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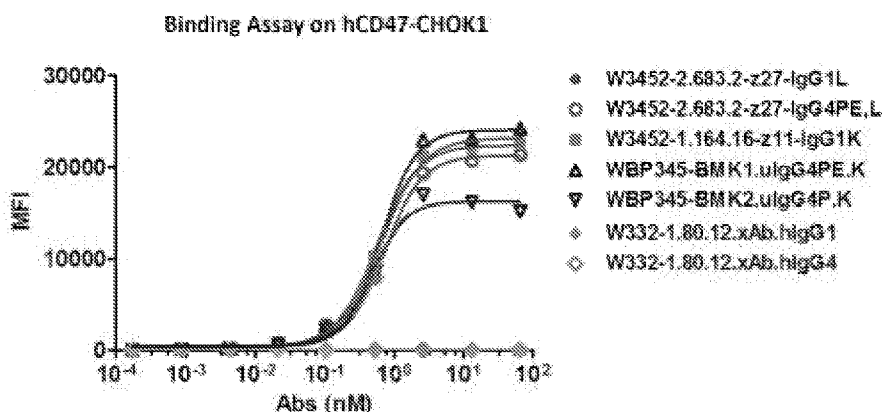


Figure 1

(57) Abstract: The present disclosure provides anti-CD47 antibodies or antigen-binding fragments thereof, isolated polynucleotides encoding the same, pharmaceutical compositions comprising the same, and the uses thereof.



NOVEL ANTI-CD47 ANTIBODIES

PRIORITY CLAIM

[0001] The present application claims the priority to PCT Application Number PCT/CN2018/079673, filed on March 20, 2018.

FIELD OF THE INVENTION

[0002] The present disclosure generally relates to novel anti-human CD47 antibodies.

BACKGROUND

[0003] From Paul Ehrlich proposing ‘magic bullet’ hypothesis to the approval of rituximab in 1997, monoclonal antibody-based therapeutics had made impressive progress in oncology, autoimmune disease, and many other diseases over the past decades. The major mechanisms of mAbs effective against cancers are rely on they interacts with components of the immune system through antibody-dependent cellular cytotoxicity (ADCC), complement-dependent cytotoxicity (CDC), antibody-dependent cellular phagocytosis (ADCP) and alter signal transduction to kill cancer cells. Among these mechanisms, macrophage-mediated cell phagocytosis has been proved an important mechanism of eliminating diseased and damaged cells.

[0004] Cluster of differentiation 47 (CD47), also known as integrin-associated protein (IAP), is an immunoglobulin superfamily membrane protein in the size of approximately 50kDa. CD47 interacts with its ligand on macrophages, signal regulatory protein alpha (SIRP α), to send an anti-phagocytic or “don’t eat me” signal to evade immune surveillance. Analysis of patient tumor and matched adjacent normal tissue reveals that CD47 is overexpressed on AML, NHL, breast cancer, NSCC and ovarian cells, and the increased CD47 expression correlated with a worse clinical prognosis. These data showed CD47 may serve as a new immune checkpoint for cancer therapy by blocking CD47-SIRP α interaction. Several anti-CD47 mAbs and SIRP α fusion protein had achieved effective macrophage involved phagocytosis against AML, NHL, breast cells, and ovarian cells. Besides, anti-CD47 monoclonal antibody combined with approved antibodies (anti-tumor-associated antigen) have efficiently enhanced anti-tumor activity. Based on these preclinical studies, two anti-CD47 mAbs (Hu5F9-G4 and CC-90002) and one SIRP α engineered fusion protein (TTI-621)

are in active phase I or II clinical trials, covering hematological malignancies and human solid tumors.

[0005] There is a significant need for novel anti- CD47 antibodies.

BRIEF SUMMARY OF THE INVENTION

[0004] Throughout the present disclosure, the articles “a,” “an,” and “the” are used herein to refer to one or to more than one (i.e., to at least one) of the grammatical object of the article. By way of example, “an antibody” means one antibody or more than one antibody.

[0005] The present disclosure provides an isolated anti-CD47 antibody or an antigen-binding fragment thereof, comprising:

a) 1, 2, or 3 heavy chain complementarity determining region (CDR) sequences selected from the group consisting of: SEQ ID NO: 1, SEQ ID NO: 3, SEQ ID NO: 5, SEQ ID NO: 7, SEQ ID NO: 9, and SEQ ID NO: 11; and/or

b) 1, 2, or 3 light chain CDR sequences selected from the group consisting of: SEQ ID NO: 2, SEQ ID NO: 4, SEQ ID NO: 6, SEQ ID NO: 8, SEQ ID NO: 10, SEQ ID NO: 12, and SEQ ID NO: 29.

[0006] In certain embodiments, the anti-CD47 antibody or an antigen-binding fragment thereof comprises a heavy chain variable region selected from the group consisting of:

a) a heavy chain variable region comprising 1, 2, or 3 CDR sequences selected from SEQ ID NO: 1, SEQ ID NO: 3, and SEQ ID NO: 5; and

b) a heavy chain variable region comprising 1, 2, or 3 CDR sequences selected from SEQ ID NO: 7, SEQ ID NO: 9, and SEQ ID NO: 11.

[0007] In certain embodiments, the anti-CD47 antibody or an antigen-binding fragment thereof comprises a light chain variable region selected from the group consisting of:

a) a light chain variable region comprising 1, 2, or 3 CDR sequences selected from SEQ ID NO: 2, SEQ ID NO: 4, and SEQ ID NO: 6;

b) a light chain variable region comprising 1, 2, or 3 CDR sequences selected from SEQ ID NO: 29, SEQ ID NO: 4, and SEQ ID NO: 6; and

c) a light chain variable region comprising 1, 2, or 3 CDR sequences selected from SEQ ID NO: 8, SEQ ID NO: 10, and SEQ ID NO: 12.

[0008] In certain embodiments, the anti-CD47 antibody or an antigen-binding fragment thereof comprises:

a) a heavy chain variable region comprising 1, 2, or 3 CDR sequences selected from SEQ ID NO: 1, SEQ ID NO: 3, and SEQ ID NO: 5; and a light chain variable region comprising 1, 2, or 3 CDR sequences selected from SEQ ID NO: 2, SEQ ID NO: 4, and SEQ ID NO: 6;

b) a heavy chain variable region comprising 1, 2, or 3 CDR sequences selected from SEQ ID NO: 1, SEQ ID NO: 3, and SEQ ID NO: 5; and a light chain variable region comprising 1, 2, or 3 CDR sequences selected from SEQ ID NO: 29, SEQ ID NO: 4, and SEQ ID NO: 6; and

c) a heavy chain variable region comprising 1, 2, or 3 CDR sequences selected from SEQ ID NO: 7, SEQ ID NO: 9, and SEQ ID NO: 11; and a light chain variable region comprising 1, 2, or 3 CDR sequences selected from SEQ ID NO: 8, SEQ ID NO: 10, and SEQ ID NO: 12.

[0009] In certain embodiments, the anti-CD47 antibody or an antigen-binding fragment thereof comprises a heavy chain variable region selected from the group consisting of SEQ ID NO: 13, SEQ ID NO: 17, SEQ ID NO: 21, and SEQ ID NO: 25, and a homologous sequence thereof having at least 80% sequence identity yet retaining specific binding affinity to CD47.

[00010] In certain embodiments, the anti-CD47 antibody or an antigen-binding fragment thereof comprises a light chain variable region selected from the group consisting of SEQ ID NO: 15, SEQ ID NO: 19, SEQ ID NO: 23, and SEQ ID NO: 27, and a homologous sequence thereof having at least 80% sequence identity yet retaining specific binding affinity to CD47.

[00011] In certain embodiments, the anti-CD47 antibody or an antigen-binding fragment thereof comprises:

a) a heavy chain variable region comprising SEQ ID NO: 13 and a light chain variable region comprising SEQ ID NO: 15;

b) a heavy chain variable region comprising SEQ ID NO: 17 and a light chain variable region comprising SEQ ID NO: 19;

c) a heavy chain variable region comprising SEQ ID NO: 21 and a light chain variable region comprising SEQ ID NO: 23; and

d) a heavy chain variable region comprising SEQ ID NO: 25 and a light chain

variable region comprising SEQ ID NO: 27.

[00012] In certain embodiments, the isolated anti-CD47 antibody or an antigen-binding fragment thereof further comprises one or more amino acid residue substitutions or modifications yet retains specific binding affinity to CD47. In certain embodiments, at least one of the substitutions or modifications is in one or more of the CDR sequences, and/or in one or more of the VH or VL sequences but not in any of the CDR sequences.

[00013] In certain embodiments, the isolated anti-CD47 antibody or an antigen-binding fragment thereof further comprises an immunoglobulin constant region, optionally a constant region of IgG, or optionally a constant region of human IgG.

[00014] In certain embodiments, the isolated anti-CD47 antibody or an antigen-binding fragment thereof is humanized.

[00015] In certain embodiments, the isolated anti-CD47 antibody or an antigen-binding fragment thereof is a camelized single domain antibody, a diabody, a scFv, an scFv dimer, a BsFv, a dsFv, a (dsFv)₂, an Fv fragment, a Fab, a Fab', a F(ab')₂, a ds diabody, a nanobody, a domain antibody, or a bivalent domain antibody.

[00016] In certain embodiments, the isolated anti-CD47 antibody or an antigen-binding fragment thereof is capable of specifically binding to human CD47 at a K_D value of no more than 10^{-9} M (e.g. no more than 9×10^{-10} M, no more than 8×10^{-10} M, no more than 7×10^{-10} M, no more than 6×10^{-10} M, no more than 5×10^{-10} M, no more than 4×10^{-10} M, no more than 3×10^{-10} M, no more than 2×10^{-10} M, no more than 10^{-10} M, no more than 9×10^{-11} M, no more than 8×10^{-11} M, or no more than 7.5×10^{-11} M) as measured by flow cytometer assay.

[00017] In certain embodiments, the isolated anti-CD47 antibody or an antigen-binding fragment thereof is capable of specifically binding to cynomolgus monkey CD47.

[00018] In certain embodiments, the isolated anti-CD47 antibody or an antigen-binding fragment thereof is linked to one or more conjugate moieties. In certain embodiments, the conjugate moiety comprises a clearance-modifying agent, a chemotherapeutic agent, a toxin, a radioactive isotope, a lanthanide, a luminescent label, a fluorescent label, an enzyme-substrate label, a DNA-alkylators, a topoisomerase inhibitor, a tubulin-binders, or other anticancer drugs.

[00019] The present disclosure also provides herein an antibody or an antigen-binding fragment thereof, which competes for the same epitope with the antibody or antigen-binding fragment thereof provided herein.

[00020] The present disclosure also provides herein a pharmaceutical composition comprising the antibody or antigen-binding fragment thereof provided herein, and a pharmaceutically acceptable carrier.

[00021] The present disclosure also provides herein an isolated polynucleotide encoding the antibody or an antigen-binding fragment thereof as provided herein. In certain embodiments, the isolated polynucleotide comprises a nucleotide sequence selecting from a group consisting of SEQ ID NO: 14, SEQ ID NO: 16, SEQ ID NO: 18, SEQ ID NO: 20, SEQ ID NO: 22, SEQ ID NO: 24, SEQ ID NO: 26, and SEQ ID NO: 28.

[00022] The present disclosure also provides herein a vector comprising the isolated polynucleotide as provided herein.

[00023] The present disclosure also provides herein a host cell comprising the vector as provided herein.

[00024] The present disclosure also provides herein a method of expressing the antibody or antigen-binding fragment thereof of as provided herein, comprising culturing the host cell as provided herein under the condition at which the vector as provided herein is expressed.

[00025] The present disclosure also provides herein a method of treating a disease or condition in a subject that would benefit from modulation of CD47 activity, comprising administering to the subject a therapeutically effective amount of the antibody or antigen-binding fragment thereof or the pharmaceutical composition as provided herein. In certain embodiments, the disease or condition is a CD47 related disease or condition. In certain embodiments, the disease or condition is cancer, autoimmune disease, fibrotic disease, inflammatory disease, or infectious disease. In certain embodiments, the subject is human. In certain embodiments, the administration is via oral, nasal, intravenous, subcutaneous, sublingual, or intramuscular administration.

[00026] In certain embodiments, the cancer is lung cancer, bronchial cancer, bone cancer, liver and bile duct cancer, pancreatic cancer, breast cancer, liver cancer, ovarian cancer, testicle cancer, kidney cancer, bladder cancer, head and neck cancer, spine cancer, brain cancer, cervix cancer, uterine cancer, endometrial cancer, colon cancer, colorectal cancer, rectal cancer, anal cancer, esophageal cancer, gastrointestinal cancer, skin cancer, prostate cancer, pituitary cancer, stomach cancer, vagina cancer, thyroid cancer, glioblastoma, astrocytoma, melanoma, myelodysplastic syndrome, sarcoma, teratoma, adenocarcinoma, leukemia, myeloma and lymphoma.

[00027] In certain embodiments, the disease or condition is hematological cancer selected from non-Hodgkin's lymphoma (NHL), acute lymphocytic leukemia (ALL), acute myeloid leukemia (AML), chronic lymphocytic leukemia (CLL), chronic myelogenous leukemia (CML), multiple myeloma (MM), diffuse large B cell lymphoma (DLBCL), Richter's syndrome, Burkitt's lymphoma or follicular lymphoma.

[00028] The present disclosure also provides herein a method of modulating CD47 activity in a CD47-expressing cell, comprising exposing the CD47-expressing cell to the antibody or antigen-binding fragment thereof as provided herein.

[00029] The present disclosure also provides herein a method of detecting presence or amount of CD47 in a sample, comprising contacting the sample with the antibody or antigen-binding fragment thereof as provided herein, and determining the presence or the amount of CD47 in the sample.

[00030] The present disclosure also provides herein a method of diagnosing a CD47 related disease or condition in a subject, comprising: a) contacting a sample obtained from the subject with the antibody or antigen-binding fragment thereof as provided herein; b) determining presence or amount of CD47 in the sample; and c) correlating the presence or the amount of CD47 to existence or status of the CD47 related disease or condition in the subject.

[00031] The present disclosure also provides herein use of the antibody or antigen-binding fragment thereof as provided herein in the manufacture of a medicament for treating a CD47 related disease or condition in a subject.

[00032] The present disclosure also provides herein use of the antibody or antigen-binding fragment thereof as provided herein in the manufacture of a diagnostic reagent for diagnosing a CD47 related disease or condition.

[00033] The present disclosure also provides herein a kit comprising the antibody or antigen-binding fragment thereof as provided herein, useful in detecting CD47.

BRIEF DESCRIPTION OF FIGURES

[00034] Figure 1 shows FACS binding assay results of W3452-1.164.16-z11-IgG1K, W3452-2.683.2-z27-IgG1L and W3452-2.683.2-z27-IgG4PE,L, to CHOK1 transfected with human CD47, in comparison to benchmark antibodies WBP345-BMK1.uIgG4PE.K, WBP345-BMK2.uIgG4P.K, and isotype control antibodies W332-1.80.12xAb.hIgG1

(Antibody isotype IgG1 negative control, made by WuXi Biologics) and W332-1.80.12xAb.hIgG4 (Antibody isotype IgG4 negative control, made by WuXi Biologics).

[00035] Figure 2 shows FACS binding assay results of W3452-1.164.16-z11-IgG1K, W3452-2.683.2-z27-IgG1L and W3452-2.683.2-z27-IgG4PE,L, to cyno PBMC, in comparison to benchmark antibodies WBP345-BMK1.uIgG4PE.K, WBP345-BMK2.uIgG4P.K, and isotype control antibodies W332-1.80.12xAb.hIgG1 and W332-1.80.12xAb.hIgG4.

[00036] Figure 3 shows FACS ligand (SIRP α) competition assay results of W3452-1.164.16-z11-IgG1K, W3452-2.683.2-z27-IgG1L and W3452-2.683.2-z27-IgG4PE,L, using CHOK1 transfected with human CD47, in comparison to benchmark antibodies WBP345-BMK1.uIgG4PE.K, WBP345-BMK2.uIgG4P.K, and isotype control antibodies W332-1.80.12xAb.hIgG1 and W332-1.80.12xAb.hIgG4.

[00037] Figure 4 shows hemagglutination activity of W3452-1.164.16-z11-IgG1K, W3452-2.683.2-z27-IgG1L and W3452-2.683.2-z27-IgG4PE,L, in comparison to benchmark antibodies WBP345-BMK1.uIgG4PE.K, WBP345-BMK2.uIgG4P.K, and isotype control antibodies W332-1.80.12xAb.hIgG1 and W332-1.80.12xAb.hIgG4.

[00038] Figures 5A and 5B show macrophage-mediated phagocytosis activity of W3452-1.164.16-z11-IgG1K, W3452-2.683.2-z27-IgG1L and W3452-2.683.2-z27-IgG4PE,L, on Jurkat.2B8 cells (Figure 5A) and Raji cells (Figure 5B), respectively, in comparison to benchmark antibodies WBP345-BMK1.uIgG4PE.K, WBP345-BMK2.uIgG4P.K, WBP345-BMK3.uIgG1K, WBP345-BMK4.uIgG4.SPK, and isotype control antibodies W332-1.80.12xAb.hIgG1 and W332-1.80.12xAb.hIgG4.

[00039] Figures 6A-6D show ADCC (Figure 6A and 6B) and CDC (Figure 6C and 6D) activity, respectively, of W3452-1.164.16-z11-IgG1K, W3452-2.683.2-z27-IgG1L and W3452-2.683.2-z27-IgG4PE,L, against Raji cells and CCRF-CEM cells, in comparison to benchmark antibodies WBP345-BMK1.uIgG4PE.K, WBP345-BMK2.uIgG4P.K, and isotype control antibodies W332-1.80.12xAb.hIgG1 and W332-1.80.12xAb.hIgG4.

[00040] Figure 7 shows thermal stability results of W3452-1.164.16-z11-IgG1K, W3452-2.683.2-z27-IgG1L and W3452-2.683.2-z27-IgG4PE,L.

[00041] Figures 8A, 8B and 8C show human serum stability of W3452-1.164.16-z11-IgG1K, W3452-2.683.2-z27-IgG1L and W3452-2.683.2-z27-IgG4PE,L, respectively.

[00042] Figure 9 shows anti-tumor activity of W3452-1.164.16-z11-IgG1K, W3452-2.683.2-z27-IgG1L and W3452-2.683.2-z27-IgG4PE,L, at doses of 0.5mg/kg and 3 mg/kg, respectively, in comparison to a benchmark antibody WBP345-BMK2.uIgG4P.K at the dose of 3mg/kg.

[00043] Figures 10A-10D show red blood cells (RBC, Figure 10A), hemoglobins (HGB, Figure 10B), hematocrits (HCT, Figure 10C) and reticulocyte (RET, Figure 10D) changes in cynomolgus monkey after antibody administration in clinical toxicology study. Each individual male cynomolgus monkey was administered with either the antibody of IgG4 format (W3452-2.683.2-z27-IgG4PE,L) or IgG1 format (W3452-2.683.2-z27-IgG1L) in different dosages (30 mg/kg or 150 mg/kg). C1001 and C1002 represent two individual animals given with W3452-2.683.2-z27-IgG1L, C2001 and C2002 represent two individual animals given with W3452-2.683.2-z27-IgG1L, C3001 and C3002 represent two individual animals given with W3452-2.683.2-z27-IgG4PE,L, and C4001 and C4002 represent two individual animals given with W3452-2.683.2-z27-IgG4PE,L.

[00044] Figure 11 shows serum antibody concentration after W3452-2.683.2-z27-IgG4PE,L administration to individual cynomolgus monkey with different dosages (30 mg/kg or 150 mg/kg).

DETAILED DESCRIPTION OF THE INVENTION

[00045] The following description of the disclosure is merely intended to illustrate various embodiments of the disclosure. As such, the specific modifications discussed are not to be construed as limitations on the scope of the disclosure. It will be apparent to one skilled in the art that various equivalents, changes, and modifications may be made without departing from the scope of the disclosure, and it is understood that such equivalent embodiments are to be included herein. All references cited herein, including publications, patents and patent applications are incorporated herein by reference in their entirety.

[00046] Definitions

[00047] The term “antibody” as used herein includes any immunoglobulin, monoclonal antibody, polyclonal antibody, multivalent antibody, bivalent antibody, monovalent antibody, antibody that binds to a specific antigen. A native intact antibody comprises two heavy (H) chains and two light (L) chains. Mammalian heavy chains are classified as alpha, delta, epsilon, gamma, and mu, each heavy chain consists of a variable region (V_H) and a first, second, and third constant region (C_{H1}, C_{H2}, C_{H3}, respectively); mammalian light chains are

classified as λ or κ , while each light chain consists of a variable region (V_L) and a constant region. The antibody has a “Y” shape, with the stem of the Y consisting of the second and third constant regions of two heavy chains bound together via disulfide bonding. Each arm of the Y includes the variable region and first constant region of a single heavy chain bound to the variable and constant regions of a single light chain. The variable regions of the light and heavy chains are responsible for antigen binding. The variable regions in both chains generally contain three highly variable loops called the complementarity determining regions (CDRs) (light chain CDRs including LCDR1, LCDR2, and LCDR3, heavy chain CDRs including HCDR1, HCDR2, HCDR3). CDR boundaries for the antibodies and antigen-binding fragments disclosed herein may be defined or identified by the conventions of Kabat, IMGT, Chothia, or Al-Lazikani (Al-Lazikani, B., Chothia, C., Lesk, A. M., J. Mol. Biol., 273(4), 927 (1997); Chothia, C. et al., J Mol Biol. Dec 5;186(3):651-63 (1985); Chothia, C. and Lesk, A.M., J.Mol.Biol., 196,901 (1987); Chothia, C. et al., Nature. Dec 21-28;342(6252):877-83 (1989) ; Kabat E.A. et al., National Institutes of Health, Bethesda, Md. (1991); Marie-Paule Lefranc et al, Developmental and Comparative Immunology, 27: 55-77 (2003); Marie-Paule Lefranc et al, Immunome Research, 1(3), (2005); Marie-Paule Lefranc, Molecular Biology of B cells (second edition), chapter 26, 481-514, (2015)). The three CDRs are interposed between flanking stretches known as framework regions (FRs), which are more highly conserved than the CDRs and form a scaffold to support the hypervariable loops. The constant regions of the heavy and light chains are not involved in antigen-binding, but exhibit various effector functions. Antibodies are assigned to classes based on the amino acid sequence of the constant region of their heavy chain. The five major classes or isotypes of antibodies are IgA, IgD, IgE, IgG, and IgM, which are characterized by the presence of alpha, delta, epsilon, gamma, and mu heavy chains, respectively. Several of the major antibody classes are divided into subclasses such as IgG1 (gamma1 heavy chain), IgG2 (gamma2 heavy chain), IgG3 (gamma3 heavy chain), IgG4 (gamma4 heavy chain), IgA1 (alpha1 heavy chain), or IgA2 (alpha2 heavy chain).

[00048] The term “bivalent” as used herein refers to an antibody or an antigen-binding fragment having two antigen-binding sites; the term “monovalent” refers to an antibody or an antigen-binding fragment having only one single antigen-binding site; and the term “multivalent” refers to an antibody or an antigen-binding fragment having multiple antigen-binding sites. In some embodiments, the antibody or antigen-binding fragment thereof is bivalent.

[00049] The term “antigen-binding fragment” as used herein refers to an antibody fragment formed from a portion of an antibody comprising one or more CDRs, or any other antibody fragment that binds to an antigen but does not comprise an intact native antibody structure. Examples of antigen-binding fragment include, without limitation, a diabody, a Fab, a Fab', a $F(ab')_2$, an Fv fragment, a disulfide stabilized Fv fragment (dsFv), a $(dsFv)_2$, a disulfide stabilized diabody (ds diabody), a single-chain antibody molecule (scFv), an scFv dimer (bivalent diabody), a camelized single domain antibody, a nanobody, a domain antibody, and a bivalent domain antibody. An antigen-binding fragment is capable of binding to the same antigen to which the parent antibody binds.

[00050] “Fab” with regard to an antibody refers to that portion of the antibody consisting of a single light chain (both variable and constant regions) bound to the variable region and first constant region of a single heavy chain by a disulfide bond.

[00051] “Fab'” refers to a Fab fragment that includes a portion of the hinge region.

[00052] “ $F(ab')_2$ ” refers to a dimer of Fab'. “Fv” with regard to an antibody refers to the smallest fragment of the antibody to bear the complete antigen-binding site. An Fv fragment consists of the variable region of a single light chain bound to the variable region of a single heavy chain.

[00053] A “dsFv” refers to a disulfide-stabilized Fv fragment that the linkage between the variable region of a single light chain and the variable region of a single heavy chain is a disulfide bond. In some embodiments, a “ $(dsFv)_2$ ” comprises three peptide chains: two V_H moieties linked by a peptide linker (*e.g.*, a long flexible linker) and bound to two V_L moieties, respectively, via disulfide bridges.

[00054] “Single-chain Fv antibody” or “scFv” refers to an engineered antibody consisting of a light chain variable region and a heavy chain variable region connected to one another directly or via a peptide linker sequence (Huston JS *et al.* Proc Natl Acad Sci USA, 85:5879(1988)).

[00055] “Fc” with regard to an antibody refers to that portion of the antibody consisting of the second and third constant regions of a first heavy chain bound to the second and third constant regions of a second heavy chain via disulfide bonding. The Fc portion of the antibody is responsible for various effector functions such as antibody-dependent cell-mediated cytotoxicity (ADCC), and complement dependent cytotoxicity (CDC), but does not function in antigen binding.

[00056] “Single-chain Fv-Fc antibody” or “scFv-Fc” refers to an engineered antibody consisting of a scFv connected to the Fc region of an antibody.

[00057] “Camelized single domain antibody,” “heavy chain antibody,” or “HCAb” refers to an antibody that contains two V_H domains and no light chains (Riechmann L. and Muyldermans S., J Immunol Methods. Dec 10;231(1-2):25-38 (1999); Muyldermans S., J Biotechnol. Jun;74(4):277-302 (2001); WO94/04678; WO94/25591; U.S. Patent No. 6,005,079). Heavy chain antibodies were originally derived from *Camelidae* (camels, dromedaries, and llamas). Although devoid of light chains, camelized antibodies have an authentic antigen-binding repertoire (Hamers-Casterman C. *et al.*, Nature. Jun 3;363(6428):446-8 (1993); Nguyen VK. *et al.* “Heavy-chain antibodies in Camelidae; a case of evolutionary innovation,” Immunogenetics. Apr;54(1):39-47 (2002); Nguyen VK. *et al.* Immunology. May;109(1):93-101 (2003)). The variable domain of a heavy chain antibody (VHH domain) represents the smallest known antigen-binding unit generated by adaptive immune responses (Koch-Nolte F. *et al.*, FASEB J. Nov;21(13):3490-8. Epub 2007 Jun 15 (2007)).

[00058] A “nanobody” refers to an antibody fragment that consists of a VHH domain from a heavy chain antibody and two constant domains, CH2 and CH3.

[00059] “Diabodies” or “dAbs” include small antibody fragments with two antigen-binding sites, wherein the fragments comprise a V_H domain connected to a V_L domain in the same polypeptide chain (V_H - V_L or V_L - V_H) (see, *e.g.*, Holliger P. *et al.*, Proc Natl Acad Sci U S A. Jul 15;90(14):6444-8 (1993); EP404097; WO93/11161). By using a linker that is too short to allow pairing between the two domains on the same chain, the domains are forced to pair with the complementary domains of another chain, thereby creating two antigen-binding sites. The antigen-binding sites may target the same antigen (or epitope). In certain embodiments, an “scFv dimer” is a bivalent diabody or bivalent ScFv (BsFv) comprising V_H - V_L (linked by a peptide linker) dimerized with another V_H - V_L moiety such that V_H 's of one moiety coordinate with the V_L 's of the other moiety and form two binding sites which can target the same antigen (or epitope).

[00060] A “domain antibody” refers to an antibody fragment containing only the variable region of a heavy chain or the variable region of a light chain. In certain instances, two or more V_H domains are covalently joined with a peptide linker to create a bivalent or

multivalent domain antibody. The two V_H domains of a bivalent domain antibody may target the same or different antigens.

[00061] The term “chimeric” as used herein, means an antibody or antigen-binding fragment, having a portion of heavy and/or light chain derived from one species, and the rest of the heavy and/or light chain derived from a different species. In an illustrative example, a chimeric antibody may comprise a constant region derived from human and a variable region from a non-human animal, such as from mouse. In some embodiments, the non-human animal is a mammal, for example, a mouse, a rat, a rabbit, a goat, a sheep, a guinea pig, or a hamster.

[00062] The term “humanized” as used herein means that the antibody or antigen-binding fragment comprises CDRs derived from non-human animals, FR regions derived from human, and when applicable, the constant regions derived from human.

[00063] “CD47” as used herein, is derived from any vertebrate source, including mammals such as primates (e.g. humans, monkeys) and rodents (e.g., mice and rats). Exemplary sequence of human CD47 includes human CD47 protein (NCBI accession number GI: 4502673). Exemplary sequence of CD47 includes *Mus musculus* (mouse) CD47 protein (NCBI accession number GI: 76364104), and *Macaca fascicularis* (monkey) CD47 protein (NCBI accession number 544416831).

[00064] The term “CD47” as used herein is intended to encompass any form of CD47, for example, 1) native unprocessed CD47 molecule, “full-length” CD47 chain or naturally occurring variants of CD47, including, for example, splice variants or allelic variants; 2) any form of CD47 that results from processing in the cell; or 3) full length, a fragment (e.g., a truncated form, an extracellular/transmembrane domain) or a modified form (e.g. a mutated form, a glycosylated/PEGylated, a His-tag/immunofluorescence fused form) of CD47 subunit generated through recombinant method.

[00065] The term “anti-CD47 antibody” refers to an antibody that is capable of specific binding CD47 (e.g. human or monkey or mouse or rat CD47).

[00066] The term “specific binding” or “specifically binds” as used herein refers to a non-random binding reaction between two molecules, such as for example between an antibody and an antigen. In certain embodiments, the antibodies or antigen-binding fragments provided herein specifically bind to human and/or CD47 with a binding affinity (K_D) of $\leq 10^{-6}$ M (e.g., $\leq 5 \times 10^{-7}$ M, $\leq 2 \times 10^{-7}$ M, $\leq 10^{-7}$ M, $\leq 5 \times 10^{-8}$ M, $\leq 2 \times 10^{-8}$ M, $\leq 10^{-8}$ M, $\leq 5 \times 10^{-9}$ M, $\leq 4 \times 10^{-9}$ M).

^9M , $\leq 3 \times 10^{-9}\text{M}$, $\leq 2 \times 10^{-9}\text{M}$, $\leq 10^{-9}\text{M}$, $5 \times 10^{-10}\text{M}$, or $5 \times 10^{-11}\text{M}$). K_D used herein refers to the ratio of the dissociation rate to the association rate ($k_{\text{off}}/k_{\text{on}}$), which may be determined by using any conventional method known in the art, including but are not limited to surface plasmon resonance method, microscale thermophoresis method, HPLC-MS method and flow cytometry (such as FACS) method. In certain embodiments, the K_D value can be appropriately determined by using flow cytometry.

[00067] The ability to “block binding” or “compete for the same epitope” as used herein refers to the ability of an antibody or antigen-binding fragment to inhibit the binding interaction between two molecules (e.g. human CD47 and an anti-CD47 antibody) to any detectable degree. In certain embodiments, an antibody or antigen-binding fragment that blocks binding between two molecules inhibits the binding interaction between the two molecules by at least 85%, or at least 90%. In certain embodiments, this inhibition may be greater than 85%, or greater than 90%.

[00068] The term “epitope” as used herein refers to the specific group of atoms or amino acids on an antigen to which an antibody binds. Two antibodies may bind the same or a closely related epitope within an antigen if they exhibit competitive binding for the antigen. For example, if an antibody or antigen-binding fragment blocks binding of a reference antibody to the antigen by at least 85%, or at least 90%, or at least 95%, then the antibody or antigen-binding fragment may be considered to bind the same/closely related epitope as the reference antibody.

[00069] Those skilled in the art will recognize that it is possible to determine, without undue experimentation, if a given antibody binds to the same epitope as the antibody of present disclosure (e.g., rodent monoclonal antibody W3452-1.164.16 and W3452-2.683.2, and humanized antibody W3452-1.164.16-z11 and W3452-2.683.2-z27) by ascertaining whether the former prevents the latter from binding to a CD47 antigen polypeptide. If the given antibody competes with the antibody of present disclosure, as shown by a decrease in binding by the antibody of present disclosure to the CD47 antigen polypeptide, then the two antibodies bind to the same, or a closely related, epitope. Or if the binding of a given antibody to the CD47 antigen polypeptide was inhibited by the antibody of present disclosure, then the two antibodies bind to the same, or a closely related, epitope.

[00070] The antibody names as used herein may include one or more suffix symbols which usually indicates the type of the antibody or particular modifications made to the antibody.

For example, “IgG1” or “IgG4” means a human (unless otherwise indicated) antibody constant region of IgG1 isotype or an IgG4 isotype, “z” means humanized antibody, “K” means Kappa light chain, “P” and “E” respectively means S228P and L235E in human IgG4 constant region.

[00071] A “conservative substitution” with reference to amino acid sequence refers to replacing an amino acid residue with a different amino acid residue having a side chain with similar physiochemical properties. For example, conservative substitutions can be made among amino acid residues with hydrophobic side chains (e.g. Met, Ala, Val, Leu, and Ile), among residues with neutral hydrophilic side chains (e.g. Cys, Ser, Thr, Asn and Gln), among residues with acidic side chains (e.g. Asp, Glu), among amino acids with basic side chains (e.g. His, Lys, and Arg), or among residues with aromatic side chains (e.g. Trp, Tyr, and Phe). As known in the art, conservative substitution usually does not cause significant change in the protein conformational structure, and therefore could retain the biological activity of a protein.

[00072] The term “homologue” and “homologous” as used herein are interchangeable and refer to nucleic acid sequences (or its complementary strand) or amino acid sequences that have sequence identity of at least 80% (e.g., at least 85%, 88%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%) to another sequences when optimally aligned.

[00073] “Percent (%) sequence identity” with respect to amino acid sequence (or nucleic acid sequence) is defined as the percentage of amino acid (or nucleic acid) residues in a candidate sequence that are identical to the amino acid (or nucleic acid) residues in a reference sequence, after aligning the sequences and, if necessary, introducing gaps, to achieve the maximum number of identical amino acids (or nucleic acids). Conservative substitution of the amino acid residues may or may not be considered as identical residues. Alignment for purposes of determining percent amino acid (or nucleic acid) sequence identity can be achieved, for example, using publicly available tools such as BLASTN, BLASTp (available on the website of U.S. National Center for Biotechnology Information (NCBI), see also, Altschul S.F. et al, J. Mol. Biol., 215:403–410 (1990); Stephen F. et al, Nucleic Acids Res., 25:3389–3402 (1997)), ClustalW2 (available on the website of European Bioinformatics Institute, see also, Higgins D.G. et al, Methods in Enzymology, 266:383-402 (1996); Larkin M.A. et al, Bioinformatics (Oxford, England), 23(21): 2947-8 (2007)), and ALIGN or Megalign (DNASTAR) software. Those skilled in the art may use the default

parameters provided by the tool, or may customize the parameters as appropriate for the alignment, such as for example, by selecting a suitable algorithm.

[00074] “Effector functions” as used herein refer to biological activities attributable to the binding of Fc region of an antibody to its effectors such as C1 complex and Fc receptor. Exemplary effector functions include: complement dependent cytotoxicity (CDC) induced by interaction of antibodies and C1q on the C1 complex; antibody-dependent cell-mediated cytotoxicity (ADCC) induced by binding of Fc region of an antibody to Fc receptor on an effector cell; and phagocytosis.

[00075] “Treating” or “treatment” of a condition as used herein includes preventing or alleviating a condition, slowing the onset or rate of development of a condition, reducing the risk of developing a condition, preventing or delaying the development of symptoms associated with a condition, reducing or ending symptoms associated with a condition, generating a complete or partial regression of a condition, curing a condition, or some combination thereof.

[00076] An “isolated” substance has been altered by the hand of man from the natural state. If an “isolated” composition or substance occurs in nature, it has been changed or removed from its original environment, or both. For example, a polynucleotide or a polypeptide naturally present in a living animal is not “isolated,” but the same polynucleotide or polypeptide is “isolated” if it has been sufficiently separated from the coexisting materials of its natural state so as to exist in a substantially pure state.

[00077] The term “vector” as used herein refers to a vehicle into which a polynucleotide encoding a protein may be operably inserted so as to bring about the expression of that protein. A vector may be used to transform, transduce, or transfect a host cell so as to bring about expression of the genetic element it carries within the host cell. Examples of vectors include plasmids, phagemids, cosmids, artificial chromosomes such as yeast artificial chromosome (YAC), bacterial artificial chromosome (BAC), or P1-derived artificial chromosome (PAC), bacteriophages such as lambda phage or M13 phage, and animal viruses. Categories of animal viruses used as vectors include retrovirus (including lentivirus), adenovirus, adeno-associated virus, herpesvirus (*e.g.*, herpes simplex virus), poxvirus, baculovirus, papillomavirus, and papovavirus (*e.g.*, SV40). A vector may contain a variety of elements for controlling expression, including promoter sequences, transcription initiation sequences, enhancer sequences, selectable elements, and reporter genes. In addition, the

vector may contain an origin of replication. A vector may also include materials to aid in its entry into the cell, including but not limited to a viral particle, a liposome, or a protein coating. A vector can be an expression vector or a cloning vector. The present disclosure provides vectors (e.g., expression vectors) containing the nucleic acid sequence provided herein encoding the antibody or antigen-binding fragment thereof, at least one promoter (e.g., SV40, CMV, EF-1 α) operably linked to the nucleic acid sequence, and at least one selection marker. Examples of vectors include, but are not limited to, retrovirus (including lentivirus), adenovirus, adeno-associated virus, herpesvirus (e.g., herpes simplex virus), poxvirus, baculovirus, papillomavirus, papovavirus (e.g., SV40), lambda phage, and M13 phage, plasmid pcDNA3.3, pMD18-T, pOptivec, pCMV, pEGFP, pIRES, pQD-Hyg-GSeu, pALTER, pBAD, pcDNA, pCal, pL, pET, pGEMEX, pGEX, pCI, pEGFT, pSV2, pFUSE, pVITRO, pVIVO, pMAL, pMONO, pSELECT, pUNO, pDUO, Psg5L, pBABE, pWPXL, pBI, p15TV-L, pPro18, pTD, pRS10, pLexA, pACT2.2, pCMV-SCRIPT.RTM., pCDM8, pCDNA1.1/amp, pcDNA3.1, pRc/RSV, PCR 2.1, pEF-1, pFB, pSG5, pXT1, pCDEF3, pSVSPORT, pEF-Bos etc.

[00078] The phrase “host cell” as used herein refers to a cell into which an exogenous polynucleotide and/or a vector has been introduced.

[00079] A “CD47-related” disease or condition as used herein refers to any disease or condition caused by, exacerbated by, or otherwise linked to increased or decreased expression or activities of CD47. In some embodiments, the CD47 related condition is immune-related disorder, such as, for example, cancer, autoimmune disease, inflammatory disease or infectious disease.

[00080] “Cancer” as used herein refers to any medical condition characterized by malignant cell growth or neoplasm, abnormal proliferation, infiltration or metastasis, and includes both solid tumors and non-solid cancers (hematologic malignancies) such as leukemia. As used herein “solid tumor” refers to a solid mass of neoplastic and/or malignant cells. Examples of cancer or tumors include hematological malignancies, oral carcinomas (for example of the lip, tongue or pharynx), digestive organs (for example esophagus, stomach, small intestine, colon, large intestine, or rectum), peritoneum, liver and biliary passages, pancreas, respiratory system such as larynx or lung (small cell and non-small cell), bone, connective tissue, skin (e.g., melanoma), breast, reproductive organs (fallopian tube, uterus, cervix, testicles, ovary, or prostate), urinary tract (e.g., bladder or kidney), brain and endocrine glands such as the thyroid. In certain embodiments, the cancer is selected from ovarian cancer, breast cancer,

head and neck cancer, renal cancer, bladder cancer, hepatocellular cancer, and colorectal cancer. In certain embodiments, the cancer hematological cancer. In certain embodiments, the hematological cancer is selected from non-Hodgkin's lymphoma (NHL), acute lymphocytic leukemia (ALL), acute myeloid leukemia (AML), chronic lymphocytic leukemia (CLL), chronic myelogenous leukemia (CML), multiple myeloma (MM), diffuse large B cell lymphoma (DLBCL), Richter's syndrome, Burkitt's lymphoma or follicular lymphoma.

[00081] The term “pharmaceutically acceptable” indicates that the designated carrier, vehicle, diluent, excipient(s), and/or salt is generally chemically and/or physically compatible with the other ingredients comprising the formulation, and physiologically compatible with the recipient thereof.

[00082] Anti-CD47 antibody

[00083] The present disclosure provides anti-CD47 antibodies and antigen-binding fragments thereof comprising one or more (e.g. 1, 2, 3, 4, 5, or 6) CDR sequences of an anti-CD47 antibody W3452-1.164.16, W3452-1.164.16-z11, W3452-2.683.2, or W3452-2.683.2-z27 .

[00084] “W3452-1.164.16” as used herein refers to a rodent monoclonal antibody having a heavy chain variable region of SEQ ID NO: 13, and a kappa light chain variable region of SEQ ID NO: 15.

[00085] “W3452-1.164.16-z11” as used herein refers to a humanized monoclonal antibody having a heavy chain variable region of SEQ ID NO: 17, and a kappa light chain variable region of SEQ ID NO: 19.

[00086] “W3452-2.683.2” as used herein refers to a rodent monoclonal antibody having a heavy chain variable region of SEQ ID NO: 21, and a lambda light chain variable region of SEQ ID NO: 23.

[00087] “W3452-2.683.2-z27” as used herein refers to a humanized monoclonal antibody having a heavy chain variable region of SEQ ID NO: 25, and a lambda light chain variable region of SEQ ID NO: 27.

[00088] Table 1 shows the CDR sequences of these anti-CD47 antibodies. The heavy chain and light chain variable region sequences are also provided below in Table 2 and Table 3.

[00089] Table 1. CDR amino acid sequences

		CDR1	CDR2	CDR3
W3452-1.164.16	HCDR	SEQ ID NO: 1	SEQ ID NO: 3	SEQ ID NO: 5
		GFSLTNYHVY	VMWNHGDTSYN SPLKS	GGYVPDY
	LCDR	SEQ ID NO: 2	SEQ ID NO: 4	SEQ ID NO: 6
		RSSQSLVHSNGKT YLH	RVSNRFS	LQTTHFPYT
W3452-1.164.16- z11	HCDR	SEQ ID NO: 1	SEQ ID NO: 3	SEQ ID NO: 5
		GFSLTNYHVY	VMWNHGDTSYN SPLKS	GGYVPDY
	LCDR	SEQ ID NO: 29	SEQ ID NO: 4	SEQ ID NO: 6
		RSSQSLVHSNAKT YLH	RVSNRFS	LQTTHFPYT
W3452-2.683.2, and W3452-2.683.2- z27	HCDR	SEQ ID NO: 7	SEQ ID NO: 9	SEQ ID NO: 11
		GFTFSTYGMA	SISTSGGSTYYRD SVQG	KGGRTYRLAY
	LCDR	SEQ ID NO: 8	SEQ ID NO: 10	SEQ ID NO: 12
		SGIELSNKYAH	KDSERPS	LSTNSDDDLPV

[00090] Table 2. Variable region amino acid sequences

	VH	VL
W3452-1.164.16	SEQ ID NO: 13	SEQ ID NO: 15
	QVQLKESGPGLVQPSQTLSTCTV SGFSLTNYHVYWVRQPPGQGLEW MGVMWNHGDTSYNSPLKSRLNIS RDTSKSQVFLEMSGLQTEDSATY YCARGGYVPDYWGQGVMVTVSS	DVVMQTPTVSLPVSPGGQASISC RSSQSLVHSNGKTYLHWYLQKP GQSPQLLIYRVSNRFSGLPDRFSG SGSGDFTLKISRVEDLDGYY CLQTTHFPYTFGAGTKLELK
W3452-1.164.16- z11	SEQ ID NO: 17	SEQ ID NO: 19
	QVQLQESGPGLVKPSSETLSLTCTV SGFSLTNYHVYWRQPPGKGLEWI GVMWNHGDTSYNSPLKSRVTISV DTSKSKFSLKLSSVTAADTAVYYC ARGGYVPDYWGQGTMTVTSS	DIVMTQSPLSLPVTPGEPASISCR SSQSLVHSNAKTYLHWYLQKPG QSPQLLIYRVSNRFSGLPDRFSGS GSGDFTLKISRVEAEDVGVYYC LQTTHFPYTFGQGTKEIK
W3452-2.683.2	SEQ ID NO: 21	SEQ ID NO: 23
	EVELVESGGDLVQPGRSMKLSCA ASGFTFSTYGMAWVRQTPTKGLE WVASISTSGGSTYYRDSVQGRFTI SRDNAKSTLYLQMDSLTSSETAT YYCTTKGGRTYRLAYWGQGTLV TVSS	SYELIQPPSASVTLENTVSLTCSGI ELSNKYAHWFQKPKDKTILEVM YKDSERPSGISDRFSGSRSGTTAI LTIRDAQAEDADYYCLSTNSDD DLPVFGGGTELTVL
W3452-2.683.2- z27	SEQ ID NO: 25	SEQ ID NO: 27
	EVQLVESGGGLVQPGRSLRLSCA ASGFTFSTYGMAWVRQAPGKGLE WVSSISTSGGSTYYRDSVQGRFTIS RDNAKSSLYLQMNSLRAEDTATY YCTTKGGRTYRLAYWGQGTLV VSS	SYELMQPPSVSVSPGQTARITCS GIELSNKYAHWYQKPGQAPVE VMYKDSERPSGIPERFSGSRSGTT VTLTISGVQAEDADYYCLSTNS DDDLPVFGGGTKLTVL

[00091] Table 3. Variable region nucleotide sequences

	VHnu	VLnu
W3452-1.164.16	SEQ ID NO: 14	SEQ ID NO: 16
	caggtgcaactgaaggagtcaggacctggcctggt gcagccctcacagacctgtctctacctgcactgtc tctgggttctctttaaccaactatcatgtgtactgggttc gacagcctccagggaagggtctggaatggatggga gtaatgtggaatcatggagacacatcatataattcacc tctcaaatcccgaactgaacatcagcagggaacacctc caagagccaagtttcttagaaatgagcgggtctgcaa actgaagactcagccacttactactgtgccagagggtg gtactcgtccctgattactggggccaaggagtcatggt cacagtctcctca	gatgttgatgacacagactccagtctccctgc ctgtcagccctggagggtcaagcctctatctcttgc cgggtcaagtcagagcctgggtacacagtaatgga aaaacctatttgattggtagctgcagaagccag gccagtctccacagctcctcatctatcgggttcc aacagattttctgggctgccagacagggtcagt gcagtgggtcagggaacagatttaccctcaaga tcagcagagtagagactgaggacttgggagatt attactgcttacaactacacattttccgtacacgtt tggagctgggaccaagctggaactgaaa
W3452-1.164.16-z11	SEQ ID NO: 18	SEQ ID NO: 20
	caggtgcagctgcaggagagcggacctggcctggt gaagcccagcgagacctgagcctgacctgcaccg tgtccggcttcagcctgaccaactaccagctgtactg gatcaggcagcccccggaagggtggaatgg atcggcgtgatgtggaaccacggcgacaccagcta caacagccccctgaagagcagggtgacctcagcg tggacaccagcaagagccagttcagcctgaagctg agcagcgtgaccgctgccgataccgctgtactac tgcgccagaggcggctatgtgcccactactgggg ccaggggcaccatggtgacctgagcagc	gacatcgtgatgaccagagccctctgagcctg cctgtgaccttgagagcctgccagcatcagc tgtaggagcagccagagcctgggtcacagcaa cgccaagacctacctccactggtagctgcagaa gccccggcagagccctcagctgctgatctacag gggtgagcaacagggtcagcggcgtgccccgaca gattcagcgggaagcggcagcggcaccgacttc acctgaagatctccagggtggaggccgagga tgtgggctgtactactgctcgcagaccaccac tccccctacacctcggccagggcaccaagctg gagatcaag
W3452-2.683.2	SEQ ID NO: 22	SEQ ID NO: 24
	gaggtagaactgggtggagctctgggggagcacttagtg cagcctggaaggtccatgaaactctcctgtgcagcct caggattcactttcagtagctatggcatggcctgggtc cgccagactccaacgaagggtctggagtgggtcgc atccattagtactagtgggtggcagcacttactatcgag actccgtgcaggggcgattcactatttcagagataa tgcaaaagagcaccctatacctacaaatggacagtct gacgtctgaggacacggccacttattactgtacaact aaggggggggagaacctataggcttgcttactgggg ccaaggcactctggtcactgtctcttca	agctatgagctgatccaaccaccatcagcatcag tactctggaaaatactgtctactcacttgttctg gaattgaattatcaaaataatgtcatttgggttc aacaaaagccagacaagaccattttggaagtga tgtacaaagatagtgcggccctcaggcatctc tgaccgattctctgggtccaggtcagggaac agccattctgacaatccgtgatgccagggtgag gatgaggctgattattactgtttgtcaacaaatagt gatgatgatctccctgtttcgggtggtggaaccga gctcactgtccta
W3452-2.683.2-z27	SEQ ID NO: 26	SEQ ID NO: 28
	gaggtgcagctgggtggagagcggaggaggactgg tgcagccccggaagaagcctgagactgagctgcgcc gccagcggcttaccttcagcacctacggcatggcct gggtgagacaggccccggcaaggactggagtg ggtgagcagcatcagcaccagcggcggcagcacc tactacaggacagcgtgcagggaagggtcaccatc agcagggaacgccaagagcagcctgtatctgca gatgaacagcctgagggccgaggacaccgccacc tactactgcaccaccaaggggcggcaggacctacag gctggcctattggggccagggcaccctggtgacagt gagctcc	agctacgagctgatgcagcctcctagcgtgagc gtgagccccggacagacagccaggatcacctg cagcggcatcgagctgagcaacaagtacgccc actggtagcagagaagcctggccaggccccct gtggagggtgatgtacaaggacagcagagacc cagcggcatccccgagaggttagcggcagca ggagcggcaccaccgtgacctgacctcagc ggagtgcaggccgaggatgaggccgactacta ctgctgagcaccacagcagatgacgacctgcc tgtgttcggcggcggcaccaggttaaccgtccta

[00092] CDRs are known to be responsible for antigen binding, however, it has been found that not all of the 6 CDRs are indispensable or unchangeable. In other words, it is possible to replace or change or modify one or more CDRs in anti-CD47 antibody W3452-1.164.16, W3452-1.164.16-z11, or W3452-2.683.2 yet substantially retain the specific binding affinity to CD47.

[00093] In certain embodiments, the anti-CD47 antibodies and the antigen-binding fragments provided herein comprise a heavy chain CDR3 sequence of one of the anti-CD47 antibodies W3452-1.164.16, W3452-1.164.16-z11, or W3452-2.683.2. In certain embodiments, the anti-CD47 antibodies and the antigen-binding fragments provided herein comprise a heavy chain CDR3 sequence of SEQ ID NOs: 5 and 11. Heavy chain CDR3 regions are located at the center of the antigen-binding site, and therefore are believed to make the most contact with antigen and provide the most free energy to the affinity of antibody to antigen. It is also believed that the heavy chain CDR3 is by far the most diverse CDR of the antigen-binding site in terms of length, amino acid composition and conformation by multiple diversification mechanisms (Tonegawa S. *Nature*. 302:575-81). The diversity in the heavy chain CDR3 is sufficient to produce most antibody specificities (Xu JL, Davis MM. *Immunity*. 13:37-45) as well as desirable antigen-binding affinity (Schier R, etc. *J Mol Biol*. 263:551-67).

[00094] In certain embodiments, the antibodies and antigen-binding fragments thereof provided herein comprise suitable framework region (FR) sequences, as long as the antibodies and antigen-binding fragments thereof can specifically bind to CD47. The CDR sequences provided in Table 1 are obtained from rat or mouse antibodies, but they can be grafted to any suitable FR sequences of any suitable species such as mouse, human, rat, rabbit, among others, using suitable methods known in the art such as recombinant techniques.

[00095] In certain embodiments, the antibodies and antigen-binding fragments thereof provided herein are humanized. A humanized antibody or antigen-binding fragment is desirable in its reduced immunogenicity in human. A humanized antibody is chimeric in its variable regions, as non-human CDR sequences are grafted to human or substantially human FR sequences. Humanization of an antibody or antigen-binding fragment can be essentially performed by substituting the non-human (such as murine) CDR genes for the corresponding human CDR genes in a human immunoglobulin gene (see, for example, Jones et al. (1986) *Nature* 321:522-525; Riechmann et al. (1988) *Nature* 332:323-327; Verhoeven et al. (1988) *Science* 239:1534-1536).

[00096] Suitable human heavy chain and light chain variable domains can be selected to achieve this purpose using methods known in the art. In an illustrative example, “best-fit” approach can be used, where a non-human (e.g. rodent) antibody variable domain sequence is screened or BLASTed against a database of known human variable domain sequences, and the human sequence closest to the non-human query sequence is identified and used as the human scaffold for grafting the non-human CDR sequences (see, for example, Sims et al, (1993) J. Immunol. 151:2296; Chothia et al. (1987) J. Mol. Biol. 196:901). Alternatively, a framework derived from the consensus sequence of all human antibodies may be used for the grafting of the non-human CDRs (see, for example, Carter et al. (1992) Proc. Natl. Acad. Sci. USA, 89:4285; Presta et al. (1993) J. Immunol., 151:2623).

[00097] In certain embodiments, the humanized antibodies or antigen-binding fragments provided herein are composed of substantially all human sequences except for the CDR sequences which are non-human. In some embodiments, the variable region FRs, and constant regions if present, are entirely or substantially from human immunoglobulin sequences. The human FR sequences and human constant region sequences may be derived different human immunoglobulin genes, for example, FR sequences derived from one human antibody and constant region from another human antibody. In some embodiments, the humanized antibody or antigen-binding fragment comprise human FR1-4.

[00098] In certain embodiments, the humanized antibodies and antigen-binding fragment thereof provided herein comprise one or more FR sequences of W3452-1.164.16-z11 or W3452-2.683.2-z27.

[00099] The exemplary humanized anti-CD47 antibodies W3452-1.164.16-z11, and W3452-2.683.2-z27 retained the specific binding affinity to CD47-expressing cell, and are at least comparable to, or even better than, the parent rat antibodies in that aspect. The two exemplary humanized antibodies retained their functional interaction with CD47-expressing cells, such as CCRF-CEM cells, in that both can block CD47- SIRP α interaction activity and induce potent macrophage-mediated tumor cells phagocytosis.

[000100] In some embodiments, the FR regions derived from human may comprise the same amino acid sequence as the human immunoglobulin from which it is derived. In some embodiments, one or more amino acid residues of the human FR are substituted with the corresponding residues from the parent non-human antibody. This may be desirable in certain embodiments to make the humanized antibody or its fragment closely approximate the non-

human parent antibody structure. In certain embodiments, the humanized antibody or antigen-binding fragment provided herein comprises no more than 10, 9, 8, 7, 6, 5, 4, 3, 2, or 1 amino acid residue substitutions in each of the human FR sequences, or no more than 10, 9, 8, 7, 6, 5, 4, 3, 2, or 1 amino acid residue substitutions in all the FRs of a heavy or a light chain variable domain. In some embodiments, such change in amino acid residue could be present in heavy chain FR regions only, in light chain FR regions only, or in both chains.

[000101] In certain embodiments, the antibodies and antigen-binding fragments thereof provided herein comprise a heavy chain variable domain sequence selected from the group consisting of SEQ ID NO: 13, SEQ ID NO: 17, SEQ ID NO: 21 or SEQ ID NO: 25. In certain embodiments, the antibodies and antigen-binding fragments thereof provided herein comprise a light chain variable domain sequence selected from the group consisting of SEQ ID NO: 15, SEQ ID NO: 19, SEQ ID NO: 23 or SEQ ID NO: 27.

[000102] In some embodiments, the anti-CD47 antibodies and the antigen-binding fragments provided herein comprise all or a portion of the heavy chain variable domain and/or all or a portion of the light chain variable domain. In one embodiment, the anti-CD47 antibodies and the antigen-binding fragments provided herein is a single domain antibody which consists of all or a portion of the heavy chain variable domain provided herein. More information of such a single domain antibody is available in the art (see, e.g., U.S. Pat. No. 6,248,516).

[000103] In certain embodiments, the anti-CD47 antibodies and the fragments thereof provided herein further comprise an immunoglobulin constant region. In some embodiments, an immunoglobulin constant region comprises a heavy chain and/or a light chain constant region. The heavy chain constant region comprises CH1, hinge, and/or CH2-CH3 regions. In certain embodiments, the heavy chain constant region comprises an Fc region. In certain embodiments, the light chain constant region comprises C κ or C λ .

[000104] In some embodiments, the anti-CD47 antibodies and antigen-binding fragments thereof provided herein have a constant region of an immunoglobulin (Ig), optionally a human Ig, optionally a human IgG. In certain embodiments, the anti-CD47 antibodies and antigen-binding fragments thereof provided herein comprises a constant region of IgG1 isotype, which could induce ADCC or CDC, or a constant region of IgG4 or IgG2 isotype, which has reduced or depleted effector function. Effector functions such as ADCC and CDC can lead to cytotoxicity to cells expressing CD47. Effector functions can be evaluated using various assays such as Fc receptor binding assay, C1q binding assay, and cell lysis assay.

[000105] Binding affinity of the antibody and antigen-binding fragment provided herein can be represented by K_D value, which represents the ratio of dissociation rate to association rate (k_{off}/k_{on}) when the binding between the antigen and antigen-binding molecule reaches equilibrium. The antigen-binding affinity (e.g. K_D) can be appropriately determined using suitable methods known in the art, including, for example, flow cytometry assay. In some embodiments, binding of the antibody to the antigen at different concentrations can be determined by flow cytometry, the determined mean fluorescence intensity (MFI) can be firstly plotted against antibody concentration, K_D value can then be calculated by fitting the dependence of specific binding fluorescence intensity (Y) and the concentration of antibodies (X) into the one site saturation equation: $Y=B_{max}*X/(K_D + X)$ using Prism version 5 (GraphPad Software, San Diego, CA), wherein B_{max} refers to the maximum specific binding of the tested antibody to the antigen.

[000106] In some embodiments, the anti-CD47 antibodies and antigen-binding fragments thereof provided herein are capable of specifically binding to human CD47 with a binding affinity (K_D) of no more than $10^{-9}M$, no more than $9 \times 10^{-10}M$, no more than 8×10^{-10} , no more than 7×10^{-10} , no more than 6×10^{-10} , no more than $5 \times 10^{-10}M$, no more than $4 \times 10^{-10}M$, no more than $3 \times 10^{-10}M$, no more than $2 \times 10^{-10}M$, no more than $10^{-10}M$, no more than $9 \times 10^{-11}M$, no more than $8 \times 10^{-11}M$, or no more than $7.5 \times 10^{-11}M$ as measured by flow cytometer assay.

[000107] In certain embodiments, the anti-CD47 antibodies and antigen-binding fragments thereof provided herein cross-react with cynomolgus monkey CD47.

[000108] Binding of the antibodies to human CD47 can also be represented by “half maximal effective concentration” (EC_{50}) value, which refers to the concentration of an antibody where 50% of its maximal effect (e.g., binding or inhibition etc.) is observed. The EC_{50} value can be measured by methods known in the art, for example, sandwich assay such as ELISA, Western Blot, flow cytometry assay, and other binding assay. In certain embodiments, the antibodies and the fragments thereof provided herein specifically bind to human CD47 at an EC_{50} (i.e. 50% binding concentration) of no more than 0.45 nM, no more than 0.5 nM, no more than 0.55 nM, no more than 0.6 nM, no more than 0.65 nM, no more than 0.7 nM, no more than 0.75, or no more than 0.8 nM by flow cytometer assay.

[000109] In certain embodiments, the antibodies and antigen-binding fragments thereof bind to cynomolgus monkey CD47 with a binding affinity lower than that of human CD47. For

example, binding of the exemplary antibody W3452-1.164.16-z11 to cynomolgus monkey CD47 is at a lower EC₅₀ value to that of human CD47.

[000110] In certain embodiments, the antibodies and the fragments thereof provided herein specifically bind to recombinant cynomolgus monkey CD47 with an EC₅₀ of no more than 0.1 nM, no more than 0.15 nM, no more than 0.2 nM, no more than 0.25 nM, no more than 0.3 nM, no more than 0.35 nM, no more than 0.4 nM or no more than 0.45 nM by flow cytometer assay.

[000111] In certain embodiments, the antibodies and the fragments thereof provided herein have a specific binding affinity to human CD47 which is sufficient to provide for diagnostic and/or therapeutic use.

[000112] In certain embodiments, the antibodies and the fragments thereof provided herein block binding of human CD47 to its ligand and thereby promoting (e.g., inducing or increasing) phagocytosis, as well as anti-tumor activity without promoting (e.g., inducing or increasing) hemagglutination of erythrocytes.

[000113] The antibodies or antigen-binding fragments thereof provided herein can be a monoclonal antibody, polyclonal antibody, humanized antibody, chimeric antibody, recombinant antibody, labeled antibody, bivalent antibody, or anti-idiotypic antibody. A recombinant antibody is an antibody prepared in vitro using recombinant methods rather than in animals.

[000114] Antibody Variants

[000115] The antibodies and antigen-binding fragments thereof provided herein also encompass various variants thereof. In certain embodiments, the antibodies and antigen-binding fragments thereof encompasses various types of variants of an exemplary antibody provided herein, i.e., W3452-1.164.16, W3452-1.164.16-z11, W3452-2.683.2, or W3452-2.683.2-z27.

[000116] In certain embodiments, the antibody variants comprise one or more modifications or substitutions in one or more CDR sequences as provided in Table 1, one or more variable region sequences (but not in any of the CDR sequences) provided in Table 2, and/or the constant region (e.g. Fc region). Such variants retain specific binding affinity to CD47 of their parent antibodies, but have one or more desirable properties conferred by the modification(s) or substitution(s). For example, the antibody variants may have improved antigen-binding affinity, improved productivity, improved stability, improved glycosylation

pattern, reduced risk of glycosylation, reduced deamination, reduced or depleted effector function(s), improved FcRn receptor binding, increased pharmacokinetic half-life, pH sensitivity, and/or compatibility to conjugation (e.g. one or more introduced cysteine residues).

[000117] The parent antibody sequence may be screened to identify suitable or preferred residues to be modified or substituted, using methods known in the art, for example “alanine scanning mutagenesis” (see, for example, Cunningham and Wells (1989) Science, 244:1081-1085). Briefly, target residues (e.g., charged residues such as Arg, Asp, His, Lys, and Glu) can be identified and replaced by a neutral or negatively charged amino acid (e.g., alanine or polyalanine), and the modified antibodies are produced and screened for the interested property. If substitution at a particular amino acid location demonstrates an interested functional change, then the position can be identified as a potential residue for modification or substitution. The potential residues may be further assessed by substituting with a different type of residue (e.g. cysteine residue, positively charged residue, etc.).

[000118] Affinity variant

[000119] Affinity variant may contain modifications or substitutions in one or more CDR sequences as provided in Table 1, one or more FR sequences, or the heavy or light chain variable region sequences provided in Table 2. FR sequences can be readily identified by a skilled person in the art based on the CDR sequences in Table 1 and variable region sequences in Table 2, as it is well-known in the art that a CDR region is flanked by two FR regions in the variable region. The affinity variants retain specific binding affinity to CD47 of the parent antibody, or even have improved CD47 specific binding affinity over the parent antibody. In certain embodiments, at least one (or all) of the substitution(s) in the CDR sequences, FR sequences, or variable region sequences comprises a conservative substitution.

[000120] A skilled artisan will understand that in the CDR sequences and variable region sequences provided in Table 1 and Table 2, one or more amino acid residues may be substituted yet the resulting antibody or antigen-binding fragment still retain the binding affinity to CD47, or even have an improved binding affinity. Various methods known in the art can be used to achieve this purpose. For example, a library of antibody variants (such as Fab or scFv variants) can be generated and expressed with phage display technology, and then screened for the binding affinity to human CD47. For another example, computer software can be used to virtually simulate the binding of the antibodies to human CD47, and

identify the amino acid residues on the antibodies which form the binding interface. Such residues may be either avoided in the substitution so as to prevent reduction in binding affinity, or targeted for substitution to provide for a stronger binding.

[000121] In certain embodiments, the humanized antibody or antigen-binding fragment provided herein comprises one or more amino acid residue substitutions in one or more CDR sequences, and/or one or more FR sequences. In certain embodiments, an affinity variant comprises no more than 10, 9, 8, 7, 6, 5, 4, 3, 2, or 1 substitutions in the CDR sequences and/or FR sequences in total.

[000122] In certain embodiments, the anti-CD47 antibodies and antigen-binding fragments thereof comprise 1, 2, or 3 CDR sequences having at least 80% (e.g. at least 85%, 88%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%) sequence identity to that (or those) listed in Table 1, and in the meantime retain the binding affinity to CD47 at a level similar to or even higher than its parent antibody.

[000123] In certain embodiments, the anti-CD47 antibodies and antigen-binding fragments thereof comprise one or more variable region sequences having at least 80% (e.g. at least 85%, 88%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%) sequence identity to that (or those) listed in Table 2, and in the meantime retain the binding affinity to CD47 at a level similar to or even higher than its parent antibody. In some embodiments, a total of 1 to 10 amino acids have been substituted, inserted, or deleted in a variable region sequence listed in Table 2. In some embodiments, the substitutions, insertions, or deletions occur in regions outside the CDRs (e.g., in the FRs).

[000124] Glycosylation variant

[000125] The anti-CD47 antibodies and antigen-binding fragments provided herein also encompass a glycosylation variant, which can be obtained to either increase or decrease the extent of glycosylation of the antibody or antigen binding fragment.

[000126] The antibody or antigen binding fragment thereof may comprise one or more amino acid residues with a side chain to which a carbohydrate moiety (e.g. an oligosaccharide structure) can be attached. Glycosylation of antibodies is typically either N-linked or O-linked. N-linked refers to the attachment of the carbohydrate moiety to the side chain of an asparagine residue, for example, an asparagine residue in a tripeptide sequence such as asparagine-X-serine and asparagine-X-threonine, where X is any amino acid except proline. O-linked glycosylation refers to the attachment of one of the sugars N-acylgalactosamine,

galactose, or xylose to a hydroxyamino acid, most commonly to serine or threonine. Removal of a native glycosylation site can be conveniently accomplished, for example, by altering the amino acid sequence such that one of the above-described tripeptide sequences (for N-linked glycosylation sites) or serine or threonine residues (for O-linked glycosylation sites) present in the sequence is substituted. A new glycosylation site can be created in a similar way by introducing such a tripeptide sequence or serine or threonine residue.

[000127] Cysteine-engineered variant

[000128] The anti-CD47 antibodies and antigen-binding fragments provided herein also encompass a cysteine-engineered variant, which comprises one or more introduced free cysteine amino acid residues.

[000129] A free cysteine residue is one which is not part of a disulfide bridge. A cysteine-engineered variant is useful for conjugation with for example, a cytotoxic and/or imaging compound, a label, or a radioisotope among others, at the site of the engineered cysteine, through for example a maleimide or haloacetyl. Methods for engineering antibodies or antigen-binding fragments to introduce free cysteine residues are known in the art, see, for example, WO2006/034488.

[000130] Fc Variant

[000131] The anti-CD47 antibodies and antigen-binding fragments provided herein also encompass an Fc variant, which comprises one or more amino acid residue modifications or substitutions at its Fc region and/or hinge region.

[000132] In certain embodiments, the anti-CD47 antibodies or antigen-binding fragments comprise one or more amino acid substitution(s) that improves pH-dependent binding to neonatal Fc receptor (FcRn). Such a variant can have an extended pharmacokinetic half-life, as it binds to FcRn at acidic pH which allows it to escape from degradation in the lysosome and then be translocated and released out of the cell. Methods of engineering an antibody and antigen-binding fragment thereof to improve binding affinity with FcRn are well-known in the art, see, for example, Vaughn, D. et al, Structure, 6(1): 63-73, 1998; Kontermann, R. et al, Antibody Engineering, Volume 1, Chapter 27: Engineering of the Fc region for improved PK, published by Springer, 2010; Yeung, Y. et al, Cancer Research, 70: 3269-3277 (2010); and Hinton, P. et al, J. Immunology, 176:346-356 (2006).

[000133] In certain embodiments, the anti-CD47 antibodies or antigen-binding fragments comprise one or more amino acid substitution(s) that alters the antibody-dependent cellular

cytotoxicity (ADCC). Certain amino acid residues at CH2 domain of the Fc region can be substituted to provide for enhanced ADCC activity. Alternatively or additionally, carbohydrate structures on the antibody can be changed to enhance ADCC activity. Methods of altering ADCC activity by antibody engineering have been described in the art, see for example, Shields RL. et al., J Biol Chem. 2001, 276(9): 6591-604; Idusogie EE. et al., J Immunol. 2000, 164(8):4178-84; Steurer W. et al., J Immunol. 1995, 155(3): 1165- 74; Idusogie EE. et al., J Immunol. 2001, 166(4): 2571-5; Lazar GA. et al., PNAS, 2006, 103(11): 4005-4010; Ryan MC. et al., Mol. Cancer Ther., 2007, 6: 3009-3018; Richards JO., et al., Mol Cancer Ther. 2008, 7(8): 2517-27; Shields R. L. et al, J. Biol. Chem, 2002, 277: 26733-26740; Shinkawa T. et al, J. Biol. Chem, 2003, 278: 3466-3473.

[000134] In certain embodiments, the anti-CD47 antibodies or antigen-binding fragments comprise one or more amino acid substitution(s) that alters Complement Dependent Cytotoxicity (CDC), for example, by improving or diminishing C1q binding and/or CDC (see, for example, WO99/51642; Duncan & Winter Nature 322:738-40 (1988); U.S. Pat. No. 5,648,260; U.S. Pat. No. 5,624,821); and WO94/29351 concerning other examples of Fc region variants. In certain embodiments, the anti-anti-CD47 antibody polypeptides comprise a human IgG4 constant region in which the 228th amino acid residue is altered, for example from Ser228Pro (S228P, which may prevent or reduce strand exchange), and/or the 235th amino acid residue is altered, for example from Leu235Glu (L235E), which may alter Fc receptor interactions. In certain other embodiments, the anti-anti-CD47 antibody polypeptides comprise a human IgG1 constant region.

[000135] In certain embodiments, the anti-CD47 antibodies or antigen-binding fragments comprise one or more amino acid substitution(s) in the interface of the Fc region to facilitate and/or promote heterodimerization. These modifications comprise introduction of a protuberance into a first Fc polypeptide and a cavity into a second Fc polypeptide, wherein the protuberance can be positioned in the cavity so as to promote interaction of the first and second Fc polypeptides to form a heterodimer or a complex. Methods of generating antibodies with these modifications are known in the art, e.g., as described in U.S. Pat. No. 5,731,168.

[000136] Antigen-binding fragments

[000137] Provided herein are also anti-CD47 antigen-binding fragments. Various types of antigen-binding fragments are known in the art and can be developed based on the anti-CD47

antibodies provided herein, including for example, the exemplary antibodies whose CDR and variable sequences are shown in Tables 1 and 2, and their different variants (such as affinity variants, glycosylation variants, Fc variants, cysteine-engineered variants and so on).

[000138] In certain embodiments, an anti-CD47 antigen-binding fragment provided herein is a camelid single domain antibody, a diabody, a single chain Fv fragment (scFv), an scFv dimer, a BsFv, a dsFv, a (dsFv)₂, an Fv fragment, a Fab, a Fab', a F(ab')₂, a ds diabody, a nanobody, a domain antibody, a single domain antibody, or a bivalent domain antibody.

[000139] Various techniques can be used for the production of such antigen-binding fragments. Illustrative methods include, enzymatic digestion of intact antibodies (see, e.g., Morimoto et al., *Journal of Biochemical and Biophysical Methods* 24:107-117 (1992); and Brennan et al., *Science*, 229:81 (1985)), recombinant expression by host cells such as *E. Coli* (e.g. for Fab, Fv and ScFv antibody fragments), screening from a phage display library as discussed above (e.g. for ScFv), and chemical coupling of two Fab'-SH fragments to form F(ab')₂ fragments (Carter et al., *Bio/Technology* 10:163-167 (1992)). Other techniques for the production of antibody fragments will be apparent to a skilled practitioner.

[000140] In certain embodiments, the antigen-binding fragment is a scFv. Generation of scFv is described in, for example, WO 93/16185; U.S. Pat. Nos. 5,571,894; and 5,587,458. scFv may be fused to an effector protein at either the amino or the carboxyl terminus to provide for a fusion protein (see, for example, *Antibody Engineering*, ed. Borrebaeck).

[000141] **Conjugates**

[000142] In some embodiments, the anti-CD47 antibodies and antigen-binding fragments thereof further comprise a conjugate moiety. The conjugate moiety can be linked to the antibodies and antigen-binding fragments thereof. A conjugate moiety is a non-proteinaceous moiety that can be attached to the antibody or antigen-binding fragment thereof. It is contemplated that a variety of conjugate moieties may be linked to the antibodies or antigen-binding fragments provided herein (see, for example, "Conjugate Vaccines", *Contributions to Microbiology and Immunology*, J. M. Cruse and R. E. Lewis, Jr. (eds.), Carger Press, New York, (1989)). These conjugate moieties may be linked to the antibodies or antigen-binding fragments by covalent binding, affinity binding, intercalation, coordinate binding, complexation, association, blending, or addition, among other methods.

[000143] In certain embodiments, the antibodies and antigen-binding fragments disclosed herein may be engineered to contain specific sites outside the epitope binding portion that

may be utilized for binding to one or more conjugate moieties. For example, such a site may include one or more reactive amino acid residues, such as for example cysteine or histidine residues, to facilitate covalent linkage to a conjugate moiety.

[000144] In certain embodiments, the antibodies may be linked to a conjugate moiety indirectly, or through another conjugate moiety. For example, the antibody or antigen-binding fragments may be conjugated to biotin, then indirectly conjugated to a second conjugate moiety that is conjugated to avidin. The conjugate moiety can be a clearance-modifying agent, a toxin (e.g., a chemotherapeutic agent), a detectable label (e.g., a radioactive isotope, a lanthanide, a luminescent label, a fluorescent label, or an enzyme-substrate label), or purification moiety.

[000145] A “toxin” can be any agent that is detrimental to cells or that can damage or kill cells. Examples of toxin include, without limitation, taxol, cytochalasin B, gramicidin D, ethidium bromide, emetine, mitomycin, etoposide, tenoposide, vincristine, MMAE, MMAF, DM1, vinblastine, colchicin, doxorubicin, daunorubicin, dihydroxy anthracin dione, mitoxantrone, mithramycin, actinomycin D, 1-dehydrotestosterone, glucocorticoids, procaine, tetracaine, lidocaine, propranolol, puromycin and analogs thereof, antimetabolites (e.g., methotrexate, 6-mercaptopurine, 6-thioguanine, cytarabine, 5-fluorouracil decarbazine), alkylating agents (e.g., mechlorethamine, thioepa chlorambucil, melphalan, carmustine (BSNU) and lomustine (CCNU), cyclophosphamide, busulfan, dibromomannitol, streptozotocin, mitomycin C, and cis-dichlorodiamine platinum (II) (DDP) cisplatin), anthracyclines (e.g., daunorubicin (formerly daunomycin) and doxorubicin), antibiotics (e.g., dactinomycin (formerly actinomycin), bleomycin, mithramycin, and anthramycin (AMC)), anti-mitotic agents (e.g., vincristine and vinblastine), a topoisomerase inhibitor, and a tubulin-binders.

[000146] Examples of detectable label may include a fluorescent labels (e.g. fluorescein, rhodamine, dansyl, phycoerythrin, or Texas Red), enzyme-substrate labels (e.g. horseradish peroxidase, alkaline phosphatase, luciferases, glucoamylase, lysozyme, saccharide oxidases or β -D-galactosidase), radioisotopes (e.g. ^{123}I , ^{124}I , ^{125}I , ^{131}I , ^{35}S , ^3H , ^{111}In , ^{112}In , ^{14}C , ^{64}Cu , ^{67}Cu , ^{86}Y , ^{88}Y , ^{90}Y , ^{177}Lu , ^{211}At , ^{186}Re , ^{188}Re , ^{153}Sm , ^{212}Bi , and ^{32}P , other lanthanides), luminescent labels, chromophoric moiety, digoxigenin, biotin/avidin, a DNA molecule or gold for detection.

[000147] In certain embodiments, the conjugate moiety can be a clearance-modifying agent

which helps increase half-life of the antibody. Illustrative example include water-soluble polymers, such as PEG, carboxymethylcellulose, dextran, polyvinyl alcohol, polyvinyl pyrrolidone, copolymers of ethylene glycol/propylene glycol, and the like. The polymer may be of any molecular weight, and may be branched or unbranched. The number of polymers attached to the antibody may vary, and if more than one polymer are attached, they can be the same or different molecules.

[000148] In certain embodiments, the conjugate moiety can be a purification moiety such as a magnetic bead.

[000149] In certain embodiments, the antibodies and antigen-binding fragments thereof provided herein is used for a base for a conjugate moiety.

[000150] Polynucleotides and Recombinant Methods

[000151] The present disclosure provides isolated polynucleotides that encode the anti-CD47 antibodies and antigen-binding fragments thereof.

[000152] The term “nucleic acid” or “polynucleotide” as used herein refers to deoxyribonucleic acids (DNA) or ribonucleic acids (RNA) and polymers thereof in either single- or double-stranded form. Unless specifically limited, the term encompasses polynucleotides containing known analogues of natural nucleotides that have similar binding properties as the reference nucleic acid and are metabolized in a manner similar to naturally occurring nucleotides. Unless otherwise indicated, a particular polynucleotide sequence also implicitly encompasses conservatively modified variants thereof (e.g., degenerate codon substitutions), alleles, orthologs, SNPs, and complementary sequences as well as the sequence explicitly indicated. Specifically, degenerate codon substitutions may be achieved by generating sequences in which the third position of one or more selected (or all) codons is substituted with mixed-base and/or deoxyinosine residues (see Batzer et al., Nucleic Acid Res. 19:5081 (1991); Ohtsuka et al., J. Biol. Chem. 260:2605-2608 (1985); and Rossolini et al., Mol. Cell. Probes 8:91-98 (1994)).

[000153] In certain embodiments, the isolated polynucleotides comprise one or more nucleotide sequences as shown in SEQ ID NO: 14, 16, 18, 20, 22, 24, 26 and/or 28, and/or a homologous sequence thereof having at least 80% (e.g. at least 85%, 88%, 90%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, or 99%) sequence identity, and/or a variant thereof having only degenerate substitutions, and encodes the variable region of the exemplary antibodies provided herein. DNA encoding the monoclonal antibody is readily isolated and sequenced

using conventional procedures (e.g., by using oligonucleotide probes that are capable of binding specifically to genes encoding the heavy and light chains of the antibody). The encoding DNA may also be obtained by synthetic methods.

[000154] The isolated polynucleotide that encodes the anti-CD47 antibodies and antigen-binding fragments thereof (e.g. including the sequences as shown in Table 3) can be inserted into a vector for further cloning (amplification of the DNA) or for expression, using recombinant techniques known in the art. Many vectors are available. The vector components generally include, but are not limited to, one or more of the following: a signal sequence, an origin of replication, one or more marker genes, an enhancer element, a promoter (e.g. SV40, CMV, EF-1 α), and a transcription termination sequence.

[000155] The present disclosure provides vectors (e.g., expression vectors) containing the nucleic acid sequence provided herein encoding the antibodies or antigen-binding fragments, at least one promoter (e.g., SV40, CMV, EF-1 α) operably linked to the nucleic acid sequence, and at least one selection marker. Examples of vectors include, but are not limited to, retrovirus (including lentivirus), adenovirus, adeno-associated virus, herpesvirus (e.g., herpes simplex virus), poxvirus, baculovirus, papillomavirus, papovavirus (e.g., SV40), lambda phage, and M13 phage, plasmid pcDNA3.3, pMD18-T, pOptivec, pCMV, pEGFP, pIRES, pQD-Hyg-GSeu, pALTER, pBAD, pcDNA, pCal, pL, pET, pGEMEX, pGEX, pCI, pEGFT, pSV2, pFUSE, pVITRO, pVIVO, pMAL, pMONO, pSELECT, pUNO, pDUO, Psg5L, pBABE, pWPXL, pBI, p15TV-L, pPro18, pTD, pRS10, pLexA, pACT2.2, pCMV-SCRIPT.RTM., pCDM8, pCDNA1.1/amp, pcDNA3.1, pRc/RSV, PCR 2.1, pEF-1, pFB, pSG5, pXT1, pCDEF3, pSVSPORT, pEF-Bos etc.

[000156] Vectors comprising the polynucleotide sequence encoding the antibody or antigen-binding fragment can be introduced to a host cell for cloning or gene expression. Suitable host cells for cloning or expressing the DNA in the vectors herein are the prokaryote, yeast, or higher eukaryote cells described above. Suitable prokaryotes for this purpose include eubacteria, such as Gram-negative or Gram-positive organisms, for example, *Enterobacteriaceae* such as *Escherichia*, e.g., *E. coli*, *Enterobacter*, *Erwinia*, *Klebsiella*, *Proteus*, *Salmonella*, e.g., *Salmonella typhimurium*, *Serratia*, e.g., *Serratia marcescans*, and *Shigella*, as well as *Bacilli* such as *B. subtilis* and *B. licheniformis*, *Pseudomonas* such as *P. aeruginosa*, and *Streptomyces*.

[000157] In addition to prokaryotes, eukaryotic microbes such as filamentous fungi or yeast are suitable cloning or expression hosts for anti-CD47 antibody-encoding vectors. *Saccharomyces cerevisiae*, or common baker's yeast, is the most commonly used among lower eukaryotic host microorganisms. However, a number of other genera, species, and strains are commonly available and useful herein, such as *Schizosaccharomyces pombe*; *Kluyveromyces* hosts such as, e.g., *K. lactis*, *K. fragilis* (ATCC 12,424), *K. bulgaricus* (ATCC 16,045), *K. wickerhamii* (ATCC 24,178), *K. waltii* (ATCC 56,500), *K. drosophilae* (ATCC 36,906), *K. thermotolerans*, and *K. marxianus*; *Yarrowia* (EP 402,226); *Pichia pastoris* (EP 183,070); *Candida*; *Trichoderma reesei* (EP 244,234); *Neurospora crassa*; *Schwanniomyces* such as *Schwanniomyces occidentalis*; and filamentous fungi such as, e.g., *Neurospora*, *Penicillium*, *Tolypocladium*, and *Aspergillus* hosts such as *A. nidulans* and *A. niger*.

[000158] Suitable host cells for the expression of glycosylated antibodies or antigen-fragment provided here are derived from multicellular organisms. Examples of invertebrate cells include plant and insect cells. Numerous baculoviral strains and variants and corresponding permissive insect host cells from hosts such as *Spodoptera frugiperda* (caterpillar), *Aedes aegypti* (mosquito), *Aedes albopictus* (mosquito), *Drosophila melanogaster* (fruitfly), and *Bombyx mori* have been identified. A variety of viral strains for transfection are publicly available, e.g., the L-1 variant of *Autographa californica* NPV and the Bm-5 strain of *Bombyx mori* NPV, and such viruses may be used as the virus herein according to the present invention, particularly for transfection of *Spodoptera frugiperda* cells. Plant cell cultures of cotton, corn, potato, soybean, petunia, tomato, and tobacco can also be utilized as hosts.

[000159] However, interest has been greatest in vertebrate cells, and propagation of vertebrate cells in culture (tissue culture) has become a routine procedure. Examples of useful mammalian host cell lines are monkey kidney CV1 line transformed by SV40 (COS-7, ATCC CRL 1651); human embryonic kidney line (293 or 293 cells subcloned for growth in suspension culture, Graham et al., *J. Gen. Virol.* 36:59 (1977)); baby hamster kidney cells (BHK, ATCC CCL 10); Chinese hamster ovary cells/-DHFR (CHO, Urlaub et al., *Proc. Natl. Acad. Sci. USA* 77:4216 (1980)); mouse sertoli cells (TM4, *Mather, Biol. Reprod.* 23:243-251 (1980)); monkey kidney cells (CV1 ATCC CCL 70); African green monkey kidney cells (VERO-76, ATCC CRL-1587); human cervical carcinoma cells (HELA, ATCC CCL 2); canine kidney cells (MDCK, ATCC CCL 34); buffalo rat liver cells (BRL 3A, ATCC CRL 1442); human lung cells (W138, ATCC CCL 75); human liver cells (Hep G2, HB 8065);

mouse mammary tumor (MMT 060562, ATCC CCL51); TRI cells (Mather *et al.*, *Annals N.Y. Acad. Sci.* 383:44-68 (1982)); MRC 5 cells; FS4 cells; and a human hepatoma line (Hep G2). In some preferable embodiments, the host cell is 293F cell.

[000160] Host cells are transformed with the above-described expression or cloning vectors for anti-CD47 antibody production and cultured in conventional nutrient media modified as appropriate for inducing promoters, selecting transformants, or amplifying the genes encoding the desired sequences. In another embodiment, the antibody may be produced by homologous recombination known in the art.

[000161] The host cells used to produce the antibodies or antigen-binding fragments provided herein may be cultured in a variety of media. Commercially available media such as Ham's F10 (Sigma), Minimal Essential Medium (MEM), (Sigma), RPMI-1640 (Sigma), and Dulbecco's Modified Eagle's Medium (DMEM), Sigma) are suitable for culturing the host cells. In addition, any of the media described in Ham *et al.*, *Meth. Enz.* 58:44 (1979), Barnes *et al.*, *Anal. Biochem.* 102:255 (1980), U.S. Pat. No. 4,767,704; 4,657,866; 4,927,762; 4,560,655; or 5,122,469; WO 90/03430; WO 87/00195; or U.S. Pat. Re. 30,985 may be used as culture media for the host cells. Any of these media may be supplemented as necessary with hormones and/or other growth factors (such as insulin, transferrin, or epidermal growth factor), salts (such as sodium chloride, calcium, magnesium, and phosphate), buffers (such as HEPES), nucleotides (such as adenosine and thymidine), antibiotics (such as GENTAMYCINTM drug), trace elements (defined as inorganic compounds usually present at final concentrations in the micromolar range), and glucose or an equivalent energy source. Any other necessary supplements may also be included at appropriate concentrations that would be known to those skilled in the art. The culture conditions, such as temperature, pH, and the like, are those previously used with the host cell selected for expression, and will be apparent to the ordinarily skilled artisan.

[000162] When using recombinant techniques, the antibody can be produced intracellularly, in the periplasmic space, or directly secreted into the medium. If the antibody is produced intracellularly, as a first step, the particulate debris, either host cells or lysed fragments, is removed, for example, by centrifugation or ultrafiltration. Carter *et al.*, *Bio/Technology* 10:163-167 (1992) describe a procedure for isolating antibodies which are secreted to the periplasmic space of *E. coli*. Briefly, cell paste is thawed in the presence of sodium acetate (pH 3.5), EDTA, and phenylmethylsulfonylfluoride (PMSF) over about 30 min. Cell debris can be removed by centrifugation. Where the antibody is secreted into the medium,

supernatants from such expression systems are generally first concentrated using a commercially available protein concentration filter, for example, an Amicon or Millipore Pellicon ultrafiltration unit. A protease inhibitor such as PMSF may be included in any of the foregoing steps to inhibit proteolysis and antibiotics may be included to prevent the growth of adventitious contaminants.

[000163] The anti-CD47 antibodies and antigen-binding fragments thereof prepared from the cells can be purified using, for example, hydroxylapatite chromatography, gel electrophoresis, dialysis, DEAE-cellulose ion exchange chromatography, ammonium sulfate precipitation, salting out, and affinity chromatography, with affinity chromatography being the preferred purification technique.

[000164] In certain embodiments, Protein A immobilized on a solid phase is used for immunoaffinity purification of the antibody and antigen-binding fragment thereof. The suitability of protein A as an affinity ligand depends on the species and isotype of any immunoglobulin Fc domain that is present in the antibody. Protein A can be used to purify antibodies that are based on human gamma1, gamma2, or gamma4 heavy chains (Lindmark *et al.*, *J. Immunol. Meth.* 62:1-13 (1983)). Protein G is recommended for all mouse isotypes and for human gamma3 (Guss *et al.*, *EMBO J.* 5:1567-1575 (1986)). The matrix to which the affinity ligand is attached is most often agarose, but other matrices are available. Mechanically stable matrices such as controlled pore glass or poly(styrenedivinyl)benzene allow for faster flow rates and shorter processing times than can be achieved with agarose. Where the antibody comprises a CH3 domain, the Bakerbond ABXTM resin (J. T. Baker, Phillipsburg, N.J.) is useful for purification. Other techniques for protein purification such as fractionation on an ion-exchange column, ethanol precipitation, Reverse Phase HPLC, chromatography on silica, chromatography on heparin SEPHAROSETM chromatography on an anion or cation exchange resin (such as a polyaspartic acid column), chromatofocusing, SDS-PAGE, and ammonium sulfate precipitation are also available depending on the antibody to be recovered.

[000165] Following any preliminary purification step(s), the mixture comprising the antibody of interest and contaminants may be subjected to low pH hydrophobic interaction chromatography using an elution buffer at a pH between about 2.5-4.5, preferably performed at low salt concentrations (e.g., from about 0-0.25M salt).

[000166] Pharmaceutical Composition

[000167] The present disclosure further provides pharmaceutical compositions comprising a anti-CD47 antibody or antigen-binding fragment thereof provided herein and one or more pharmaceutically acceptable carriers.

[000168] Pharmaceutical acceptable carriers for use in the pharmaceutical compositions disclosed herein may include, for example, pharmaceutically acceptable liquid, gel, or solid carriers, aqueous vehicles, nonaqueous vehicles, antimicrobial agents, isotonic agents, buffers, antioxidants, anesthetics, suspending/dispersing agents, sequestering or chelating agents, diluents, adjuvants, excipients, or non-toxic auxiliary substances, other components known in the art, or various combinations thereof.

[000169] Suitable components may include, for example, antioxidants, fillers, binders, disintegrants, buffers, preservatives, lubricants, flavorings, thickeners, coloring agents, emulsifiers or stabilizers such as sugars and cyclodextrins. Suitable antioxidants may include, for example, methionine, ascorbic acid, EDTA, sodium thiosulfate, platinum, catalase, citric acid, cysteine, thioglycerol, thioglycolic acid, thiosorbitol, butylated hydroxyanisole, butylated hydroxytoluene, and/or propyl gallate. As disclosed herein, inclusion of one or more antioxidants such as methionine in a composition comprising an antibody or antigen-binding fragment and conjugates as provided herein decreases oxidation of the antibody or antigen-binding fragment. This reduction in oxidation prevents or reduces loss of binding affinity, thereby improving antibody stability and maximizing shelf-life. Therefore, in certain embodiments compositions are provided that comprise one or more antibodies or antigen-binding fragments as disclosed herein and one or more antioxidants such as methionine. Further provided are methods for preventing oxidation of, extending the shelf-life of, and/or improving the efficacy of an antibody or antigen-binding fragment as provided herein by mixing the antibody or antigen-binding fragment with one or more antioxidants such as methionine.

[000170] To further illustrate, pharmaceutical acceptable carriers may include, for example, aqueous vehicles such as sodium chloride injection, Ringer's injection, isotonic dextrose injection, sterile water injection, or dextrose and lactated Ringer's injection, nonaqueous vehicles such as fixed oils of vegetable origin, cottonseed oil, corn oil, sesame oil, or peanut oil, antimicrobial agents at bacteriostatic or fungistatic concentrations, isotonic agents such as sodium chloride or dextrose, buffers such as phosphate or citrate buffers, antioxidants such as sodium bisulfate, local anesthetics such as procaine hydrochloride, suspending and dispersing agents such as sodium carboxymethylcellulose, hydroxypropyl methylcellulose, or

polyvinylpyrrolidone, emulsifying agents such as Polysorbate 80 (TWEEN-80), sequestering or chelating agents such as EDTA (ethylenediaminetetraacetic acid) or EGTA (ethylene glycol tetraacetic acid), ethyl alcohol, polyethylene glycol, propylene glycol, sodium hydroxide, hydrochloric acid, citric acid, or lactic acid. Antimicrobial agents utilized as carriers may be added to pharmaceutical compositions in multiple-dose containers that include phenols or cresols, mercurials, benzyl alcohol, chlorobutanol, methyl and propyl p-hydroxybenzoic acid esters, thimerosal, benzalkonium chloride and benzethonium chloride. Suitable excipients may include, for example, water, saline, dextrose, glycerol, or ethanol. Suitable non-toxic auxiliary substances may include, for example, wetting or emulsifying agents, pH buffering agents, stabilizers, solubility enhancers, or agents such as sodium acetate, sorbitan monolaurate, triethanolamine oleate, or cyclodextrin.

[000171] The pharmaceutical compositions can be a liquid solution, suspension, emulsion, pill, capsule, tablet, sustained release formulation, or powder. Oral formulations can include standard carriers such as pharmaceutical grades of mannitol, lactose, starch, magnesium stearate, polyvinyl pyrrolidone, sodium saccharine, cellulose, magnesium carbonate, etc.

[000172] In certain embodiments, the pharmaceutical compositions are formulated into an injectable composition. The injectable pharmaceutical compositions may be prepared in any conventional form, such as for example liquid solution, suspension, emulsion, or solid forms suitable for generating liquid solution, suspension, or emulsion. Preparations for injection may include sterile and/or non-pyretic solutions ready for injection, sterile dry soluble products, such as lyophilized powders, ready to be combined with a solvent just prior to use, including hypodermic tablets, sterile suspensions ready for injection, sterile dry insoluble products ready to be combined with a vehicle just prior to use, and sterile and/or non-pyretic emulsions. The solutions may be either aqueous or nonaqueous.

[000173] In certain embodiments, unit-dose parenteral preparations are packaged in an ampoule, a vial or a syringe with a needle. All preparations for parenteral administration should be sterile and not pyretic, as is known and practiced in the art.

[000174] In certain embodiments, a sterile, lyophilized powder is prepared by dissolving an antibody or antigen-binding fragment as disclosed herein in a suitable solvent. The solvent may contain an excipient which improves the stability or other pharmacological components of the powder or reconstituted solution, prepared from the powder. Excipients that may be used include, but are not limited to, water, dextrose, sorbitol, fructose, corn syrup, xylitol,

glycerin, glucose, sucrose or other suitable agent. The solvent may contain a buffer, such as citrate, sodium or potassium phosphate or other such buffer known to those of skill in the art at, in one embodiment, about neutral pH. Subsequent sterile filtration of the solution followed by lyophilization under standard conditions known to those of skill in the art provides a desirable formulation. In one embodiment, the resulting solution will be apportioned into vials for lyophilization. Each vial can contain a single dosage or multiple dosages of the anti-CD47 antibody or antigen-binding fragment thereof or composition thereof. Overfilling vials with a small amount above that needed for a dose or set of doses (e.g., about 10%) is acceptable so as to facilitate accurate sample withdrawal and accurate dosing. The lyophilized powder can be stored under appropriate conditions, such as at about 4 °C to room temperature.

[000175] Reconstitution of a lyophilized powder with water for injection provides a formulation for use in parenteral administration. In one embodiment, for reconstitution the sterile and/or non-pyretic water or other liquid suitable carrier is added to lyophilized powder. The precise amount depends upon the selected therapy being given, and can be empirically determined.

[000176] Methods of Use

[000177] The present disclosure also provides therapeutic methods comprising: administering a therapeutically effective amount of the antibody or antigen-binding fragment as provided herein to a subject in need thereof, thereby treating or preventing a CD47-related condition or a disorder. In some embodiment, the CD47-related condition or a disorder is cancer, autoimmune disease, fibrotic disease, inflammatory disease, or infectious disease.

[000178] Examples of cancer include but are not limited to, lung cancer, bronchial cancer, bone cancer, liver and bile duct cancer, pancreatic cancer, breast cancer, liver cancer, ovarian cancer, testicle cancer, kidney cancer, bladder cancer, head and neck cancer, spine cancer, brain cancer, cervix cancer, uterine cancer, endometrial cancer, colon cancer, colorectal cancer, rectal cancer, anal cancer, esophageal cancer, gastrointestinal cancer, skin cancer, prostate cancer, pituitary cancer, stomach cancer, vagina cancer, thyroid cancer, glioblastoma, astrocytoma, melanoma, myelodysplastic syndrome, sarcoma, teratoma, adenocarcinoma, leukemia, myeloma and lymphoma.

[000179] Examples of autoimmune diseases include, but are not limited to, Acquired Immunodeficiency Syndrome (AIDS, which is a viral disease with an autoimmune

component), alopecia areata, ankylosing spondylitis, antiphospholipid syndrome, autoimmune Addison's disease, autoimmune hemolytic anemia, autoimmune hepatitis, autoimmune inner ear disease (AIED), autoimmune lymphoproliferative syndrome (ALPS), autoimmune thrombocytopenic purpura (ATP), Behcet's disease, cardiomyopathy, celiac sprue-dermatitis hepeticiformis; chronic fatigue immune dysfunction syndrome (CFIDS), chronic inflammatory demyelinating polyneuropathy (CIPD), cicatricial pemphigoid, cold agglutinin disease, crest syndrome, Crohn's disease, Degos' disease, dermatomyositis-juvenile, discoid lupus, essential mixed cryoglobulinemia, fibromyalgia-fibromyositis, Graves' disease, Guillain-Barre syndrome, Hashimoto's thyroiditis, idiopathic pulmonary fibrosis, idiopathic thrombocytopenia purpura (ITP), IgA nephropathy, insulin-dependent diabetes mellitus, juvenile chronic arthritis (Still's disease), juvenile rheumatoid arthritis, Meniere's disease, mixed connective tissue disease, multiple sclerosis, myasthenia gravis, pernicious anemia, polyarteritis nodosa, polychondritis, polyglandular syndromes, polymyalgia rheumatica, polymyositis and dermatomyositis, primary agammaglobulinemia, primary biliary cirrhosis, psoriasis, psoriatic arthritis, Raynaud's phenomena, Reiter's syndrome, rheumatic fever, rheumatoid arthritis, sarcoidosis, scleroderma (progressive systemic sclerosis (PSS), also known as systemic sclerosis (SS)), Sjogren's syndrome, stiff-man syndrome, systemic lupus erythematosus, Takayasu arteritis, temporal arteritis/giant cell arteritis, ulcerative colitis, uveitis, vitiligo and Wegener's granulomatosis.

[000180] Inflammatory disorders, include, for example, chronic and acute inflammatory disorders. Examples of inflammatory disorders include Alzheimer's disease, asthma, atopic allergy, allergy, atherosclerosis, bronchial asthma, eczema, glomerulonephritis, graft vs. host disease, hemolytic anemias, osteoarthritis, sepsis, stroke, transplantation of tissue and organs, vasculitis, diabetic retinopathy and ventilator induced lung injury.

[000181] Examples of infectious disease include, but are not limited to, fungus infection, parasite/protozoan infection or chronic viral infection, for example, malaria, coccidioiodomycosis immitis, histoplasmosis, onychomycosis, aspergilosis, blastomycosis, candidiasis albicans, paracoccidioiodomycosis, microsporidiosis, Acanthamoeba keratitis, Amoebiasis, Ascariasis, Babesiosis, Balantidiasis, Baylisascariasis, Chagas disease, Clonorchiasis, Cochliomyia, Cryptosporidiosis, Diphyllbothriasis, Dracunculiasis, Echinococcosis, Elephantiasis, Enterobiasis, Fascioliasis, Fasciolopsiasis, Filariasis, Giardiasis, Gnathostomiasis, Hymenolepiasis, Isosporiasis, Katayama fever, Leishmaniasis, Lyme disease, Metagonimiasis, Myiasis, Onchocerciasis, Pediculosis, Scabies,

Schistosomiasis, Sleeping sickness, Strongyloidiasis, Taeniasis, Toxocariasis, Toxoplasmosis, Trichinosis, Trichuriasis, Trypanosomiasis, helminth infection, infection of hepatitis B (HBV), hepatitis C (HCV), herpes virus, Epstein-Barr virus, HIV, cytomegalovirus, herpes simplex virus type I, herpes simplex virus type II, human papilloma virus, adenovirus, human immunodeficiency virus I, human immunodeficiency virus II, Kaposi West sarcoma associated herpes virus epidemics, thin ring virus (Torquetenovirus), human T lymphotropic virus I, human T lymphotropic virus II, varicella zoster, JC virus or BK virus.

[000182] The therapeutically effective amount of an antibody or antigen-binding fragment as provided herein will depend on various factors known in the art, such as for example body weight, age, past medical history, present medications, state of health of the subject and potential for cross-reaction, allergies, sensitivities and adverse side-effects, as well as the administration route and extent of disease development. Dosages may be proportionally reduced or increased by one of ordinary skill in the art (e.g., physician or veterinarian) as indicated by these and other circumstances or requirements.

[000183] In certain embodiments, the antibody or antigen-binding fragment as provided herein may be administered at a therapeutically effective dosage of about 0.01 mg/kg to about 100 mg/kg. In certain of these embodiments, the antibody or antigen-binding fragment is administered at a dosage of about 50 mg/kg or less, and in certain of these embodiments the dosage is 10 mg/kg or less, 5 mg/kg or less, 3 mg/kg or less, 1 mg/kg or less, 0.5 mg/kg or less, or 0.1 mg/kg or less. In certain embodiments, the administration dosage may change over the course of treatment. For example, in certain embodiments the initial administration dosage may be higher than subsequent administration dosages. In certain embodiments, the administration dosage may vary over the course of treatment depending on the reaction of the subject.

[000184] Dosage regimens may be adjusted to provide the optimum desired response (e.g., a therapeutic response). For example, a single dose may be administered, or several divided doses may be administered over time.

[000185] The antibodies and antigen-binding fragments disclosed herein may be administered by any route known in the art, such as for example parenteral (e.g., subcutaneous, intraperitoneal, intravenous, including intravenous infusion, intramuscular, or intradermal injection) or non-parenteral (e.g., oral, intranasal, intraocular, sublingual, rectal, or topical) routes.

[000186] In some embodiments, the antibodies or antigen-binding fragments disclosed herein may be administered alone or in combination with one or more additional therapeutic means or agents. For example, the antibodies or antigen-binding fragments disclosed herein may be administered in combination with another therapeutic agent, for example, a chemotherapeutic agent or an anti-cancer drug.

[000187] In certain of these embodiments, an antibody or antigen-binding fragment as disclosed herein that is administered in combination with one or more additional therapeutic agents may be administered simultaneously with the one or more additional therapeutic agents, and in certain of these embodiments the antibody or antigen-binding fragment and the additional therapeutic agent(s) may be administered as part of the same pharmaceutical composition. However, an antibody or antigen-binding fragment administered “in combination” with another therapeutic agent does not have to be administered simultaneously with or in the same composition as the agent. An antibody or antigen-binding fragment administered prior to or after another agent is considered to be administered “in combination” with that agent as the phrase is used herein, even if the antibody or antigen-binding fragment and second agent are administered via different routes. Where possible, additional therapeutic agents administered in combination with the antibodies or antigen-binding fragments disclosed herein are administered according to the schedule listed in the product information sheet of the additional therapeutic agent, or according to the Physicians' Desk Reference 2003 (Physicians' Desk Reference, 57th Ed; Medical Economics Company; ISBN: 1563634457; 57th edition (November 2002)) or protocols well known in the art.

[000188] The present disclosure further provides methods of using the anti-CD47 antibodies or antigen-binding fragments thereof.

[000189] In some embodiments, the present disclosure provides methods of detecting presence or amount of CD47 in a sample, comprising contacting the sample with the antibody or antigen-binding fragment thereof, and determining the presence or the amount of CD47 in the sample.

[000190] In some embodiments, the present disclosure provides methods of diagnosing a CD47 related disease or condition in a subject, comprising: a) contacting a sample obtained from the subject with the antibody or antigen-binding fragment thereof provided herein; b) determining presence or amount of CD47 in the sample; and c) correlating the existence of the CD47 to the CD47 related disease or condition in the subject.

[000191] In some embodiments, the present disclosure provides kits comprising the antibody or antigen-binding fragment thereof provided herein, optionally conjugated with a detectable moiety. The kits may be useful in detection of CD47 or diagnosis of CD47 related disease.

[000192] In some embodiments, the present disclosure also provides use of the antibody or antigen-binding fragment thereof provided herein in the manufacture of a medicament for treating a CD47 related disease or condition in a subject, in the manufacture of a diagnostic reagent for diagnosing a CD47 related disease or condition.

[000193] Advantages

[000194] The antibodies and antigen-binding fragments provided herein have high affinity binding to CD47. They can block CD47-SIRP α interaction, induce potent macrophage-mediated tumor cells phagocytosis under in vitro and in vivo conditions. Due to CD47 ubiquitous expression in human normal cells, side effects such as anemia and platelet depletion have been reported for prior art antibodies. Interestingly, the antibodies and antigen-binding fragments provided herein do not cause hemagglutination and platelet depletion, which indicate they may not cause side effect and have advantages in clinical trials.

[000195] The following examples are provided to better illustrate the claimed invention and are not to be interpreted as limiting the scope of the invention. All specific compositions, materials, and methods described below, in whole or in part, fall within the scope of the present invention. These specific compositions, materials, and methods are not intended to limit the invention, but merely to illustrate specific embodiments falling within the scope of the invention. One skilled in the art may develop equivalent compositions, materials, and methods without the exercise of inventive capacity and without departing from the scope of the invention. It will be understood that many variations can be made in the procedures herein described while still remaining within the bounds of the present invention. It is the intention of the inventors that such variations are included within the scope of the invention.

EXAMPLES

EXAMPLE 1: Generation of Hybridoma Antibody

[000196] 1. Animal immunization

[000197] Extra-cellular domains (ECD) proteins of human CD47 was used as immunogens for animal immunization. SD rats were purchased from Shanghai SLAC laboratory animal Co, Ltd. and were housed in an IACUC approved animal facility. The animal bleed were

collected before and after immunization and serum titers against target proteins were monitored by ELISA according to general ELISA procedures.

[000198] 2. Hybridoma generation

[000199] The SD rat with the highest serum titer was chosen for cell fusion. The B cells from spleen and lymph nodes were fused with SP2/0 myeloma cells by electro-fusion according to general electro-fusion procedures. After cell fusion, the cells were plated in 96-well plates with DMEM medium supplemented with 20% FBS and 1% HAT selective reagents.

[000200] 3. Antibody humanization

[000201] “Best Fit” approach was used to humanize antibody light and heavy chains. For light chains amino acid sequences of W3452-1.164.16 and W3452-2.683.2 corresponding V-genes were blasted against in-house human germline V-gene database. The sequence of humanized VL-gene was derived by replacing human CDR sequences in the top hit with mouse CDR sequences using Kabat CDR definition. For heavy chains, humanized sequences were derived. First sequence was derived as for light chain. additional sequences were created by blasting mouse frameworks against human germline V-gene database. Frameworks were defined using extended CDR definition where Kabat CDR1 was extended by 5 amino acids at N-terminus. Top three hits were used to derive sequences of humanized VH-genes. Humanized genes were back-translated, codon-optimized for mammalian expression, and synthesized by GeneWiz (Suzhou). Synthetic genes were re-cloned into human IgG expression vectors respectively, expressed, and purified.

[000202] Two humanized clones W3452-2.683.2-z27 and W3452-1.164.16-z11 were obtained, and expressed with different human constant regions. “W3452-2.683.2-z27-IgG1L” as used herein refers to antibody W3452-2.683.2-z27 further comprising a human IgG1 constant region. “W3452-2.683.2-z27-IgG4PE,L” as used herein refers to antibody W3452-2.683.2-z27 further comprising a human IgG4 constant region containing mutations of S228P and L235E. “W3452-1.164.16-z11-IgG1K” as used herein refers to antibody W3452-1.164.16-z11 further comprising a human IgG1 constant region.

[000203] Benchmark antibodies were also prepared and used in the characterization assays and studies as controls. A total of four benchmark antibodies were made, namely: WBP345-BMK1.uIgG4PE.K (sequence disclosed in WO 2016/109415 A1), WBP345-BMK2.uIgG4P.K (disclosed as Hu5F9-G4 in Liu J. et al., PLoS One, 2015, 10: e0137345), WBP345-BMK3.uIgG1K (C47B222-IgG1, the sequence of C47B222 is disclosed in WO

2016/081423 A1), WBP345-BMK4.uIgG4.SPK (disclosed as 2.3D11 IgG4 in US 2017/0081407 A1).

[000204] Isotype control antibodies W332-1.80.12xAb.hIgG1 and W332-1.80.12xAb.hIgG4 do not bind to CD47 and were used as negative controls.

EXAMPLE 2: *In vitro* characterization

[000205] 1. Target binding (ELISA/FACS)

[000206] 1.1 Target binding (FACS)

[000207] Human CD47-expressing cell lines (W345-CHOK1.hPro1.D1), CCRF-CEM (ATCC#CRM-CCL-119) were prepared or purchased by WuXi Biologics.

[000208] Binding of CD47 antibodies to CD47 expressing cells (W345-CHOK1.hPro1.D1, CCRF-CEM) was determined by flow cytometry. Briefly, 1×10^5 cells/well were added to each well of a 96-well plate and centrifuged at 1500rpm for 5 minutes at 4°C before removing the supernatant. Serial dilutions of test antibodies, as well as positive and negative control antibodies were added to the pelleted cells and incubated for 1 hour at 4°C. The cells were washed two times with 200 µl PBS with 1% BSA. PE conjugated goat anti-human IgG Fc (Jackson, #109-115-098) 1:100 diluted in FACS buffer were added to the cells and incubated at 4°C for 30 minutes. Additional washing steps were performed two times with 200 µl FACS buffer followed by centrifugation at 1500rpm for 5 minutes at 4°C. Finally, the cells were resuspended in 100 µl FACS buffer and fluorescence values were measured by flow cytometry and analyzed by FlowJo.

[000209] Antigen binding activity of W3452-1.164.16-z11-IgG1K and W3452-2.683.2-z27-IgG1L and W3452-2.683.2-z27-IgG4PE,L were carried out by FACS using CHOK1 cells stably transfected with human CD47. As shown in Figure 1, W3452-1.164.16-z11-IgG1K and W3452-2.683.2-z27-IgG1L and W3452-2.683.2-z27-IgG4PE,L bind to CD47-transfected cells, but not to parental CHOK1 blank cells. The specific data is shown in Table 4 below.

[000210] Table 4. Results of human CD47 binding by FACS

Abs	Hu-CD47-CHOK1	
	EC50 (nM)	Max MFI
W3452-2.683.2-z27-IgG1L	0.66	22900
W3452-2.683.2-z27-IgG4PE,L	0.73	21300
W3452-1.164.16-z11-IgG1K	0.57	22500
WBP345-BMK1.ulIgG4PE.K	0.65	24200
WBP345-BMK2.ulIgG4P.K	0.49	16900
W332-1.80.12.xAb.hIgG1	>66.7	233
W332-1.80.12.xAb.hIgG4	37.9	38.6

[000211] 1.2 Cross binding (FACS)

[000212] Cynomolgus PBMC was used for cyno CD47 expression cells, which was isolated from Cyno fresh blood

[000213] Binding of CD47 antibodies to CD47 expressing cells (Cynomolgus PBMC) was determined by flow cytometry. Briefly, 1×10^5 cells/well were added to each well of a 96-well plate and centrifuged at 1500rpm for 5 minutes at 4°C before removing the supernatant. Serial dilutions of test antibodies, as well as positive and negative control antibodies were added to the pelleted cells and incubated for 1 hour at 4°C. The cells were washed two times with 200 µl PBS with 1% BSA. PE conjugated goat anti-human IgG Fc (Jackson, #109-115-098) 1:100 diluted in FACS buffer were added to the cells and incubated at 4°C for 30 minutes. Additional washing steps were performed two times with 200 µl FACS buffer followed by centrifugation at 1500 rpm for 5 minutes at 4°C. Finally, the cells were resuspended in 100 µl FACS buffer and fluorescence values were measured by flow cytometry and analyzed by FlowJo.

[000214] Antigen binding activity of W3452-1.164.16-z11-IgG1K and W3452-2.683.2-z27-IgG1L and W3452-2.683.2-z27-IgG4PE,L were carried out by FACS using cyno PBMC. Results showed that W3452-1.164.16-z11-IgG1K binds cyno PBMC well with low EC₅₀ and max MFI. W3452-2.683.2-z27-IgG1L and W3452-2.683.2-z27-IgG4PE,L showed similar EC₅₀ and MFI (see Table 5 below and Figure 2). W332-1.80.12.xAb.hIgG1 and W332-1.80.12.xAb.hIgG4 in the Table are IgG1 and IgG4 isotype control antibodies, respectively.

[000215] Table 5. Results of cyno CD47 binding by FACS

Abs	cyno-PBMC	
	EC50 (nM)	Max MFI
W3452-2.683.2-z27-IgG1L	0.2	15300
W3452-2.683.2-z27-IgG4PE,L	0.21	14500
W3452-1.164.16-z11-IgG1K	0.09	16400
WBP345-BMK1.ulgG4PE.K	0.2	13600
WBP345-BMK2.ulgG4P.K	0.12	12700
W332-1.80.12.xAb.hlgG1	36.2	912
W332-1.80.12.xAb.hlgG4	NA	364

[000216] 2. Affinity (FACS)

[000217] Harvest cells and count viable cells (W345.CHO-K1.hPro1.FL.G4(P1)), centrifuge cells and resuspend in an appropriate volume of 1%BSA/1XPBS to at the concentration of 1×10^6 cell/ml, and add 50 ul cell suspension to each well of 96-well U-plate. Then centrifuge at 1500 rpm for 4 min and discard supernatant.

[000218] Dilute W3452-1.164.16-z11-IgG1K and W3452-2.683.2-z27-IgG1L and W3452-2.683.2-z27-IgG4PE,L (initial con. 10 μ g/ml, 2-fold diluted) with 1%BSA/1XPBS, and add 100 ul/well to FACS plates, incubate at 4°C for 1 hour. Then centrifuge at 1500 rpm for 4 min and discard supernatant, add 100 ul/well Goat Anti-Human IgG Fc FITC (diluted to 14 μ g/ml with 1%BSA/1XPBS) to resuspend cells, incubate at 4°C for 30 mins. Following that, wash cells once with 180 ul/well 1%BSA/1XPBS, and discard supernatant. After final wash, resuspend cells in 100 ul/well 1%BSA/1XPBS. Keep cells at 4°C in the dark until running. The fluorescence intensity is measured by flow cytometry (BD Canto II) and analyzed by FlowJo.

[000219] Antigen binding affinity of W3452-1.164.16-z11-IgG1K and W3452-2.683.2-z27-IgG1L and W3452-2.683.2-z27-IgG4PE,L was carried out by FACS using CHOK1 cells stably transfected with human CD47 (Table 6). All three antibodies showed high affinity with human CD47.

[000220] Table 6. Results of human CD47 binding affinity by FACS

Abs	W3452-1.164.16-z11-IgG1K	W3452-2.683.2-z27-IgG1L	W3452-2.683.2-z27-IgG4PE,L	WBP345BMK1.ulgG4PE.K	WBP345BMK2.ulgG4P.K
Bmax	2.30E-10	2.50E-10	2.10E-10	2.30E-10	1.80E-10
KD	7.40E-11	4.20E-10	4.90E-10	3.70E-10	2.90E-10
r ²	0.99	0.99	0.99	0.99	0.99

[000221] 3. Cell based ligand competition assay

[000222] Human CD47-expressing cell lines (W345-CHOK1.hPro1.D1), CCRF-CEM (ATCC#CRM-CCL-119) were prepared or purchased by WuXi Biologics. Human SIRP α (W345-hPro1L1.His(sino)) was purchased from Sino Biological(Cat# 11612-H08H-50).

[000223] Competition of CD47 antibodies with the ligand human SIRP α on CD47 expressing cells (W345-CHOK1.hPro1.D1, CCRF-CEM) was determined by flow cytometry. Briefly, 1×10^5 cells/well were added to each well of a 96-well round bottom plates and centrifuged at 1500rpm for 5 minutes at 4°C before removing the supernatant. Serial dilutions of test antibodies, as well as positive and negative control antibodies with 1 μ g/ml Human SIRP α were added to the pelleted cells and incubated for 2 hours at 4°C. The cells were washed two times with 200 μ l PBS with 1% BSA. Biotin conjugated anti-His Tag Antibody (GenScript, # A00613) 1:400 diluted in FACS buffer were added to the cells and incubated at 4°C for 30 minutes. PE conjugated Streptavidin (Affymetrix, # 12-4317) 1:200 diluted in FACS buffer were added to the cells and incubated at 4°C for 30 minutes. Additional washing steps were performed two times with 200 μ l FACS buffer followed by centrifugation at 1500rpm for 5 minutes at 4°C. Finally, the cells were resuspended in 100 μ l FACS buffer and fluorescence values were measured by flow cytometry and analyzed by FlowJo.

[000224] To assess ligand (SIRP α) blocking activity of W3452-1.164.16-z11-IgG1K and W3452-2.683.2-z27-IgG1L and W3452-2.683.2-z27-IgG4PE,L, competition binding was carried out to test interaction activity of the above three antibodies to block CD47- SIRP α by FACS using CHOK1 stably transfected with human CD47. The results show that the blocking rates of W3452-1.164.16-z11-IgG1K and W3452-2.683.2-z27-IgG1L and W3452-2.683.2-z27-IgG4PE,L, exceed 90%. (see Table 7 below and Figure 3). W332-1.80.12.xAb.hIgG1 and W332-1.80.12.xAb.hIgG4 in the Table are IgG1 and IgG4 isotype control antibodies, respectively.

[000225] Table 7. Results of ligand (SIRP α) competition assay by FACS

Abs	SIRP α Blocking	
	EC50 (nM)	Blocking rate%
W3452-2.683.2-z27-IgG1L	0.94	95.00%
W3452-2.683.2-z27-IgG4PE,L	1.24	94.60%
W3452-1.164.16-z11-IgG1K	0.62	94.50%
WBP345-BMK1.ulgG4PE.K	0.83	95.20%
WBP345-BMK2.ulgG4P.K	0.68	95.70%
W332-1.80.12.xAb.hIgG1	NA	NA
W332-1.80.12.xAb.hIgG4	NA	NA

[000226] 4. Human RBC hemagglutination assay (HA)

[000227] To evaluate the hemagglutinating capacity of CD47 antibodies (i.e. W3452-1.164.16-z11-IgG1K, W3452-2.683.2-z27-IgG1L and W3452-2.683.2-z27-IgG4PE,L), human RBCs obtained from the fresh human blood after washing three times with PBS and centrifugation at 2000 rpm for 5 minutes, were diluted to 4% in PBS and incubated at 37°C for 1 h with a titration of CD47 antibodies, as well as positive and negative control antibodies in around bottom 96 well plate. The graph of results was recorded by camera. Evidence of hemagglutination is demonstrated by the presence of non-settled RBCs, appearing as a haze compared to a punctuate red dot of non-hemagglutinated RBCs. Results showed that W3452-1.164.16-z11-IgG1K, W3452-2.683.2-z27-IgG1L and W3452-2.683.2-z27-IgG4PE did not exhibit hemagglutinating activity at any tested concentration (see Figure 4). In comparison, the benchmark control antibody WBP345-BMK2.uIgG4P.K showed hemagglutinating activity.

[000228] 5. Cell based phagocytosis assay

[000229] PBMCs were isolated from human blood, and the CD14 positive monocytes were isolated from PBMC by hCD14 Microbeads (Miltenyi Biotec, # 130-050-201). The CD14 positive monocytes were differentiated into macrophages by incubating them in the 10%FBS RPIM1640 medium with 100ng/ml rhM-CSF (R&D Systems , #216-MC/CF) for 7 days. These monocyte derived macrophages(MDMs) become adherent allowing other cells to be washed away. MDMs were scraped and re-plated in 96 well dishes. The human tumor cell line Jurkat.2B8, CCRF-CEM, Raji and hRBCs with high CD47 expression were chosen as a target cell type. Target cells were labeled with 1uM CFSE (Life-technology, # C34570) at 37°C for 30 minutes, then washed and added to MDMs at a ratio of 1:1 tumor cells per phagocyte, and CD47 antibody was added at various concentrations. Phagocytosis of target cells was allowed for 2 hours. Subsequently, stained with an antibody to the macrophage marker CD14 conjugated to APC (BD Pharmingen, # 561708), and analyzed by flow cytometry. Phagocytosis was measured by gating on live cells that were FL4 positive (CD14+), and then assessing the percent of FL1 (CFSE+) positive cells.

[000230] Phagocytic activity was measured by using human PBMC-derived macrophages and Jurkat.2B8 and Raji cells, counting the ratio of the number of macrophages that ingested tumor cells to the total number of macrophages. Results showed W3452-1.164.16-z11-IgG1K, W3452-2.683.2-z27-IgG1L and W3452-2.683.2-z27-IgG4PE,L induced potent phagocytosis of tumor cells (see Tables 8 and 9 below, and Figures 5A and 5B). W332-1.80.12.xAb.hIgG1

and W332-1.80.12.xAb.hIgG4 in the Table are IgG1 and IgG4 isotype control antibodies, respectively.

[000231] Table 8. Results of phagocytic assay on Jurkat.2B8 cells

Abs	EC50	Phagocytosis Index% on Jurkat.2B8			
	(nM)	(%CFSE+/CD14+ of Total CD14+)			
		1ug/ml	0.2ug/ml	0.1ug/ml	0.04ug/ml
W3452-2.683.2-z27-IgG1L	0.135	30.55	36.86	31.17	24.23
W3452-2.683.2-z27-IgG4PE,L	1.123	25.95	16.55	9.91	4.52
W3452-1.164.16-z11-IgG1K	0.154	31.93	30.85	27.79	21.21
WBP345-BMK1.uIgG4PE.K	2.471	16.67 (1.67ug/ml)	19.44 (0.56ug/ml)	9.70 (0.37ug/ml)	2.78 (0.18ug/ml)
WBP345-BMK2.uIgG4P.K	0.18	35.4	35.75	33.56	24.22
WBP345-BMK3.uIgG1K	0.211	35.14	37.35	33.29	22.11
WBP345-BMK4.uIgG4.SPK	0.796	39.18	27.17	17.81	9.66
W332-1.80.12.xAb.hIgG1	NA	2.58	2.16	2.43	2.93
W332-1.80.12.xAb.hIgG4	NA	2.73	3.36	2.74	2.85

[000232] Table 9. Results of phagocytic assay on Raji cells

Abs	EC50 (nM)	Phagocytosis Index% on Raji (%CFSE+/CD14+ of Total CD14+)		
		5ug/ml	1ug/ml	0.2ug/ml
W3452-2.683.2-z27-IgG1L	0.07	66.6	65.3	65.5
W3452-2.683.2-z27-IgG4PE,L	0.48	54.9	46.9	44.6
W3452-1.164.16-z11-IgG1K	0.05	66.5	66.6	68.9
WBP345-BMK1.uIgG4PE.K	0.39	18.3 (5ug/ml)	20.2 (1.7ug/ml)	22.6 (0.6ug/ml)
WBP345-BMK2.uIgG4P.K	0.11	49.4	54.4	57.7
WBP345-BMK3.uIgG1K	0.1	64	65.2	64.4
WBP345-BMK4.uIgG4.SPK	1.18	32.9	33.7	25.8
W332-1.80.12 xAb.hIgG1	NA	17.1	17.7	18.3
W332-1.80.12 xAb.hIgG4	NA	19	19.4	21.7

[000233] 6. ADCC and CDC assays

[000234] 6.1 ADCC assay

[000235] In brief, human PBMC were incubated overnight in RPMI1640 medium containing 10% fetal bovine serum, 1% penicillin/streptomycin solution and 50 unit/mL hIL-2. The next day, PBMC were used as effector cells and CCRF-CEM or Raji were used as target cells. 2×10^4 target cells in 50 μ L RPMI1640 (no phenol) medium containing 1% FBS were added per well in a 96-well U-bottom plate. Then, serial-diluted antibodies in 10 μ L RPMI1640 (no phenol) medium containing 1% FBS were added to each well. After 15 minutes incubation at 37°C, 4×10^5 PBMC in 40 μ L RPMI1640 (no phenol) medium containing 1% FBS were added to each well to give a 20:1 E/T ratio. After incubation at 37°C for 4 h, mixtures were centrifuged at 1500 rpm for 5 min and 70 μ L of supernatant were transferred. Cell death was evaluated using LDH Cytotoxicity Detection Kit (Roche) according to manufacturer's instructions.

[000236] 6.2 CDC assay

[000237] 5×10^4 target cells in 50 μ L RPMI1640 (no phenol) medium containing 1% FBS were added per well in a 96-well U-bottom plate. Then, serial-diluted antibodies in 10 μ L RPMI1640 (no phenol) medium containing 1% FBS were added to each well. After 15 minutes incubation at 37°C, human serum in 40 μ L RPMI1640 (no phenol) medium containing 1% FBS were added to each well to give a final concentration of 20%. After incubation at 37°C for 2 h, 10 μ L propidium iodide (Invitrogen) was added into each well and incubated at room temperature for 10 min. Cell death was evaluated by FACS analysis.

[000238] 6.3 Results

[000239] As shown in Figures 6A-6D, W3452-1.164.16-z11-IgG1K and W3452-2.683.2-z27-IgG1L induced potent ADCC activity on both CCRF-CEM and Raji cells, while only W3452-1.164.16-z11-IgG1K induced CDC activity on tumor cells. W3452-2.683.2-z27-IgG4PE,L induced no or weak ADCC and CDC activity.

[000240] 7. Thermal stability

[000241] A differential scanning fluorometry (DSF) assay was performed for testing thermal stability of W3452-1.164.16-z11-IgG1K, W3452-2.683.2-z27-IgG1L and W3452-2.683.2-z27-IgG4PE,L, using 7500 Fast Real-Time PCR system (Applied Biosystems). Briefly, 19 μ L of antibody solution was mixed with 1 μ L of 62.5 X SYPRO Orange solution (Invitrogen) and added to a 96 well plate (Biosystems). The plate was heated from 26 °C to 95 °C at a rate of 2 °C/min, and the resulting fluorescence data were collected. The negative derivatives of the fluorescence changes with respect to different temperatures were calculated, and the maximal value was defined as melting temperature T_h . If a protein has multiple unfolding transitions, the first two T_h were reported, named as T_{h1} and T_{h2} . T_{h1} is always interpreted as the formal melting temperature T_m to facilitate comparisons between different proteins. Data collection and T_h calculation were conducted automatically by its operation software. Once the plot of negative derivatives of different temperatures was reported by the software, the point in the plot where the curve starts to decrease from a pre-transition baseline could be roughly estimated as the onset temperature T_{on} .

[000242] As shown in Figure 7 and Table 10 below, W3452-1.164.16-z11-IgG1K, W3452-2.683.2-z27-IgG1L and W3452-2.683.2-z27-IgG4PE,L showed good thermal stability compared to human mAb normal values and as good as or better than benchmark control antibodies (WBP345-BMK1.uIgG4PE.K and WBP345-BMK2.uIgG4P.K).

[000243] Table 10. Results of thermal stability

Protein Name	PI	Buffer	T_{on} (°C)	T_{h1} (°C)	T_{h2} (°C)	Comments
W3452-1.164.16-z11-IgG1K	7.84	PBS	58	68.7	80.7	Human mAb reference values : Ton: 54.4 $\pm$ 3.0 °C Th1: 66.5 $\pm$ 3.2 °C Th2: 75.9 $\pm$ 3.3 °C
W3452-2.683.2-z27-IgG1L	8.01	PBS	58	65.4	—	
W3452-2.683.2-z27-IgG4PE,L	7.08	PBS	56	64.3	—	
WBP345-BMK1.uIgG4PE.K	7.55	PBS	56	63.4	71	
WBP345-BMK2.uIgG4P.K	7.9	PBS	54	65.3	77.4	

[000244] 8. Serum stability

[000245] In order to facilitate *in vivo* study, serum stability of W3452-1.164.16-z11-IgG1K, W3452-2.683.2-z27-IgG1L and W3452-2.683.2-z27-IgG4PE,L was carried out on human serum. Freshly collected human blood was incubated in polystyrene tubes without anticoagulant for 30 minutes at room temperature. Serum was collected after centrifugation the blood at 4000 rpm for 10 minutes. Repeat the centrifugation step until the serum was clarifying. Gently mix antibodies with serum, and aliquots were drawn at 37°C for the indicated times: 0 day, 1 day, 4 days, 7 days and 14 days. Quickly-freeze the samples at the indicated time in liquid nitrogen and store them at -80°C until use. The samples were used to assess their binding ability on W345-CHOK1.hPro1.D1 cells and CCRF-CEM cells by FACS. Briefly, serial dilutions of antibodies were added to W345-CHOK1.hPro1.D1 cells or CCRF-CEM cells and incubated for 1 hour at 4°C. The cells were washed two times with 200 µl PBS with 1% BSA. PE conjugated goat anti-human IgG Fc (Jackson, #109-115-098) diluted 1:100 with FACS buffer were added to the cells and incubated at 4°C for 30 minutes. Additional washing steps were performed two times with 200 µl FACS buffer followed by Centrifugation at 1500rpm for 4 minutes at 4°C. Finally, the cells were resuspended in 100 µl FACS buffer and fluorescence values were measured by flow cytometry and analyzed by FlowJo.

[000246] As showed in Figures 8A-8C, 14 days serum culturing has no effect on human CD47 binding of the tested antibodies as measured by FACS.

[000247] 9. Effect of anti-CD47 mAbs against Raji cells in B-NSG mice

[000248] The anti-tumor activity of W3452-1.164.16-z11-IgG1K, W3452-2.683.2-z27-IgG1L and W3452-2.683.2-z27-IgG4PE,L was evaluated in a Raji-Luc model of lymphoma in B-NSG mice. 0.5 million Raji cells/mouse were engrafted into mice through intravenous injection. They were imaged *in vivo* to determine the level of fluorescence and randomized into 9 groups (6 mice per group, day0), treatments were given twice a week at day0, day4, day7, day 11 with 3 mg/kg or 0.5 mg/kg.

[000249] As shown in Figure 9, W3452-1.164.16-z11-IgG1K, W3452-2.683.2-z27-IgG1L, and in particular W3452-2.683.2-z27-IgG4PE,L demonstrated the most potent anti-tumor activity in this animal model of lymphoma. 3 mg/kg treatments showed the best anti-tumor ability, much better than WBP345-BMK2.uIgG4P.K (3 mg/kg). W3452-2.683.2-z27-IgG4PE,L showed better anti-tumor ability than W3452-2.683.2-z27-IgG1L at 0.5 mg/kg.

Since CD47 is ubiquitously expressed in normal human cells, especially in human red blood cells, IgG1 format may induce severe side effect, such as anemia. In order further reduce Fc-based effector function, we engineered IgG4 version with site mutation technology. W3452-1.164.16-z11-IgG1K induced mild anti-tumor ability, which showed as good as binding CD47 and blocking CD47 interaction with its ligand, and even induced better phagocytosis ability, indicating *in vivo* anti-tumor ability is inconsistent with *in vitro* performance.

[000250] 10. Pharmacokinetics of W3452-2.683.2-z27-IgG1L and W3452-2.683.2-z27-IgG4PE,L in Non-Naïve Male Cynomolgus Monkeys

[000251] 10.1 Clinical toxicology study

[000252] To determine the pharmacokinetics of W3452-2.683.2-z27-IgG1L and W3452-2.683.2-z27-IgG4PE,L in non-naïve male cynomolgus monkeys following a single intravenous bolus administration, eight animals (2 animal/group) are divided in 4 groups: low and high dose groups (30 and 150 mg/kg) for the 2 antibodies with one single dose (see Table 11). Animals are intravenously administered with W3452-2.683.2-z27-IgG1L and W3452-2.683.2-z27-IgG4PE,L respectively. The antibodies are formulated in 20 mM Histidine, 5% sucrose solution at pH 5.0. The blood samples are collected at pre-dose (Day-1), 0.25h, 0.5h, 1h, 4h, 8h, 24h, Day 3, Day 7, Day 14, Day 21 and Day 28, and the antibody concentration is determined with ELISA method and pharmacokinetics (PK) data and analyzed with WinNonlin software. Cage-side observations for general health, appearance, and especially skin irritation are performed regularly. Whole blood sample analyses for hematology (CBC) and serum chemistry are determined by hematology analyzer (ADVIA2120) and chemistry (HITACHI 7180), respectively.

[000253] Table 11. Grouping and dosing information

Test article	Number. of animals	Dose Level	Dose Conc.	Dose Volume	Vehicle	Dosing Route
	Male	(mg/kg)	(mg/mL)	(mL/kg)		
W3452-2.683.2-z27-IgG1L	2 (C1001/C1002)	30	32.546	0.92	20 mM Histidine, 5% sucrose, pH 5.0	IV bolus
W3452-2.683.2-z27-IgG1L	2 (C2001/C2002)	150	32.546	4.61		
W3452-2.683.2-z27-IgG4PE,L	2 (C3001/C3002)	30	38.931	0.77	20 mM Histidine, 5% sucrose, pH 5.0	IV bolus
W3452-2.683.2-z27-IgG4PE,L	2 (C4001/C4002)	150	38.931	3.85		

[000254] After early antibody administration, no abnormal signs for general health, appearance, and skin irritation was found. The animals in W3452-2.683.2-z27-IgG4PE,L group (IgG4 format) showed normal sign and normal RBC count in clinical observation. But on Day 7, RBC and HGB counts were reduced, indicating a milder anemia in both low and high dose groups. As shown in Figure 10A-10B, red blood cell (RBC) and hemoglobin (HGB) decreased 7 days after the antibody administration. However, RBC and HGB counts started to recover as early as Day 7 and reach to normal range around Day 28. In addition, reticulocyte (RET) counts were significantly increased as early as day 3 and compensated the RBC loss in blood (Figure 10D). Thus, we conclude that the anemia was transient and well-tolerated, or compensated.

[000255] Compared with IgG4 format, the parameters of RBC, HGB and hematocrit (HCT) in hematology study for W3452-2.683.2-z27-IgG1L at 30 and 150 mg/kg were all decreased at Day 7, and caused an anemia in all animals leading to one animal death in Day 8. Two animals were euthanized on Day 12 due to severe anemia (Figures10A-10C). This severe anemia was probably due to the IgG format or Fc function.

[000256] 10.2 Pharmacokinetics parameters of W3452-2.683.2-z27-IgG4PE,L

[000257] The average half-life of W3452-2.683.2-z27-IgG4PE,L is 147 and 79 hours for 30 mg/kg and 150 mg/kg groups, respectively, as shown in Figure 11 and in Table 12. The systemic exposure for C₀ increased 5.99 fold for W3452-2.683.2-z27-IgG4PE,L as the

dosage increased from 30 to 150 mg/kg. The half-life for W3452-2.683.2-z27-IgG4PE,L is longer than that of Hu5F9-G4 (Hu5F9-G4 is an antibody as described in Liu, J. et al. (2015). Pre-Clinical Development of a Humanized Anti-CD47 Antibody with Anti-Cancer Therapeutic Potential. PLoS ONE 10, e0137345, which is also tested herein as WBP345-BMK2.uIgG4P.K), indicating W3452-2.683.2-z27-IgG4PE,L PK is comparable to or better than Hu5F9-G4 (as shown in Table 13).

[000258] Table 12. The summary for PK parameters was listed.

PK parameters	30mg/kg	150mg/kg
	W3452-2.683.2-z27-IgG4PE,L	
C_0 (μg/mL)	1071.488	6417.401
$T_{1/2}$ (h)	147.254	78.907
$V_{d_{ss}}$ (L/kg)	0.075	0.048
CL (mL/min/kg)	0.008	0.006
AUC_{0-last} (h*μg/mL)	57017.325	442717.350
AUC_{0-inf} (h*μg/mL)	59109.130	444231.400
MRT_{0-last} (h)	118.565	139.647
MRT_{0-inf} (h)	146.608	141.903

[000259] Table 13. Pharmacokinetics parameters of Hu5F9-G4

Dose	$T_{1/2}$ (hr)	T_{max} (hr)	C_{max} (ug/ml)	AUC_{0-43} (hr*ug/ml)	CL (ml/hr/kg)
PD1/MD30	65.9	2	886	83758	0.26
PD3/MD30	94.0	25	854	100063	0.17

[000260] Note: A priming dose (PD) of either 1 or 3 mg/kg was administered on Day 1, followed one week later by weekly maintenance dosing (MD) of 30 mg/kg for six weeks.

[000261] Abbreviations

AUC	The area under the serum concentration-time curve
AUC_{0-last}	The area under the serum concentration-time curve from time zero to the last quantifiable concentration
AUC_{0-inf}	The area under the serum concentration-time curve from time zero extrapolated to infinity were calculated using the linear/log trapezoidal rule
C_0	Maximum serum concentration
CL	Total body clearance
MRT	Mean residence time
MRT_{0-last}	Mean residence time from time zero to the last quantifiable concentration
MRT_{0-inf}	Mean residence time from time zero to infinity
$T_{1/2}$	Half-life
T_{max}	Time to reach C_{max}
Vdss	Volume of distribution at steady state

WHAT IS CLAIMED IS:

1. An isolated anti-CD47 antibody or an antigen-binding fragment thereof, comprising:
1, 2, or 3 heavy chain complementarity determining region (CDR) sequences selected from the group consisting of: SEQ ID NO: 1, SEQ ID NO: 3, SEQ ID NO: 5, SEQ ID NO: 7, SEQ ID NO: 9, and SEQ ID NO: 11; and/or
1, 2, or 3 light chain CDR sequences selected from the group consisting of: SEQ ID NO: 2, SEQ ID NO: 4, SEQ ID NO: 6, SEQ ID NO: 8, SEQ ID NO: 10, SEQ ID NO: 12, and SEQ ID NO: 29.
2. The antibody or an antigen-binding fragment thereof of claim 1, comprising a heavy chain variable region selected from the group consisting of:
 - a) a heavy chain variable region comprising 1, 2, or 3 CDR sequences selected from SEQ ID NO: 1, SEQ ID NO: 3, and SEQ ID NO: 5; and
 - b) a heavy chain variable region comprising 1, 2, or 3 CDR sequences selected from SEQ ID NO: 7, SEQ ID NO: 9, and SEQ ID NO: 11.
3. The antibody or an antigen-binding fragment thereof of any of the preceding claims, comprising a light chain variable region selected from the group consisting of:
 - a) a light chain variable region comprising 1, 2, or 3 CDR sequences selected from SEQ ID NO: 2, SEQ ID NO: 4, and SEQ ID NO: 6;
 - b) a light chain variable region comprising 1, 2, or 3 CDR sequences selected from SEQ ID NO: 29, SEQ ID NO: 4, and SEQ ID NO: 6; and
 - c) a light chain variable region comprising 1, 2, or 3 CDR sequences selected from SEQ ID NO: 8, SEQ ID NO: 10, and SEQ ID NO: 12.
4. The antibody or an antigen-binding fragment thereof of any of the preceding claims, comprising:
 - a) a heavy chain variable region comprising 1, 2, or 3 CDR sequences selected from SEQ ID NO: 1, SEQ ID NO: 3, and SEQ ID NO: 5; and a light chain variable region comprising 1, 2, or 3 CDR sequences selected from SEQ ID NO: 2, SEQ ID NO: 4, and SEQ ID NO: 6;
 - b) a heavy chain variable region comprising 1, 2, or 3 CDR sequences selected from SEQ ID NO: 1, SEQ ID NO: 3, and SEQ ID NO: 5; and a light chain variable region comprising 1, 2, or 3 CDR sequences selected from SEQ ID NO: 29, SEQ ID NO: 4, and SEQ ID NO: 6; and
 - c) a heavy chain variable region comprising 1, 2, or 3 CDR sequences selected from

SEQ ID NO: 7, SEQ ID NO: 9, and SEQ ID NO: 11; and a light chain variable region comprising 1, 2, or 3 CDR sequences selected from SEQ ID NO: 8, SEQ ID NO: 10, and SEQ ID NO: 12.

5. The antibody or an antigen-binding fragment thereof of any of the preceding claims, comprising a heavy chain variable region selected from the group consisting of SEQ ID NO: 13, SEQ ID NO: 17, SEQ ID NO: 21, and SEQ ID NO: 25, and a homologous sequence thereof having at least 80% sequence identity yet retaining specific binding affinity to CD47.
6. The antibody or an antigen-binding fragment thereof of any of the preceding claims, comprising a light chain variable region selected from the group consisting of SEQ ID NO: 15, SEQ ID NO: 19, SEQ ID NO: 23, and SEQ ID NO: 27, and a homologous sequence thereof having at least 80% sequence identity yet retaining specific binding affinity to CD47.
7. The antibody or an antigen-binding fragment thereof of any of the preceding claims, comprising:
 - a) a heavy chain variable region comprising SEQ ID NO: 13 and a light chain variable region comprising SEQ ID NO: 15;
 - b) a heavy chain variable region comprising SEQ ID NO: 17 and a light chain variable region comprising SEQ ID NO: 19;
 - c) a heavy chain variable region comprising SEQ ID NO: 21 and a light chain variable region comprising SEQ ID NO: 23; and
 - d) a heavy chain variable region comprising SEQ ID NO: 25 and a light chain variable region comprising SEQ ID NO: 27.
8. The antibody or antigen-binding fragment thereof of any of the preceding claims, further comprising one or more amino acid residue substitutions or modifications yet retains specific binding affinity to CD47.
9. The antibody or antigen-binding fragment thereof of claim 8, wherein at least one of the substitutions or modifications is in one or more of the CDR sequences, and/or in one or more of the VH or VL sequences but not in any of the CDR sequences.
10. The antibody or antigen-binding fragment thereof of any of the preceding claims, further comprising an immunoglobulin constant region, optionally a constant region of human Ig, or optionally a constant region of human IgG.
11. The antibody or an antigen-binding fragment thereof of any of the preceding claims, which is humanized.

12. The antibody or antigen-binding fragment thereof of any of the preceding claims, which is a camelized single domain antibody, a diabody, a scFv, an scFv dimer, a BsFv, a dsFv, a (dsFv)₂, an Fv fragment, a Fab, a Fab', a F(ab')₂, a ds diabody, a nanobody, a domain antibody, or a bivalent domain antibody.
13. The antibody or an antigen-binding fragment thereof of any of the preceding claims, capable of specifically binding to human CD47 at a K_D value of no more than 10⁻⁹ M, 5×10⁻¹⁰ M, 10⁻¹⁰ M, or 7.5×10⁻¹¹ M as measured by flow cytometer assay.
14. The antibody or an antigen-binding fragment thereof of any of the preceding claims, capable of specifically binding to cynomolgus monkey CD47.
15. The antibody or antigen-binding fragment thereof of any of the preceding claims linked to one or more conjugate moieties.
16. The antibody or antigen-binding fragment thereof of claim 15, wherein the conjugate moiety comprises a clearance-modifying agent, a chemotherapeutic agent, a toxin, a radioactive isotope, a lanthanide, a luminescent label, a fluorescent label, an enzyme-substrate label, a DNA-alkylators, a topoisomerase inhibitor, a tubulin-binders, or other anticancer drugs.
17. An antibody or an antigen-binding fragment thereof, which competes for the same epitope with the antibody or antigen-binding fragment thereof of any of the preceding claims.
18. A pharmaceutical composition comprising the antibody or antigen-binding fragment thereof of any of the preceding claims, and a pharmaceutically acceptable carrier.
19. An isolated polynucleotide encoding the antibody or an antigen-binding fragment thereof of claims 1-17.
20. The isolated polynucleotide of claim 19, comprising a nucleotide sequence selecting from a group consisting of SEQ ID NO: 14, SEQ ID NO: 16, SEQ ID NO: 18, SEQ ID NO: 20, SEQ ID NO: 22, SEQ ID NO: 24, SEQ ID NO: 26, and SEQ ID NO: 28, and/or a homologous sequence thereof having at least 80% (e.g. at least 85%, 88%, 90%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, or 99%) sequence identity, and/or a variant thereof having only degenerate substitutions.
21. A vector comprising the isolated polynucleotide of claim 19 or 20.
22. A host cell comprising the vector of claim 21.
23. A method of expressing the antibody or antigen-binding fragment thereof of any of claims 1-17, comprising culturing the host cell of claim 22 under the condition at which the vector of claim 21 is expressed.

24. A method of treating a disease or condition in a subject that would benefit from modulation of CD47 activity, comprising administering to the subject a therapeutically effective amount of the antibody or antigen-binding fragment thereof of any of claims 1-17 or the pharmaceutical composition of claim 18.
25. The method of claim 24, wherein the disease or condition is a CD47 related disease or condition.
26. The method of claim 25, wherein the disease or condition is cancer, autoimmune disease, fibrotic disease, inflammatory disease, or infectious disease.
27. The method of claim 26, wherein the cancer is lung cancer, bronchial cancer, bone cancer, liver and bile duct cancer, pancreatic cancer, breast cancer, liver cancer, ovarian cancer, testicle cancer, kidney cancer, bladder cancer, head and neck cancer, spine cancer, brain cancer, cervix cancer, uterine cancer, endometrial cancer, colon cancer, colorectal cancer, rectal cancer, anal cancer, esophageal cancer, gastrointestinal cancer, skin cancer, prostate cancer, pituitary cancer, stomach cancer, vagina cancer, thyroid cancer, glioblastoma, astrocytoma, melanoma, myelodysplastic syndrome, sarcoma, teratoma, adenocarcinoma, leukemia, myeloma and lymphoma.
28. The method of any of claims 23-27, wherein the disease or condition is hematological cancer chosen from non-Hodgkin's lymphoma (NHL), acute lymphocytic leukemia (ALL), acute myeloid leukemia (AML), chronic lymphocytic leukemia (CLL), chronic myelogenous leukemia (CML), multiple myeloma (MM), diffuse large B cell lymphoma (DLBCL), Richter's syndrome, Burkitt's lymphoma or follicular lymphoma.
29. The method of any of claims 23-28, wherein the subject is human.
30. The method of any of claims 23-29, wherein the administration is via oral, nasal, intravenous, subcutaneous, sublingual, or intramuscular administration.
31. A method of modulating CD47 activity in a CD47-expressing cell, comprising exposing the CD47-expressing cell to the antibody or antigen-binding fragment thereof of any of claims 1-17.
32. A method of detecting presence or amount of CD47 in a sample, comprising contacting the sample with the antibody or antigen-binding fragment thereof of any of claims 1-17, and determining the presence or the amount of CD47 in the sample.
33. A method of diagnosing a CD47 related disease or condition in a subject, comprising: a) contacting a sample obtained from the subject with the antibody or antigen-binding fragment thereof of any of claims 1-17; b) determining presence or amount of CD47 in the sample; and c) correlating the presence or the amount of CD47 to existence or status

of the CD47 related disease or condition in the subject.

34. Use of the antibody or antigen-binding fragment thereof of any of claims 1-17 in the manufacture of a medicament for treating a CD47 related disease or condition in a subject.
35. Use of the antibody or antigen-binding fragment thereof of any of claims 1-17 in the manufacture of a diagnostic reagent for diagnosing a CD47 related disease or condition.
36. A kit comprising the antibody or antigen-binding fragment thereof of any of claims 1-17, useful in detecting CD47.

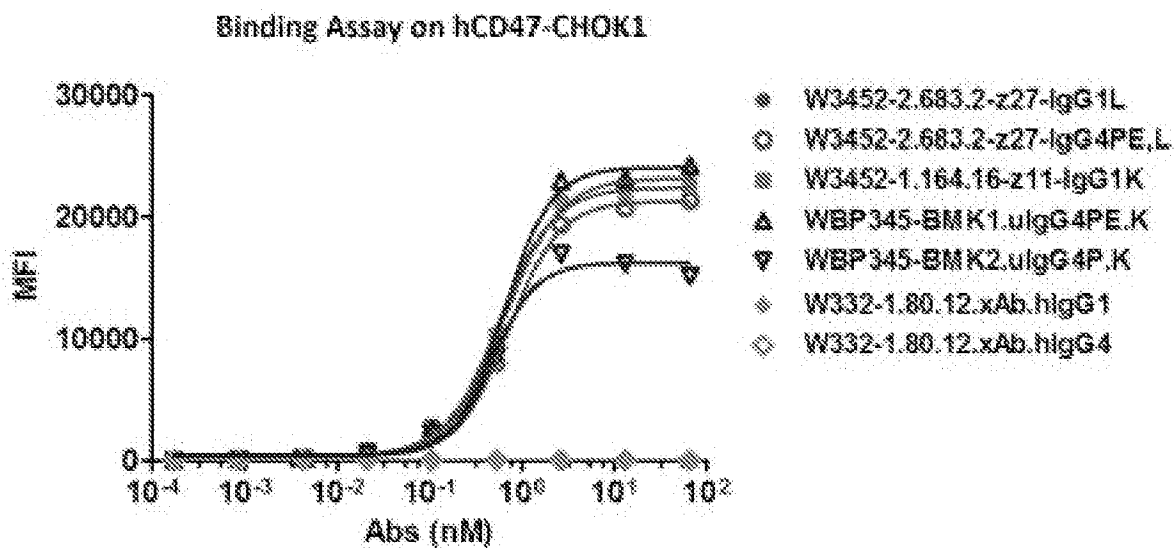


Figure 1

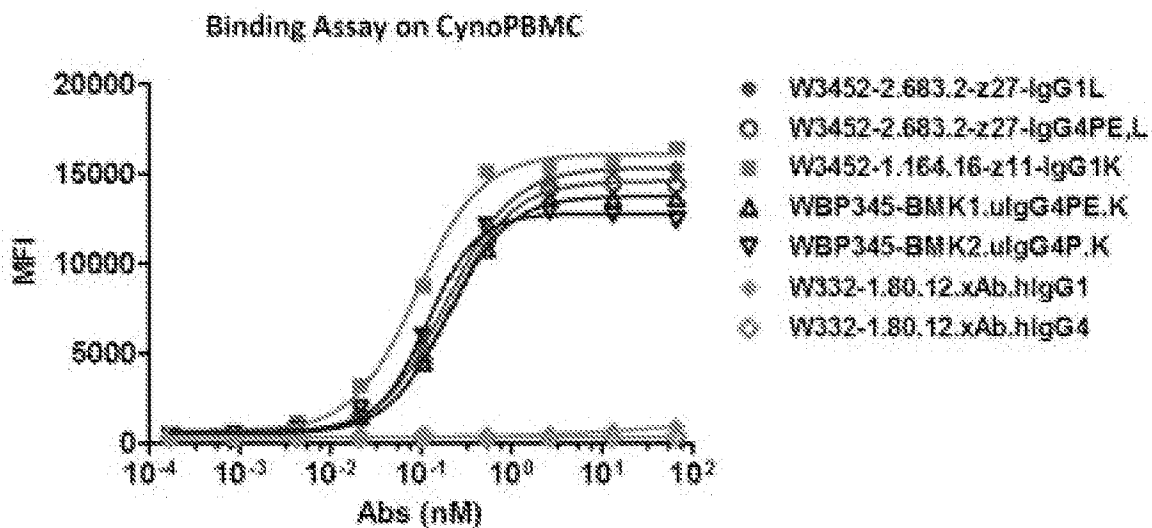


Figure 2

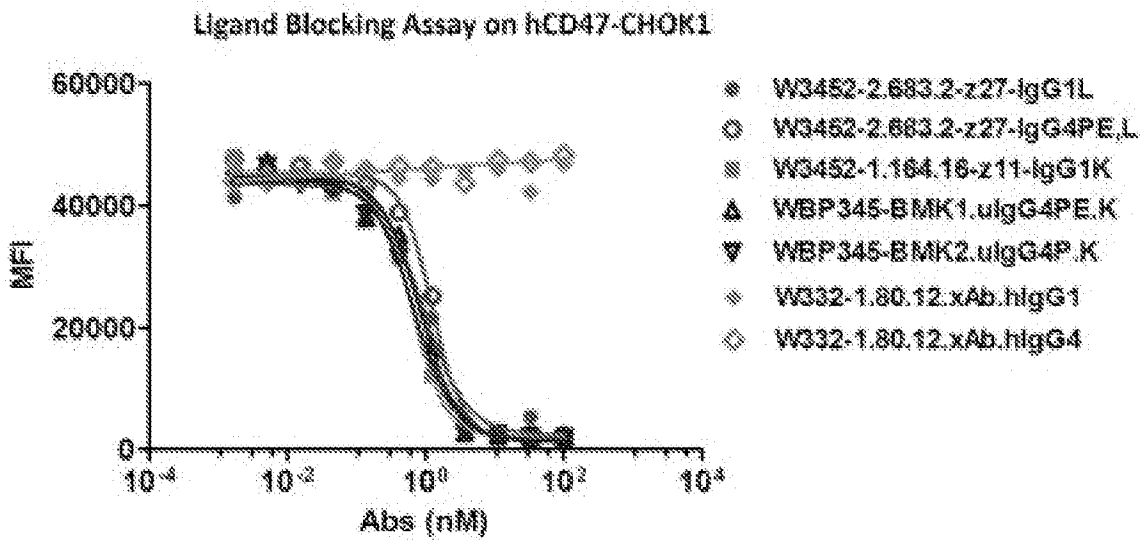


Figure 3

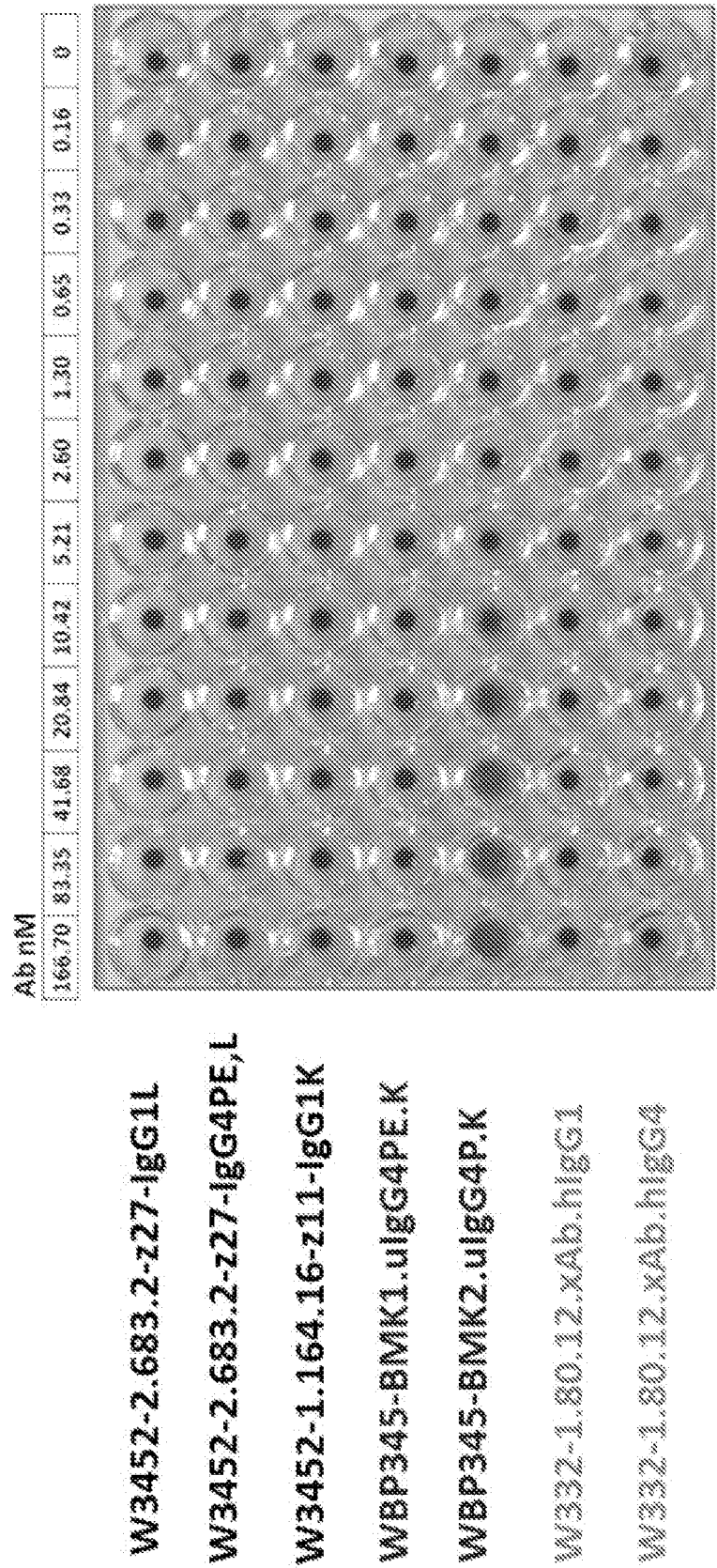


Figure 4

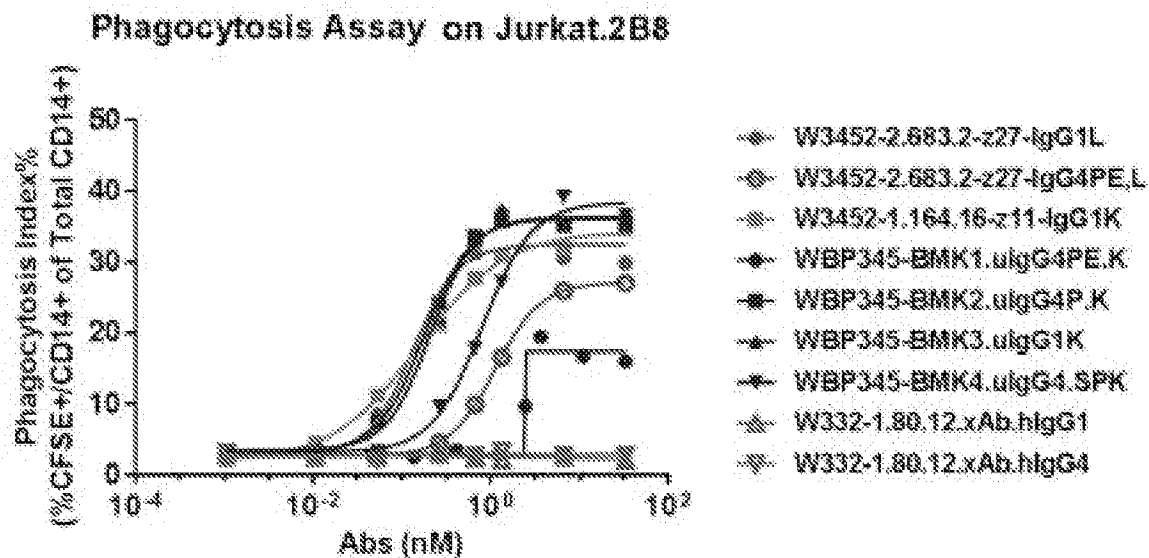


Figure 5A

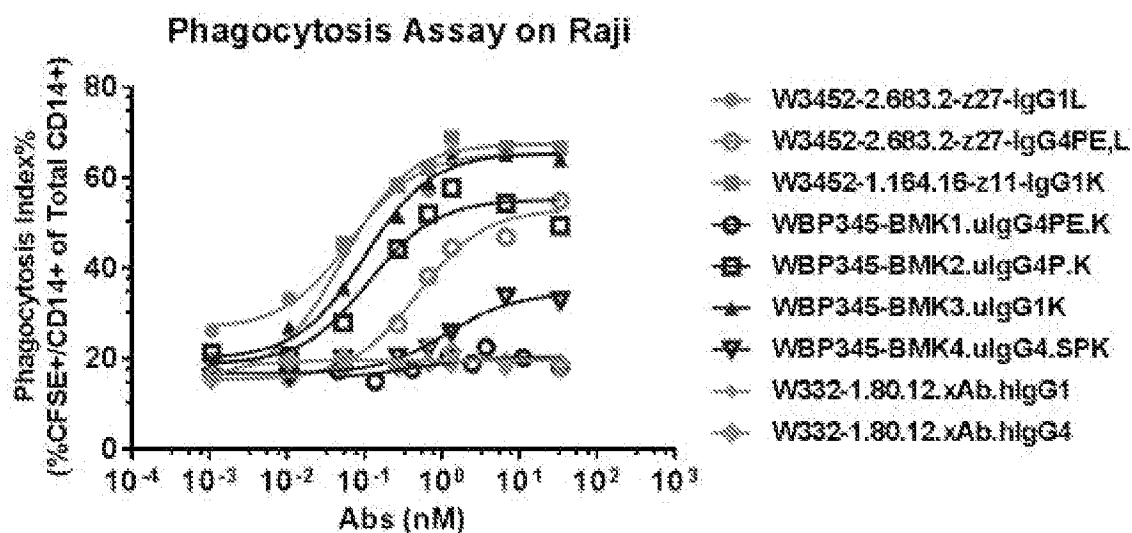


Figure 5B

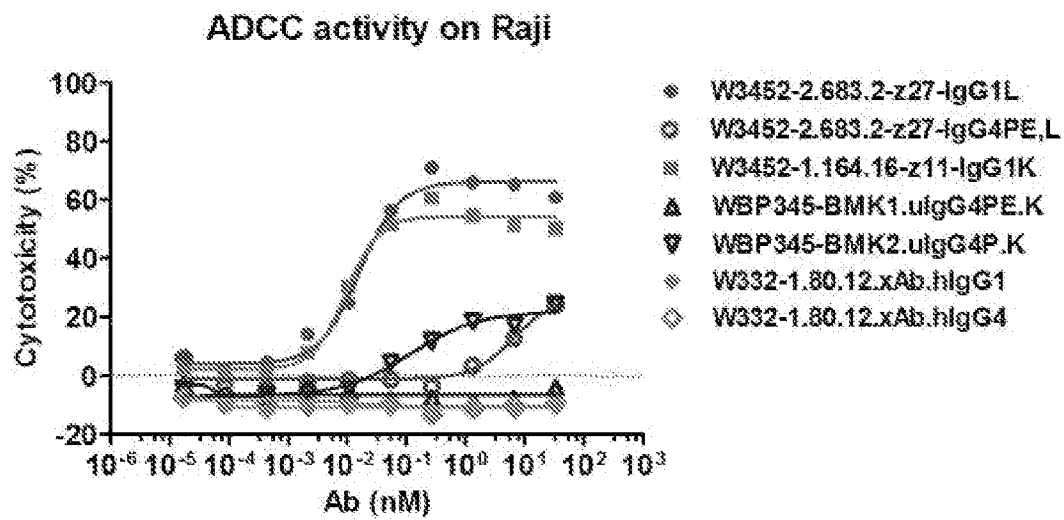


Figure 6A

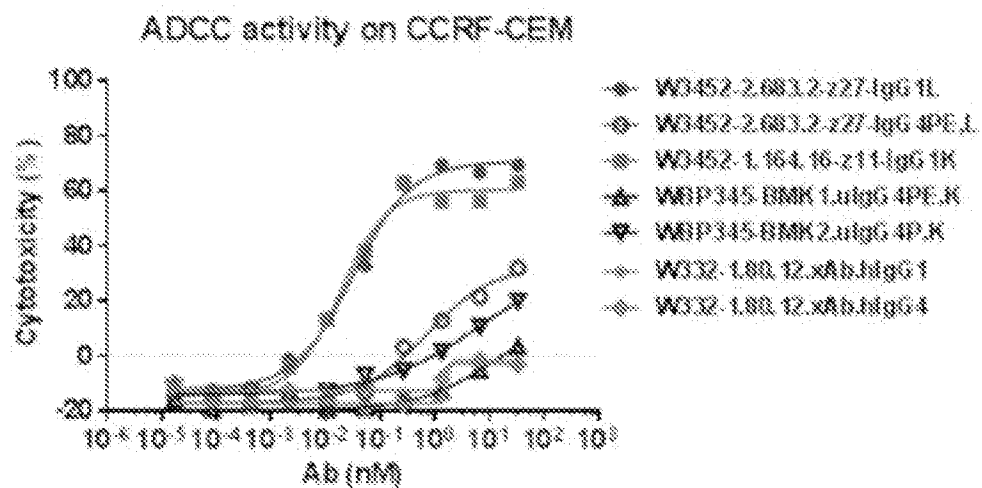


Figure 6B

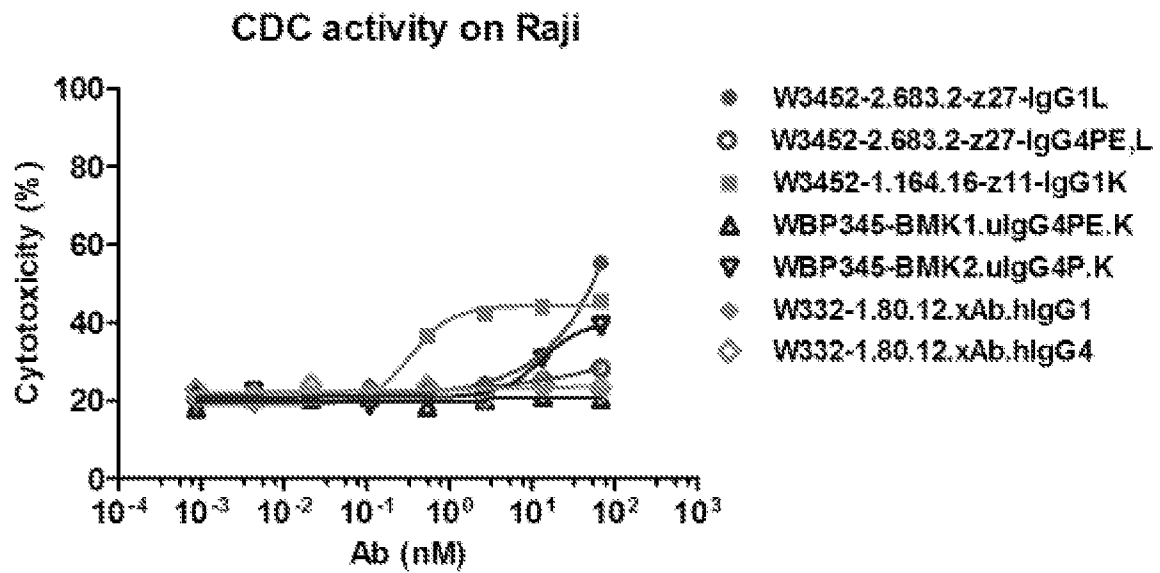


Figure 6C

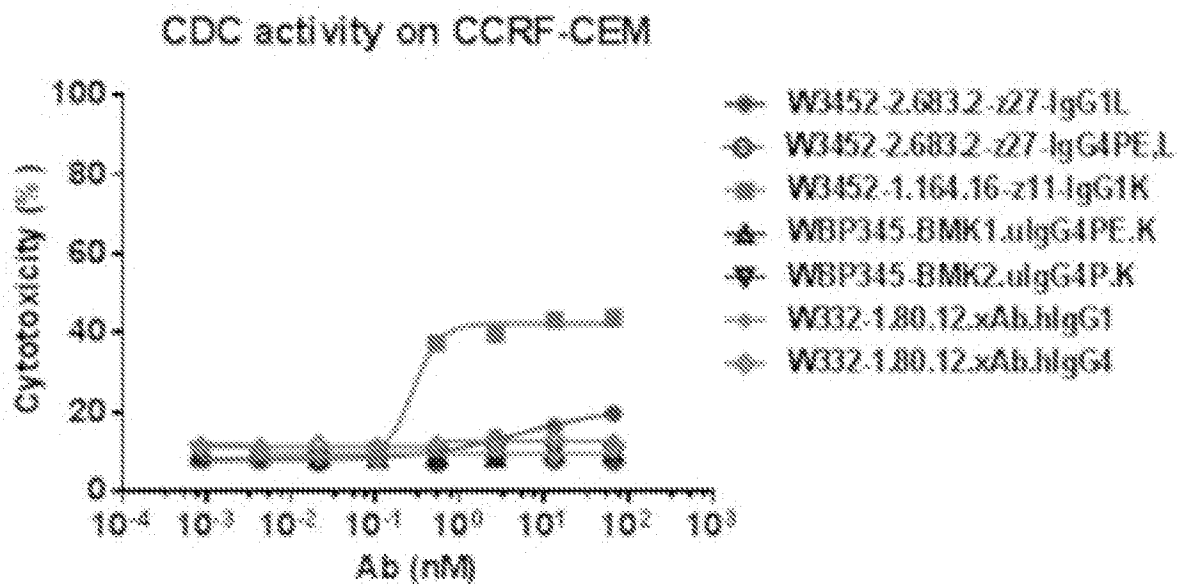


Figure 6D

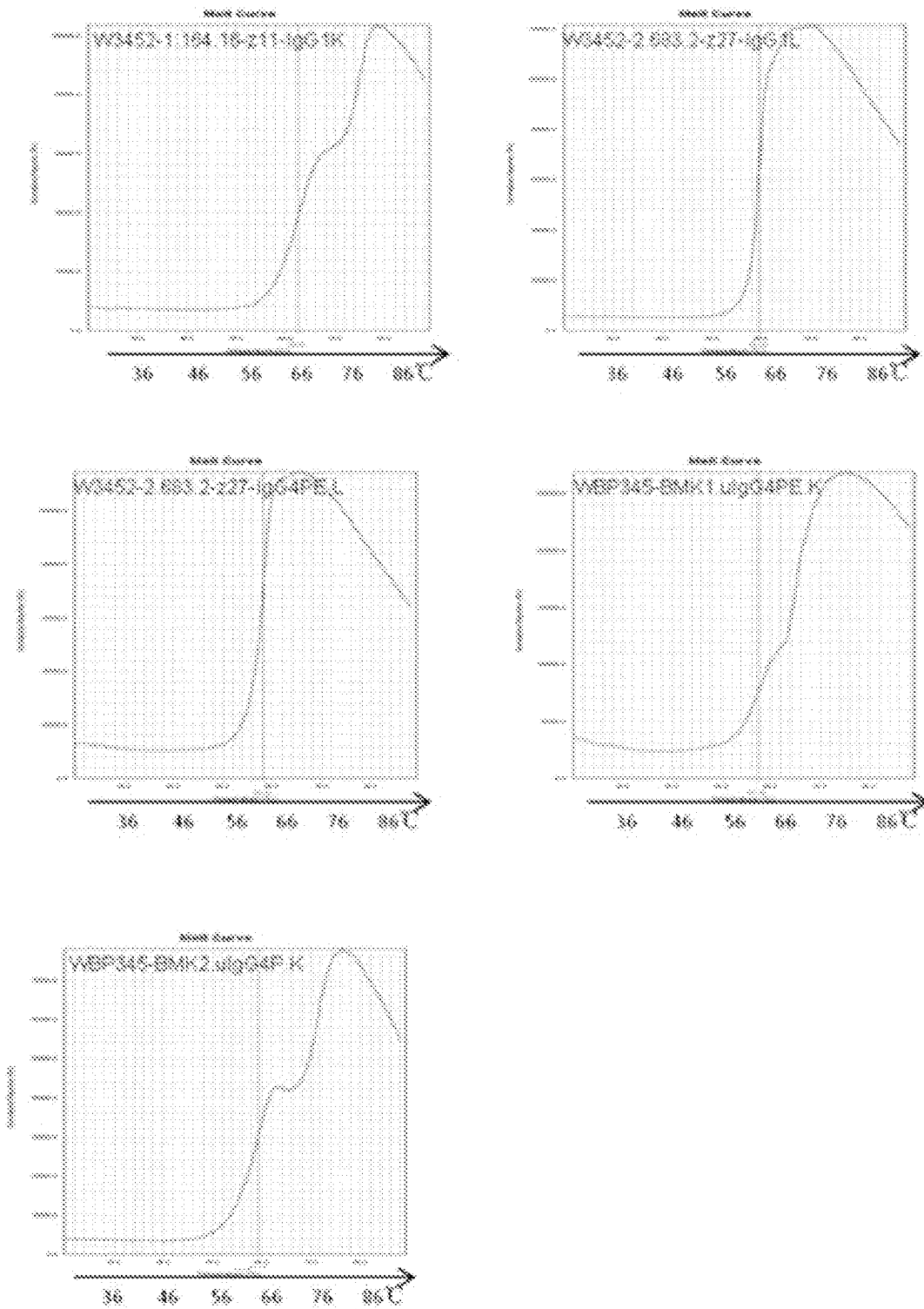


Figure 7

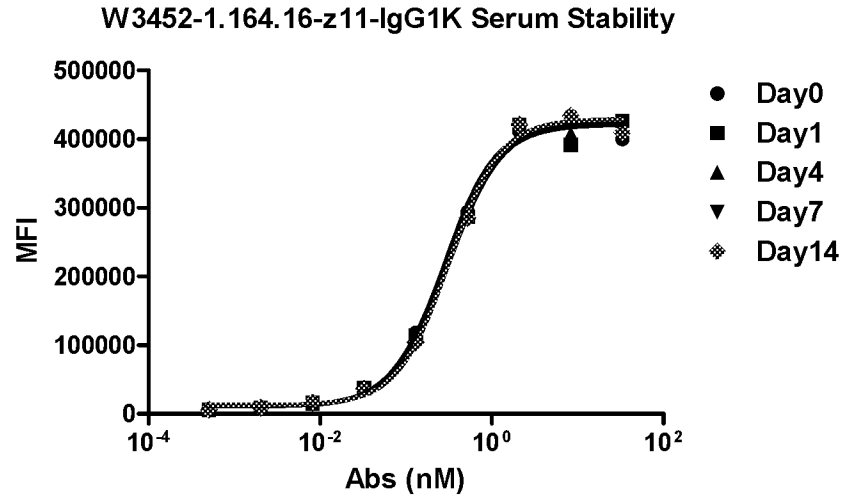


Figure 8A

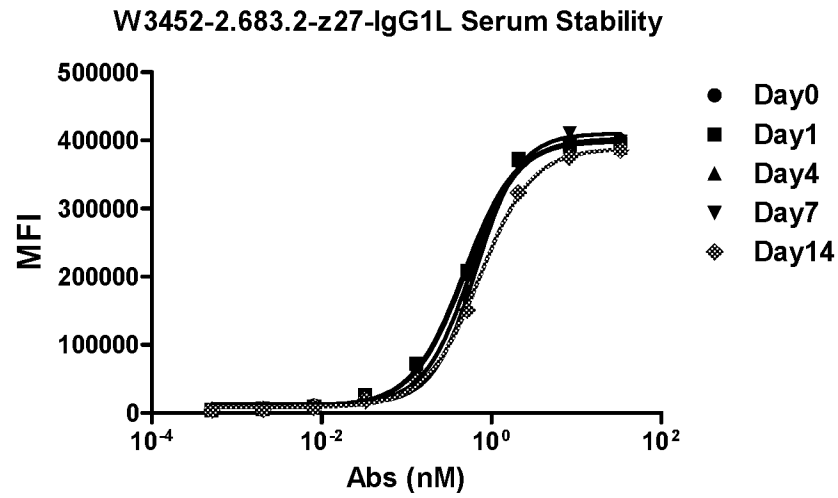


Figure 8B

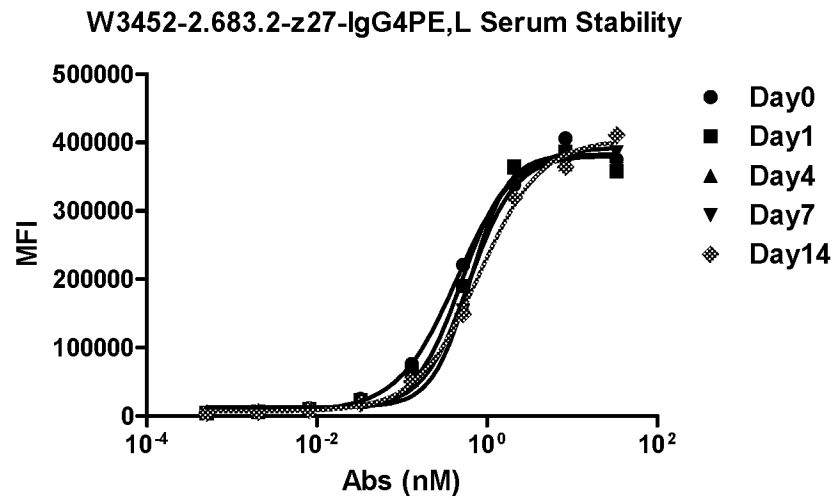


Figure 8C

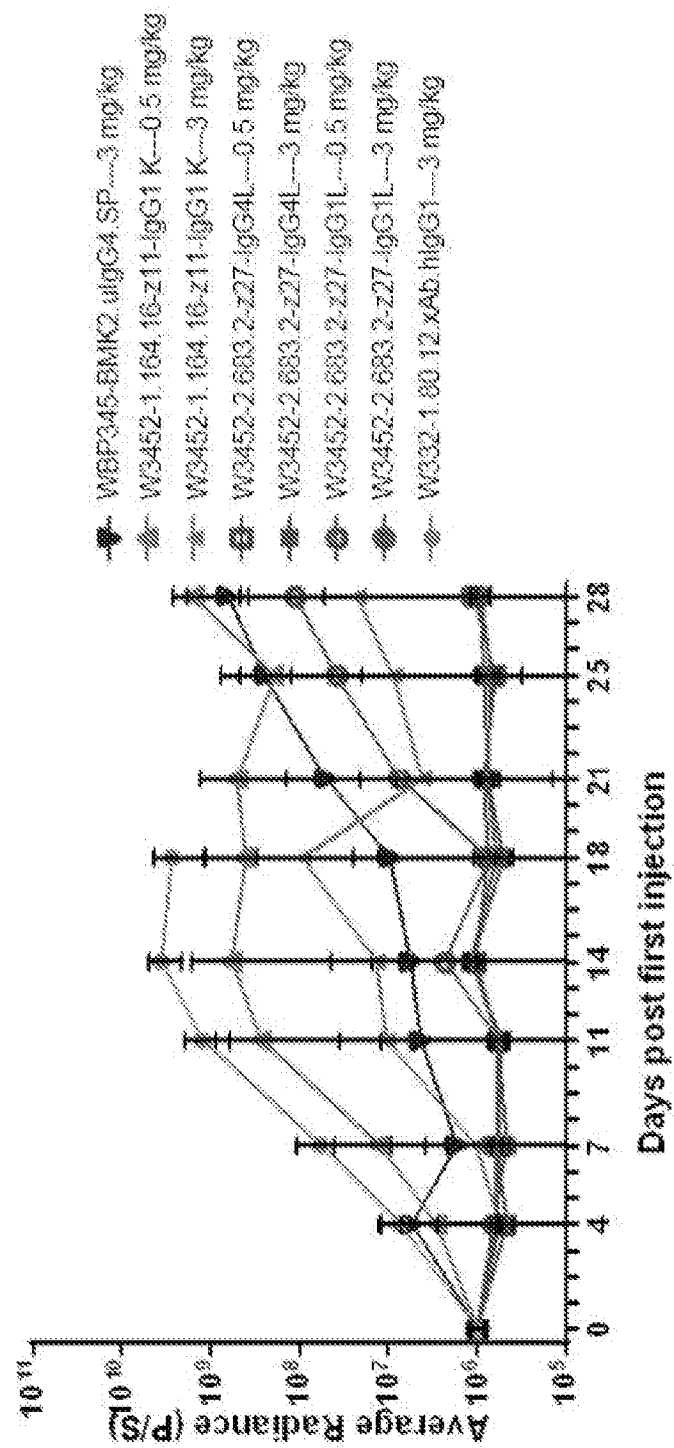


Figure 9

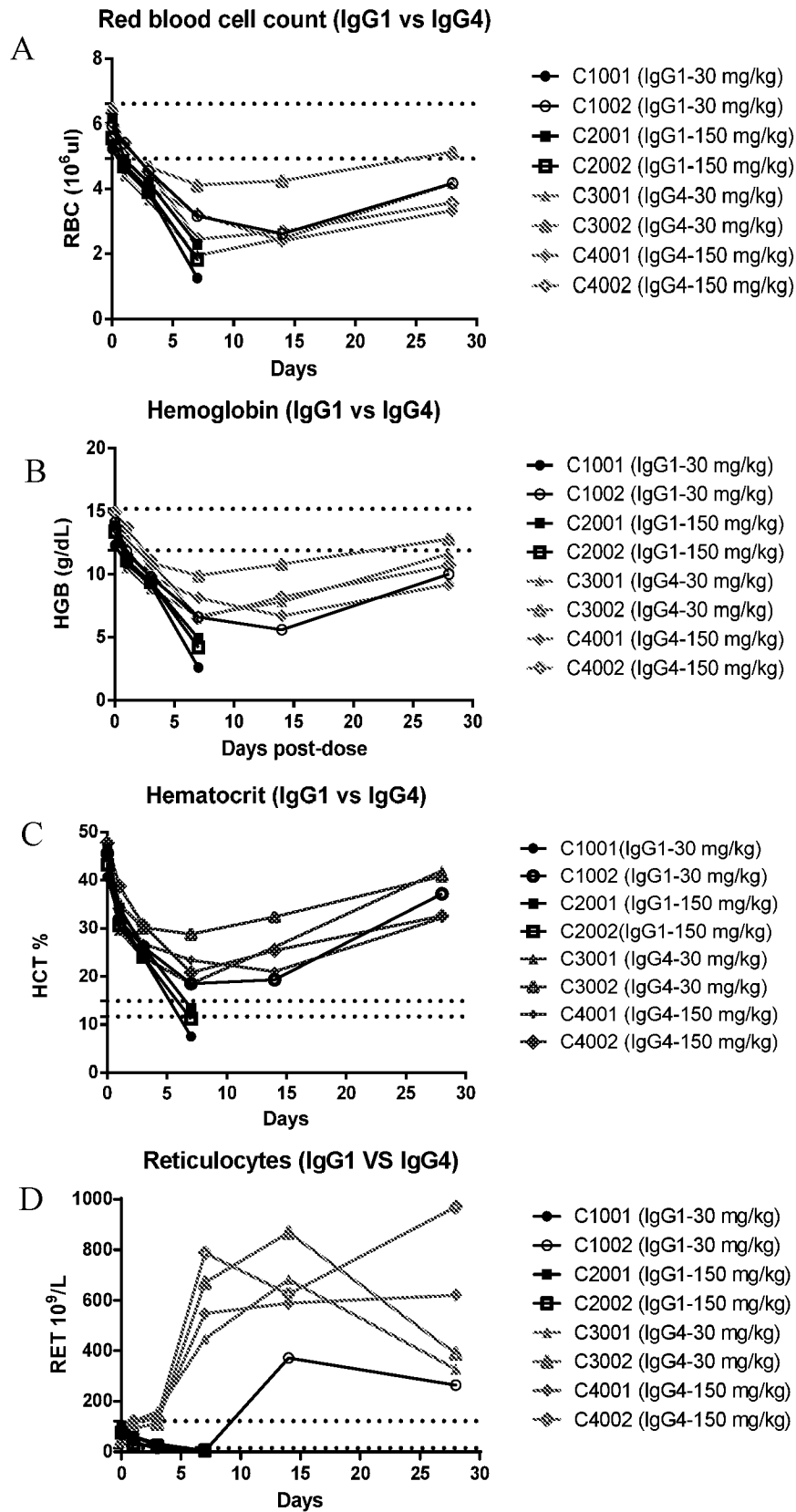


Figure 10

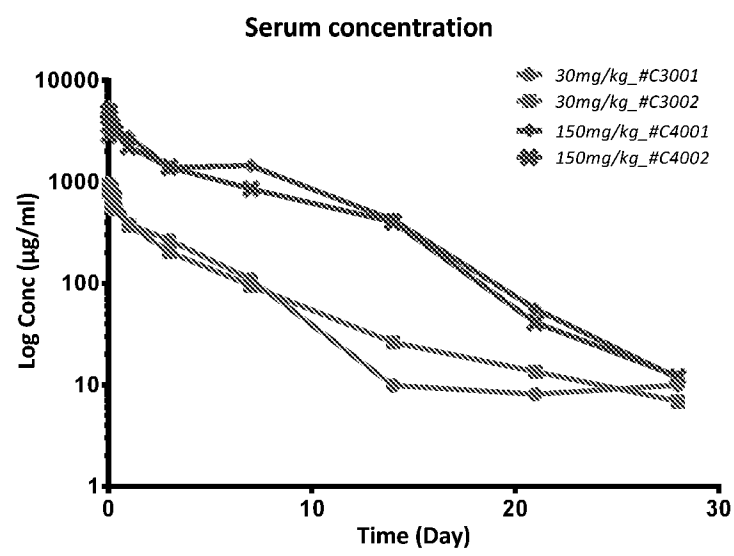


Figure 11

INTERNATIONAL SEARCH REPORT

International application No.

PCT/CN2019/078316

A. CLASSIFICATION OF SUBJECT MATTER

C07K 16/30(2006.01)i; C12N 15/12(2006.01)i; A61K 39/395(2006.01)i; A61P 35/00(2006.01)i

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)

C07K; C12N; A61K; A61P

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)

CNABS, CPRS, DWPI, SIPOABS, CNKI, NCBI, ISI Web of Science, GenBank: antibody, monoclonal, CD47, SEQ ID NOs: 1-29

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	WO 2017049251 A2 (TIOMA THERAPEUTICS INC) 23 March 2017 (2017-03-23) the whole document** especially claims** examples and the abstract	1-36
X	CN 106117354 A (ANHUI SINOBIOWAY CELL THERAPY CO LTD) 16 November 2016 (2016-11-16) the whole document** especially claims ** description** paragraph [0072] and the abstract	1-36

☐ Further documents are listed in the continuation of Box C.☒ See patent family annex.

* Special categories of cited documents:

“A” document defining the general state of the art which is not considered to be of particular relevance

“E” earlier application or patent but published on or after the international filing date

“L” document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)

“O” document referring to an oral disclosure, use, exhibition or other means

“P” document published prior to the international filing date but later than the priority date claimed

“T” later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention

“X” document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone

“Y” document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art

“&” document member of the same patent family

Date of the actual completion of the international search

23 April 2019

Date of mailing of the international search report

22 May 2019

Name and mailing address of the ISA/CN

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INTERNATIONAL SEARCH REPORT
Information on patent family members

International application No.

PCT/CN2019/078316

Patent document cited in search report			Publication date (day/month/year)	Patent family member(s)			Publication date (day/month/year)
WO	2017049251	A2	23 March 2017	MX	2018003374	A	09 November 2018
				US	10239945	B2	26 March 2019
				IL	258168	D0	31 May 2018
				PH	12018500515	A1	29 August 2018
				AU	2016324316	A1	05 April 2018
				BR	112018005322	A2	11 December 2018
				WO	2017049251	A3	20 April 2017
				EP	3349787	A4	27 March 2019
				CN	108348589	A	31 July 2018
				EP	3349787	A2	25 July 2018
				US	2018171014	A1	21 June 2018
				CA	2998644	A1	23 March 2017
				JP	2018536624	A	13 December 2018
CN	106117354	A	16 November 2016	None			