

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 SIRP $\alpha$  ECD-hFc-Biotin for binding to CD47, effectively blocking the binding of SIRP $\alpha$  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 disclosure 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 examples, 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 example of the present disclosure 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 example of the present disclosure 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 example of the present disclosure 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 example of the present disclosure 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 example of the present disclosure, the antigen-binding fragment is selected from Fab, Fab', F(ab')<sub>2</sub>, Fd, Fv, dAb, Fab/c, complementarity determining region (CDR) fragment, single-chain antibody (e.g., scFv), bivalent antibody and domain antibody.

In one example of the present disclosure, 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 example of the present disclosure, the antibody that specifically binds to CD47 binds to human CD47 protein with a  $K_D$  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  $K_D$  is measured by a Fortebio system.

In one example of the present disclosure, 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 example of the present disclosure, 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 example of the present disclosure, 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 example of the present disclosure 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 example of the present disclosure 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 example of the present disclosure 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 example of the present disclosure 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 example of the present disclosure 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.

In one aspect, the present disclosure refers to 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 (GGGS)<sub>n</sub>, 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.

In one aspect, the present disclosure refers to 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 disclosed herein; 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 disclosed herein (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 disclosed herein, and the product B3 is the anti-PD-1 monoclonal antibody or the antigen-binding fragment thereof as disclosed herein, 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 disclosed herein, and the product C is different from the product B;

preferably, the kit further comprises a package insert.

In one aspect, the present disclosure refers to 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 disclosed herein, wherein preferably, the method further comprises administering to the subject in need an effective amount of the component C as disclosed herein;

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.

In one aspect, the present disclosure refers to 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 disclosed herein,

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 disclosed herein;

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 disclosed herein;

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

In one aspect, the present disclosure refers to 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 disclosed herein,

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 disclosed herein;

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.

The present disclosure further relates to the following examples:

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:

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 example, the anti-tumor chemotherapeutic is selected from azacitidine, an anthracycline, cytarabine, gemcitabine and cycloctidine.

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,

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-A167, CBT-502, STI-A1014, REMD-290, BGB-A333, BCD-135 and MCLA-1450.

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

In one example of the present disclosure, 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 disclosure, 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 disclosure are the routine operations widely used in the corresponding fields. Meanwhile, in order to better understand the present disclosure, 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 example, such fragments will retain at least one CDR present in the full-length light or heavy chain of the antibody, and in some examples, will comprise a single heavy and/or light chain or a portion thereof. Such biologically active fragments can be produced by

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 examples, 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 examples, 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 disclosure, 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 examples of the present disclosure, 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 examples, 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:  $K_D = k_{dis}/k_{on}$ . A smaller equilibrium dissociation constant indicates a more intensive antibody-antigen binding and a higher affinity between the antibody

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 disclosure to be administered to a subject at time points of a regimen, preferably per kg body weight of the subject. In specific examples of the present disclosure, 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 disclosure, 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 examples, 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 disclosure has the following advantages:

The anti-CD47 monoclonal antibody involved in the present disclosure can effectively block the binding of SIRP $\alpha$  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