggaatagcggcgctctg
acaagcggagtccatacattccctgctgtgctgcaaagcagcggactctattccctgtccagcgtcgtcacagtgcccagcagcagcc
tgggcacccagacctacatctgtaacgtcaaccacaagccctccaacaccaaggtggacaagaaagtggagcccaaatcctgcgac
aagacacacacctgtccccctgtcctgctcccgaaGCTGCTggagCccctagcgtcttcctctttcctcccaaacccaaggaca
ccctcatgatcagcagaacccctgaagtcacctgtgtcgtcgtggatgtcagccatgaggaccccgaggtgaaattcaactggtatgtc
gatggcgtcgaggtgcacaacgccaaaaccaagcccagggaggaacagtacaactccacctacagggtggtgtccgtgctgacag
tcctccaccaggactggctgaacggcaaggagtacaagtgcaaggtgtccaacaaggctctccctgcccccattgagaagaccatca
gcaaggccaaaggccaacccagggagccccaggtctatacactgcctccctccagggacgaactcaccaagaaccaggtgtccct
gacctgcctggtcaagggcttttatcccagcgacatcgccgtcgagtgggagtccaacggacagcccgagaataactacaagaccac
ccctcctgtcctcgactccgacggctccttcttcctgtacagcAAACTGACCGTGGATAAGTCCAGATGGCA
GCAGGGCAATGTCTTTTCATGTTCCGTGATGCACGAGGCACTGCACAACCACTATA
CCCAGAAGTCTCTGAGTCTGTCACCAGGAAAAGGAGGAGGAGGCTCTGGAGGAG
GCGGAAGTGGAGGCGGAGGATCAGGAGGGGGAGGATCTCAGGTGCAGCTGGTCG
AATCCGGAGCCGAGGTGAAGAAACCCGGCGCTTCCGTGAAGGTCTCTTGCAAAG
CATCAGGCTACAGCTTCACAGGGTATACTATGAACTGGGTGCGGCAGGCACCTGG
ACAGTGTCTGGAATGGATCGGCCTGATTAACCCATACAACAACATCACTAACTACG
CCCAGAAGTTCCAGGGCCGGGTGACTTTTACCGTGGACACTAGCATTTCCACCGC
TTACATGGAGCTGAGTCGGCTGAGATCAGACGATACCGGCGTGTATTTTTGCGCAA
GGCTGGATTACAGAAGTTATTGGGGACAGGGAACACTTGTTACAGTCTCTGCTGG
AGGAGGCGGATCTGGAGGAGGAGGATCTGGCGGAGGAGGCAGTGGAGGAGGAG
GATCACAGGCTGTGGTTACTCAGGAACCAAGCCTGACCGTGAGCCCCGGAGGCA
CAGTCACTCTGACCTGTGGGAGCTCCACAGGAGCTGTGACCACATCTAACTTCCC

TAATTGGGTGCAGCAGAAGCCAGGACAGGCACCTCGATCCCTGATCGGGGGAACC
AACAACAAGGCCAGCTGGACACCCGCCAGATTTTCTGGCAGTCTGCTGGGCGGG
AAAGCCGCTCTGACCATTAGCGGCGCTCAGCCTGAGGACGAAGCAGAGTACTATT
GCGCCCTGTGGTATAGTAATCATTGGGTGTTCGGGTGTGGGACAAAACTGACCGT
GCTGAGA

SEQ ID NO: 78 amino acid sequence (full-length) of heavy chain of CP004(hG1TM)
EVQLVESGGGLVQPGGSLRLSCAASGFAFSSYDMSWVRQAPGKGLDWVATISGGGR
YTYYPDSVKGRFTISRDNSKNNLYLQMNSLRAEDTALYYCANRYGEAWFAYWGQGT
LVTVSSASTKGPSVFPLAPSSKSTSGGTAALGCLVKDYFPEPVTVSWNSGALTSGVHT
FPAVLQSSGLYSLSSVVTVPSSSLGTQTYICNVNHKPSNTKVDKKVEPKSCDKTHTCP
PCPAPEAAGAPSVFLFPPKPKDTLMISRTPEVTCVVVDVSHEDPEVKFNWYVDGVEV
HNAKTKPREEQYNSTYRVVSVLTVLHQDWLNGKEYKCKVSNKALPAPIEKTISKAK
GQPREPQVYTLPPSRDELTKNQVSLTCLVKGFYPSDIAVEWESNGQPENNYKTTPPVL
DSDGSFFLYSKLTVDKSRWQQGNVFSCSVMHEALHNHYTQKSLSLSPGKGGGGSGG
GGSGGGGSGGGGSQVQLVESGAEVKKPGASVKVSCKASGYSFTGYTMNWVRQAPG
QCLEWIGLINPYNNITNYAQKFQGRVTFTVDTSISTAYMELSRLRSDDTGVYFCARLD
YRSYWGQGTLVTVSAGGGGSGGGGSGGGGSGGGGSQAVVTQEPSLTVSPGGTVTLT
CGSSTGAVTTSNFPNWVQQKPGQAPRSLIGGTNNKASWTPARFSGSLLGGKAALTIS
GAQPEDEAEYYCALWYSNHWVFGCGTKLTVLR (Note: the underlined parts represent CDR sequences)

SEQ ID NO: 79 nucleic acid sequence of light chain of CP004(hG1TM)
GACATTCAGATGACTCAGAGCCCCTCCTCCATGTCCGCCTCTGTGGGCGACAGGG
TCACCTTCACATGCCGCGCTAGTCAGGATATCAACACCTACCTGAGCTGGTTTCAG
CAGAAGCCAGGGAAAAGCCCCAAGACACTGATCTACCGGGCTAATAGACTGGTGT
CTGGAGTCCCAAGTCGGTTCAGTGGCTCAGGGAGCGGACAGGACTACACTCTGA
CCATCAGCTCCCTGCAGCCTGAGGACATGGCAACCTACTATTGCCTGCAGTATGAT
GAGTTCCCACTGACCTTTGGCGCCGGGACAAAACTGGAGCTGAAGCGAACTGTG

GCCGCTCCCTCCGTCTTCATTTTTCCCCCTTCTGACGAACAGCTGAAATCAGGCAC
AGCCAGCGTGGTCTGTCTGCTGAACAATTTCTACCCTAGAGAGGCAAAAGTGCAG
TGGAAGGTCGATAACGCCCTGCAGTCCGGCAACAGCCAGGAGAGTGTGACTGAA
CAGGACTCAAAAGATAGCACCTATTCCCTGTCTAGTACACTGACTCTGTCCAAGGC
TGATTACGAGAAGCACAAAGTGTATGCATGCGAAGTGACACATCAGGGACTGTCA
AGCCCCGTGACTAAGTCTTTTAACCGGGGCGAATGT

SEQ ID NO: 80 amino acid sequence (full-length) of light chain of CP004(hG1TM)
DIQMTQSPSSMSASVGDRVTFTCRASQDINTYLSWFQQKPGKSPKTLIYRANRLVSG
VPSRFSGSGSGQDYTLTISSLQPEDMATYYCLQYDEFPLTFGAGTKLELKRTVAAPSVF
IFPPSDEQLKSGTASVVCLLNNFYPREAKVQWKVDNALQSGNSQESVTEQDSKDSTY
SLSSTLTLSKADYEKHKVYACEVTHQGLSSPVTKSFNRGEC

SEQ ID NO: 81 nucleic acid sequence of heavy chain of penpulimab: (1344 bp)
GAAGTGCAGCTGGTCGAGTCTGGGGGAGGGCTGGTGCAGCCCGGCGGGTCACTG
CGACTGAGCTGCGCAGCTTCCGGATTCGCCTTTAGCTCCTACGACATGTCCTGGGT
GCGACAGGCACCAGGAAAGGGACTGGATTGGGTCGCTACTATCTCAGGAGGCGG
GAGATACACCTACTATCCTGACAGCGTCAAGGGCCGGTTCACAATCTCTAGAGATA
ACAGTAAGAACAATCTGTATCTGCAGATGAACAGCCTGAGGGCTGAGGACACCGC
ACTGTACTATTGTGCCAACCGCTACGGGGAAGCATGGTTTGCCTATTGGGGGCAGG
GAACCCTGGTGACAGTCTCTAGTGCCAGCACCAAAGGGCCCAGCGTGTTTCCTCT
CGCCCCCTCCTCCAAAAGCACCAGCGGAGGAACCGCTGCTCTCGGATGTCTGGTG
AAGGACTACTTCCCTGAACCCGTCACCGTGAGCTGGAATAGCGGCGCTCTGACAA
GCGGAGTCCATACATTCCCTGCTGTGCTGCAAAGCAGCGGACTCTATTCCCTGTCC
AGCGTCGTCACAGTGCCCAGCAGCAGCCTGGGCACCCAGACCTACATCTGTAACG
TCAACCACAAGCCCTCCAACACCAAGGTGGACAAGAAAGTGGAGCCCAAATCCT
GCGACAAGACACACACCTGTCCCCCCTGTCCTGCTCCCGAAGCTGCTGGAGCCCC
TAGCGTCTTCCTCTTTCCTCCCAAACCCAAGGACACCCTCATGATCAGCAGAACCC
CTGAAGTCACCTGTGTCGTCGTGGATGTCAGCCATGAGGACCCCGAGGTGAAATT

CAACTGGTATGTCGATGGCGTCGAGGTGCACAACGCCAAAACCAAGCCCAGGGA
GGAACAGTACAACTCCACCTACAGGGTGGTGTCCGTGCTGACAGTCCTCCACCAG
GACTGGCTGAACGGCAAGGAGTACAAGTGCAAGGTGTCCAACAAGGCTCTCCCT
GCCCCCATTGAGAAGACCATCAGCAAGGCCAAAGGCCAACCCAGGGAGCCCCAG
GTCTATACACTGCCTCCCTCCAGGGACGAACTCACCAAGAACCAGGTGTCCCTGA
CCTGCCTGGTCAAGGGCTTTTATCCCAGCGACATCGCCGTCGAGTGGGAGTCCAA
CGGACAGCCCGAGAATAACTACAAGACCACCCCTCCTGTCCTCGACTCCGACGGC
TCCTTCTTCCTGTACAGCAAGCTGACCGTGGACAAAAGCAGGTGGCAGCAGGGA
AACGTGTTCTCCTGCAGCGTGATGCACGAAGCCCTCCACAACCACTACACCCAGA
AAAGCCTGTCCCTGAGCCCCGGCAAA

SEQ ID NO: 82 amino acid sequence of heavy chain of penpulimab: (448 aa)

EVQLVESGGGLVQPGGSLRLSCAASGFAFSSYDMSWVRQAPGKGLDWVATISGGGR
YTYYPDSVKGRFTISRDNSKNNLYLQMNSLRAEDTALYYCANRYGEAWFAYWGQGT
LVTVSSASTKGPSVFPLAPSSKSTSGGTAALGCLVKDYFPEPVTVSWNSGALTSGVHT
FPAVLQSSGLYSLSSVVTVPSSSLGTQTYICNVNHKPSNTKVDKKVEPKSCDKTHTCP
PCPAPEAAGAPSVFLFPPKPKDTLMISRTPEVTCVVVDVSHEDPEVKFNWYVDGVEV
HNAKTKPREEQYNSTYRVVSVLTVLHQDWLNGKEYKCKVSNKALPAPIEKTISKAK
GQPREPQVYTLPPSRDELTKNQVSLTCLVKGFYPSDIAVEWESNGQPENNYKTTPPVL
DSDGSFFLYSKLTVDKSRWQQGNVFSCSVMHEALHNHYTQKSLSLSPGK

SEQ ID NO: 83 nucleic acid sequence of light chain of penpulimab: (642 bp)

GACATTCAGATGACTCAGAGCCCCTCCTCCATGTCCGCCTCTGTGGGCGACAGGG
TCACCTTCACATGCCGCGCTAGTCAGGATATCAACACCTACCTGAGCTGGTTTCAG
CAGAAGCCAGGGAAAAGCCCCAAGACACTGATCTACCGGGCTAATAGACTGGTGT
CTGGAGTCCCAAGTCGGTTCAGTGGCTCAGGGAGCGGACAGGACTACACTCTGA
CCATCAGCTCCCTGCAGCCTGAGGACATGGCAACCTACTATTGCCTGCAGTATGAT
GAGTTCCCACTGACCTTTGGCGCCGGGACAAAACTGGAGCTGAAGCGAACTGTG
GCCGCTCCCTCCGTCTTCATTTTTCCCCCTTCTGACGAACAGCTGAAATCAGGCAC

AGCCAGCGTGGTCTGTCTGCTGAACAATTTCTACCCTAGAGAGGCAAAAGTGCAG
TGGAAGGTCGATAACGCCCTGCAGTCCGGCAACAGCCAGGAGAGTGTGACTGAA
CAGGACTCAAAAGATAGCACCTATTCCCTGTCTAGTACACTGACTCTGTCCAAGGC
TGATTACGAGAAGCACAAAGTGTATGCATGCGAAGTGACACATCAGGGACTGTCA
AGCCCCGTGACTAAGTCTTTTAACCGGGGCGAATGT

SEQ ID NO: 84 amino acid sequence of light chain of penpulimab: (214 aa)
DIQMTQSPSSMSASVGDRVTFTCRASQDINTYLSWFQQKPGKSPKTLIYRANRLVSG
VPSRFSGSGSGQDYTLTISSLQPEDMATYYCLQYDEFPLTFGAGTKLELKRTVAAPSVF
IFPPSDEQLKSGTASVVCLLNNFYPREAKVQWKVDNALQSGNSQESVTEQDSKDSTY
SLSSTLTLSKADYEKHKVYACEVTHQGLSSPVTKSFNRGEC

SEQ ID NO: 121 amino acid sequence of heavy chain variable region of penpulimab
EVQLVESGGGLVQPGGSLRLSCAASGFAFSSYDMSWVRQAPGKGLDWVATISG
GGRYTYYPDSVKGRFTISRDNSKNNLYLQMNSLRAEDTALYYCANRYGEAWFAYWG
QGTLVTVSS

SEQ ID NO: 122 amino acid sequence of light chain variable region of penpulimab
DIQMTQSPSSMSASVGDRVTFTCRASQDINTYLSWFQQKPGKSPKTLIYRANRLVSG
VPSRFSGSGSGQDYTLTISSLQPEDMATYYCLQYDEFPLTFGAGTKLELK

SEQ ID NO: 123 HCDR1 of penpulimab
GFAFSSYD
SEQ ID NO: 124 HCDR2 of penpulimab
ISGGGRYT
SEQ ID NO: 125 HCDR3 of penpulimab
ANRYGEAWFAY
SEQ ID NO: 126 LCDR1 of penpulimab
QDINTY

SEQ ID NO: 127 LCDR2 of penpulimab

RAN

SEQ ID NO: 128 LCDR3 of penpulimab

LQYDEFPLT

SEQ ID NO: 85 amino acid sequence of heavy chain of Hu5F9-G4

QVQLVQSGAEVKKPGASVKVSCKASGYTFTNYNMHWVRQAPGQRLEWMGTIYPG
NDDTSYNQKFKDRVTITADTSASTAYMELSSLRSEDTAVYYCARGGYRAMDYWGQG
TLVTVSSASTKGPSVFPLAPCSRSTSESTAALGCLVKDYFPEPVTVSWNSGALTSGVH
TFPAVLQSSGLYSLSSVVTVPSSSLGTKTYTCNVDHKPSNTKVDKRVESKYGPPCPPCP
APEFLGGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSQEDPEVQFNWYVDGVEVHNA
KTKPREEQFNSTYRVVSVLTVLHQDWLNGKEYKCKVSNKGLPSSIEKTISKAKGQPR
EPQVYTLPPSQEEMTKNQVSLTCLVKGFYPSDIAVEWESNGQPENNYKTTPPVLDSD
GSFFLYSRLTVDKSRWQEGNVFSCSVMHEALHNHYTQKSLSLSLGK

SEQ ID NO: 86 amino acid sequence of light chain of Hu5F9-G4

DIVMTQSPLSLPVTPGEPASISCRSSQSIVYSNGNTYLGWYLQKPGQSPQLLIYKVSNR
FSGVPDRFSGSGSGTDFTLKISRVEAEDVGVYYCFQGSHVPYTFGQGTKLEIKRTVAA
PSVFIFPPSDEQLKSGTASVVCLLNNFYPREAKVQWKVDNALQSGNSQESVTEQDSK
DSTYSLSSTLTLSKADYEKHKVYACEVTHQGLSSPVTKSFNRGEC

SEQ ID NO: 87 amino acid sequence of heavy chain variable region of 14C12

EVKLVESGGGLVKPGGSLKLSCAASGFAFSSYDMSWVRQTPEKRLEWVATISGGGRY
TYYPDSVKGRFTISRDNARNTLYLQMSSLRSEDTALYYCANRYGEAWFAYWGQGTL
VTVSA

SEQ ID NO: 88 amino acid sequence of light chain variable region of 14C12

DIKMTQSPSSMYASLGERVTFTCKASQDINTYLSWFQQKPGKSPKTLIYRANRLVDG
VPSRFSGSGSGQDYSLTISSLEYEDMGIYYCLQYDEFPLTFGAGTKLELK

SEQ ID NO: 89 HCDR1 of 14C12

GFAFSSYD

SEQ ID NO: 90 HCDR2 of 14C12

ISGGGRYT

SEQ ID NO: 91 HCDR3 of 14C12

ANRYGEAWFAY

SEQ ID NO: 92 LCDR1 of 14C12

QDINTY

SEQ ID NO: 93 LCDR2 of 14C12

RAN

SEQ ID NO: 94 LCDR3 of 14C12

LQYDEFPLT

SEQ ID NO: 95 amino acid sequence of heavy chain variable region of 4G10

QVKLQESGPELVKPGASMKISCKASGYSFTGYTMNWVKQSHGKNLEWIGLINPYNNI
TNYNQKFMGKATFTVDKSSSTAYMELLRLTSEDSGVYFCARLDYRSYWGQGTLVTV
SAAKTTPPSVY

SEQ ID NO: 96 amino acid sequence of light chain variable region of 4G10

QAVVTQESALTTSPGETVTLTCRSSTGAVTTSNFANWVQEKPDHLFTSLIGGTNNRAP
GVPARFSGSLIGDKAALTITGAQTEDEAIYFCALWYSNHWVFGGGTKLTVLGQPKSSP
SVTLFQGQFC

SEQ ID NO: 97 HCDR1 of 4G10

GYSFTGYT

SEQ ID NO: 98 HCDR2 of 4G10

INPYNNIT

SEQ ID NO: 99 HCDR3 of 4G10

ARLDYRSY

SEQ ID NO: 100 LCDR1 of 4G10

TGAVTTSNF

SEQ ID NO: 101 LCDR2 of 4G10

GTN

SEQ ID NO: 102 LCDR3 of 4G10

ALWYSNHWV

SEQ ID NO: 103 amino acid sequence of heavy chain variable region of humanized monoclonal antibody 14C12H1L1: (118 aa)

EVQLVESGGGLVQPGGSLRLSCAASGFAFSSYDMSWVRQAPGKGLDWVATISGGGR
YTYYPDSVKGRFTISRDNSKNNLYLQMNSLRAEDTALYYCANRYGEAWFAYWGQGT
LVTVSS

SEQ ID NO: 104 amino acid sequence of light chain variable region of 14C12H1L1(M): (108 aa, mutation position based on 14C12H1L1 underlined in the amino acid sequence)

DIQMTQSPSSMSASVGDRVTFTCRASQDINTYLSWFQQKPGKSPKTLIYRANRLVSG
VPSRFSGSGSGQDYTLTISSLQPEDMATYYCLQYDEFPLTFGAGTKLELKR

SEQ ID NO: 105 amino acid sequence of HCDR1 of heavy chain variable region of 14C12H1L1(M)

GFAFSSYD

SEQ ID NO: 106 amino acid sequence of HCDR2 of heavy chain variable region of 14C12H1L1(M)

ISGGGRYT

SEQ ID NO: 107 amino acid sequence of HCDR3 of heavy chain variable region of 14C12H1L1(M)

ANRYGEAWFAY

SEQ ID NO: 108 amino acid sequence of LCDR1 of light chain variable region of 14C12H1L1(M)
QDINTY

SEQ ID NO: 109 amino acid sequence of LCDR2 of light chain variable region of 14C12H1L1(M)
RAN

SEQ ID NO: 110 amino acid sequence of LCDR3 of light chain variable region of 14C12H1L1(M)
LQYDEFPLT

SEQ ID NO: 111 amino acid sequence of heavy chain variable region of bevacizumab (Bevacizumab-Hv): (123 aa)
EVQLVESGGGLVQPGGSLRLSCAASGYTFTNYGMNWVRQAPGKGLEWVGWINTYT
GEPTYAADFKRRFTFSLDTSKSTAYLQMNSLRAEDTAVYYCAKYPHYYGSSHWYFD
VWGQGTLVTVSS

SEQ ID NO: 112 amino acid sequence of light chain variable region of bevacizumab (Bevacizumab-Lv): (107 aa)
DIQMTQSPSSLSASVGDRVTITCSASQDISNYLNWYQQKPGKAPKVLIYFTSSLHSGV
PSRFSGSGSGTDFTLTISSLQPEDFATYYCQQYSTVPWTFGQGTKVEIK

SEQ ID NO: 113 amino acid sequence of HCDR1 of heavy chain variable region of bevacizumab
GYTFTNYG

SEQ ID NO: 114 amino acid sequence of HCDR2 of heavy chain variable region of

bevacizumab

INTYTGEP

SEQ ID NO: 115 amino acid sequence of HCDR3 of heavy chain variable region of bevacizumab

AKYPHYYGSSHWYFDV

SEQ ID NO: 116 amino acid sequence of LCDR1 of light chain variable region of bevacizumab

QDISNY (SEQ ID NO: 116)

SEQ ID NO: 117 amino acid sequence of LCDR2 of light chain variable region of bevacizumab

FTS

SEQ ID NO: 118 amino acid sequence of LCDR3 of light chain variable region of bevacizumab

QQYSTVPWT

SEQ ID NO: 119: amino acid sequence of linker 1

GGGGSGGGGSGGGGS

SEQ ID NO: 120: amino acid sequence of linker 2

GGGGSGGGGSGGGGSGGGGS

SEQ ID NO: 129: amino acid sequence of heavy chain variable region of 14C12H1L1

EVQLVESGGGLVQPGGSLRLSCAASGFAFSSYDMSWVRQAPGKGLDWVATISGGGR
YTYYPDSVKGRFTISRDNSKNNLYLQMNSLRAEDTALYYCANRYGEAWFAYWGQGT
LVTVSS

SEQ ID NO: 130: amino acid sequence of light chain variable region of 14C12H1L1

DIQMTQSPSSMSASVGDRVTFTCRASQDINTYLSWFQQKPGKSPKTLIYRANRLVSG
VPSRFSGSGSGQDYTLTISSLQPEDMATYYCLQYDEFPLTFGAGTKLELK

SEQ ID NO: 131: amino acid sequence of heavy chain variable region of 4G10H1L1
QVQLVESGAELVKPGASMKISCKASGYSFTGYTMNWVKQAPGQGLEWIGLINPYNN
ITNYNQKFMGKATFTVDKSISTAYMELSRLTSDDSGVYFCARLDYRSYWGQGTLVTV
SA

SEQ ID NO: 132: amino acid sequence of light chain variable region of 4G10H1L1
QAVVTQEPSLTVSPGGTVTLTCGSSTGAVTTSNFANWVQEKPGQAFRSLIGGTNNRAS
WVPARFSGSLLGGKAALTISGAQPEDEAEYFCALWYSNHWVFGGGTKLTVL

SEQ ID NO: 133: amino acid sequence of heavy chain variable region of 4G10H3L3
QVQLVESGAEVKKPGASVKVSCKASGYSFTGYTMNWVRQAPGQGLEWIGLINPYN
NITNYAQKFQGRVTFTVDTSISTAYMELSRLRSDDTGVYFCARLDYRSYWGQGTLVT
VSA

SEQ ID NO: 134: amino acid sequence of light chain variable region of 4G10H3L3
QAVVTQEPSLTVSPGGTVTLTCGSSTGAVTTSNFPNWVQQKPGQAPRSLIGGTNNKAS
WTPARFSGSLLGGKAALTISGAQPEDEAEYYCALWYSNHWVFGGGTKLTVL

SEQ ID NO: 135: amino acid sequence of 4G10H1V(M)
QVQLVESGAELVKPGASMKISCKASGYSFTGYTMNWVKQAPGQCLEWIGLINPYNNI
TNYNQKFMGKATFTVDKSISTAYMELSRLTSDDSGVYFCARLDYRSYWGQGTLVTV
SA

SEQ ID NO: 136: amino acid sequence of 4G10L1V(M)

QAVVTQEPSLTVSPGGTVTLTCGSSTGAVTTSNFANWVQEKPGQAFRSLIGGTNNRAS
WVPARFSGSLLGGKAALTISGAQPEDEAEYFCALWYSNHWVFGCGTKLTVLR

SEQ ID NO: 137: amino acid sequence of 4G10H3V(M)

QVQLVESGAEVKKPGASVKVSCKASGYSFTGYTMNWVRQAPGQCLEWIGLINPYN
NITNYAQKFQGRVTFTVDTSISTAYMELSRLRSDDTGVYFCARLDYRSYWGQGTLVT
VSA

SEQ ID NO: 138: amino acid sequence of 4G10L3V(M)

QAVVTQEPSLTVSPGGTVTLTCGSSTGAVTTSNFPNWVQQKPGQAPRSLIGGTNNKAS
WTPARFSGSLLGGKAALTISGAQPEDEAEYYCALWYSNHWVFGCGTKLTVLR

SEQ ID NO: 139: amino acid sequence of heavy chain of immunoglobulin moiety in CP004(hG1TM)

EVQLVESGGGLVQPGGSLRLSCAASGFAFSSYDMSWVRQAPGKGLDWVATISGGGR
YTYYPDSVKGRFTISRDNSKNNLYLQMNSLRAEDTALYYCANRYGEAWFAYWGQGT
LVTVSSASTKGPSVFPLAPSSKSTSGGTAALGCLVKDYFPEPVTVSWNSGALTSGVHT
FPAVLQSSGLYSLSSVVTVPSSSLGTQTYICNVNHKPSNTKVDKKVEPKSCDKTHTCP
PCPAPEAAGAPSVFLFPPKPKDTLMISRTPEVTCVVVDVSHEDPEVKFNWYVDGVEV
HNAKTKPREEQYNSTYRVVSVLTVLHQDWLNGKEYKCKVSNKALPAPIEKTISKAK
GQPREPQVYTLPPSRDELTKNQVSLTCLVKGFYPSDIAVEWESNGQPENNYKTTPPVL
DSDGSFFLYSKLTVDKSRWQQGNVFSCSVMHEALHNHYTQKSLSLSPGK

SEQ ID NO: 140 amino acid sequence of heavy chain of bevacizumab

EVQLVESGGGLVQPGGSLRLSCAASGYTFTNYGMNWVRQAPGKGLEWVGWINTYT
GEPTYAADFKRRFTFSLDTSKSTAYLQMNSLRAEDTAVYYCAKYPHYYGSSHWYFD
VWGQGTLVTVSSASTKGPSVFPLAPSSKSTSGGTAALGCLVKDYFPEPVTVSWNSGA
LTSGVHTFPAVLQSSGLYSLSSVVTVPSSSLGTQTYICNVNHKPSNTKVDKKVEPKSC

DKTHTCPPCPAPELLGGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSHEDPEVKFNWY
VDGVEVHNAKTKPREEQYNSTYRVVSVLTVLHQDWLNGKEYKCKVSNKALPAPIEK
TISKAKGQPREPQVYTLPPSRDELTKNQVSLTCLVKGFYPSDIAVEWESNGQPENNYK
TTPPVLDSDGSFFLYSKLTVDKSRWQQGNVFSCSVMHEALHNHYTQKSLSLSPGK

SEQ ID NO: 141 amino acid sequence of heavy chain of VP101(hG1DM)
EVQLVESGGGLVQPGGSLRLSCAASGYTFTNYGMNWVRQAPGKGLEWVGWINTYT
GEPTYAADFKRRFTFSLDTSKSTAYLQMNSLRAEDTAVYYCAKYPHYYGSSHWYFD
VWGQGTLVTVSSASTKGPSVFPLAPSSKSTSGGTAALGCLVKDYFPEPVTVSWNSGA
LTSGVHTFPAVLQSSGLYSLSSVVTVPSSSLGTQTYICNVNHKPSNTKVDKKVEPKSC
DKTHTCPPCPAPEAAGGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSHEDPEVKFNW
YVDGVEVHNAKTKPREEQYNSTYRVVSVLTVLHQDWLNGKEYKCKVSNKALPAPIE
KTISKAKGQPREPQVYTLPPSRDELTKNQVSLTCLVKGFYPSDIAVEWESNGQPENNY
KTTPPVLDSDGSFFLYSKLTVDKSRWQQGNVFSCSVMHEALHNHYTQKSLSLSPGKG
GGGSGGGGSGGGGSGGGGSEVQLVESGGGLVQPGGSLRLSCAASGFAFSSYDMSWV
RQAPGKGLDWVATISGGGRYTYYPDSVKGRFTISRDNSKNNLYLQMNSLRAEDTALY
YCANRYGEAWFAYWGQGTLVTVSSGGGGSGGGGSGGGGSGGGGSDIQMTQSPSSM
SASVGDRVTFTCRASQDINTYLSWFQQKPGKSPKTLIYRANRLVSGVPSRFSGSGSGQ
DYTLTISSLQPEDMATYYCLQYDEFPLTFGAGTKLELKR (SEQ ID NO: 141)

SEQ ID NO: 142 nucleic acid sequence of heavy chain of VP101(hG1DM)
GAGGTGCAGCTGGTCGAGTCCGGGGGGGGGCTGGTGCAGCCAGGCGGGTCTCTG
AGGCTGAGTTGCGCCGCTTCAGGGTACACCTTCACAAACTATGGAATGAATTGGG
TGCGCCAGGCACCAGGAAAGGGACTGGAGTGGGTCGGCTGGATCAACACTTACA
CCGGGGAACCTACCTATGCAGCCGACTTTAAGCGGCGGTTCACCTTCAGCCTGGA
TACAAGCAAATCCACTGCCTACCTGCAGATGAACAGCCTGCGAGCTGAGGACACC
GCAGTCTACTATTGTGCTAAATATCCCCACTACTATGGGAGCAGCCATTGGTATTTT
GACGTGTGGGGGCAGGGGACTCTGGTGACAGTGAGCAGCGCAAGCACCAAAGG
GCCCAGCGTGTTTCCTCTCGCCCCCTCCTCCAAAAGCACCAGCGGAGGAACCGCT

GCTCTCGGATGTCTGGTGAAGGACTACTTCCCTGAACCCGTCACCGTGAGCTGGA

ATAGCGGCGCTCTGACAAGCGGAGTCCATACATTCCCTGCTGTGCTGCAAAGCAG

CGGACTCTATTCCCTGTCCAGCGTCGTCACAGTGCCCAGCAGCAGCCTGGGCACC

CAGACCTACATCTGTAACGTCAACCACAAGCCCTCCAACACCAAGGTGGACAAG

AAAGTGGAGCCCAAATCCTGCGACAAGACACACACCTGTCCCCCCTGTCCTGCTC

CCGAAGCTGCTGGAGGCCCTAGCGTCTTCCTCTTTCCTCCCAAACCCAAGGACAC

CCTCATGATCAGCAGAACCCCTGAAGTCACCTGTGTCGTCGTGGATGTCAGCCAT

GAGGACCCCGAGGTGAAATTCAACTGGTATGTCGATGGCGTCGAGGTGCACAACG

CCAAAACCAAGCCCAGGGAGGAACAGTACAACTCCACCTACAGGGTGGTGTCCG

TGCTGACAGTCCTCCACCAGGACTGGCTGAACGGCAAGGAGTACAAGTGCAAGG

TGTCCAACAAGGCTCTCCCTGCCCCCATTGAGAAGACCATCAGCAAGGCCAAAGG

CCAACCCAGGGAGCCCCAGGTCTATACACTGCCTCCCTCCAGGGACGAACTCACC

AAGAACCAGGTGTCCCTGACCTGCCTGGTCAAGGGCTTTTATCCCAGCGACATCG

CCGTCGAGTGGGAGTCCAACGGACAGCCCGAGAATAACTACAAGACCACCCCTC

CTGTCCTCGACTCCGACGGCTCCTTCTTCCTGTACAGCAAACTGACCGTCGATAAA

TCTAGGTGGCAGCAGGGCAACGTGTTCTCTTGTTCCGTGATGCATGAAGCACTGC

ACAACCATTATACCCAGAAGTCTCTGAGCCTGTCCCCCGGCAAGGGCGGCGGCGG

CTCTGGAGGAGGAGGCAGCGGCGGAGGAGGCTCCGGAGGCGGCGGCTCTGAGG

TGCAGCTGGTGGAGTCTGGAGGAGGACTGGTGCAGCCTGGAGGCTCCCTGAGGC

TGTCTTGCGCAGCAAGCGGATTCGCCTTTAGCTCCTACGACATGAGCTGGGTGCG

GCAGGCACCTGGCAAGGGTCTGGATTGGGTGGCAACCATCAGCGGAGGCGGCAG

ATACACATACTATCCCGACTCCGTGAAGGGCAGGTTCACCATCTCCCGCGATAACT

CTAAGAACAATCTGTATCTGCAGATGAACAGCCTGAGGGCCGAGGACACAGCCCT

GTACTATTGCGCCAACCGCTACGGCGAGGCCTGGTTTGCCTATTGGGGCCAGGGC

ACCCTGGTGACAGTGTCTAGCGGCGGCGGCGGCAGCGGCGGCGGCGGCTCCGGA

GGAGGCGGCTCTGGCGGCGGCGGCAGCGATATCCAGATGACCCAGTCCCCCTCCT

CTATGTCTGCCAGCGTGGGCGACCGGGTGACCTTCACATGTAGAGCCTCCCAGGA

TATCAACACCTACCTGTCTTGGTTTCAGCAGAAGCCCGGCAAGAGCCCTAAGACA

CTGATCTATCGGGCCAATAGACTGGTGAGCGGAGTGCCTTCCCGGTTCTCCGGCTC

TGGCAGCGGACAGGACTATACCCTGACAATCAGCTCCCTGCAGCCAGAGGATATG
GCCACATACTATTGCCTGCAGTATGACGAGTTCCCCCTGACCTTCGGGGCTGGCAC
TAAGCTGGAGCTGAAAAGA (SEQ ID NO: 142)

SEQ ID NO: 143 amino acid sequence of light chain of VP101(hG1DM)
DIQMTQSPSSLSASVGDRVTITCSASQDISNYLNWYQQKPGKAPKVLIYFTSSLHSGV
PSRFSGSGSGTDFTLTISSLQPEDFATYYCQQYSTVPWTFGQGTKVEIKRTVAAPSVFIF
PPSDEQLKSGTASVVCLLNNFYPREAKVQWKVDNALQSGNSQESVTEQDSKDSTYS
LSSTLTLSKADYEKHKVYACEVTHQGLSSPVTKSFNRGEC (SEQ ID NO: 143)

SEQ ID NO: 144 nucleic acid sequence of light chain of VP101(hG1DM)
GATATTCAGATGACTCAGAGCCCCTCCTCCCTGTCCGCCTCTGTGGGCGACAGGGT
CACCATCACATGCAGTGCTTCACAGGATATTTCCAACTACCTGAATTGGTATCAGC
AGAAGCCAGGAAAAGCACCCAAGGTGCTGATCTACTTCACTAGCTCCCTGCACTC
AGGAGTGCCAAGCCGGTTCAGCGGATCCGGATCTGGAACCGACTTTACTCTGACC
ATTTCTAGTCTGCAGCCTGAGGATTTCGCTACATACTATTGCCAGCAGTATTCTACC
GTGCCATGGACATTTGGCCAGGGGACTAAAGTCGAGATCAAGCGGACCGTGGCCG
CTCCCAGTGTCTTCATTTTTCCCCCTAGCGACGAACAGCTGAAATCCGGGACAGCC
TCTGTGGTCTGTCTGCTGAACAACTTCTACCCTAGAGAGGCAAAAGTGCAGTGGA
AGGTCGATAACGCCCTGCAGAGTGGCAATTCACAGGAGAGCGTGACAGAACAGG
ACTCCAAAGATTCTACTTATAGTCTGTCAAGCACACTGACTCTGAGCAAGGCTGAC
TACGAAAAGCATAAAGTGTATGCATGTGAGGTCACCCACCAGGGGCTGAGCAGTC
CAGTCACCAAGTCATTCAACAGAGGCGAGTGC (SEQ ID NO: 144)

SEQ ID NO: 145 nucleic acid sequence of heavy chain variable region of bevacizumab: (369 bp)
GAGGTGCAGCTGGTCGAGTCCGGGGGGGGGCTGGTGCAGCCAGGCGGGTCTCTG
AGGCTGAGTTGCGCCGCTTCAGGGTACACCTTCACAAACTATGGAATGAATTGGG
TGCGCCAGGCACCAGGAAAGGGACTGGAGTGGGTCGGCTGGATCAACACTTACA

CCGGGGAACCTACCTATGCAGCCGACTTTAAGCGGCGGTTCACCTTCAGCCTGGA
TACAAGCAAATCCACTGCCTACCTGCAGATGAACAGCCTGCGAGCTGAGGACACC
GCAGTCTACTATTGTGCTAAATATCCCCACTACTATGGGAGCAGCCATTGGTATTTT
GACGTGTGGGGGCAGGGGACTCTGGTGACAGTGAGCAGC (SEQ ID NO: 145)

SEQ ID NO: 146 nucleic acid sequence of light chain variable region of bevacizumab: (321 bp)
GATATTCAGATGACTCAGAGCCCCTCCTCCCTGTCCGCCTCTGTGGGCGACAGGGT
CACCATCACATGCAGTGCTTCACAGGATATTTCCAACTACCTGAATTGGTATCAGC
AGAAGCCAGGAAAAGCACCCAAGGTGCTGATCTACTTCACTAGCTCCCTGCACTC
AGGAGTGCCAAGCCGGTTCAGCGGATCCGGATCTGGAACCGACTTTACTCTGACC
ATTTCTAGTCTGCAGCCTGAGGATTTCGCTACATACTATTGCCAGCAGTATTCTACC
GTGCCATGGACATTTGGCCAGGGGACTAAAGTCGAGATCAAG (SEQ ID NO: 146)