(12) STANDARD PATENT (11) Application No. **AU 2018312222 B2**
(19) AUSTRALIAN PATENT OFFICE

(54) Title
Anti-CD47 antibodies and uses thereof

(51) International Patent Classification(s)
C07K 16/28 (2006.01) ***C07K 16/18*** (2006.01)
C07K 16/00 (2006.01)

(21) Application No: **2018312222** (22) Date of Filing: **2018.07.30**

(87) WIPO No: **WO19/027903**

(30) Priority Data

(31) Number	(32) Date	(33) Country
62/540,118	**2017.08.02**	**US**
62/657,094	**2018.04.13**	**US**

(43) Publication Date: **2019.02.07**
(44) Accepted Journal Date: **2024.11.21**

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(56) Related Art
WO 2017/121771 A1
WO 2017/053423 A1
WO 2016/109415 A1
WO 2016/081423 A1
WO 2013/119714 A1
WO 2014/123580 A1
WO 2017/049251 A2

(12) INTERNATIONAL APPLICATION PUBLISHED UNDER THE PATENT COOPERATION TREATY (PCT)

(19) World Intellectual Property Organization
International Bureau

(43) International Publication Date
07 February 2019 (07.02.2019)

WIPO | PCT

(10) International Publication Number
WO 2019/027903 A1

(51) International Patent Classification:
C07K 16/28 (2006.01) *C07K 16/00* (2006.01)
C07K 16/18 (2006.01)

(21) International Application Number:
PCT/US2018/044384

(22) International Filing Date:
30 July 2018 (30.07.2018)

(25) Filing Language: English

(26) Publication Language: English

(30) Priority Data:
62/540,118 02 August 2017 (02.08.2017) US
62/657,094 13 April 2018 (13.04.2018) US

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(81) Designated States *(unless otherwise indicated, for every kind of national protection available)*: AE, AG, AL, AM, AO, AT, AU, AZ, BA, BB, BG, BH, BN, BR, BW, BY, BZ, CA, CH, CL, CN, CO, CR, CU, CZ, DE, DJ, DK, DM, DO, DZ, EC, EE, EG, ES, FI, GB, GD, GE, GH, GM, GT, HN, HR, HU, ID, IL, IN, IR, IS, JO, JP, KE, KG, KH, KN, KP, KR, KW, KZ, LA, LC, LK, LR, LS, LU, LY, MA, MD, ME, MG, MK, MN, MW, MX, MY, MZ, NA, NG, NI, NO, NZ, OM, PA, PE, PG, PH, PL, PT, QA, RO, RS, RU, RW, SA, SC, SD, SE, SG, SK, SL, SM, ST, SV, SY, TH, TJ, TM, TN, TR, TT, TZ, UA, UG, US, UZ, VC, VN, ZA, ZM, ZW.

(84) Designated States *(unless otherwise indicated, for every kind of regional protection available)*: ARIPO (BW, GH, GM, KE, LR, LS, MW, MZ, NA, RW, SD, SL, ST, SZ, TZ,

(54) Title: ANTI-CD47 ANTIBODIES AND USES THEREOF

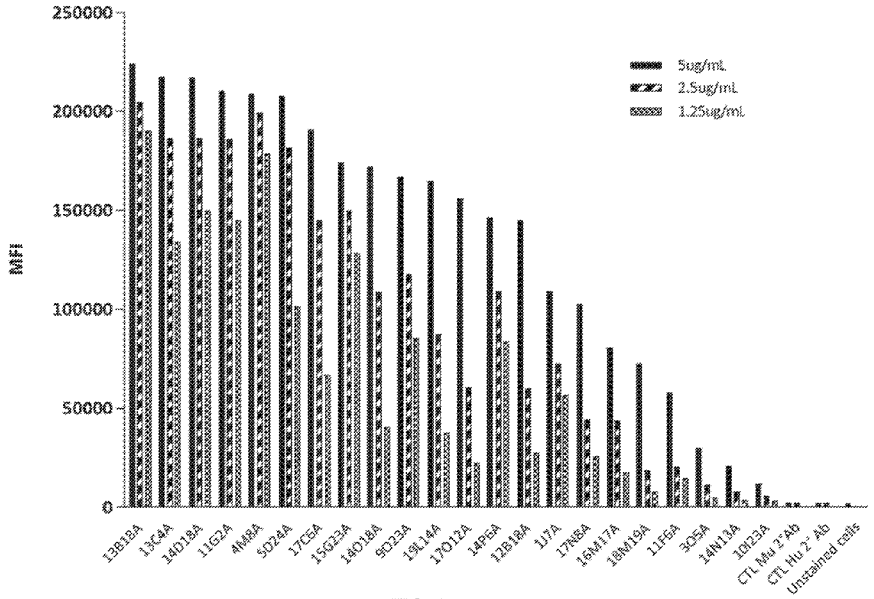

FIG. 1

(57) Abstract: Anti-CD47 antibodies and antigen-binding fragments thereof are described. Also described are nucleic acids encoding the antibodies, compositions comprising the antibodies, and methods of producing the antibodies and using the antibodies for treating or preventing diseases such as cancer, inflammatory disease, infectious disease, atherosclerosis, cardiovascular disease, metabolic disease, radiation-induced injury, and/or autoimmune disease.

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[Continued on next page]

UG, ZM, ZW), Eurasian (AM, AZ, BY, KG, KZ, RU, TJ, TM), European (AL, AT, BE, BG, CH, CY, CZ, DE, DK, EE, ES, FI, FR, GB, GR, HR, HU, IE, IS, IT, LT, LU, LV, MC, MK, MT, NL, NO, PL, PT, RO, RS, SE, SI, SK, SM, TR), OAPI (BF, BJ, CF, CG, CI, CM, GA, GN, GQ, GW, KM, ML, MR, NE, SN, TD, TG).

Published:

— *with international search report (Art. 21(3))*
— *before the expiration of the time limit for amending the claims and to be republished in the event of receipt of amendments (Rule 48.2(h))*
— *with sequence listing part of description (Rule 5.2(a))*

ANTI-CD47 ANTIBODIES AND USES THEREOF

CROSS REFERENCE TO RELATED APPLICATIONS

[0001] This application claims priority to U.S. Provisional Application No. 62/540,118, filed on August 2, 2017, and U.S. Provisional Application No. 62/657,094, filed on April 13, 2018. Each disclosure is incorporated herein by reference in its entirety.

FIELD OF THE INVENTION

[0002] This invention relates to monoclonal anti-CD47 antibodies, nucleic acids and expression vectors encoding the antibodies, recombinant cells containing the vectors, and compositions comprising the antibodies. Methods of making the antibodies, and methods of using the antibodies to treat diseases including cancer, inflammatory diseases, infectious diseases, atherosclerosis, cardiovascular disease, metabolic diseases, radiation-induced injury, and/or autoimmune diseases are also provided.

REFERENCE TO SEQUENCE LISTING SUBMITTED ELECTRONICALLY

[0003] This application contains a sequence listing, which is submitted electronically via EFS-Web as an ASCII formatted sequence listing with a file name "689204.2WO Sequence Listing" and a creation date of July 05, 2018, and having a size of 95 kb. The sequence listing submitted via EFS-Web is part of the specification and is herein incorporated by reference in its entirety.

BACKGROUND OF THE INVENTION

[0004] Cancer cells can evolve various canning capabilities to avoid the attack by the host, including that from the immune system. They either adopt the native appearance on cell surface as normal human cells or interrupt the immune attack upon capture by immune cells. The latter mechanism has been firmly vindicated by the astonishing success of therapeutic monoclonal antibodies targeting immune suppressors CTLA-4, PD-1 and PD-L1. These antibodies inactivate the immune checkpoint and allow T-cells to organize effective attacks on cancer cells, resulting in durable efficacy in some patients. The early success of these antibodies rejuvenated the field of immuno-oncology and

inspired research and development of more therapeutics to mobilize the human immune system to fight cancer.

[0005] The human immune system consists of adaptive and innate immunities. Current checkpoint blockers and tumor microenvironment modulators in clinical practice or in pharmaceutical development target the adaptive immunity. The checkpoint blockers and tumor microenvironment modulators mobilize T-cells by rescuing helper T-cells and killer T-cells from exhaustion, depleting immunosuppressive regulatory T-cells, or blocking the formation of the immune-suppressive tumor microenvironment. More recently, emerging evidence indicates that tumor cells also suppress innate immunity and alleviating such suppression has demonstrated great therapeutic potential *in vitro* and *in vivo* in treating cancers.

[0006] Innate immunity is the first line defense against invading pathogens. It is made up of defensive mechanisms and antigen engulfing leukocytes. Among them, the macrophages remove dysfunctional aged and infected host cells by phagocytosis. Tumor cells also evade macrophage attack by overexpressing cluster of differentiation 47 (CD47) (also known as integrin-associated protein), a marker that is also ubiquitously expressed on the surface of normal cells. Remarkably, in the presence of antibodies that specifically block CD47, macrophages attack tumor cells *in vitro* in phagocytosis assay and eradicate tumors *in vivo* in xenograft models. Currently, several therapeutic agents that target CD47 have entered clinical phase of drug development.

[0007] CD47, first identified as an integrin associated protein, is a receptor-ligand and interacts with many proteins. CD47 is the receptor to thrombospondin-1 (TSP1), one of the best characterized secreted ligands. On the other hand, CD47 is the ligand to signal regulatory protein alpha (SIRPα), an inhibitory receptor expressed on the surface of macrophages. It is the latter binding that prevents macrophage from ingesting cancer cells.

[0008] As of all cancer immunotherapies, blocking CD47 may induce unintended immune attack on normal cells, causing dose-limiting toxicity. Indeed, a CD47 blocking antibody, B6H12, causes hemagglutination, presumably by binding to CD47 on the red blood cells. Remarkably, this antibody blocks the binding of both TSP1 and SIRPα to CD47. It is unclear, however, whether the blocking of TSP1 interaction by B6H12 is responsible for the hemagglutination. Furthermore, it is proposed that macrophages may

attack normal cells when their surface CD47 molecules are masked by systematically administrated anti-CD47 antibodies. Therefore, it is of great importance to develop anti-CD47 antibodies that have increased specificity to tumor cells and reduced toxicity to normal cells. It is an object of the invention to go at least some way to addressing this need and/or at least to provide the public with a useful choice.

BRIEF SUMMARY OF THE INVENTION

[0008a] In a first aspect, the invention provides an isolated monoclonal antibody or antigen-binding fragment thereof comprising a heavy chain complementarity determining region 1 (HCDR1), HCDR2, HCDR3, a light chain complementarity determining region 1 (LCDR1), LCDR2, and LCDR3, having the polypeptide sequence of:

(1) SEQ ID NOs:201, 202, 203, 204, 205, and 206, respectively;

wherein the antibody or antigen-binding fragment thereof specifically binds CD47, preferably human CD47.

[0008b] In a second aspect, the invention provides a bispecific antibody comprising the monoclonal antibody or antigen-binding fragment thereof according to the first aspect of the invention.

[0008c] In a third aspect, the invention provides an isolated nucleic acid encoding the monoclonal antibody or antigen-binding fragment thereof according to the first aspect of the invention.

[0008d] In a fourth aspect, the invention provides a vector comprising the isolated nucleic acid according to the third aspect of the invention.

[0008e] In a fifth aspect, the invention provides an isolated host cell comprising the vector according to the fourth aspect of the invention.

[0008f] In a sixth aspect, the invention provides a pharmaceutical composition, comprising the isolated monoclonal antibody or antigen-binding fragment thereof according to the first aspect of the invention and a pharmaceutically acceptable carrier.

[0008g] In a seventh aspect, the invention relates to a method of blocking binding of CD47 to signal regulatory protein α (SIRPα) comprising administering to the subject the pharmaceutical composition according to the sixth aspect of the invention.

[0008h] In an eighth aspect, the invention relates to a method of treating a CD47 expressing disease and/or condition selected from the group consisting of a cancer, an inflammatory disease, an infectious disease, atherosclerosis, a cardiovascular disease, a metabolic disease, a radiation-induced injury, and an autoimmune disease in a subject in need thereof, comprising administering to the subject the pharmaceutical composition according to the sixth aspect of the invention.

[0008i] In a ninth aspect, the invention relates to a method of determining a level of CD47 in a subject, the method comprising:

a. obtaining a sample from the subject;

b. contacting the sample with an antibody or antigen-binding fragment thereof according to the first aspect;

c. determining a level of CD47 in the subject.

[0008j] In a tenth aspect, the invention relates to a method of producing the monoclonal antibody or antigen-binding fragment thereof of according to the first aspect of the invention, comprising culturing a cell comprising a nucleic acid encoding the monoclonal antibody or antigen-binding fragment thereof under conditions to produce the monoclonal antibody or antigen-binding fragment thereof, and recovering the antibody or antigen-binding fragment thereof from the cell or culture.

[0008k] In an eleventh aspect, the invention relates to a method of producing a pharmaceutical composition comprising the monoclonal antibody or antigen-binding fragment thereof according to the first aspect of the invention, comprising combining the monoclonal antibody or antigen-binding fragment thereof with a pharmaceutically acceptable carrier to obtain the pharmaceutical composition.

[0008l] The invention is defined in the claims. However, the disclosure preceding the claims may refer to additional methods and other subject matter outside the scope of the present claims. This disclosure is retained for technical purposes.

[0009] In one general aspect, the invention relates to isolated monoclonal antibodies or antigen-binding fragments thereof that bind CD47.

[0010] Provided are isolated monoclonal antibodies or antigen-binding fragments thereof comprising a heavy chain complementarity determining region 1 (HCDR1),

HCDR2, HCDR3, a light chain complementarity determining region 1 (LCDR1), LCDR2, and LCDR3, having the polypeptide sequences of:

(1) SEQ ID NOs:177, 46, 47, 178, 112, and 179, respectively;
(2) SEQ ID NOs:51, 52, 53, 117, 118, and 119, respectively;
(3) SEQ ID NOs:54, 55, 56, 120, 121, and 122, respectively;
(4) SEQ ID NOs:57, 58, 59, 123, 124, and 125, respectively;
(5) SEQ ID NOs:60, 61, 62, 126, 127, and 128, respectively;
(6) SEQ ID NOs:180, 181, 182, 129, 130, and 131, respectively;
(7) SEQ ID NOs:72, 73, 74, 138, 139, and 140, respectively;
(8) SEQ ID NOs:78, 79, 80, 144, 145, and 146, respectively;
(9) SEQ ID NOs:81, 82, 83, 147, 148, and 149, respectively;
(10) SEQ ID NOs:84, 85, 86, 150, 151, and 152, respectively;
(11) SEQ ID NOs:87, 88, 89, 153, 154, and 155, respectively;
(12) SEQ ID NOs:90, 91, 92, 156, 157, and 158, respectively;
(13) SEQ ID NOs:93, 94, 95, 159, 160, and 161, respectively;
(14) SEQ ID NOs:96, 97, 98, 162, 163, and 164, respectively;
(15) SEQ ID NOs:99, 100, 101, 165, 166, and 167, respectively;
(16) SEQ ID NOs:102, 103, 104, 168, 169, and 170, respectively;
(17) SEQ ID NOs:105, 106, 107, 171, 172, and 173, respectively;
(18) SEQ ID NOs:108, 109, 110, 174, 175, and 176, respectively; or
(19) SEQ ID NOs:201, 202, 203, 204, 205, and 206, respectively;

wherein the antibody or antigen-binding fragment thereof specifically binds CD47, preferably human CD47. SEQ ID NO:177 is represented by the amino acid sequence GYTFTX$_1$YY, wherein X$_1$ is an amino acid selected from D or A. SEQ ID NO:178 is represented by the amino acid sequence X$_1$NVGTY, wherein X$_1$ is an amino acid selected from D or E. SEQ ID NO:179 is represented by the amino acid sequence GQX$_1$YSYPLT, wherein X$_1$ is an amino acid selected from S or T. SEQ ID NO:180 is represented by the amino acid sequence GYTFTSX$_1$W, wherein X$_1$ is an amino acid selected from S or Y. SEQ ID NO:181 is represented by the amino acid sequence IDPSDSEX$_1$, wherein X$_1$ is an amino acid selected from T or A. SEQ ID NO:182 is represented by the amino acid

sequence X_1RWGYYGKSAX_2DY, wherein X_1 is an amino acid selected from A or S and X_2 is an amino acid selected from I or M.

[0011] In certain embodiments, the isolated monoclonal antibody or antigen-binding fragment comprises a heavy chain variable region having a polypeptide sequence at least 95% identical to SEQ ID NO:1, 3, 5, 7, 9, 11, 13, 15, 17, 19, 21, 23, 25, 27, 29, 31, 33, 35, 37, 39, 41, or 43, or a light chain variable region having a polypeptide sequence at least 95% identical to SEQ ID NO:2, 4, 6, 8, 10, 12, 14, 16, 18, 20, 22, 24, 26, 28, 30, 32, 34, 36, 38, 40, 42, or 44.

[0012] In certain embodiments, the isolated monoclonal antibody or antigen-binding fragment comprises:

(a) a heavy chain variable region having the polypeptide sequence of SEQ ID NO:1, and a light chain variable region having the polypeptide sequence of SEQ ID NO:2;

(b) a heavy chain variable region having the polypeptide sequence of SEQ ID NO:3, and a light chain variable region having the polypeptide sequence of SEQ ID NO:4;

(c) a heavy chain variable region having the polypeptide sequence of SEQ ID NO:5, and a light chain variable region having the polypeptide sequence of SEQ ID NO:6;

(d) a heavy chain variable region having the polypeptide sequence of SEQ ID NO:7, and a light chain variable region having the polypeptide sequence of SEQ ID NO:8;

(e) a heavy chain variable region having the polypeptide sequence of SEQ ID NO:9, and a light chain variable region having the polypeptide sequence of SEQ ID NO:10;

(f) a heavy chain variable region having the polypeptide sequence of SEQ ID NO:11, and a light chain variable region having the polypeptide sequence of SEQ ID NO:12;

(g) a heavy chain variable region having the polypeptide sequence of SEQ ID NO:13, and a light chain variable region having the polypeptide sequence of SEQ ID NO:14;

(h) a heavy chain variable region having the polypeptide sequence of SEQ ID NO:15, and a light chain variable region having the polypeptide sequence of SEQ ID NO:16;

(i) a heavy chain variable region having the polypeptide sequence of SEQ ID NO:17, and a light chain variable region having the polypeptide sequence of SEQ ID NO:18;

(j) a heavy chain variable region having the polypeptide sequence of SEQ ID NO:19, and a light chain variable region having the polypeptide sequence of SEQ ID NO:20;

(k) a heavy chain variable region having the polypeptide sequence of SEQ ID NO:21, and a light chain variable region having the polypeptide sequence of SEQ ID NO:22;

(l) a heavy chain variable region having the polypeptide sequence of SEQ ID NO:23, and a light chain variable region having the polypeptide sequence of SEQ ID NO:24;

(m) a heavy chain variable region having the polypeptide sequence of SEQ ID NO:25, and a light chain variable region having the polypeptide sequence of SEQ ID NO:26;

(n) a heavy chain variable region having the polypeptide sequence of SEQ ID NO:27, and a light chain variable region having the polypeptide sequence of SEQ ID NO:28;

(o) a heavy chain variable region having the polypeptide sequence of SEQ ID NO:29, and a light chain variable region having the polypeptide sequence of SEQ ID NO:30;

(p) a heavy chain variable region having the polypeptide sequence of SEQ ID NO:31, and a light chain variable region having the polypeptide sequence of SEQ ID NO:32;

(q) a heavy chain variable region having the polypeptide sequence of SEQ ID NO:33, and a light chain variable region having the polypeptide sequence of SEQ ID NO:34;

(r) a heavy chain variable region having the polypeptide sequence of SEQ ID NO:35, and a light chain variable region having the polypeptide sequence of SEQ ID NO:36;

(s) a heavy chain variable region having the polypeptide sequence of SEQ ID NO:37, and a light chain variable region having the polypeptide sequence of SEQ ID NO:38;

(t) a heavy chain variable region having the polypeptide sequence of SEQ ID NO:39, and a light chain variable region having the polypeptide sequence of SEQ ID NO:40;

(u) a heavy chain variable region having the polypeptide sequence of SEQ ID NO:41, and a light chain variable region having the polypeptide sequence of SEQ ID NO:42; or

(v) a heavy chain variable region having the polypeptide sequence of SEQ ID NO:43, and a light chain variable region having the polypeptide sequence of SEQ ID NO:44.

[0013] In certain embodiments, the isolated monoclonal antibody or antigen-binding fragment thereof is chimeric.

[0014] In certain embodiments, the isolated monoclonal antibody or antigen-binding fragment thereof is human or humanized. In certain embodiments, the humanized monoclonal antibody or antigen-binding fragment thereof comprises:

a. a heavy chain variable region having the polypeptide sequence of SEQ ID NO:183, and a light chain variable region having the polypeptide sequence of SEQ ID NO:191;

b. a heavy chain variable region having the polypeptide sequence of SEQ ID NO:183, and a light chain variable region having the polypeptide sequence of SEQ ID NO:192;

c. a heavy chain variable region having the polypeptide sequence of SEQ ID NO:183, and a light chain variable region having the polypeptide sequence of SEQ ID NO:193;

d. a heavy chain variable region having the polypeptide sequence of SEQ ID NO:184, and a light chain variable region having the polypeptide sequence of SEQ ID NO:190;

e. a heavy chain variable region having the polypeptide sequence of SEQ ID NO:184, and a light chain variable region having the polypeptide sequence of SEQ ID NO:192;

f. a heavy chain variable region having the polypeptide sequence of SEQ ID NO:184, and a light chain variable region having the polypeptide sequence of SEQ ID NO:193;

g. a heavy chain variable region having the polypeptide sequence of SEQ ID NO:185, and a light chain variable region having the polypeptide sequence of SEQ ID NO:190;

h. a heavy chain variable region having the polypeptide sequence of SEQ ID NO:185, and a light chain variable region having the polypeptide sequence of SEQ ID NO:191;

i. a heavy chain variable region having the polypeptide sequence of SEQ ID NO:185, and a light chain variable region having the polypeptide sequence of SEQ ID NO:193;

j. a heavy chain variable region having the polypeptide sequence of SEQ ID NO:185, and a light chain variable region having the polypeptide sequence of SEQ ID NO:198;

k. a heavy chain variable region having the polypeptide sequence of SEQ ID NO:187, and a light chain variable region having the polypeptide sequence of SEQ ID NO:194;

l. a heavy chain variable region having the polypeptide sequence of SEQ ID NO:188, and a light chain variable region having the polypeptide sequence of SEQ ID NO:194;

m. a heavy chain variable region having the polypeptide sequence of SEQ ID NO:188, and a light chain variable region having the polypeptide sequence of SEQ ID NO:196;

n. a heavy chain variable region having the polypeptide sequence of SEQ ID NO:188, and a light chain variable region having the polypeptide sequence of SEQ ID NO:197; or

o. a heavy chain variable region having the polypeptide sequence of SEQ ID NO:199, and a light chain variable region having the polypeptide sequence of SEQ ID NO:200.

[0015] In certain embodiments, the isolated monoclonal antibody or antigen-binding fragment thereof is capable of blocking binding of CD47 to thrombospondin-1 (TSP1) and/or to signal regulatory protein alpha (SIRPα).

[0016] In certain embodiments, the isolated monoclonal antibody or antigen-binding fragment thereof is capable of inducing macrophage-mediated phagocytosis of cancer cells.

[0017] In certain embodiments, the isolated monoclonal antibody or antigen-binding fragment thereof is capable of binding cancer cells with minimal to undetectable binding to red blood cells.

[0018] Also provided are isolated nucleic acids encoding the monoclonal antibodies or antigen-binding fragments thereof of the invention.

[0019] Also provided are vectors comprising the isolated nucleic acids encoding the monoclonal antibodies or antigen-binding fragments thereof of the invention.

[0020] Also provided are host cells comprising the vectors comprising the isolated nucleic acids encoding the monoclonal antibodies or antigen-binding fragments thereof of the invention.

[0021] In certain embodiments, provided is a pharmaceutical composition comprising the isolated monoclonal antibody or antigen-binding fragment thereof of the invention and a pharmaceutically acceptable carrier.

[0022] Also provided are methods of blocking binding of CD47 to thrombospondin-1 (TSP1) and/or CD47 to signal regulatory protein alpha (SIRPα) in a subject in need thereof, comprising administering to the subject the pharmaceutical compositions of the invention.

[0023] Also provided are methods of treating cancer in a subject in need thereof, comprising administering to the subject the pharmaceutical compositions of the invention.

The cancer can be any liquid or solid cancer, for example, it can be selected from but not limited to, a lung cancer, a gastric cancer, a colon cancer, a hepatocellular carcinoma, a renal cell carcinoma, a bladder urothelial carcinoma, a metastatic melanoma, a breast cancer, an ovarian cancer, a cervical cancer, a head and neck cancer, a pancreatic cancer, a glioma, a glioblastoma, and other solid tumors, and a non-Hodgkin's lymphoma (NHL), an acute lymphocytic leukemia (ALL), a chronic lymphocytic leukemia (CLL), a chronic myelogenous leukemia (CML), a multiple myeloma (MM), an acute myeloid leukemia (AML), and other liquid tumors.

[0024] Also provided are methods of treating an inflammatory disease in a subject in need thereof, comprising administering to the subject the pharmaceutical compositions of the invention.

[0025] Also provided are methods of treating an infectious disease in a subject in need thereof, comprising administering to the subject the pharmaceutical compositions of the invention.

[0026] Also provided are methods of treating atherosclerosis in a subject in need thereof, comprising administering to the subject the pharmaceutical compositions of the invention.

[0027] Also provided are methods of treating a cardiovascular disease in a subject in need thereof, comprising administering to the subject the pharmaceutical compositions of the invention.

[0028] Also provided are methods of treating a metabolic disease in a subject in need thereof, comprising administering to the subject the pharmaceutical compositions of the invention.

[0029] Also provided are methods of treating a radiation-induced injury in a subject in need thereof, comprising administering to the subject the pharmaceutical compositions of the invention.

[0030] Also provided are methods of treating an autoimmune disease in a subject in need thereof, comprising administering to the subject the pharmaceutical compositions of the invention.

[0031] Also provided are methods of determining a level of CD47 in a subject. The methods comprise (a) obtaining a sample from the subject; (b) contacting the sample with

an antibody or antigen-binding fragment thereof of the invention; and (c) determining a level of CD47 in the subject. In certain embodiments, the sample is a tissue or blood sample. The tissue sample can, for example, be a cancer tissue sample. The blood sample can, for example, comprise cancer cells.

[0032] Also provided are methods of producing the monoclonal antibody or antigen-binding fragment thereof of the invention, comprising culturing a cell comprising a nucleic acid encoding the monoclonal antibody or antigen-binding fragment under conditions to produce the monoclonal antibody or antigen-binding fragment, and recovering the antibody or antigen-binding fragment from the cell or culture.

[0033] Also provided are methods of producing a pharmaceutical composition comprising the monoclonal antibody or antigen-binding fragment thereof of the invention, comprising combining the monoclonal antibody or antigen-binding fragment with a pharmaceutically acceptable carrier to obtain the pharmaceutical composition.

BRIEF DESCRIPTION OF THE DRAWINGS

[0034] The foregoing summary, as well as the following detailed description of preferred embodiments of the present application, will be better understood when read in conjunction with the appended drawings. It should be understood, however, that the application is not limited to the precise embodiments shown in the drawings.

[0035] FIG. 1 shows a graph of the binding of anti-CD47 mAbs to RAJI cells by FACS analysis. The control groups "CTL Mu 2° Ab" and "CTL Hu 2° Ab" were not treated with primary antibodies, but were treated with AlexaFluor488-conjµgated anti-mouse and anti-human IgG secondary Abs, respectively.

[0036] FIGS. 2A-2P show graphs of the activity of the anti-CD47 mAbs blocking the interaction between CD47(ECD)-HIS and SIRPα-huFc as analyzed by ELISA. The curves were produced and IC_{50} values were calculated with Prism GraphPad, v7. FIG. 2A shows a graph of the activity of the anti-CD47 mAb 15G23A. FIG. 2B shows a graph of the activity of the anti-CD47 mAb 17C6A. FIG. 2C shows a graph of the activity of the anti-CD47 mAb 13B18A. FIG. 2D shows a graph of the activity of the anti-CD47 mAb 4M8A. FIG. 2E shows a graph of the activity of the anti-CD47 mAb 14D18A. FIG. 2F shows a graph of the activity of the anti-CD47 mAb 11G2A. FIG. 2G shows a graph of

the activity of the anti-CD47 mAb 13C4A. FIG. 2H shows a graph of the activity of the anti-CD47 mAb 5D24A. FIG. 2I shows a graph of the activity of the anti-CD47 mAb 9O23A. FIG. 2J shows a graph of the activity of the anti-CD47 mAb 17N8A. FIG. 2K shows a graph of the activity of the anti-CD47 mAb 14P6A. FIG. 2L shows a graph of the activity of the anti-CD47 mAb 19L14A. FIG. 2M shows a graph of the activity of the anti-CD47 mAb 14O18A. FIG. 2N shows a graph of the activity of the anti-CD47 mAb 1J7A. FIG. 2O shows a graph of the activity of the anti-CD47 mAb 16M17A. FIG. 2P shows a graph of the activity of the anti-CD47 mAb 18M19A (chimeric with human IgG4 heavy chain and kappa light chain).

[0037] FIGS. 3A-3B show the evaluation of anti-CD47 mAbs in a hemagglutination assay using fresh blood from a donor. Purification buffer, B6H12, and PBS were used as controls. The antibody concentrations are indicated above the panel. FIG. 3A shows the results of the hemagglutination assay for anti-CD47 mAbs 14P6A, 11F6A, 18M19A, 19L14A, 3O5A, 10I23A, 14N13A, 14O18A, 13C4A, 16M17A, and 17O12A. FIG. 3B shows the results of the hemagglutination assay for anti-CD47 mAbs 12B18A, 4M8A, 13B18A, 11G2A, 5D24A, 14D18A, 17C6A, 17N8A, 9O23A, 15G23A, and 1J7A and controls PBS and B6H12.

[0038] FIGS. 4A-4C show graphs of *in vivo* anti-tumor activity, body weight, and serum exposure in mice treated with anti-CD47 mAb 13B18A-huIgG1. FIG. 4A shows the *in vivo* anti-tumor activity of 13B18A-huIgG1 in a RAJI xenograft mouse model; rituximab was used as positive control. FIG. 4B shows the body weight data of the animals in different groups during the study. FIG. 4C shows the serum exposure of 13B18A-huIgG1 in mAb-treated groups 2 days after the final dose.

[0039] FIGS. 5A and 5B show the activity of humanized anti-CD47 mAbs H3L9 (FIG. 5A) and H5L5 (FIG. 5B) in blocking the interaction between human CD47(ECD)-HIS and huSIRPα-muFc as analyzed by ELISA.

[0040] FIG. 6 shows the results for the hemagglutination assay with humanized mAbs H3L9, H5L5 and H8L10. Mouse mAb 15G23A was used as positive control.

[0041] FIGS. 7A-7B show the results for the red blood cell (RBC) (FIG. 7A) and RAJI cell (FIG. 7B) binding assays with humanized mAbs H3L9, H5L5 and H8L10.

[0042] FIG. 8 shows the activity of humanized anti-CD47 mAbs H3L9, H5L5 and H8L10 in blocking the binding of huSIRPα-muFc to RAJI cells. "2nd Ab only' and "No mAb/no 2nd Ab control" are negative controls.

[0043] FIG. 9 shows the activity of humanized anti-CD47 mAbs H3L9, H5L5 and H8L10 in inducing macrophage-mediated phagocytosis of RAJI cells.

DETAILED DESCRIPTION OF THE INVENTION

[0044] Various publications, articles and patents are cited or described in the background and throughout the specification; each of these references is herein incorporated by reference in its entirety. Discussion of documents, acts, materials, devices, articles or the like which has been included in the present specification is for the purpose of providing context for the invention. Such discussion is not an admission that any or all of these matters form part of the prior art with respect to any inventions disclosed or claimed.

[0045] Unless defined otherwise, all technical and scientific terms used herein have the same meaning as commonly understood to one of ordinary skill in the art to which this invention pertains. Otherwise, certain terms used herein have the meanings as set forth in the specification.

[0046] It must be noted that as used herein and in the appended claims, the singular forms "a," "an," and "the" include plural reference unless the context clearly dictates otherwise.

[0047] Unless otherwise stated, any numerical values, such as a concentration or a concentration range described herein, are to be understood as being modified in all instances by the term "about." Thus, a numerical value typically includes ± 10% of the recited value. For example, a concentration of 1 mg/mL includes 0.9 mg/mL to 1.1 mg/mL. Likewise, a concentration range of 1% to 10% (w/v) includes 0.9% (w/v) to 11% (w/v). As used herein, the use of a numerical range expressly includes all possible subranges, all individual numerical values within that range, including integers within such ranges and fractions of the values unless the context clearly indicates otherwise.

[0048] Unless otherwise indicated, the term "at least" preceding a series of elements is to be understood to refer to every element in the series. Those skilled in the art will

recognize, or be able to ascertain using no more than routine experimentation, many equivalents to the specific embodiments of the invention described herein. Such equivalents are intended to be encompassed by the invention.

[0049] As used herein, the terms "comprises," "comprising," "includes," "including," "has," "having," "contains" or "containing," or any other variation thereof, will be understood to imply the inclusion of a stated integer or group of integers but not the exclusion of any other integer or group of integers and are intended to be non-exclusive or open-ended. For example, a composition, a mixture, a process, a method, an article, or an apparatus that comprises a list of elements is not necessarily limited to only those elements but can include other elements not expressly listed or inherent to such composition, mixture, process, method, article, or apparatus. Further, unless expressly stated to the contrary, "or" refers to an inclusive or and not to an exclusive or. For example, a condition A or B is satisfied by any one of the following: A is true (or present) and B is false (or not present), A is false (or not present) and B is true (or present), and both A and B are true (or present).

[0050] As used herein, the conjunctive term "and/or" between multiple recited elements is understood as encompassing both individual and combined options. For instance, where two elements are conjoined by "and/or," a first option refers to the applicability of the first element without the second. A second option refers to the applicability of the second element without the first. A third option refers to the applicability of the first and second elements together. Any one of these options is understood to fall within the meaning, and therefore satisfy the requirement of the term "and/or" as used herein. Concurrent applicability of more than one of the options is also understood to fall within the meaning, and therefore satisfy the requirement of the term "and/or."

[0051] As used herein, the term "consists of," or variations such as "consist of" or "consisting of," as used throughout the specification and claims, indicate the inclusion of any recited integer or group of integers, but that no additional integer or group of integers can be added to the specified method, structure, or composition.

[0052] As used herein, the term "consists essentially of," or variations such as "consist essentially of" or "consisting essentially of," as used throughout the specification and claims, indicate the inclusion of any recited integer or group of integers, and the optional

inclusion of any recited integer or group of integers that do not materially change the basic or novel properties of the specified method, structure or composition. See M.P.E.P. § 2111.03.

[0052a] The term "comprising" as used in this specification and claims means "consisting at least in part of". When interpreting statements in this specification and claims which include the term "comprising", other features besides the features prefaced by this term in each statement can also be present. Related terms such as "comprise" and "comprised" are to be interpreted in similar manner.

[0053] As used herein, "subject" means any animal, preferably a mammal, most preferably a human. The term "mammal" as used herein, encompasses any mammal. Examples of mammals include, but are not limited to, cows, horses, sheep, pigs, cats, dogs, mice, rats, rabbits, guinea pigs, monkeys, humans, etc., more preferably a human.

[0054] The words "right," "left," "lower," and "upper" designate directions in the drawings to which reference is made.

[0055] It should also be understood that the terms "about," "approximately," "generally," "substantially," and like terms, used herein when referring to a dimension or characteristic of a component of the preferred invention, indicate that the described dimension/characteristic is not a strict boundary or parameter and does not exclude minor variations therefrom that are functionally the same or similar, as would be understood by one having ordinary skill in the art. At a minimum, such references that include a numerical parameter would include variations that, using mathematical and industrial principles accepted in the art (e.g., rounding, measurement or other systematic errors, manufacturing tolerances, etc.), would not vary the least significant digit.

[0056] The terms "identical" or percent "identity," in the context of two or more nucleic acids or polypeptide sequences (e.g., anti-CD47 antibodies, CD47 polypeptides, and polynucleotides that encode them), refer to two or more sequences or subsequences that are the same or have a specified percentage of amino acid residues or nucleotides that are the same, when compared and aligned for maximum correspondence, as measured using one of the following sequence comparison algorithms or by visual inspection.

[0057] For sequence comparison, typically one sequence acts as a reference sequence, to which test sequences are compared. When using a sequence comparison algorithm, test and reference sequences are input into a computer, subsequence coordinates are designated, if necessary, and sequence algorithm program parameters are designated. The sequence comparison algorithm then calculates the percent sequence identity for the test sequence(s) relative to the reference sequence, based on the designated program parameters.

[0058] Optimal alignment of sequences for comparison can be conducted, e.g., by the local homology algorithm of Smith & Waterman, Adv. Appl. Math. 2:482 (1981), by the homology alignment algorithm of Needleman & Wunsch, J. Mol. Biol. 48:443 (1970), by the search for similarity method of Pearson & Lipman, Proc. Nat'l. Acad. Sci. USA 85:2444 (1988), by computerized implementations of these algorithms (GAP, BESTFIT, FASTA, and TFASTA in the Wisconsin Genetics Software Package, Genetics Computer Group, 575 Science Dr., Madison, WI), or by visual inspection (see generally, Current Protocols in Molecular Biology, F.M. Ausubel et al., eds., Current Protocols, a joint venture between Greene Publishing Associates, Inc. and John Wiley & Sons, Inc., (1995 Supplement) (Ausubel)).

[0059] Examples of algorithms that are suitable for determining percent sequence identity and sequence similarity are the BLAST and BLAST 2.0 algorithms, which are described in Altschul et al. (1990) J. Mol. Biol. 215: 403-410 and Altschul et al. (1997) Nucleic Acids Res. 25: 3389-3402, respectively. Software for performing BLAST analyses is publicly available through the National Center for Biotechnology Information. This algorithm involves first identifying high scoring sequence pairs (HSPs) by identifying short words of length W in the query sequence, which either match or satisfy some positive-valued threshold score T when aligned with a word of the same length in a database sequence. T is referred to as the neighborhood word score threshold (Altschul et al, supra). These initial neighborhood word hits act as seeds for initiating searches to find longer HSPs containing them. The word hits are then extended in both directions along each sequence for as far as the cumulative alignment score can be increased.

[0060] Cumulative scores are calculated using, for nucleotide sequences, the parameters M (reward score for a pair of matching residues; always > 0) and N (penalty

score for mismatching residues; always < 0). For amino acid sequences, a scoring matrix is used to calculate the cumulative score. Extension of the word hits in each direction are halted when: the cumulative alignment score falls off by the quantity X from its maximum achieved value; the cumulative score goes to zero or below, due to the accumulation of one or more negative-scoring residue alignments; or the end of either sequence is reached. The BLAST algorithm parameters W, T, and X determine the sensitivity and speed of the alignment. The BLASTN program (for nucleotide sequences) uses as defaults a wordlength (W) of 11, an expectation (E) of 10, M=5, N=-4, and a comparison of both strands. For amino acid sequences, the BLASTP program uses as defaults a wordlength (W) of 3, an expectation (E) of 10, and the BLOSUM62 scoring matrix (see Henikoff & Henikoff, Proc. Natl. Acad. Sci. USA 89:10915 (1989)).

[0061] In addition to calculating percent sequence identity, the BLAST algorithm also performs a statistical analysis of the similarity between two sequences (see, e.g., Karlin & Altschul, Proc. Nat'l. Acad. Sci. USA 90:5873-5787 (1993)). One measure of similarity provided by the BLAST algorithm is the smallest sum probability (P(N)), which provides an indication of the probability by which a match between two nucleotide or amino acid sequences would occur by chance. For example, a nucleic acid is considered similar to a reference sequence if the smallest sum probability in a comparison of the test nucleic acid to the reference nucleic acid is less than about 0.1, more preferably less than about 0.01, and most preferably less than about 0.001.

[0062] A further indication that two nucleic acid sequences or polypeptides are substantially identical is that the polypeptide encoded by the first nucleic acid is immunologically cross reactive with the polypeptide encoded by the second nucleic acid, as described below. Thus, a polypeptide is typically substantially identical to a second polypeptide, for example, where the two peptides differ only by conservative substitutions. Another indication that two nucleic acid sequences are substantially identical is that the two molecules hybridize to each other under stringent conditions.

[0063] As used herein, the terms "inhibit," "inhibiting," and "inhibition," mean to decrease an activity, response, condition, disease or other biological parameter. This can include, but is not limited to complete ablation of the activity, response, condition, or disease. This may also include, for example, a 10% reduction in the activity, response,

condition, or disease as compared to the native or control level. Thus, the reduction can be a 10, 20, 30, 40, 50, 60, 70, 80, 90, 100%, or any amount of reduction in between, as compared to native or control levels. By way of a non-limiting example, an antibody of the invention can inhibit the activity of a CD47 protein. The activity of the CD47 protein can be reduced or ablated relative to the native CD47 protein activity.

[0064] Antibodies

[0065] The invention generally relates to isolated anti-CD47 antibodies, nucleic acids and expression vectors encoding the antibodies, recombinant cells containing the vectors, and compositions comprising the antibodies. Methods of making the antibodies, and methods of using the antibodies to treat diseases including cancer, inflammatory diseases, autoimmune diseases, atherosclerosis, cardiovascular disease, metabolic diseases, radiation-induced injury, and/or infectious diseases are also provided. The antibodies of the invention possess one or more desirable functional properties, including but not limited to high-affinity binding to CD47, high specificity to CD47, the ability and/or inability to block the binding of CD47 to thrombospondin-1 (TSP1), the ability to block the binding of CD47 to signal regulatory protein alpha (SIRPα), the ability to induce phagocytosis of CD47 expressing cells associated with disease or disorder (including, but not limited to, cancer and atherosclerosis), and the ability to inhibit tumor growth in animal models and subjects when administered alone or in combination with other anti-cancer therapies, and the inability to induce hemagglutination.

[0066] In a general aspect, the invention relates to isolated monoclonal antibodies or antigen-binding fragments thereof that bind CD47.

[0067] As used herein, the term “antibody” is used in a broad sense and includes immunoglobulin or antibody molecules including human, humanized, composite and chimeric antibodies and antibody fragments that are monoclonal or polyclonal. In general, antibodies are proteins or peptide chains that exhibit binding specificity to a specific antigen. Antibody structures are well known. Immunoglobulins can be assigned to five major classes (i.e., IgA, IgD, IgE, IgG and IgM), depending on the heavy chain constant domain amino acid sequence. IgA and IgG are further sub-classified as the isotypes IgA1, IgA2, IgG1, IgG2, IgG3 and IgG4. Accordingly, the antibodies of the invention can be of any of the five major classes or corresponding sub-classes. Preferably, the antibodies of

the invention are IgG1, IgG2, IgG3 or IgG4. Antibody light chains of vertebrate species can be assigned to one of two clearly distinct types, namely kappa and lambda, based on the amino acid sequences of their constant domains. Accordingly, the antibodies of the invention can contain a kappa or lambda light chain constant domain. According to particular embodiments, the antibodies of the invention include heavy and/or light chain constant regions from rat or human antibodies. In addition to the heavy and light constant domains, antibodies contain an antigen-binding region that is made up of a light chain variable region and a heavy chain variable region, each of which contains three domains (i.e., complementarity determining regions 1-3; (CDR1, CDR2, and CDR3)). The light chain variable region domains are alternatively referred to as LCDR1, LCDR2, and LCRD3, and the heavy chain variable region domains are alternatively referred to as HCDR1, HCRD2, and HCDR3.

[0068] As used herein, the term an "isolated antibody" refers to an antibody which is substantially free of other antibodies having different antigenic specificities (e.g., an isolated antibody that specifically binds to CD47 is substantially free of antibodies that do not bind to CD47). In addition, an isolated antibody is substantially free of other cellular material and/or chemicals.

[0069] As used herein, the term "monoclonal antibody" refers to an antibody obtained from a population of substantially homogeneous antibodies, i.e., the individual antibodies comprising the population are identical except for possible naturally occurring mutations that may be present in minor amounts. The monoclonal antibodies of the invention can be made by the hybridoma method, phage display technology, single lymphocyte gene cloning technology, or by recombinant DNA methods. For example, the monoclonal antibodies can be produced by a hybridoma which includes a B cell obtained from a transgenic nonhuman animal, such as a transgenic mouse or rat, having a genome comprising a human heavy chain transgene and a light chain transgene.

[0070] As used herein, the term "antigen-binding fragment" refers to an antibody fragment such as, for example, a diabody, a Fab, a Fab', a F(ab')2, an Fv fragment, a disulfide stabilized Fv fragment (dsFv), a $(dsFv)_2$, a bispecific dsFv (dsFv-dsFv'), a disulfide stabilized diabody (ds diabody), a single-chain antibody molecule (scFv), a single domain antibody (sdab) an scFv dimer (bivalent diabody), a multispecific antibody

formed from a portion of an antibody comprising one or more CDRs, a camelized single domain antibody, a nanobody, a domain antibody, a bivalent domain antibody, or any other antibody fragment that binds to an antigen but does not comprise a complete antibody structure. An antigen-binding fragment is capable of binding to the same antigen to which the parent antibody or a parent antibody fragment binds. According to particular embodiments, the antigen-binding fragment comprises a light chain variable region, a light chain constant region, and an Fd segment of the heavy chain. According to other particular embodiments, the antigen-binding fragment comprises Fab and F(ab').

[0071] As used herein, the term "single-chain antibody" refers to a conventional single-chain antibody in the field, which comprises a heavy chain variable region and a light chain variable region connected by a short peptide of about 15 to about 20 amino acids. As used herein, the term "single domain antibody" refers to a conventional single domain antibody in the field, which comprises a heavy chain variable region and a heavy chain constant region or which comprises only a heavy chain variable region.

[0072] As used herein, the term "human antibody" refers to an antibody produced by a human or an antibody having an amino acid sequence corresponding to an antibody produced by a human made using any technique known in the art. This definition of a human antibody includes intact or full-length antibodies, fragments thereof, and/or antibodies comprising at least one human heavy and/or light chain polypeptide.

[0073] As used herein, the term "humanized antibody" refers to a non-human antibody that is modified to increase the sequence homology to that of a human antibody, such that the antigen-binding properties of the antibody are retained, but its antigenicity in the human body is reduced.

[0074] As used herein, the term "chimeric antibody" refers to an antibody wherein the amino acid sequence of the immunoglobulin molecule is derived from two or more species. The variable region of both the light and heavy chains often corresponds to the variable region of an antibody derived from one species of mammal (e.g., mouse, rat, rabbit, etc.) having the desired specificity, affinity, and capability, while the constant regions correspond to the sequences of an antibody derived from another species of mammal (e.g., human) to avoid eliciting an immune response in that species.

[0075] As used herein, the term "multispecific antibody" refers to an antibody that comprises a plurality of immunoglobulin variable domain sequences, wherein a first immunoglobulin variable domain sequence of the plurality has binding specificity for a first epitope and a second immunoglobulin variable domain sequence of the plurality has binding specificity for a second epitope. In an embodiment, the first and second epitopes are on the same antigen, *e.g.,* the same protein (or subunit of a multimeric protein). In an embodiment, the first and second epitopes overlap or substantially overlap. In an embodiment, the first and second epitopes do not overlap or do not substantially overlap. In an embodiment, the first and second epitopes are on different antigens, *e.g.*, the different proteins (or different subunits of a multimeric protein). In an embodiment, a multispecific antibody comprises a third, fourth, or fifth immunoglobulin variable domain. In an embodiment, a multispecific antibody is a bispecific antibody molecule, a trispecific antibody, or a tetraspecific antibody molecule.

[0076] As used herein, the term "bispecifc antibody" refers to a multispecific antibody that binds no more than two epitopes or two antigens. A bispecific antibody is characterized by a first immunoglobulin variable domain sequence which has binding specificity for a first epitope and a second immunoglobulin variable domain sequence that has binding specificity for a second epitope. In an embodiment, the first and second epitopes are on the same antigen, *e.g.*, the same protein (or subunit of a multimeric protein). In an embodiment, the first and second epitopes overlap or substantially overlap. In an embodiment the first and second epitopes are on different antigens, *e.g.*, the different proteins (or different subunits of a multimeric protein). In an embodiment a bispecific antibody comprises a heavy chain variable domain sequence and a light chain variable domain sequence which have binding specificity for a first epitope and a heavy chain variable domain sequence and a light chain variable domain sequence which have binding specificity for a second epitope. In an embodiment, a bispecific antibody comprises a half antibody, or fragment thereof, having binding specificity for a first epitope and a half antibody, or fragment thereof, having binding specificity for a second epitope. In an embodiment, a bispecific antibody comprises a scFv, or fragment thereof, having binding specificity for a first epitope, and a scFv, or fragment thereof, having binding specificity for a second epitope. In an embodiment, the first epitope is located on

CD47 and the second epitope is located on PD-1, PD-L1, LAG-3, TIM-3, CTLA-4, EGFR, HER-2, CD19, CD20, CD33, CD73, apelin, DLL3, claudin18.2, TIP-1, folate receptor alpha, CD3 and/or other tumor associated immune suppressors or surface antigens.

[0077] As used herein, the term "CD47" refers to a multi-spanning transmembrane receptor belonging to the immunoglobulin superfamily, which has been indicated to be involved in multiple cellular process, including cell migration, adhesion, and T cell function. CD47, also known as integrin-associated protein (IAP), ovarian cancer antigen (OA3), Rh-related antigen, and MER6, was originally identified as a tumor antigen on human ovarian cancer and was subsequently shown to be expressed on multiple human tumor types, including both hematologic and solid tumors. The interaction between CD47 and signal regulatory protein alpha (SIRPα), an inhibitory protein expressed on macrophages, prevents phagocytosis of CD47-expressing cells. CD47 is additionally expressed at low levels on virtually all non-malignant cells. The term "human CD47" refers to a CD47 originated from a human. An exemplary amino acid sequence of a human CD47 is represented in GenBank Accession No. NP_001768.1 (SEQ ID NO:207).

[0078] As used herein, an antibody that "specifically binds to CD47" refers to an antibody that binds to a CD47, preferably a human CD47, with a KD of 1×10^{-7} M or less, preferably 1×10^{-8} M or less, more preferably 5×10^{-9} M or less, 1×10^{-9} M or less, 5×10^{-10} M or less, or 1×10^{-10} M or less. The term "KD" refers to the dissociation constant, which is obtained from the ratio of Kd to Ka (i.e., Kd/Ka) and is expressed as a molar concentration (M). KD values for antibodies can be determined using methods in the art in view of the present disclosure. For example, the KD of an antibody can be determined by using surface plasmon resonance, such as by using a biosensor system, e.g., a Biacore® system, or by using bio-layer interferometry technology, such as a Octet RED96 system.

[0079] The smaller the value of the KD of an antibody, the higher affinity that the antibody binds to a target antigen.

[0080] According to a particular aspect, the invention relates to an isolated monoclonal antibody or antigen-binding fragment thereof comprising a heavy chain complementarity determining region 1 (HCDR1), a HCDR2, a HCDR3, a light chain complementarity

determining region 1 (LCDR1), a LCDR2, and a LCDR3, having the polypeptide sequences of:

(1) SEQ ID NOs:177, 46, 47, 178, 112, and 179, respectively;
(2) SEQ ID NOs:51, 52, 53, 117, 118, and 119, respectively;
(3) SEQ ID NOs:54, 55, 56, 120, 121, and 122, respectively;
(4) SEQ ID NOs:57, 58, 59, 123, 124, and 125, respectively;
(5) SEQ ID NOs:60, 61, 62, 126, 127, and 128, respectively;
(6) SEQ ID NOs:180, 181, 182, 129, 130, and 131, respectively;
(7) SEQ ID NOs:72, 73, 74, 138, 139, and 140, respectively;
(8) SEQ ID NOs:78, 79, 80, 144, 145, and 146, respectively;
(9) SEQ ID NOs:81, 82, 83, 147, 148, and 149, respectively;
(10) SEQ ID NOs:84, 85, 86, 150, 151, and 152, respectively;
(11) SEQ ID NOs:87, 88, 89, 153, 154, and 155, respectively;
(12) SEQ ID NOs:90, 91, 92, 156, 157, and 158, respectively;
(13) SEQ ID NOs:93, 94, 95, 159, 160, and 161, respectively;
(14) SEQ ID NOs:96, 97, 98, 162, 163, and 164, respectively;
(15) SEQ ID NOs:99, 100, 101, 165, 166, and 167, respectively;
(16) SEQ ID NOs:102, 103, 104, 168, 169, and 170, respectively;
(17) SEQ ID NOs:105, 106, 107, 171, 172, and 173, respectively;
(18) SEQ ID NOs:108, 109, 110, 174, 175, and 176, respectively; or
(19) SEQ ID NOs:201, 202, 203, 204, 205, and 206, respectively;

wherein the antibody or antigen-binding fragment thereof specifically binds CD47, preferably human CD47.

[0081] SEQ ID NO:177 is represented by the amino acid sequence GYTFTX$_1$YY, wherein X$_1$ is an amino acid selected from D or A.

[0082] SEQ ID NO:178 is represented by the amino acid sequence X$_1$NVGTY, wherein X$_1$ is an amino acid selected from D or E.

[0083] SEQ ID NO:179 is represented by the amino acid sequence GQX$_1$YSYPLT, wherein X$_1$ is an amino acid selected from S or T.

[0084] SEQ ID NO:180 is represented by the amino acid sequence GYTFTSX$_1$W, wherein X$_1$ is an amino acid selected from S or Y.

[0085] SEQ ID NO:181 is represented by the amino acid sequence IDPSDSEX$_1$, wherein X$_1$ is an amino acid selected from T or A.

[0086] SEQ ID NO:182 is represented by the amino acid sequence X$_1$RWGYYGKSAX$_2$DY, wherein X$_1$ is an amino acid selected from A or S and X$_2$ is an amino acid selected from I or M.

[0087] According to another particular aspect, the invention relates to an isolated monoclonal antibody or antigen-binding fragment thereof comprising a heavy chain variable region having a polypeptide sequence at least 85%, preferably 90%, more preferably 95% identical to one of SEQ ID NOs:1, 3, 5, 7, 9, 11, 13, 15, 17, 19, 21, 23, 25, 27, 29, 31, 33, 35, 37, 39, 41, or 43, or a light chain variable region having a polypeptide sequence at least 85%, preferably 90%, more preferably 95% identical to one of SEQ ID NOs:2, 4, 6, 8, 10, 12, 14, 16, 18, 20, 22, 24, 26, 28, 30, 32, 34, 36, 38, 40, 42, or 44. According to one preferred embodiment, the isolated monoclonal antibody or antigen-binding fragment thereof of the invention comprises a heavy chain variable region having the polypeptide sequence at least 85%, preferably 90%, more preferably 95% identical to SEQ ID NO:1, 3, 5, 7, 9, 11, 13, 15, 17, 19, 21, 23, 25, 27, 29, 31, 33, 35, 37, 39, 41, or 43, and a light chain variable region having a polypeptide sequence at least 85%, preferably 90%, more preferably 95% identical to SEQ ID NO:2, 4, 6, 8, 10, 12, 14, 16, 18, 20, 22, 24, 26, 28, 30, 32, 34, 36, 38, 40, 42, or 44, respectively.

[0088] According to another particular aspect, the invention relates to an isolated monoclonal antibody or antigen-binding fragment thereof of the invention, comprising:

a. a heavy chain variable region having the polypeptide sequence of SEQ ID NO:1, and a light chain variable region having the polypeptide sequence of SEQ ID NO:2;
b. a heavy chain variable region having the polypeptide sequence of SEQ ID NO:3, and a light chain variable region having the polypeptide sequence of SEQ ID NO:4;
c. a heavy chain variable region having the polypeptide sequence of SEQ ID NO:5, and a light chain variable region having the polypeptide sequence of SEQ ID NO:6;

d. a heavy chain variable region having the polypeptide sequence of SEQ ID NO:7, and a light chain variable region having the polypeptide sequence of SEQ ID NO:8;

e. a heavy chain variable region having the polypeptide sequence of SEQ ID NO:9, and a light chain variable region having the polypeptide sequence of SEQ ID NO:10;

f. a heavy chain variable region having the polypeptide sequence of SEQ ID NO:11, and a light chain variable region having the polypeptide sequence of SEQ ID NO:12;

g. a heavy chain variable region having the polypeptide sequence of SEQ ID NO:13, and a light chain variable region having the polypeptide sequence of SEQ ID NO:14;

h. a heavy chain variable region having the polypeptide sequence of SEQ ID NO:15, and a light chain variable region having the polypeptide sequence of SEQ ID NO:16;

i. a heavy chain variable region having the polypeptide sequence of SEQ ID NO:17, and a light chain variable region having the polypeptide sequence of SEQ ID NO:18;

j. a heavy chain variable region having the polypeptide sequence of SEQ ID NO:19, and a light chain variable region having the polypeptide sequence of SEQ ID NO:20;

k. a heavy chain variable region having the polypeptide sequence of SEQ ID NO:21, and a light chain variable region having the polypeptide sequence of SEQ ID NO:22;

l. a heavy chain variable region having the polypeptide sequence of SEQ ID NO:23, and a light chain variable region having the polypeptide sequence of SEQ ID NO:24;

m. a heavy chain variable region having the polypeptide sequence of SEQ ID NO:25, and a light chain variable region having the polypeptide sequence of SEQ ID NO:26;

n. a heavy chain variable region having the polypeptide sequence of SEQ ID NO:27, and a light chain variable region having the polypeptide sequence of SEQ ID NO:28;

o. a heavy chain variable region having the polypeptide sequence of SEQ ID NO:29, and a light chain variable region having the polypeptide sequence of SEQ ID NO:30;

p. a heavy chain variable region having the polypeptide sequence of SEQ ID NO:31, and a light chain variable region having the polypeptide sequence of SEQ ID NO:32;

q. a heavy chain variable region having the polypeptide sequence of SEQ ID NO:33, and a light chain variable region having the polypeptide sequence of SEQ ID NO:34;

r. a heavy chain variable region having the polypeptide sequence of SEQ ID NO:35, and a light chain variable region having the polypeptide sequence of SEQ ID NO:36;

s. a heavy chain variable region having the polypeptide sequence of SEQ ID NO:37, and a light chain variable region having the polypeptide sequence of SEQ ID NO:38;

t. a heavy chain variable region having the polypeptide sequence of SEQ ID NO:39, and a light chain variable region having the polypeptide sequence of SEQ ID NO:40;

u. a heavy chain variable region having the polypeptide sequence of SEQ ID NO:41, and a light chain variable region having the polypeptide sequence of SEQ ID NO:42; or

v. a heavy chain variable region having the polypeptide sequence of SEQ ID NO:43, and a light chain variable region having the polypeptide sequence of SEQ ID NO:44.

[0089] In one embodiment, the invention relates to an isolated monoclonal antibody or antigen-binding fragment thereof, comprising HCDR1, HCDR2, HCDR3, LCDR1, LCDR2 and LCDR3, having the polypeptide sequences of SEQ ID NOs:45, 46, 47, 111, 112, and 113, respectively. In another embodiment, the isolated monoclonal antibody or

antigen-binding fragment thereof comprises a heavy chain variable region having a polypeptide sequence at least 85%, preferably 90%, more preferably 95% identical to SEQ ID NO:1, and a light chain variable region having a polypeptide sequence at least 85%, preferably 90%, more preferably 95% identical to SEQ ID NO:2. Preferably, the isolated monoclonal antibody or antigen-binding fragment thereof comprises a heavy chain variable region having the polypeptide sequence of SEQ ID NO:1; and a light chain variable region having the polypeptide sequence of SEQ ID NO:2.

[0090] In one embodiment, the invention relates to an isolated monoclonal antibody or antigen-binding fragment thereof, comprising HCDR1, HCDR2, HCDR3, LCDR1, LCDR2 and LCDR3, having the polypeptide sequences of SEQ ID NOs:48, 49, 50, 114, 115, and 116, respectively. In another embodiment, the isolated monoclonal antibody or antigen-binding fragment thereof comprises a heavy chain variable region having a polypeptide sequence at least 85%, preferably 90%, more preferably 95% identical to SEQ ID NO:3, and a light chain variable region having a polypeptide sequence at least 85%, preferably 90%, more preferably 95% identical to SEQ ID NO:4. Preferably, the isolated monoclonal antibody or antigen-binding fragment thereof comprises a heavy chain variable region having the polypeptide sequence of SEQ ID NO:3; and a light chain variable region having the polypeptide sequence of SEQ ID NO:4.

[0091] In one embodiment, the invention relates to an isolated monoclonal antibody or antigen-binding fragment thereof, comprising HCDR1, HCDR2, HCDR3, LCDR1, LCDR2 and LCDR3, having the polypeptide sequences of SEQ ID NOs:51, 52, 53, 117, 118, and 119, respectively. In another embodiment, the isolated monoclonal antibody or antigen-binding fragment thereof comprises a heavy chain variable region having a polypeptide sequence at least 85%, preferably 90%, more preferably 95% identical to SEQ ID NO:5, and a light chain variable region having a polypeptide sequence at least 85%, preferably 90%, more preferably 95% identical to SEQ ID NO:6. Preferably, the isolated monoclonal antibody or antigen-binding fragment thereof comprises a heavy chain variable region having the polypeptide sequence of SEQ ID NO:5; and a light chain variable region having the polypeptide sequence of SEQ ID NO:6.

[0092] In one embodiment, the invention relates to an isolated monoclonal antibody or antigen-binding fragment thereof, comprising HCDR1, HCDR2, HCDR3, LCDR1,

LCDR2 and LCDR3, having the polypeptide sequences of SEQ ID NOs:54, 55, 56, 120, 121, and 122, respectively. In another embodiment, the isolated monoclonal antibody or antigen-binding fragment thereof comprises a heavy chain variable region having a polypeptide sequence at least 85%, preferably 90%, more preferably 95% identical to SEQ ID NO:7, and a light chain variable region having a polypeptide sequence at least 85%, preferably 90%, more preferably 95% identical to SEQ ID NO:8. Preferably, the isolated monoclonal antibody or antigen-binding fragment thereof comprises a heavy chain variable region having the polypeptide sequence of SEQ ID NO:7; and a light chain variable region having the polypeptide sequence of SEQ ID NO:8.

[0093] In one embodiment, the invention relates to an isolated monoclonal antibody or antigen-binding fragment thereof, comprising HCDR1, HCDR2, HCDR3, LCDR1, LCDR2 and LCDR3, having the polypeptide sequences of SEQ ID NOs:57, 58, 59, 123, 124, and 125, respectively. In another embodiment, the isolated monoclonal antibody or antigen-binding fragment thereof comprises a heavy chain variable region having a polypeptide sequence at least 85%, preferably 90%, more preferably 95% identical to SEQ ID NO:9, and a light chain variable region having a polypeptide sequence at least 85%, preferably 90%, more preferably 95% identical to SEQ ID NO:10. Preferably, the isolated monoclonal antibody or antigen-binding fragment thereof comprises a heavy chain variable region having the polypeptide sequence of SEQ ID NO:9; and a light chain variable region having the polypeptide sequence of SEQ ID NO:10.

[0094] In one embodiment, the invention relates to an isolated monoclonal antibody or antigen-binding fragment thereof, comprising HCDR1, HCDR2, HCDR3, LCDR1, LCDR2 and LCDR3, having the polypeptide sequences of SEQ ID NOs:60, 61, 62, 126, 127, and 128, respectively. In another embodiment, the isolated monoclonal antibody or antigen-binding fragment thereof comprises a heavy chain variable region having a polypeptide sequence at least 85%, preferably 90%, more preferably 95% identical to SEQ ID NO:11, and a light chain variable region having a polypeptide sequence at least 85%, preferably 90%, more preferably 95% identical to SEQ ID NO:12. Preferably, the isolated monoclonal antibody or antigen-binding fragment thereof comprises a heavy chain variable region having the polypeptide sequence of SEQ ID NO:11; and a light chain variable region having the polypeptide sequence of SEQ ID NO:12.

[0095] In one embodiment, the invention relates to an isolated monoclonal antibody or antigen-binding fragment thereof, comprising HCDR1, HCDR2, HCDR3, LCDR1, LCDR2 and LCDR3, having the polypeptide sequences of SEQ ID NOs:63, 64, 65, 129, 130, and 131, respectively. In another embodiment, the isolated monoclonal antibody or antigen-binding fragment thereof comprises a heavy chain variable region having a polypeptide sequence at least 85%, preferably 90%, more preferably 95% identical to SEQ ID NO:13, and a light chain variable region having a polypeptide sequence at least 85%, preferably 90%, more preferably 95% identical to SEQ ID NO:14. Preferably, the isolated monoclonal antibody or antigen-binding fragment thereof comprises a heavy chain variable region having the polypeptide sequence of SEQ ID NO:13; and a light chain variable region having the polypeptide sequence of SEQ ID NO:14.

[0096] In one embodiment, the invention relates to an isolated monoclonal antibody or antigen-binding fragment thereof, comprising HCDR1, HCDR2, HCDR3, LCDR1, LCDR2 and LCDR3, having the polypeptide sequences of SEQ ID NOs:66, 67, 68, 132, 133, and 134, respectively. In another embodiment, the isolated monoclonal antibody or antigen-binding fragment thereof comprises a heavy chain variable region having a polypeptide sequence at least 85%, preferably 90%, more preferably 95% identical to SEQ ID NO:15, and a light chain variable region having a polypeptide sequence at least 85%, preferably 90%, more preferably 95% identical to SEQ ID NO:16. Preferably, the isolated monoclonal antibody or antigen-binding fragment thereof comprises a heavy chain variable region having the polypeptide sequence of SEQ ID NO:15; and a light chain variable region having the polypeptide sequence of SEQ ID NO:16.

[0097] In one embodiment, the invention relates to an isolated monoclonal antibody or antigen-binding fragment thereof, comprising HCDR1, HCDR2, HCDR3, LCDR1, LCDR2 and LCDR3, having the polypeptide sequences of SEQ ID NOs:69, 70, 71, 135, 136, and 137, respectively. In another embodiment, the isolated monoclonal antibody or antigen-binding fragment thereof comprises a heavy chain variable region having a polypeptide sequence at least 85%, preferably 90%, more preferably 95% identical to SEQ ID NO:17, and a light chain variable region having a polypeptide sequence at least 85%, preferably 90%, more preferably 95% identical to SEQ ID NO:18. Preferably, the isolated monoclonal antibody or antigen-binding fragment thereof comprises a heavy

chain variable region having the polypeptide sequence of SEQ ID NO:17; and a light chain variable region having the polypeptide sequence of SEQ ID NO:18.

[0098] In one embodiment, the invention relates to an isolated monoclonal antibody or antigen-binding fragment thereof, comprising HCDR1, HCDR2, HCDR3, LCDR1, LCDR2 and LCDR3, having the polypeptide sequences of SEQ ID NOs:72, 73, 74, 138, 139, and 140, respectively. In another embodiment, the isolated monoclonal antibody or antigen-binding fragment thereof comprises a heavy chain variable region having a polypeptide sequence at least 85%, preferably 90%, more preferably 95% identical to SEQ ID NO:19, and a light chain variable region having a polypeptide sequence at least 85%, preferably 90%, more preferably 95% identical to SEQ ID NO:20. Preferably, the isolated monoclonal antibody or antigen-binding fragment thereof comprises a heavy chain variable region having the polypeptide sequence of SEQ ID NO:19; and a light chain variable region having the polypeptide sequence of SEQ ID NO:20.

[0099] In one embodiment, the invention relates to an isolated monoclonal antibody or antigen-binding fragment thereof, comprising HCDR1, HCDR2, HCDR3, LCDR1, LCDR2 and LCDR3, having the polypeptide sequences of SEQ ID NOs:75, 76, 77, 141, 142, and 143, respectively. In another embodiment, the isolated monoclonal antibody or antigen-binding fragment thereof comprises a heavy chain variable region having a polypeptide sequence at least 85%, preferably 90%, more preferably 95% identical to SEQ ID NO:21, and a light chain variable region having a polypeptide sequence at least 85%, preferably 90%, more preferably 95% identical to SEQ ID NO:22. Preferably, the isolated monoclonal antibody or antigen-binding fragment thereof comprises a heavy chain variable region having the polypeptide sequence of SEQ ID NO:21; and a light chain variable region having the polypeptide sequence of SEQ ID NO:22.

[00100] In one embodiment, the invention relates to an isolated monoclonal antibody or antigen-binding fragment thereof, comprising HCDR1, HCDR2, HCDR3, LCDR1, LCDR2 and LCDR3, having the polypeptide sequences of SEQ ID NOs:78, 79, 80, 144, 145, and 146, respectively. In another embodiment, the isolated monoclonal antibody or antigen-binding fragment thereof comprises a heavy chain variable region having a polypeptide sequence at least 85%, preferably 90%, more preferably 95% identical to SEQ ID NO:23, and a light chain variable region having a polypeptide sequence at least

85%, preferably 90%, more preferably 95% identical to SEQ ID NO:24. Preferably, the isolated monoclonal antibody or antigen-binding fragment thereof comprises a heavy chain variable region having the polypeptide sequence of SEQ ID NO:23; and a light chain variable region having the polypeptide sequence of SEQ ID NO:24.

[00101] In one embodiment, the invention relates to an isolated monoclonal antibody or antigen-binding fragment thereof, comprising HCDR1, HCDR2, HCDR3, LCDR1, LCDR2 and LCDR3, having the polypeptide sequences of SEQ ID NOs:81, 82, 83, 147, 148, and 149, respectively. In another embodiment, the isolated monoclonal antibody or antigen-binding fragment thereof comprises a heavy chain variable region having a polypeptide sequence at least 85%, preferably 90%, more preferably 95% identical to SEQ ID NO:25, and a light chain variable region having a polypeptide sequence at least 85%, preferably 90%, more preferably 95% identical to SEQ ID NO:26. Preferably, the isolated monoclonal antibody or antigen-binding fragment thereof comprises a heavy chain variable region having the polypeptide sequence of SEQ ID NO:25; and a light chain variable region having the polypeptide sequence of SEQ ID NO:26.

[00102] In one embodiment, the invention relates to an isolated monoclonal antibody or antigen-binding fragment thereof, comprising HCDR1, HCDR2, HCDR3, LCDR1, LCDR2 and LCDR3, having the polypeptide sequences of SEQ ID NOs:84, 85, 86, 150, 151, and 152, respectively. In another embodiment, the isolated monoclonal antibody or antigen-binding fragment thereof comprises a heavy chain variable region having a polypeptide sequence at least 85%, preferably 90%, more preferably 95% identical to SEQ ID NO:27, and a light chain variable region having a polypeptide sequence at least 85%, preferably 90%, more preferably 95% identical to SEQ ID NO:28. Preferably, the isolated monoclonal antibody or antigen-binding fragment thereof comprises a heavy chain variable region having the polypeptide sequence of SEQ ID NO:27; and a light chain variable region having the polypeptide sequence of SEQ ID NO:28.

[00103] In one embodiment, the invention relates to an isolated monoclonal antibody or antigen-binding fragment thereof, comprising HCDR1, HCDR2, HCDR3, LCDR1, LCDR2 and LCDR3, having the polypeptide sequences of SEQ ID NOs:87, 88, 89, 153, 154, and 155, respectively. In another embodiment, the isolated monoclonal antibody or antigen-binding fragment thereof comprises a heavy chain variable region having a

polypeptide sequence at least 85%, preferably 90%, more preferably 95% identical to SEQ ID NO:29, and a light chain variable region having a polypeptide sequence at least 85%, preferably 90%, more preferably 95% identical to SEQ ID NO:30. Preferably, the isolated monoclonal antibody or antigen-binding fragment thereof comprises a heavy chain variable region having the polypeptide sequence of SEQ ID NO:29; and a light chain variable region having the polypeptide sequence of SEQ ID NO:30.

[00104] In one embodiment, the invention relates to an isolated monoclonal antibody or antigen-binding fragment thereof, comprising HCDR1, HCDR2, HCDR3, LCDR1, LCDR2 and LCDR3, having the polypeptide sequences of SEQ ID NOs:90, 91, 92, 156, 157, and 158, respectively. In another embodiment, the isolated monoclonal antibody or antigen-binding fragment thereof comprises a heavy chain variable region having a polypeptide sequence at least 85%, preferably 90%, more preferably 95% identical to SEQ ID NO:31, and a light chain variable region having a polypeptide sequence at least 85%, preferably 90%, more preferably 95% identical to SEQ ID NO:32. Preferably, the isolated monoclonal antibody or antigen-binding fragment thereof comprises a heavy chain variable region having the polypeptide sequence of SEQ ID NO:31; and a light chain variable region having the polypeptide sequence of SEQ ID NO:32.

[00105] In one embodiment, the invention relates to an isolated monoclonal antibody or antigen-binding fragment thereof, comprising HCDR1, HCDR2, HCDR3, LCDR1, LCDR2 and LCDR3, having the polypeptide sequences of SEQ ID NOs:93, 94, 95, 159, 160, and 161, respectively. In another embodiment, the isolated monoclonal antibody or antigen-binding fragment thereof comprises a heavy chain variable region having a polypeptide sequence at least 85%, preferably 90%, more preferably 95% identical to SEQ ID NO:33, and a light chain variable region having a polypeptide sequence at least 85%, preferably 90%, more preferably 95% identical to SEQ ID NO:34. Preferably, the isolated monoclonal antibody or antigen-binding fragment thereof comprises a heavy chain variable region having the polypeptide sequence of SEQ ID NO:33; and a light chain variable region having the polypeptide sequence of SEQ ID NO:34.

[00106] In one embodiment, the invention relates to an isolated monoclonal antibody or antigen-binding fragment thereof, comprising HCDR1, HCDR2, HCDR3, LCDR1, LCDR2 and LCDR3, having the polypeptide sequences of SEQ ID NOs:96, 97, 98, 162,

163, and 164, respectively. In another embodiment, the isolated monoclonal antibody or antigen-binding fragment thereof comprises a heavy chain variable region having a polypeptide sequence at least 85%, preferably 90%, more preferably 95% identical to SEQ ID NO:35, and a light chain variable region having a polypeptide sequence at least 85%, preferably 90%, more preferably 95% identical to SEQ ID NO:36. Preferably, the isolated monoclonal antibody or antigen-binding fragment thereof comprises a heavy chain variable region having the polypeptide sequence of SEQ ID NO:35; and a light chain variable region having the polypeptide sequence of SEQ ID NO:36.

[00107] In one embodiment, the invention relates to an isolated monoclonal antibody or antigen-binding fragment thereof, comprising HCDR1, HCDR2, HCDR3, LCDR1, LCDR2 and LCDR3, having the polypeptide sequences of SEQ ID NOs:99, 100, 101, 165, 166, and 167, respectively. In another embodiment, the isolated monoclonal antibody or antigen-binding fragment thereof comprises a heavy chain variable region having a polypeptide sequence at least 85%, preferably 90%, more preferably 95% identical to SEQ ID NO:37, and a light chain variable region having a polypeptide sequence at least 85%, preferably 90%, more preferably 95% identical to SEQ ID NO:38. Preferably, the isolated monoclonal antibody or antigen-binding fragment thereof comprises a heavy chain variable region having the polypeptide sequence of SEQ ID NO:37; and a light chain variable region having the polypeptide sequence of SEQ ID NO:38.

[00108] In one embodiment, the invention relates to an isolated monoclonal antibody or antigen-binding fragment thereof, comprising HCDR1, HCDR2, HCDR3, LCDR1, LCDR2 and LCDR3, having the polypeptide sequences of SEQ ID NOs:102, 103, 104, 168, 169, and 170, respectively. In another embodiment, the isolated monoclonal antibody or antigen-binding fragment thereof comprises a heavy chain variable region having a polypeptide sequence at least 85%, preferably 90%, more preferably 95% identical to SEQ ID NO:39, and a light chain variable region having a polypeptide sequence at least 85%, preferably 90%, more preferably 95% identical to SEQ ID NO:40. Preferably, the isolated monoclonal antibody or antigen-binding fragment thereof comprises a heavy chain variable region having the polypeptide sequence of SEQ ID

NO:39; and a light chain variable region having the polypeptide sequence of SEQ ID NO:40.

[00109] In one embodiment, the invention relates to an isolated monoclonal antibody or antigen-binding fragment thereof, comprising HCDR1, HCDR2, HCDR3, LCDR1, LCDR2 and LCDR3, having the polypeptide sequences of SEQ ID NOs:105, 106, 107, 171, 172, and 173, respectively. In another embodiment, the isolated monoclonal antibody or antigen-binding fragment thereof comprises a heavy chain variable region having a polypeptide sequence at least 85%, preferably 90%, more preferably 95% identical to SEQ ID NO:41, and a light chain variable region having a polypeptide sequence at least 85%, preferably 90%, more preferably 95% identical to SEQ ID NO:42. Preferably, the isolated monoclonal antibody or antigen-binding fragment thereof comprises a heavy chain variable region having the polypeptide sequence of SEQ ID NO:41; and a light chain variable region having the polypeptide sequence of SEQ ID NO:42.

[00110] In one embodiment, the invention relates to an isolated monoclonal antibody or antigen-binding fragment thereof, comprising HCDR1, HCDR2, HCDR3, LCDR1, LCDR2 and LCDR3, having the polypeptide sequences of SEQ ID NOs:108, 109, 110, 174, 175, and 176, respectively. In another embodiment, the isolated monoclonal antibody or antigen-binding fragment thereof comprises a heavy chain variable region having a polypeptide sequence at least 85%, preferably 90%, more preferably 95% identical to SEQ ID NO:43, and a light chain variable region having a polypeptide sequence at least 85%, preferably 90%, more preferably 95% identical to SEQ ID NO:44. Preferably, the isolated monoclonal antibody or antigen-binding fragment thereof comprises a heavy chain variable region having the polypeptide sequence of SEQ ID NO:43; and a light chain variable region having the polypeptide sequence of SEQ ID NO:44.

[00111] In one embodiment, the invention relates to an isolated monoclonal antibody or antigen-binding fragment thereof, comprising HCDR1, HCDR2, HCDR3, LCDR1, LCDR2 and LCDR3, having the polypeptide sequences of SEQ ID NOs:201, 202, 203, 204, 205, and 206, respectively. In another embodiment, the isolated monoclonal antibody or antigen-binding fragment thereof comprises a heavy chain variable region

having a polypeptide sequence at least 85%, preferably 90%, more preferably 95% identical to SEQ ID NO:199, and a light chain variable region having a polypeptide sequence at least 85%, preferably 90%, more preferably 95% identical to SEQ ID NO:200. Preferably, the isolated monoclonal antibody or antigen-binding fragment thereof comprises a heavy chain variable region having the polypeptide sequence of SEQ ID NO:199; and a light chain variable region having the polypeptide sequence of SEQ ID NO:200.

[00112] According to another particular aspect, the invention relates to an isolated monoclonal antibody or antigen-binding fragment thereof of the invention, wherein the antibody or antigen-binding fragment thereof is chimeric.

[00113] According to another particular aspect, the invention relates to an isolated monoclonal antibody or antigen-binding fragment thereof of the invention, wherein the antibody or antigen-binding fragment thereof is human or humanized.

[00114] According to another particular aspect, the invention relates to an isolated humanized monoclonal antibody or antigen-binding fragment thereof, wherein the isolated humanized antibody or antigen-binding fragment thereof comprises:

a. a heavy chain variable region having the polypeptide sequence of SEQ ID NO:183, and a light chain variable region having the polypeptide sequence of SEQ ID NO:191;
b. a heavy chain variable region having the polypeptide sequence of SEQ ID NO:183, and a light chain variable region having the polypeptide sequence of SEQ ID NO:192;
c. a heavy chain variable region having the polypeptide sequence of SEQ ID NO:183, and a light chain variable region having the polypeptide sequence of SEQ ID NO:193;
d. a heavy chain variable region having the polypeptide sequence of SEQ ID NO:184, and a light chain variable region having the polypeptide sequence of SEQ ID NO:190;
e. a heavy chain variable region having the polypeptide sequence of SEQ ID NO:184, and a light chain variable region having the polypeptide sequence of SEQ ID NO:192;

f. a heavy chain variable region having the polypeptide sequence of SEQ ID NO:184, and a light chain variable region having the polypeptide sequence of SEQ ID NO:193;

g. a heavy chain variable region having the polypeptide sequence of SEQ ID NO:185, and a light chain variable region having the polypeptide sequence of SEQ ID NO:190;

h. a heavy chain variable region having the polypeptide sequence of SEQ ID NO:185, and a light chain variable region having the polypeptide sequence of SEQ ID NO:191;

i. a heavy chain variable region having the polypeptide sequence of SEQ ID NO:185, and a light chain variable region having the polypeptide sequence of SEQ ID NO:193;

j. a heavy chain variable region having the polypeptide sequence of SEQ ID NO:185, and a light chain variable region having the polypeptide sequence of SEQ ID NO:198;

k. a heavy chain variable region having the polypeptide sequence of SEQ ID NO:187, and a light chain variable region having the polypeptide sequence of SEQ ID NO:194;

l. a heavy chain variable region having the polypeptide sequence of SEQ ID NO:188, and a light chain variable region having the polypeptide sequence of SEQ ID NO:194;

m. a heavy chain variable region having the polypeptide sequence of SEQ ID NO:188, and a light chain variable region having the polypeptide sequence of SEQ ID NO:196;

n. a heavy chain variable region having the polypeptide sequence of SEQ ID NO:188, and a light chain variable region having the polypeptide sequence of SEQ ID NO:197; or

o. a heavy chain variable region having the polypeptide sequence of SEQ ID NO:199, and a light chain variable region having the polypeptide sequence of SEQ ID NO:200.

[00115] According to another particular aspect, the invention relates to an isolated monoclonal antibody or antigen-binding fragment thereof, wherein the antibody or antigen-binding fragment thereof is capable of blocking binding of CD47 to thrombospondin-1 (TSP1) and/or to signal regulatory protein alpha (SIRPα).

[00116] According to another particular aspect, the invention relates to an isolated monoclonal antibody or antigen-binding fragment thereof, wherein the antibody or antigen-binding fragment thereof is capable of inducing macrophage-mediated phagocytosis of cancer cells.

[00117] According to another particular aspect, the invention relates to an isolated monoclonal antibody or antigen-binding fragment thereof, wherein the antibody or antigen-binding fragment thereof is capable of binding cancer cells with minimal to undetectable binding to red blood cells. Binding of cancer cells by the isolated monoclonal antibody or antigen-binding fragment thereof of the invention can be determined using methods known in the art.

[00118] In another general aspect, the invention relates to an isolated nucleic acid encoding a monoclonal antibody or antigen-binding fragment thereof of the invention. It will be appreciated by those skilled in the art that the coding sequence of a protein can be changed (e.g., replaced, deleted, inserted, etc.) without changing the amino acid sequence of the protein. Accordingly, it will be understood by those skilled in the art that nucleic acid sequences encoding monoclonal antibodies or antigen-binding fragments thereof of the invention can be altered without changing the amino acid sequences of the proteins.

[00119] In another general aspect, the invention relates to a vector comprising an isolated nucleic acid encoding a monoclonal antibody or antigen-binding fragment thereof of the invention. Any vector known to those skilled in the art in view of the present disclosure can be used, such as a plasmid, a cosmid, a phage vector or a viral vector. In some embodiments, the vector is a recombinant expression vector such as a plasmid. The vector can include any element to establish a conventional function of an expression vector, for example, a promoter, ribosome binding element, terminator, enhancer, selection marker, and origin of replication. The promoter can be a constitutive, inducible, or repressible promoter. A number of expression vectors capable of delivering nucleic acids to a cell are known in the art and can be used herein for production of an

antibody or antigen-binding fragment thereof in the cell. Conventional cloning techniques or artificial gene synthesis can be used to generate a recombinant expression vector according to embodiments of the invention.

[00120] In another general aspect, the invention relates to a host cell comprising an isolated nucleic acid encoding a monoclonal antibody or antigen-binding fragment thereof of the invention. Any host cell known to those skilled in the art in view of the present disclosure can be used for recombinant expression of antibodies or antigen-binding fragments thereof of the invention. In some embodiments, the host cells are E. coli TG1 or BL21 cells (for expression of, e.g., an scFv or Fab antibody), CHO-DG44 or CHO-K1 cells or HEK293 cells (for expression of, e.g., a full-length IgG antibody). According to particular embodiments, the recombinant expression vector is transformed into host cells by conventional methods such as chemical transfection, heat shock, or electroporation, where it is stably integrated into the host cell genome such that the recombinant nucleic acid is effectively expressed.

[00121] In another general aspect, the invention relates to a method of producing a monoclonal antibody or antigen-binding fragment thereof of the invention, comprising culturing a cell comprising a nucleic acid encoding the monoclonal antibody or antigen-binding fragment thereof under conditions to produce a monoclonal antibody or antigen-binding fragment thereof of the invention, and recovering the antibody or antigen-binding fragment thereof from the cell or cell culture (e.g., from the supernatant). Expressed antibodies or antigen-binding fragments thereof can be harvested from the cells and purified according to conventional techniques known in the art and as described herein.

[00122] **Pharmaceutical Compositions**

[00123] In another general aspect, the invention relates to a pharmaceutical composition, comprising an isolated monoclonal antibody or antigen-binding fragment thereof of the invention and a pharmaceutically acceptable carrier. The term "pharmaceutical composition" as used herein means a product comprising an antibody of the invention together with a pharmaceutically acceptable carrier. Antibodies of the invention and compositions comprising them are also useful in the manufacture of a medicament for therapeutic applications mentioned herein.

[00124] As used herein, the term "carrier" refers to any excipient, diluent, filler, salt, buffer, stabilizer, solubilizer, oil, lipid, lipid containing vesicle, microsphere, liposomal encapsulation, or other material well known in the art for use in pharmaceutical formulations. It will be understood that the characteristics of the carrier, excipient or diluent will depend on the route of administration for a particular application. As used herein, the term "pharmaceutically acceptable carrier" refers to a non-toxic material that does not interfere with the effectiveness of a composition according to the invention or the biological activity of a composition according to the invention. According to particular embodiments, in view of the present disclosure, any pharmaceutically acceptable carrier suitable for use in an antibody pharmaceutical composition can be used in the invention.

[00125] The formulation of pharmaceutically active ingredients with pharmaceutically acceptable carriers is known in the art, e.g., Remington: The Science and Practice of Pharmacy (e.g. 21st edition (2005), and any later editions). Non-limiting examples of additional ingredients include: buffers, diluents, solvents, tonicity regulating agents, preservatives, stabilizers, and chelating agents. One or more pharmaceutically acceptable carrier may be used in formulating the pharmaceutical compositions of the invention.

[00126] In one embodiment of the invention, the pharmaceutical composition is a liquid formulation. A preferred example of a liquid formulation is an aqueous formulation, i.e., a formulation comprising water. The liquid formulation may comprise a solution, a suspension, an emulsion, a microemulsion, a gel, and the like. An aqueous formulation typically comprises at least 50% w/w water, or at least 60%, 70%, 75%, 80%, 85%, 90%, or at least 95% w/w of water.

[00127] In one embodiment, the pharmaceutical composition may be formulated as an injectable which can be injected, for example, via an injection device (e.g., a syringe or an infusion pump). The injection may be delivered subcutaneously, intramuscularly, intraperitoneally, intravitreally, or intravenously, for example.

[00128] In another embodiment, the pharmaceutical composition is a solid formulation, e.g., a freeze-dried or spray-dried composition, which may be used as is, or whereto the physician or the patient adds solvents, and/or diluents prior to use. Solid dosage forms may include tablets, such as compressed tablets, and/or coated tablets, and capsules (e.g.,

hard or soft gelatin capsules). The pharmaceutical composition may also be in the form of sachets, dragees, powders, granules, lozenges, or powders for reconstitution, for example.

[00129] The dosage forms may be immediate release, in which case they may comprise a water-soluble or dispersible carrier, or they may be delayed release, sustained release, or modified release, in which case they may comprise water-insoluble polymers that regulate the rate of dissolution of the dosage form in the gastrointestinal tract or under the skin.

[00130] In other embodiments, the pharmaceutical composition may be delivered intranasally, intrabuccally, or sublingually.

[00131] The pH in an aqueous formulation can be between pH 3 and pH 10. In one embodiment of the invention, the pH of the formulation is from about 7.0 to about 9.5. In another embodiment of the invention, the pH of the formulation is from about 3.0 to about 7.0.

[00132] In another embodiment of the invention, the pharmaceutical composition comprises a buffer. Non-limiting examples of buffers include: arginine, aspartic acid, bicine, citrate, disodium hydrogen phosphate, fumaric acid, glycine, glycylglycine, histidine, lysine, maleic acid, malic acid, sodium acetate, sodium carbonate, sodium dihydrogen phosphate, sodium phosphate, succinate, tartaric acid, tricine, and tris(hydroxymethyl)-aminomethane, and mixtures thereof. The buffer may be present individually or in the aggregate, in a concentration from about 0.01 mg/ml to about 50 mg/ml, for example from about 0.1 mg/ml to about 20 mg/ml. Pharmaceutical compositions comprising each one of these specific buffers constitute alternative embodiments of the invention.

[00133] In another embodiment of the invention, the pharmaceutical composition comprises a preservative. Non-limiting examples of preservatives include: benzethonium chloride, benzoic acid, benzyl alcohol, bronopol, butyl 4-hydroxybenzoate, chlorobutanol, chlorocresol, chlorohexidine, chlorphenesin, o-cresol, m-cresol, p-cresol, ethyl 4-hydroxybenzoate, imidurea, methyl 4-hydroxybenzoate, phenol, 2-phenoxyethanol, 2-phenylethanol, propyl 4-hydroxybenzoate, sodium dehydroacetate, thiomerosal, and mixtures thereof. The preservative may be present individually or in the aggregate, in a concentration from about 0.01 mg/ml to about 50 mg/ml, for example from about 0.1

mg/ml to about 20 mg/ml. Pharmaceutical compositions comprising each one of these specific preservatives constitute alternative embodiments of the invention.

[00134] In another embodiment of the invention, the pharmaceutical composition comprises an isotonic agent. Non-limiting examples of the embodiment include a salt (such as sodium chloride), an amino acid (such as glycine, histidine, arginine, lysine, isoleucine, aspartic acid, tryptophan, and threonine), an alditol (such as glycerol, 1,2-propanediol propyleneglycol), 1,3-propanediol, and 1,3-butanediol), polyethyleneglycol (e.g. PEG400), and mixtures thereof. Another example of an isotonic agent includes a sugar. Non-limiting examples of sugars may be mono-, di-, or polysaccharides, or water-soluble glucans, including for example fructose, glucose, mannose, sorbose, xylose, maltose, lactose, sucrose, trehalose, dextran, pullulan, dextrin, cyclodextrin, alpha and beta- HPCD, soluble starch, hydroxyethyl starch, and sodium carboxymethylcellulose. Another example of an isotonic agent is a sugar alcohol, wherein the term "sugar alcohol" is defined as a C(4-8) hydrocarbon having at least one —OH group. Non-limiting examples of sugar alcohols include mannitol, sorbitol, inositol, galactitol, dulcitol, xylitol, and arabitol. Pharmaceutical compositions comprising each isotonic agent listed in this paragraph constitute alternative embodiments of the invention. The isotonic agent may be present individually or in the aggregate, in a concentration from about 0.01 mg/ml to about 50 mg/ml, for example from about 0.1 mg/ml to about 20 mg/ml. Pharmaceutical compositions comprising each one of these specific isotonic agents constitute alternative embodiments of the invention.

[00135] In another embodiment of the invention, the pharmaceutical composition comprises a chelating agent. Non-limiting examples of chelating agents include citric acid, aspartic acid, salts of ethylenediaminetetraacetic acid (EDTA), and mixtures thereof. The chelating agent may be present individually or in the aggregate, in a concentration from about 0.01 mg/ml to about 50 mg/ml, for example from about 0.1 mg/ml to about 20 mg/ml. Pharmaceutical compositions comprising each one of these specific chelating agents constitute alternative embodiments of the invention.

[00136] In another embodiment of the invention, the pharmaceutical composition comprises a stabilizer. Non-limiting examples of stabilizers include one or more

aggregation inhibitors, one or more oxidation inhibitors, one or more surfactants, and/or one or more protease inhibitors.

[00137] In another embodiment of the invention, the pharmaceutical composition comprises a stabilizer, wherein said stabilizer is carboxy-/hydroxycellulose and derivates thereof (such as HPC, HPC-SL, HPC-L and HPMC), cyclodextrins, 2-methylthioethanol, polyethylene glycol (such as PEG 3350), polyvinyl alcohol (PVA), polyvinyl pyrrolidone, salts (such as sodium chloride), sulphur-containing substances such as monothioglycerol), or thioglycolic acid. The stabilizer may be present individually or in the aggregate, in a concentration from about 0.01 mg/ml to about 50 mg/ml, for example from about 0.1 mg/ml to about 20 mg/ml. Pharmaceutical compositions comprising each one of these specific stabilizers constitute alternative embodiments of the invention.

[00138] In further embodiments of the invention, the pharmaceutical composition comprises one or more surfactants, preferably a surfactant, at least one surfactant, or two different surfactants. The term "surfactant" refers to any molecules or ions that are comprised of a water-soluble (hydrophilic) part, and a fat-soluble (lipophilic) part. The surfactant may, for example, be selected from the group consisting of anionic surfactants, cationic surfactants, nonionic surfactants, and/or zwitterionic surfactants. The surfactant may be present individually or in the aggregate, in a concentration from about 0.1 mg/ml to about 20 mg/ml. Pharmaceutical compositions comprising each one of these specific surfactants constitute alternative embodiments of the invention.

[00139] In a further embodiment of the invention, the pharmaceutical composition comprises one or more protease inhibitors, such as, e.g., EDTA, and/or benzamidine hydrochloric acid (HCl). The protease inhibitor may be present individually or in the aggregate, in a concentration from about 0.1 mg/ml to about 20 mg/ml. Pharmaceutical compositions comprising each one of these specific protease inhibitors constitute alternative embodiments of the invention.

[00140] In another general aspect, the invention relates to a method of producing a pharmaceutical composition comprising a monoclonal antibody or antigen-binding fragment thereof of the invention, comprising combining a monoclonal antibody or antigen-binding fragment thereof with a pharmaceutically acceptable carrier to obtain the pharmaceutical composition.

[00141] Methods of use

[00142] In another general aspect, the invention relates to a method of blocking the binding of CD47 to thrombospondin-1 (TSP1), or a method of blocking the binding of CD47 to signal regulatory protein alpha (SIRPα), the method comprising administering to the subject a pharmaceutical composition of the invention.

[00143] The functional activity of antibodies and antigen-binding fragments thereof that bind CD47 can be characterized by methods known in the art and as described herein. Methods for characterizing antibodies and antigen-binding fragments thereof that bind CD47 include, but are not limited to, affinity and specificity assays including Biacore, ELISA, and OctetRed analysis; receptor ligand binding assays to detect blocking of the binding of CD47 to TSP1 and/or SIRPα; phagocytosis assays where CD47-expressing cells are fluorescently labeled and incubated with macrophages to detect the effect of blocking CD47 binding to SIRPα on the phagocytosis of the CD47-expressing cells by macrophages; hemagglutination assays to detect the effect of anti-CD47 on red blood cells, and cell-based assays to detect the effect of blocking the TSP1-CD47 interaction on downstream eNOS/NO/cGMP signaling in endothelial cells. According to particular embodiments, the methods for characterizing antibodies and antigen-binding fragments thereof that bind CD47 include those described below.

[00144] In another general aspect, the invention relates to a method of treating a cancer in a subject in need thereof, comprising administering to the subject a pharmaceutical composition of the invention. The cancer can be any liquid or solid cancer, for example, it can be selected from but not limited to, a lung cancer, a gastric cancer, a colon cancer, a hepatocellular carcinoma, a renal cell carcinoma, a bladder urothelial carcinoma, a metastatic melanoma, a breast cancer, an ovarian cancer, a cervical cancer, a head and neck cancer, a pancreatic cancer, a glioma, a glioblastoma, and other solid tumors, and a non-Hodgkin's lymphoma (NHL), an acute lympocytic leukemia (ALL), a chronic lymphocytic leukemia (CLL), a chronic myelogenous leukemia (CML), a multiple myeloma (MM), an acute myeloid leukemia (AML), and other liquid tumors.

[00145] In another general aspect, the invention relates to a method of treating an inflammatory disease in a subject in need thereof, comprising administering to the subject a pharmaceutical composition of the invention.

[00146] In another general aspect, the invention relates to a method of treating an infectious disease in a subject in need thereof, comprising administering to the subject a pharmaceutical composition of the invention.

[00147] In another general aspect, the invention relates to a method of treating atherosclerosis in a subject in need thereof, comprising administering to the subject a pharmaceutical composition of the invention.

[00148] In another general aspect, the invention relates to a method of treating a cardiovascular disease in a subject in need thereof, comprising administering to the subject a pharmaceutical composition of the invention.

[00149] In another general aspect, the invention relates to a method of treating a metabolic disease in a subject in need thereof, comprising administering to the subject a pharmaceutical composition of the invention.

[00150] In another general aspect, the invention relates to a method of a radiation-induced injury in a subject in need thereof, comprising administering to the subject a pharmaceutical composition of the invention.

[00151] In another general aspect, the invention relates to a method of treating an autoimmune disease in a subject in need thereof, comprising administering to the subject a pharmaceutical composition of the invention.

[00152] According to embodiments of the invention, the pharmaceutical composition comprises a therapeutically effective amount of the anti-CD47 antibody or antigen-binding fragment thereof. As used herein, the term "therapeutically effective amount" refers to an amount of an active ingredient or component that elicits the desired biological or medicinal response in a subject. A therapeutically effective amount can be determined empirically and in a routine manner, in relation to the stated purpose.

[00153] As used herein with reference to anti-CD47 antibodies or antigen-binding fragments thereof, a therapeutically effective amount means an amount of the anti-CD47 antibody or antigen-binding fragment thereof that modulates an immune response in a subject in need thereof. Also as used herein with reference to anti-CD47 antibodies or antigen-binding fragments thereof, a therapeutically effective amount means an amount of the anti-CD47 antibody or antigen-binding fragment thereof that results in treatment of a disease, disorder, or condition; prevents or slows the progression of the disease,

disorder, or condition; or reduces or completely alleviates symptoms associated with the disease, disorder, or condition.

[00154] According to particular embodiments, the disease, disorder or condition to be treated is cancer, preferably a cancer selected from the group consisting of lung cancer, gastric cancer, colon cancer, hepatocellular carcinoma, renal cell carcinoma, bladder urothelial carcinoma, metastatic melanoma, breast cancer, ovarian cancer, cervical cancer, head and neck cancer, pancreatic cancer, glioma, glioblastoma, and other solid tumors, and non-Hodgkin's lymphoma (NHL), acute lymphocytic leukemia (ALL), chronic lymphocytic leukemia (CLL), chronic myelogenous leukemia (CML), multiple myeloma (MM), acute myeloid leukemia (AML), and other liquid tumors. According to other particular embodiments, the disease, disorder or condition to be treated is an inflammatory disease, an infectious disease, atherosclerosis, cardiovascular disease, metabolic diseases, radiation-induced injury, an immune disease, and/or an autoimmune disease.

[00155] According to particular embodiments, a therapeutically effective amount refers to the amount of therapy which is sufficient to achieve one, two, three, four, or more of the following effects: (i) reduce or ameliorate the severity of the disease, disorder or condition to be treated or a symptom associated therewith; (ii) reduce the duration of the disease, disorder or condition to be treated, or a symptom associated therewith; (iii) prevent the progression of the disease, disorder or condition to be treated, or a symptom associated therewith; (iv) cause regression of the disease, disorder or condition to be treated, or a symptom associated therewith; (v) prevent the development or onset of the disease, disorder or condition to be treated, or a symptom associated therewith; (vi) prevent the recurrence of the disease, disorder or condition to be treated, or a symptom associated therewith; (vii) reduce hospitalization of a subject having the disease, disorder or condition to be treated, or a symptom associated therewith; (viii) reduce hospitalization length of a subject having the disease, disorder or condition to be treated, or a symptom associated therewith; (ix) increase the survival of a subject with the disease, disorder or condition to be treated, or a symptom associated therewith; (xi) inhibit or reduce the disease, disorder or condition to be treated, or a symptom associated therewith

in a subject; and/or (xii) enhance or improve the prophylactic or therapeutic effect(s) of another therapy.

[00156] The therapeutically effective amount or dosage can vary according to various factors, such as the disease, disorder or condition to be treated, the means of administration, the target site, the physiological state of the subject (including, e.g., age, body weight, health), whether the subject is a human or an animal, other medications administered, and whether the treatment is prophylactic or therapeutic. Treatment dosages are optimally titrated to optimize safety and efficacy.

[00157] According to particular embodiments, the compositions described herein are formulated to be suitable for the intended route of administration to a subject. For example, the compositions described herein can be formulated to be suitable for intravenous, subcutaneous, or intramuscular administration.

[00158] As used herein, the terms "treat," "treating," and "treatment" are all intended to refer to an amelioration or reversal of at least one measurable physical parameter related to a cancer, an immune disease, disorder or condition, an autoimmune disease, disorder or condition, or an inflammatory disease, disorder or condition, an infectious disease, disorder or condition, an atherosclerosis, disorder or condition, a cardiovascular disease, disorder or condition, a metabolic disease disorder or condition, a radiation-induced injury, disorder or condition, which is not necessarily discernible in the subject, but can be discernible in the subject. The terms "treat," "treating," and "treatment," can also refer to causing regression, preventing the progression, or at least slowing down the progression of the disease, disorder, or condition. In a particular embodiment, "treat," "treating," and "treatment" refer to an alleviation, prevention of the development or onset, or reduction in the duration of one or more symptoms associated with the disease, disorder, or condition, such as a tumor or more preferably a cancer. In a particular embodiment, "treat," "treating," and "treatment" refer to prevention of the recurrence of the disease, disorder, or condition. In a particular embodiment, "treat," "treating," and "treatment" refer to an increase in the survival of a subject having the disease, disorder, or condition. In a particular embodiment, "treat," "treating," and "treatment" refer to elimination of the disease, disorder, or condition in the subject.

[00159] According to particular embodiments, a composition used in the treatment of a cancer, an immune disease, disorder or condition, an autoimmune disease, disorder or condition, an inflammatory disease, disorder or condition, an infectious disease, disorder or condition, an atherosclerosis, disorder or condition, a cardiovascular disease, disorder or condition, a metabolic disease, disorder or condition, a radiation-induced injury, disorder or condition, can be used in combination with another treatment. For cancer treatment, the composition can be used in combination with another treatment including, but not limited to, a chemotherapy, an anti-CD20 mAb, an anti-CTLA-4 antibody, an anti-LAG-3 mAb, an anti-EGFR mAb, an anti-HER-2 mAb, an anti-CD19 mAb, an anti-CD33 mAb, an anti-CD73 mAb, an anti-CD47 mAb, an anti-DLL-3 mAb, an anti-apelin mAb, an anti-TIP-1 mAb, an anti-CLDN18.2 mAb, an anti-FOLR1 mAb, an anti-PD-L1 antibody, an anti-PD-1 antibody, a PD-1/PD-L1 therapy, or other immuno-oncology drug, a targeted therapy, an antiangiogenic agent, a radiation therapy, or other anticancer drugs. Anti-CD47 antibodies can be used to construct bispecific antibodies with partner mAbs against PD-1, PD-L1, LAG3, TIM-3, CTLA-4, EGFR, HER-2, CD19, CD20, CD33, CD73, apelin, DLL3, claudin18.2, TIP-1, CD3, folate receptor alpha and/or other tumor surface antigens to treat cancers/tumors that express both CD47 and the specific tumor associated antigen.

[00160] As used herein, the term "in combination," in the context of the administration of two or more therapies to a subject, refers to the use of more than one therapy. The use of the term "in combination" does not restrict the order in which therapies are administered to a subject. For example, a first therapy (e.g., a composition described herein) can be administered prior to (e.g., 5 minutes, 15 minutes, 30 minutes, 45 minutes, 1 hour, 2 hours, 4 hours, 6 hours, 12 hours, 16 hours, 24 hours, 48 hours, 72 hours, 96 hours, 1 week, 2 weeks, 3 weeks, 4 weeks, 5 weeks, 6 weeks, 8 weeks, or 12 weeks before), concomitantly with, or subsequent to (e.g., 5 minutes, 15 minutes, 30 minutes, 45 minutes, 1 hour, 2 hours, 4 hours, 6 hours, 12 hours, 16 hours, 24 hours, 48 hours, 72 hours, 96 hours, 1 week, 2 weeks, 3 weeks, 4 weeks, 5 weeks, 6 weeks, 8 weeks, or 12 weeks after) the administration of a second therapy to a subject.

[00161] In another general aspect, the invention relates to a method of determining a level of CD47 in a subject. The methods comprise (a) obtaining a sample from the subject;

(b) contacting the sample with an antibody or antigen-binding fragment thereof of the invention; and (c) determining a level of CD47 in the subject.

[00162] As used herein, "sample" refers to a biological sample isolated from a subject and can include, but is not limited to, whole blood, serum, plasma, blood cells, endothelial cells, tissue biopsies (e.g., a cancer tissue, a hepatic tissue, etc.), lymphatic fluid, ascites fluid, interstitial fluid, bone marrow, cerebrospinal fluid, saliva, mucous, sputum, sweat, urine, or any other secretion, excretion, or other bodily fluids. A "blood sample" refers to whole blood or any fraction thereof, including blood cells, serum, and plasma. A "blood sample" can, for example, comprise cancer cells.

[00163] In certain embodiments, the level of CD47 in the subject can be determined utilizing assays selected from, but not limited to, a Western blot assay, an ELISA assay, a FACS assay, and/or an immunohistochemistry (IHC). Relative protein levels can be determined by utilizing Western blot analysis, FACS assay, and immunohistochemistry (IHC), and absolute protein levels can be determined by utilizing an ELISA assay. When determining the relative levels of CD47, the levels of CD47 can be determined between at least two samples, e.g., between samples from the same subject at different time points, between samples from different tissues in the same subject, and/or between samples from different subjects. Alternatively, when determining absolute levels of CD47, such as by an ELISA assay, the absolute level of CD47 in the sample can be determined by creating a standard for the ELISA assay prior to testing the sample. A person skilled in the art would understand which analytical techniques to utilize to determine the level of CD47 in a sample from the subject utilizing the antibodies or antigen-binding fragments thereof of the invention.

[00164] Utilizing methods of determining a level of CD47 in a sample from a subject can lead to the diagnosis of abnormal (elevated, reduced, or insufficient) CD47 levels in a disease and making appropriate therapeutic decisions. Such a disease can be selected from, but not limited to, a cancer, preferably a cancer selected from the group consisting of lung cancer, gastric cancer, colon cancer, hepatocellular carcinoma, renal cell carcinoma, bladder urothelial carcinoma, metastatic melanoma, breast cancer, ovarian cancer, cervical cancer, head and neck cancer, pancreatic cancer, glioma, glioblastoma, and other solid tumors, and non-Hodgkin's lymphoma (NHL), acute lymphocytic

leukemia (ALL), chronic lymphocytic leukemia (CLL), chronic myelogenous leukemia (CML), multiple myeloma (MM), acute myeloid leukemia (AML), and other liquid tumors, an inflammatory disease, an infectious disease, atherosclerosis, cardiovascular disease, metabolic diseases, radiation-induced injury, an immune disease, and/or an autoimmune disease.

EMBODIMENTS

[00165] The invention provides also the following non-limiting embodiments.

[00166] Embodiment 1 is an isolated monoclonal antibody or antigen-binding fragment thereof comprising a heavy chain complementarity determining region 1 (HCDR1), HCDR2, HCDR3, a light chain complementarity determining region 1 (LCDR1), LCDR2, and LCDR3, having the polypeptide sequences of:

(1) SEQ ID NOs:177, 46, 47, 178, 112, and 179, respectively;
(2) SEQ ID NOs:51, 52, 53, 117, 118, and 119, respectively;
(3) SEQ ID NOs:54, 55, 56, 120, 121, and 122, respectively;
(4) SEQ ID NOs:57, 58, 59, 123, 124, and 125, respectively;
(5) SEQ ID NOs:60, 61, 62, 126, 127, and 128, respectively;
(6) SEQ ID NOs:180, 181, 182, 129, 130, and 131, respectively;
(7) SEQ ID NOs:72, 73, 74, 138, 139, and 140, respectively;
(8) SEQ ID NOs:78, 79, 80, 144, 145, and 146, respectively;
(9) SEQ ID NOs:81, 82, 83, 147, 148, and 149, respectively;
(10) SEQ ID NOs:84, 85, 86, 150, 151, and 152, respectively;
(11) SEQ ID NOs:87, 88, 89, 153, 154, and 155, respectively;
(12) SEQ ID NOs:90, 91, 92, 156, 157, and 158, respectively;
(13) SEQ ID NOs:93, 94, 95, 159, 160, and 161, respectively;
(14) SEQ ID NOs:96, 97, 98, 162, 163, and 164, respectively;
(15) SEQ ID NOs:99, 100, 101, 165, 166, and 167, respectively;
(16) SEQ ID NOs:102, 103, 104, 168, 169, and 170, respectively;
(17) SEQ ID NOs:105, 106, 107, 171, 172, and 173, respectively;
(18) SEQ ID NOs:108, 109, 110, 174, 175, and 176, respectively; or
(19) SEQ ID NOs:201, 202, 203, 204, 205, and 206, respectively

wherein the antibody or antigen-binding fragment thereof specifically binds CD47, preferably human CD47.

[00167] Embodiment 2 is the isolated monoclonal antibody or antigen-binding fragment of embodiment 1, comprising a heavy chain variable region having a polypeptide sequence at least 95% identical to SEQ ID NO:1, 3, 5, 7, 9, 11, 13, 15, 17, 19, 21, 23, 25, 27, 29, 31, 33, 35, 37, 39, 41, or 43, or a light chain variable region having a polypeptide sequence at least 95% identical to SEQ ID NO:2, 4, 6, 8, 10, 12, 14, 16, 18, 20, 22, 24, 26, 28, 30, 32, 34, 36, 38, 40, 42, or 44.

[00168] Embodiment 3 is the isolated monoclonal antibody or antigen-binding fragment of embodiment 1 or 2, comprising

(a) a heavy chain variable region having the polypeptide sequence of SEQ ID NO:1, and a light chain variable region having the polypeptide sequence of SEQ ID NO:2;

(b) a heavy chain variable region having the polypeptide sequence of SEQ ID NO:3, and a light chain variable region having the polypeptide sequence of SEQ ID NO:4;

(c) a heavy chain variable region having the polypeptide sequence of SEQ ID NO:5, and a light chain variable region having the polypeptide sequence of SEQ ID NO:6;

(d) a heavy chain variable region having the polypeptide sequence of SEQ ID NO:7, and a light chain variable region having the polypeptide sequence of SEQ ID NO:8;

(e) a heavy chain variable region having the polypeptide sequence of SEQ ID NO:9, and a light chain variable region having the polypeptide sequence of SEQ ID NO:10;

(f) a heavy chain variable region having the polypeptide sequence of SEQ ID NO:11, and a light chain variable region having the polypeptide sequence of SEQ ID NO:12;

(g) a heavy chain variable region having the polypeptide sequence of SEQ ID NO:13, and a light chain variable region having the polypeptide sequence of SEQ ID NO:14;

(h) a heavy chain variable region having the polypeptide sequence of SEQ ID NO:15, and a light chain variable region having the polypeptide sequence of SEQ ID NO:16;

(i) a heavy chain variable region having the polypeptide sequence of SEQ ID NO:17, and a light chain variable region having the polypeptide sequence of SEQ ID NO:18;

(j) a heavy chain variable region having the polypeptide sequence of SEQ ID NO:19, and a light chain variable region having the polypeptide sequence of SEQ ID NO:20;

(k) a heavy chain variable region having the polypeptide sequence of SEQ ID NO:21, and a light chain variable region having the polypeptide sequence of SEQ ID NO:22;

(l) a heavy chain variable region having the polypeptide sequence of SEQ ID NO:23, and a light chain variable region having the polypeptide sequence of SEQ ID NO:24;

(m) a heavy chain variable region having the polypeptide sequence of SEQ ID NO:25, and a light chain variable region having the polypeptide sequence of SEQ ID NO:26;

(n) a heavy chain variable region having the polypeptide sequence of SEQ ID NO:27, and a light chain variable region having the polypeptide sequence of SEQ ID NO:28;

(o) a heavy chain variable region having the polypeptide sequence of SEQ ID NO:29, and a light chain variable region having the polypeptide sequence of SEQ ID NO:30;

(p) a heavy chain variable region having the polypeptide sequence of SEQ ID NO:31, and a light chain variable region having the polypeptide sequence of SEQ ID NO:32;

(q) a heavy chain variable region having the polypeptide sequence of SEQ ID NO:33, and a light chain variable region having the polypeptide sequence of SEQ ID NO:34;

(r) a heavy chain variable region having the polypeptide sequence of SEQ ID NO:35, and a light chain variable region having the polypeptide sequence of SEQ ID NO:36;

(s) a heavy chain variable region having the polypeptide sequence of SEQ ID NO:37, and a light chain variable region having the polypeptide sequence of SEQ ID NO:38;

(t) a heavy chain variable region having the polypeptide sequence of SEQ ID NO:39, and a light chain variable region having the polypeptide sequence of SEQ ID NO:40;

(u) a heavy chain variable region having the polypeptide sequence of SEQ ID NO:41, and a light chain variable region having the polypeptide sequence of SEQ ID NO:42; or

(v) a heavy chain variable region having the polypeptide sequence of SEQ ID NO:43, and a light chain variable region having the polypeptide sequence of SEQ ID NO:44.

[00169] Embodiment 4 is the isolated monoclonal antibody or antigen-binding fragment of any one of embodiments 1 to 3, wherein the antibody or antigen-binding fragment thereof inhibits the interaction of CD47 and thrombospondin-1 (TSP-1) and/or CD47 and SIRPα.

[00170] Embodiment 5 is the isolated monoclonal antibody or antigen-binding fragment of any one of embodiments 1 to 4, wherein the antibody or antigen-binding fragment thereof is chimeric.

[00171] Embodiment 6 is the isolated monoclonal antibody or antigen-binding fragment of any one of embodiments 1 to 5, wherein the antibody or antigen-binding fragment thereof is human or humanized.

[00172] Embodiment 7 is the isolated monoclonal antibody or antigen-binding fragment thereof of embodiment 6, wherein the antibody or antigen-binding fragment thereof comprises:

a. a heavy chain variable region having the polypeptide sequence of SEQ ID NO:183, and a light chain variable region having the polypeptide sequence of SEQ ID NO:191;

b. a heavy chain variable region having the polypeptide sequence of SEQ ID NO:183, and a light chain variable region having the polypeptide sequence of SEQ ID NO:192;

c. a heavy chain variable region having the polypeptide sequence of SEQ ID NO:183, and a light chain variable region having the polypeptide sequence of SEQ ID NO:193;

d. a heavy chain variable region having the polypeptide sequence of SEQ ID NO:184, and a light chain variable region having the polypeptide sequence of SEQ ID NO:190;

e. a heavy chain variable region having the polypeptide sequence of SEQ ID NO:184, and a light chain variable region having the polypeptide sequence of SEQ ID NO:192;

f. a heavy chain variable region having the polypeptide sequence of SEQ ID NO:184, and a light chain variable region having the polypeptide sequence of SEQ ID NO:193;

g. a heavy chain variable region having the polypeptide sequence of SEQ ID NO:185, and a light chain variable region having the polypeptide sequence of SEQ ID NO:190;

h. a heavy chain variable region having the polypeptide sequence of SEQ ID NO:185, and a light chain variable region having the polypeptide sequence of SEQ ID NO:191;

i. a heavy chain variable region having the polypeptide sequence of SEQ ID NO:185, and a light chain variable region having the polypeptide sequence of SEQ ID NO:193;

j. a heavy chain variable region having the polypeptide sequence of SEQ ID NO:185, and a light chain variable region having the polypeptide sequence of SEQ ID NO:198;

k. a heavy chain variable region having the polypeptide sequence of SEQ ID NO:187, and a light chain variable region having the polypeptide sequence of SEQ ID NO:194;

l. a heavy chain variable region having the polypeptide sequence of SEQ ID NO:188, and a light chain variable region having the polypeptide sequence of SEQ ID NO:194;

m. a heavy chain variable region having the polypeptide sequence of SEQ ID NO:188, and a light chain variable region having the polypeptide sequence of SEQ ID NO:196;

n. a heavy chain variable region having the polypeptide sequence of SEQ ID NO:188, and a light chain variable region having the polypeptide sequence of SEQ ID NO:197; or

o. a heavy chain variable region having the polypeptide sequence of SEQ ID NO:199, and a light chain variable region having the polypeptide sequence of SEQ ID NO:200.

[00173] Embodiment 8 is the isolated monoclonal antibody or antigen-binding fragment of any one of embodiments 1 to 7, wherein the antibody or antigen-binding fragment thereof is capable of blocking binding of CD47 to thrombospondin-1 (TSP1) and/or to signal regulatory protein alpha (SIRPα).

[00174] Embodiment 9 is the isolated monoclonal antibody or antigen-binding fragment of any one of embodiments 1 to 7, wherein the antibody or antigen-binding fragment thereof is capable of inducing macrophage-mediated phagocytosis of cancer cells.

[00175] Embodiment 10 is the isolated monoclonal antibody or antigen-binding fragment of any one of embodiments 1 to 7, wherein the antibody or antigen-binding fragment thereof is capable of binding cancer cells with minimal to undetectable binding to red blood cells.

[00176] Embodiment 11 is an isolated nucleic acid encoding the monoclonal antibody or antigen-binding fragment of any one of embodiments 1 to 10.

[00177] Embodiment 12 is a vector comprising the isolated nucleic acid of embodiment 11.

[00178] Embodiment 13 is a host cell comprising the vector of embodiment 12.

[00179] Embodiment 14 is a pharmaceutical composition, comprising the isolated monoclonal antibody or antigen-binding fragment of any one of embodiments 1 to 10 and a pharmaceutically acceptable carrier.

[00180] Embodiment 15 is a method of blocking binding of CD47 to thrombospondin-1 (TSP1) in a subject in need thereof, comprising administering to the subject the pharmaceutical composition of embodiment 14.

[00181] Embodiment 16 is a method of blocking binding of CD47 to signal regulatory protein alpha (SIRPα) in a subject in need thereof, comprising administering to the subject the pharmaceutical composition of embodiment 14.

[00182] Embodiment 17 is a method of treating cancer in a subject in need thereof, comprising administering to the subject the pharmaceutical composition of embodiment 14.

[00183] Embodiment 18 is a method of treating an inflammatory disease in a subject in need thereof, comprising administering to the subject the pharmaceutical composition of embodiment 14.

[00184] Embodiment 19 is a method of treating an infectious disease in a subject in need thereof, comprising administering to the subject the pharmaceutical composition of embodiment 14.

[00185] Embodiment 20 is a method of treating atherosclerosis in a subject in need thereof, comprising administering to the subject the pharmaceutical composition of embodiment 14.

[00186] Embodiment 21 is a method of treating a cardiovascular disease in a subject in need thereof, comprising administering to the subject the pharmaceutical composition of embodiment 14.

[00187] Embodiment 22 is a method of treating a metabolic disease in a subject in need thereof, comprising administering to the subject the pharmaceutical composition of embodiment 14.

[00188] Embodiment 23 is a method of treating a radiation-induced injury in a subject in need thereof, comprising administering to the subject the pharmaceutical composition of embodiment 14.

[00189] Embodiment 24 is a method of treating an autoimmune disease in a subject in need thereof, comprising administering to the subject the pharmaceutical composition of embodiment 14.

[00190] Embodiment 25 is a method of determining a level of CD47 in a subject, the method comprising (a) obtaining a sample from the subject; (b) contacting the sample with an antibody or antigen-binding fragment of any one of embodiments 1 to 10; and (c) determining a level of CD47 in the subject.

[00191] Embodiment 26 is the method of embodiment 25, wherein the sample is a tissue sample.

[00192] Embodiment 27 is the method of embodiment 26, wherein the tissue sample is a cancer tissue sample.

[00193] Embodiment 28 is the method of embodiment 25, wherein the sample is a blood sample.

[00194] Embodiment 29 is a method of producing the monoclonal antibody or antigen-binding fragment of any one of embodiments 1 to 10, comprising culturing a cell comprising a nucleic acid encoding the monoclonal antibody or antigen-binding fragment under conditions to produce the monoclonal antibody or antigen-binding fragment, and recovering the antibody or antigen-binding fragment from the cell or culture.

[00195] Embodiment 30 is a method of producing a pharmaceutical composition comprising the monoclonal antibody or antigen-binding fragment of any one of embodiments 1 to 10, comprising combining the monoclonal antibody or antigen-binding fragment with a pharmaceutically acceptable carrier to obtain the pharmaceutical composition.

[00194a] In this specification where reference has been made to patent specifications, other external documents, or other sources of information, this is generally for the purpose of providing a context for discussing the features of the invention. Unless specifically stated otherwise, reference to such external documents is not to be construed as an admission that such documents, or such sources of information, in any jurisdiction, are prior art, or form part of the common general knowledge in the art.

EXAMPLES

[00196] Example 1: Identification of anti-CD47 monoclonal antibodies

[00197] Anti-CD47 monoclonal antibodies (mAbs) were generated from mice immunized with recombinant human and cynomolgus CD47-HIS. Briefly, after the immunization, the titer of antibodies in the serum was estimated by ELISA using huCD47-HIS and cyCD47-HIS coated plates. B-cells were harvested and fused with a myeloma cell line to produce hybridomas. Hybridomas were plated into 20 x 384 well plates and the supernatants from each well were screened by ELISA for their binding towards both human and cynomolgus CD47. 400 hybridomas were expanded and were further analyzed for binding to RAJI cells by FACS, blocking CD47/SIRPα interaction, binding kinetics to recombinant huCD47 on an Octet, and tested for hemagglutination activity with human blood. Top positive hybridomas were then cloned by plating parental hybridomas at 1 cell per well in 384 well plates and screening clonal supernatants by ELISA for binding to huCD47. Heavy chain and light chain variable regions from clonal hybridomas were amplified by 5' RACE and sequenced. The supernatants of these clones from scale-up culture were used to purify antibodies with protein A for further characterization.

[00198] The sequences of heavy and light chain variable regions for anti-CD47 monoclonal antibodies are provided in Tables 1 and 2, respectively, and the CDR regions for the anti-CD47 monoclonal antibodies are provided in Tables 3 and 4. The CDR regions for the anti-CD47 monoclonal antibodies were determined utilizing the IMGT method.

[00199] Table 1: Sequences of heavy chain variable regions for anti-CD47 monoclonal antibodies (mAbs)

mAb clones	VH
9O23A	QIQLQQSGPELVRPGASVKISCKASGYTFTDYYINWVKQRPGQGLEWIGWIYPG SGNTKYNEKFKGKATLTVDSSSSTAYMQLSSLTSEDSAVYFCARRGPWYFDVW GAGTTVTVSS **(SEQ ID NO:1)**
14P6A	QIQLQQSGPELVRPGASVKISCKASGYTFTAYYINWVKQRPGQGLEWIGWIYPG SGNTKYNEKFKGKATLTVDTSSSTAYIQLSSLTSEDSAVYFCARRGPWYFDVWG AGTTVTVSS **(SEQ ID NO:3)**
4M8A	QVQLQQPGAELVKPGASVKLSCKTSGYTFTSYWIHWVNQRPGQGLEWIGNIDPS DSETHYNPKFKDKATLTVDKSSSTAYMQLSSLTSEDSAVYYCARWGGWLPLDY WGQGTTLTVSS **(SEQ ID NO:5)**
16M17A	QVQLQQPGAELVKPGASVKLSCKASGYTFTNYWMHWVKQRPGQGLEWIGNID PSDSETHYNQKFKDKATLTVDKSSSTAYMQLSSLTSEDSAVYYCARMAFITTVV

	DYWGQGTTLTVSS **(SEQ ID NO:7)**
13C4A	QVQLQQPGAELVKPGASVKLSCKASGYTFTSYWMHWVKQRPGQGLEWIGNID PSDSETHYNQKFKDKATLTVDKSSSTAYMQLSSLTSEDSAVYYCARWGYYGRS PLDHWGQGTTLTVSS **(SEQ ID NO:9)**
14O18A	QVQLQQPGAELVKPGASVKLSCKASGYTFTSYWMHWVKQRPGQGLEWIGNID PSDSETHYNQKFKDKATLTLDKSSSTAYMQLSSLTSEDSAVYYCARWYYGGSG AMDYWGQGTSVTVSS **(SEQ ID NO:11)**
5D24A	QVQLQQPGAELVKPGASVKLSCKASGYTFTSSWMHWVKQRPGQGLEWIGNIDP SDSETHYNQKFKDKATLTVDKSSSTAYMQLSSLTSEDSAVYYCARWGYYGKSA IDYWGQGTSVTVSS **(SEQ ID NO:13)**
11G2A	QVQLQQPGAELVKPGASVKLSCKASGYTFTSYWMHWVKQRPGQGLEWIGNID PSDSEAHYNQKFRDKATLTVDKSSSTAYMQLSSLTSEDSAVYYCARWGYYGKS AMDYWGQGTSVTVSS **(SEQ ID NO:15)**
13B18A	QVQLQQPGAELVKPGASVKLSCKASGYTFTSYWMHWVKQRPGQGLEWIGNID PSDSETHYNQKFKDKATLTVDKSSSTAYMQLSSLTSEDSAVYYCSRWGYYGKS AMDYWGQGTSVTVSS **(SEQ ID NO:17)**
1J7A	QVQLQQPGAELVKPGASVKLSCKASGYTFTSYWMHWVKQRPGQGLEWIGNID PSDSETHYNQKFRDKATLTVDKSSNTAYMQLSSLTSEDSAVYYCARWGLRGAM DYWGQGTSVTVS **(SEQ ID NO:19)**
14D18A	QVQLQQPGAELVKPGASVKLSCKASGYTFTSYWMHWVKQRPGQGLEWIGNID PSDSETHYNQKFKDKATLTVDKSSSTAYMQLSSLTSEDSAVYYCARWGYYGRS PLDHWGQGTTLTVSS **(SEQ ID NO:21)**
3O5A	EVKLVESGGGLVQSGRSLRLSCATSGFTFSDFYMEWVRQAPGKGLEWIAASRN KANDYTTEYSASVKGRFIVSRDTSQSILYLQMNALRAEDTAIYYCARDTAYWG QGTLVTVSA **(SEQ ID NO:23)**
17C6A	QVQLQQPGAELVKPGASVKLSCKASGYTFTSYWMHWVKQRPGQGLEWIGNID PSDSETHYNQKFKDKATLTVDKSSSTAHMQLSSLTSEDSAVYYCAGTDLAYWG QGTLVTVSA **(SEQ ID NO:25)**
14N13A	QVQLQQPGAELVKPGASVKLSCKASGYIFTSYWMHWVKQRPGQGLEWIGNIDP SDSETHYNQKFKDKATLTVDKSSSTAYMQLSSLTSEDSAVYYCAKGFSDYWGQ GTTLTVSS **(SEQ ID NO:27)**
10I23A	EVQLQQSGAELVRPGASVKLSCTASGFNIKDSLMHWVKQRPEQGLEWIGWIDPE DGETKCAPKFQDKATITADTSSNTAYLQLSSLTSEDTAIYYCAVISTVVAPDYWG QGTTLTVSS **(SEQ ID NO:29)**
12B18A	EVQLQQSGPELVKPGASVKISCKASGYSFTGYFMNWVKQSHGKSLEWIGRINPY NGDTFYNQKFKGKATLTVDKSSSTAHMELRSLTSEDSAIYYCARGGVVATDYW GQGTTLTVSS **(SEQ ID NO:31)**
17O12A	EVQLQQSGPELVKPGASVKISCKASGYSFTGYFMHWVKQSHGKSLEWIGRINPY NGDTFNNQKFKGKATLAVDKSSSTAHMELRSLTSEDSTVYYCARGGYAMDYW GQGTSVTVSS **(SEQ ID NO:33)**
15G23A	EVQLQQSGPELVKPGASVKMSCKASGYTFTNYVIHWVKQKPGQGLEWIGYINP YNDGTKYNEKFKGKATLTSDKSSSTAYMELSSLTSEDSAVYYCAKGGTGTGDY WGQGTTLTVSS **(SEQ ID NO:35)**
17N8A	EVKLEESGGGMVQPGGSMKVSCVASGFTFSNYWMNWVRQSPEKGLEWVAQIR LKSDNYATHYAESVKGRFTISRDDSKSSVYLQMNNLRAEDTGIYYCTGGGKGG FAYWGQGTLVTVSA **(SEQ ID NO:37)**
18M19A	EVQLQQSGPELVKPGASVKMSCKASGYTFTSYVMHWVKQKPGQGLEWIGYINP YNDGTKYNEKFKGKATLTSDKSSSTAYMELSSLTSEDSAVYYCAKGGYYAMD YWGQGTSVTVSS **(SEQ ID NO:39)**
11F6A	EVQLQQSGAELVRPGASVKLSCTASGFNIKDSLMHWVKQRPEQGLEWIGWIDPE DGETKCAPKFQDKATITADTSSNTAYLQLSSLTSEDTAIYYCARITTVVATDYWG QGTTLTVSS **(SEQ ID NO:41)**
19L14A	QVQLQQPGAELVKPGASVKLSCKASGYTFTSYWINWVKQRPGQGLEWIGNSNP GSSSTNYNEKFKSKAILTVDKSSSTAYMQLSSLTSDDSAVYYCAREGLRRFAYW GQGTLVTVSA **(SEQ ID NO:43)**

VH: heavy chain variable region

[00200] Table 2: Sequences of light chain variable regions for anti-CD47 mAbs

mAb clones	VL
9O23A	NIVMTQSPKSMSMSVGERVTLSCKASDNVGTYVSWYQQKPEQSPKLLIYGASN RYTGVPDRFTGSGSARDFTLTITSVQAEDLADYHCGQSYSYPLTFGAGTKLELK **(SEQ ID NO:2)**
14P6A	NIVMTQSPKSMSMSVGERVTLSCKASENVGTYVSWYQQKPEQSPNLLIYGASNR YTGVPDRFTGSGSATDFTLTISSVQAEDLADYHCGQTYSYPLTFGAGTKLELK **(SEQ ID NO:4)**
4M8A	DVQITQSPSYLAASPGETITINCRASKNISKYLAWFQEKPGKTNKLLIYSGSTLQS GIPSRFSGSGSGTDFTLTISRLEPEDFAMYYCQQHNEYPWTFGGGTKLEIK **(SEQ ID NO:6)**
16M17A	DVQITQSPSYLAASPGETITINCRASKSISKYLAWYQEKPGKTNKLLIYSGSTLQS GIPSRFSGSGSGTDFTLTISSLEPEDFAMYYCQQHNEYPWTFGGGTKLEIK **(SEQ ID NO:8)**
13C4A	DVQITQSPSYLAASPGETITINCRASKSISKYLAWYQEKPGKTNKLLIYSGSSLQS GIPSRFSGSGSGTDFTLTISSLEPEDFAMYYCQQHNEYPWTFGGGTKLEIK **(SEQ ID NO:10)**
14O18A	DVQITQSPSYLAASPGETITINCRASKSISKYLAWYQEKPGKTNKLLIYSGSTLQS GIPSRFSGSGSGTDFTLTISSLEPEDFAMYYCQQHNEYPWTFGGGTKLEIK **(SEQ ID NO:12)**
5D24A	DVQITQSPSYLAASPGETITINCRASKSISKYLAWYQEKPGKTNKLLIYSGSTLQS GIPSRFSGSGSGTDFTLTISSLEPEDFAMYYCQQHNEYPWTFGGGTKLEIK **(SEQ ID NO:14)**
11G2A	DVQITQSPSYLAASPGETITINCRASKSISKYLAWYQEKPGKTNKLLIYSGSTLQS GIPSRFSGSGSGTDFTLTISSLEPEDFAMYYCQQHNEYPWTFGGGTKLEIK **(SEQ ID NO:16)**
13B18A	AVQITQFPSYLAASPGQTITINCRASKSISKYLAWYQEKPGKTNKLLIYSGSTLQS GIPSRFSGSGSGTDFTLTISSLEPEDFAMYYCQQHNEYPWTFGGGTKLEIK **(SEQ ID NO:18)**
1J7A	DVQITQSPTYLTASPGETITINCRANKSISKYLAWYQEKPGKTNKLLIYSGSTLQS GIPSRFSGSGSGTDFTLTISSLEPEDFAMYYCQQHNEYPWTFGGGTKLEIK **(SEQ ID NO:20)**
14D18A	DVQITQSPSYLAASPGETITINCRASKSISKYLAWYQEKPGKTNKLLIYSGSTLQS GIPSRFSGSGSGTDFTLTISSLEPEDFAMYYCQQHNEYPWTFGGGTKLEIK **(SEQ ID NO:22)**
3O5A	DIVMTQAAPSVPVTPGESVSISCRSSKSLLHSNGNTYLYWFLQRPGQSPQLLIYR MSNLASGVPDRFSGSGSGTAFTLRISRVEAEDVGVYYCMQHLEYPFTFGSGTKL EIK **(SEQ ID NO:24)**
17C6A	DIQMNQSPSSLSASLGDTITITCHASQNINVWLSWYQQKPGNIPKLLIYKASNLHT GVPSRFSGSGSGTGFTLTISSLQPEDIATYYCQQGQSYPWTFGGGTKLEIK **(SEQ ID NO:26)**
14N13A	DIVMSQSPSSLAVSVGEKVTMSCKSSQSLLYSSNQKNYLAWYQQKPGQSPKVLI YWASTRESGVPDRFTGSGSGTDFTLTISSVKAEDLAVYYCQQYYSYPLTFGAGT KLELK **(SEQ ID NO:28)**
10I23A	DVVMTQTPLSLPVSLGVQASISCRSSQSLVHSNGNTYLHWYLQKPGQSPKLLIY KVSNRFSGVPDRFSGSGSGTDFTLKISRVEAEDLGVYFCSQSTHVPWTFGGGTKL EIK **(SEQ ID NO:30)**
12B18A	DVVMTQTPVSLPVSLGDQASISCRSSQSLVHSNGNTYLHWYLQRPGQSPKLLIY KVSNRFSGVPDRFSGSGSGTDFTLKISRVEAEDLGVYFCSQSTHVPFTFGSGTKLE IK **(SEQ ID NO:32)**
17O12A	DVVMTQTPLSLPVSLGDQASISCRSSQSLVHSNGNTYLYWYLQKPGQSPKLLIYR

	VSNRFSGVPDRFSGSGSGTDFTLKISRVEAEDLGVYFCFQSTHVPHTFGGGTKLEI K **(SEQ ID NO:34)**
15G23A	DVVMTQTPLSLPVSLGDQASISCRSSQSLVHSNGNTYLHWYLQKPGQSPKLLIY KVSNRFSGVPDRFSGSGSGTDFTLKISRVEAEDLGVYFCSQSTHVPPLTFGAGTK LELK **(SEQ ID NO:36)**
17N8A	DVVMTQTPLSLPVSLGDQASISCRSTQSLVHSNGNTYLHWYLQKPGQSPKLLIY KVSNRFSGVPDRFSGSGSGTDFTLKISRVEAEDLGVYFCSQSTHVPLTFGAGTKL ELK **(SEQ ID NO:38)**
18M19A	DVVMTQTPLSLPVSLGDQASISCRSSQSLVHSNGNTYLHWYLQKPGQSPKLLIY KVSNRFSGVPDRFSGSGSGTDFTLKISRVEAEDLGVYFCSQSTHVPWTFGGGTKL EIK **(SEQ ID NO:40)**
11F6A	DVVMTQTPLSLPVSLGDQASISCRSSQSLVHSNGNTYLHWYLQKPGQSPKLLIY KVSNRFSGVPDRFSGSGSGTDFTLKISRVEAEDLGVYFCSQSTHVPLTFGAGTKL ELK **(SEQ ID NO:42)**
19L14A	ENVLTQSPAIMSASPGEKVTMTCRASSSVSSSYLHWYQQKSGASPKLWIYSTSN LASGVPARFSGSGSGTSYSLTISSVEAEDAATYYCQQYSGYPFTFGSGTKLEIK **(SEQ ID NO:44)**

VL: light chain variable region

[00201] Table 3: CDR Regions 1-3 of heavy chain for anti-CD47 mAbs

mAb clones	**HC**		
	CDR1 (SEQ ID NO:)	**CDR2 (SEQ ID NO:)**	**CDR3 (SEQ ID NO:)**
9O23A	GYTFTDYY (45)	IYPGSGNT (46)	ARRGPWYFDV (47)
14P6A	GYTFTAYY (48)	IYPGSGNT (49)	ARRGPWYFDV (50)
4M8A	GYTFTSYW (51)	IDPSDSET (52)	ARWGGWLPLDY (53)
16M17A	GYTFTNYW (54)	IDPSDSET (55)	ARMAFITTVVDY (56)
13C4A	GYTFTSYW (57)	IDPSDSET (58)	ARWGYYGRSPLDH (59)
14O18A	GYTFTSYW (60)	IDPSDSET (61)	ARWYYGGSGAMDY (62)
5D24A	GYTFTSSW (63)	IDPSDSET (64)	ARWGYYGKSAIDY (65)
11G2A	GYTFTSYW (66)	IDPSDSEA (67)	ARWGYYGKSAMDY (68)
13B18A	GYTFTSYW (69)	IDPSDSET (70)	SRWGYYGKSAMDY (71)
1J7A	GYTFTSYW (72)	IDPSDSET (73)	ARWGLRGAMDY (74)
14D18A	GYTFTSYW (75)	IDPSDSET (76)	ARWGYYGRSPLDH (77)
3O5A	GFTFSDFY (78)	SRNKANDYTT (79)	ARDTAY (80)
17C6A	GYTFTSYW (81)	IDPSDSET (82)	AGTDLAY (83)
14N13A	GYIFTSYW (84)	IDPSDSET (85)	AKGFSDY (86)
10I23A	GFNIKDSL (87)	IDPEDGET (88)	AVISTVVAPDY (89)
12B18A	GYSFTGYF (90)	INPYNGDT (91)	ARGGVVATDY (92)
17O12A	GYSFTGYF (93)	INPYNGDT (94)	ARGGYAMDY (95)
15G23A	GYTFTNYV (96)	INPYNDGT (97)	AKGGTGTGDY (98)
17N8A	GFTFSNYW (99)	IRLKSDNYAT (100)	TGGGKGGFAY (101)
18M19A	GYTFTSYV (102)	INPYNDGT (103)	AKGGYYAMDY (104)
11F6A	GFNIKDSL (105)	IDPEDGET (106)	ARITTVVATDY (107)
19L14A	GYTFTSYW (108)	SNPGSSST (109)	AREGLRRFAY (110)

HC: heavy chain; CDR: complementarity determining region

The HC CDRs for the anti-CD47 mAbs were determined utilizing the IMGT method (Lefranc, M.-P. et al., Nucleic Acids Res. 1999; 27:209-212).

[00202] Table 4: CDR regions 1-3 of light chain for anti-CD47 mAbs

mAb clones	LC		
	CDR1 (SEQ ID NO:)	CDR2 (SEQ ID NO:)	CDR3 (SEQ ID NO:)
9O23A	DNVGTY (111)	GAS (112)	GQSYSYPLT (113)
14P6A	ENVGTY (114)	GAS (115)	GQTYSYPLT (116)
4M8A	KNISKY (117)	SGS (118)	QQHNEYPWT (119)
16M17A	KSISKY (120)	SGS (121)	QQHNEYPWT (122)
13C4A	KSISKY (123)	SGS (124)	QQHNEYPWT (125)
14O18A	KSISKY (126)	SGS (127)	QQHNEYPWT (128)
5D24A	KSISKY (129)	SGS (130)	QQHNEYPWT (131)
11G2A	KSISKY (132)	SGS (133)	QQHNEYPWT (134)
13B18A	KSISKY (135)	SGS (136)	QQHNEYPWT (137)
1J7A	KSISKY (138)	SGS (139)	QQHNEYPWT (140)
14D18A	KSISKY (141)	SGS (142)	QQHNEYPWT (143)
3O5A	KSLLHSNGNTY (144)	RMS (145)	MQHLEYPFT (146)
17C6A	QNINVW (147)	KAS (148)	QQGQSYPWT (149)
14N13A	QSLLYSSNQKNY (150)	WAS (151)	QQYYSYPLT (152)
10I23A	QSLVHSNGNTY (153)	KVS (154)	SQSTHVPWT (155)
12B18A	QSLVHSNGNTY (156)	KVS (157)	SQSTHVPFT (158)
17O12A	QSLVHSNGNTY (159)	RVS (160)	FQSTHVPHT (161)
15G23A	QSLVHSNGNTY (162)	KVS (163)	SQSTHVPPLT (164)
17N8A	QSLVHSNGNTY (165)	KVS (166)	SQSTHVPLT (167)
18M19A	QSLVHSNGNTY (168)	KVS (169)	SQSTHVPWT (170)
11F6A	QSLVHSNGNTY (171)	KVS (172)	SQSTHVPLT (173)
19L14A	SSVSSSY (174)	STS (175)	QQYSGYPFT (176)

LC: light chain; CDR: complementarity determining region

The LC CDRs for the anti-CD47 mAbs were determined utilizing the IMGT method (Lefranc, M.-P. et al., Nucleic Acids Res. 1999; 27:209-212).

[00203] Example 2: Detection of the binding of CD47 mAbs to RAJI cells using FACS

[00204] Anti-CD47 mAbs were analyzed by flow cytometry for their ability to bind cell surface CD47. RAJI (ATCC#CCL-86) cells (20,000 cells) cultured in Hanks' Balanced Salt Solution (HBSS) were incubated with either a solution of purified mAb (1 μg/ml) in HBSS or in HBSS alone. Using AlexaFluor488-conjugated anti-mouse IgG secondary Ab, the presence of mouse anti-CD47 mAbs on RAJI cells were measured by FACS (IntelliCyt iQue® Screener; Albuquerque, NM). Results of the FACS binding analysis of the anti-CD47 mAbs are provided in FIG. 1.

[00205] Example 3: Assessment of CD47 mAbs for their ability to block the CD47/SIRPα interaction

[00206] The activity of hybridoma supernatants or purified anti-CD47 mAbs in blocking SIRPα/CD47 interaction was measured by an ELISA assay. Recombinant human CD47(ECD)-HIS (1 μg/ml) was immobilized on a 384-well ELISA plate. Binding of recombinant huSIRPα-huFc-Biotin (0.5 μg/ml final concentration) was evaluated in the presence of increasing amounts of mouse anti-CD47 mAbs in triplicate. Bound SIRPα was determined using an HRP-conjugated streptavidin secondary antibody (Thermo Fisher Scientific; Waltham, MA). Wash steps using PBS supplemented with 0.1% Tween-20 were performed after the addition of CD47, blocking solution, SIRPα protein, secondary antibody, and detection reagents. Results of the ELISA assays are provided in FIGS. 2A-2O. For the 18M19A chimeric (on IgG4 and kapa backbone) antibody (FIG. 2P), blocking activity was measured on a 96-well ELISA plate. Recombinant human CD47(ECD)-HIS (1μg/mL) was immobilized on a plate. Binding of recombinant huSIRPα-muFc (0.5 μg/mL final concentration) was measured in the presence of increasing amounts of human anti-CD47 mAb in duplicate. Bound SIRPα was measured using an HRP-conjugated anti-muFc secondary antibody (Jackson ImmunoResearch; West Grove, PA). Plates were washed as described above. Detection was performed with TMB detection substrate (Thermo Fisher Scientific; Waltham, MA). Result of the ELISA assay is provided in FIG. 2P.

[00207] Example 4: Assessing the potential of anti-CD47 mAbs for inducing hemagglutination

[00208] To evaluate the hemagglutinating capacity of anti-CD47 mAbs, purified mAbs, at two-fold serial dilutions, were added in a 96-well clear and round-bottomed plate and incubated with a 2.5% human red blood cell (huRBC) suspension in PBS (0.25% RBC final concentration) at room temperature for 1 hour. Lack of hemagglutination was evidenced by the presence of punctate dots in the center of round-bottomed plates. Evidence of hemagglutination was demonstrated by the presence of a uniform color across the well. Results for the hemagglutination assay are provided in FIGS. 3A-3B.

[00209] Example 5: Assessment of *in vivo* efficacy of chimeric 13B18A in RAJI cell xenograft model

[00210] A chimeric version of 13B18A antibody (13B18A-huIgG1) was constructed by fusing the variable regions (VH and VL) of 13B18A to the constant regions of human IgG1 heavy chain and kappa light chain, respectively. The resulting antibody was stably expressed in CHO stable pools and purified by Protein A affinity column. To test the efficacy of 13B18A-huIgG1, RAJI cells were inoculated subcutaneously at the right flank of each NOD/SCID mouse (female, n=10/group). Mice were treated with indicated doses of test articles when mean tumor size reached 100 mm^3. Doses were given intravenously 3 times per week from day 6 to day 27, and tumor volumes were measured on the same day in two dimensions using a caliper. For pharmacokinetic (PK) analysis, serum was collected 48 hours after final dose and serum levels of 13B18A-huIgG1 were measured by detecting human Fc bound on ELISA plates coated with recombinant human CD47(ECD)-HIS. A standard curve was constructed using serum spiked with a standard of 13B18A-huIgG1 of known concentration. Following washing with PBS supplemented with 0.1% Tween-20, bound antibody was detected by HRP-conjugated anti-huFc secondary antibody (Jackson ImmunoResearch, West Grove, PA) followed by TMB detection substrate (Thermo Fisher Scientific; Waltham, MA). Tumor growth curves are shown in FIG. 4A. Data of body weight are shown in FIG. 4B. The pharmacokinetics (PK) data is shown in FIG. 4C.

[00211] Example 6: Humanization of anti-CD47 mAbs

[00212] The mouse anti-CD47 mAbs 13B18A, 14P6A and 17C6A were humanized to reduce the potential of immunogenicity when used in human patients. The sequences of the variable regions of the heavy and light chains (VH and VL) were compared with the human antibody sequences in the Protein Data Bank (PDB) database and homology models were built. The CDRs in both the heavy and light chains of the mouse mAbs were grafted into human frameworks that have the highest possibility of maintaining the proper structure likely required for antigen binding. Backmutations from human residues to mouse residue or other mutations were designed when necessary. The sequences of the humanized VH and VL regions are shown in Table 5 and Table 6, respectively. The humanized VH and VL regions were fused to the constant regions of human IgG4 heavy chain and kappa light chain, respectively. Constructs corresponding to the mAb

sequences were used for transient transfection in 293E cells and purified mAbs were analyzed for their ability to block the SIRPα/CD47 interaction using ELISA. Results are shown as absorbance wherein higher absorbance indicates higher level of SIRPα/CD47 interaction. The IC_{50} values for humanized mAbs are provided in Table 7. The IC_{50} curves for humanized mAbs H3L9 and H5L5 are shown in FIGS. 5A-5B. Results for the hemagglutination assay are provided in FIG. 6. The CDR regions for the humanized mAb H8L10 are provided in Table 8.

[00213] Table 5: Sequences of heavy chain variable regions of humanized anti-CD47 mAbs

Design	**VH**	**SEQ ID NO:**
H1	QVQLVQSGAEVKKPGASVKVSCKASGYTFTSYWMHWVRQAPGQGLEW MGNIDPSDSETHYNQKFKDRVTLTVDTSTSTVYMELSSLRSEDTAVYYCSR WGYYGKSAMDYWGQGTLVTVSS	183
H2	QVQLVQSGAEVKKPGASVKLSCKASGYTFTSYWMHWVRQAPGQGLEWM GNIDPSDSETHYNQKFKDRVTLTVDTSTSTAYMELSSLRSEDTAVYYCSR WGYYGKSAMDYWGQGTLVTVSS	184
H3	QVQLVQSGAEVKKPGASVKLSCKASGYTFTSYWMHWVRQAPGQGLEWI GNIDPSDSETHYNQKFKDRATLTVDTSTSTAYMELSSLRSEDTAVYYCSR WGYYGKSAMDYWGQGTLVTVSS	185
H4	QVQLVQSGAEVKKPGASVKLSCKASGYTFTSYWMHWVRQRPGQGLEWI GNIDPSDSETHYNQKFKDRATLTVDTSTSTAYMELSSLRSEDTAVYYCSR WGYYGKSAMDYWGQGTLVTVSS	186
H5	QIQLVQSGAEVKKPGASVKVSCKASGYTFTAYYINWVRQAPGQRLEWIG WIYPGSGNTKYNEKFKGRVTLTVDTSASTAYIELSSLRSEDTAVYYCARRG PWYFDVWGQGTTVTVSS	187
H6	QIQLVQSGAEVKKPGASVKVSCKASGYTFTAYYINWVRQAPGQRLEWIG WIYPGSGNTKYNEKFKGRVTLTVDTSASTAYIELSSLRSEDTAVYFCARRG PWYFDVWGQGTTVTVSS	188
H7	QIQLVQSGAEVKKPGASVKISCKASGYTFTAYYINWVRQAPGQGLEWIGW IYPGSGNTKYNEKFKGRATLTVDTSASTAYIELSSLRSEDTAVYFCARRGP WYFDVWGQGTTVTVSS	189
H8	QVQLVQSGAEVKKPGASVKVSCKASGYTFTSYWMHWVRQAPGQGLEWI GNIDPSDSETHYAQKFQGRVTLTVDKSTSTVYMELSSLRSEDTAVYYCAG TDLAYWGQGTLVTVSS	199

[00214] Table 6: Sequences of light chain variable regions of humanized anti-CD47 mAbs

Design	**VL**	**SEQ ID NO:**
L1	AVQLTQSPSFLSASVGDRVTITCRASKSISKYLAWYQQKPGKANKLLIYSG STLQSGVPSRFSGSGSGTEFTLTISSLQPEDFAMYYCQQHNEYPWTFGGGT	190

	KVEIK	
L2	AVQLTQSPSFLSASVGQRITINCRASKSISKYLAWYQQKPGKANKLLIYSGS TLQSGVPSRFSGSGSGTEFTLTISSLQPEDFAMYYCQQHNEYPWTFGGGTK VEIK	191
L3	AVQLTQSPSFLSASVGQRITINCRASKSISKYLAWYQEKPGKANKLLIYSGS TLQSGIPSRFSGSGSGTDFTLTISSLQPEDFAMYYCQQHNEYPWTFGGGTK VEIK	192
L4	AVQITQSPSFLSASVGQTITINCRASKSISKYLAWYQEKPGKANKLLIYSGST LQSGIPSRFSGSGSGTDFTLTISSLEPEDFAMYYCQQHNEYPWTFGGGTKVE IK	193
L5	EIVMTQSPATLSLSPGERATLSCRASENVGTYVSWYQQKPGQAPNLLIYGA SNRYTGIPARFSGSGSGTDFTLTISSLQPEDFAVYHCGQTYSYPLTFGQGTK LEIK	194
L6	EIVMTQSPATLSLSPGERATLSCRASENVGTYVSWYQQKPGQSPNLLIYGA SNRYTGIPDRFSGSGSGTDFTLTISSLQPEDFAVYHCGQTYSYPLTFGQGTK LEIK	195
L7	EIVMTQSPATLSLSPGERATLSCKASENVGTYVSWYQQKPGQSPNLLIYGA SNRYTGIPDRFSGSGSGTDFTLTISSLQPEDFAVYHCGQTYSYPLTFGQGTK LEIK	196
L8	NIVMTQSPATLSLSPGERATLSCKASENVGTYVSWYQQKPGQSPNLLIYGA SNRYTGVPDRFSGSGSATDFTLTISSLQPEDFADYHCGQTYSYPLTFGQGTK LEIK	197
L9	DVQLTQSPSFLSASVGDRVTITCRASKSISKYLAWYQQKPGKANKLLIYSG STLQSGVPSRFSGSGSGTEFTLTISSLQPEDFAMYYCQQHNEYPWTFGGGT KVEIK	198
L10	EIVMTQSPGTLSLSPGERATLSCHASQNINVWLSWYQQKPGQAPRLLIYKA SNLHTGIPDRFSGSGSGTDFTLTISRLEPEDFAVYYCQQGQSYPFTFGQGTK VEIK	200

[00215] Table 7: IC_{50} values for anti-CD47 mAbs in SIRPα/CD47 interaction assay

mAb ID	**IC50 (nM)**
H1L2	6.80
H1L3	7.43
H1L4	8.09
H2L1	4.73
H2L3	9.56
H2L4	6.52
H3L1	5.62
H3L2	7.88
H3L4	5.35
H3L9	9.60
H5L5	15.06
H6L5	9.67
H6L7	39.16
H6L8	13.20
H8L10	1.05

H1L2 refers to the mAb with the H1 heavy chain variable region and the L2 light chain variable region; all the other humanized mAbs in the table adopt the same naming rule.

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[00216] Table 8: CDR regions 1-3 of heavy and light chains for humanized mAb H8L10

	CDR1 (SEQ ID NO:)	CDR2 (SEQ ID NO:)	CDR3 (SEQ ID NO:)
HC	GYTFTSYW (201)	IDPSDSET (202)	AGTDLAY (203)
LC	QNINVW (204)	KAS (205)	QQGQSYPFT (206)

[00217] Example 7: Red blood cell (RBC) and RAJI cell binding assays

[00218] Anti-CD47 mAbs H3L9, H5L5 and H8L10 were analyzed by flow cytometry for their ability to bind cell surface CD47. Purified mAbs were serially diluted (1:3) into FACS buffer (Hanks' Balanced Salt Solution (HBSS) containing 0.1% BSA and 0.05% sodium azide). The top concentration of purified mAb was 190 μg/ml. Red blood cells or RAJI cells (ATCC#CCL-86) (14,000 cells) were pelleted in U-bottom plates by centrifugation at 600 RPM (62 x g) for 5 minutes. Cells were resuspended into 20 μL of purified antibodies in FACS buffer and incubated for 30 minutes at room temperature. After incubation, cells were washed three times by FACS buffer. Using PE/Cy7-conjugated anti-human IgG (Biolegend, Cat #409316) secondary Ab, the presence of anti-CD47 mAbs on red blood cells and RAJI cells was measured by FACS (Attune NxT Flow Cytometer; Carlsbad, CA). Results of the FACS binding analysis of the anti-CD47 mAbs are provided in FIGS. 7A-7B.

[00219] Example 8: Cell-based SIRPα binding assay

[00220] RAJI cells were cultured in RPMI+10% FBS. Human SIRPα (ECD)-mFc (huSIRPα-muFc) protein (human SIRPα ECD fused to mouse Fc) at 30 nM final concentration was incubated with purified humanized anti-CD47 mAbs at 30, 90 and 300 nM. The mixture was then added to 14,000 RAJI cells in a 96-well round bottom plate, mixed and incubated on the nutator for 30 minutes at room temperature. Cells were then centrifuged at 600 rpm for 5 minutes and washed with FACS buffer (HBSS supplemented with 0.1% BSA and 0.05% Sodium Azide) three times. The cells were then incubated with FITC-conjugated donkey anti-mouse Fc polyclonal antibodies (Jackson ImmunoResearch, Cat: 715-095-150) on the nutator for 15 minutes at room temperature, washed with FACS buffer three times and then resuspended in FACS buffer. Cells were then run through the Attune NxT instrument and the data were analyzed by the Attune NxT software. Results

of humanized anti-CD47 mAbs H3L9, H5L5 and H8L10 in blocking the binding of huSIRPα-muFc to RAJI cells are shown in FIG. 8.

[00221] Example 9: Macrophage-mediated phagocytosis assay

[00222] Human monocytes were induced for 6 days in AIM-V media (Thermo Fisher, Cat: 12055091) containing 50 ng/ml GM-CSF (Shenandoah, Cat: 100-08-20ug). Macrophages were then polarized with 100 ng/ml INF-gamma (Shenandoah, Cat: 100-77-100ug) for an additional 2 days. M1 macrophages were defined as CD14+, CD80+, CD163- and CD206+ population. After detached from the tissue culture plates, macrophages were washed once with RPMI-1640 containing 10% FBS and then twice with ice-cold HBSS. The cell number was adjusted to 2 x 10^6 cells/mL in AIM-V media. 25 μL of test mAbs and 25 μL of the macrophage cell suspension (50,000 cells) were added to 50 μL of RAJI cells (100,000 cells) labeled with CFSE (Thermo Fisher, Cat: 34570) in each well of a 96-well plate and incubated for 2 hours at 37°C. The final concentration of mAb was 10 ug/ml. After co-culture, the cell mixtures were stained with PE-Cy7 conjugated anti-human CD14 mAb (Biolegend, Cat: 367111). Following staining, cells were analyzed by flow cytometry. The percentage of both CFSE and PE-Cy7 positive macrophages in the population of PE-Cy7 positive macrophages is presented as phagocytosis. Results of humanized anti-CD47 mAbs H3L9, H5L5 and H8L10 in inducing macrophage-mediated phagocytosis of RAJI cells are shown in FIG. 9.

[00223] It will be appreciated by those skilled in the art that changes could be made to the embodiments described above without departing from the broad inventive concept thereof. It is understood, therefore, that this invention is not limited to the particular embodiments disclosed, but it is intended to cover modifications within the spirit and scope of the present invention as defined by the present description.

WE CLAIM;

1. An isolated monoclonal antibody or antigen-binding fragment thereof comprising a heavy chain complementarity determining region 1 (HCDR1), HCDR2, HCDR3, a light chain complementarity determining region 1 (LCDR1), LCDR2, and LCDR3, having the polypeptide sequence of:

(1) SEQ ID NOs:201, 202, 203, 204, 205, and 206, respectively;

wherein the antibody or antigen-binding fragment thereof specifically binds CD47, preferably human CD47.

2. The isolated monoclonal antibody or antigen-binding fragment of claim 1, comprising a heavy chain variable region having a polypeptide sequence at least 95% identical to SEQ ID NO: 199, or a light chain variable region having a polypeptide sequence at least 95% identical to SEQ ID NO: 200.

3. The isolated monoclonal antibody or antigen-binding fragment thereof of claim 1 or 2, comprising:

a. a heavy chain variable region having the polypeptide sequence of SEQ ID NO:199, and a light chain variable region having the polypeptide sequence of SEQ ID NO:200.

4. The isolated monoclonal antibody or antigen-binding fragment thereof of any one of claims 1 to 3, wherein the antibody or antigen-binding fragment thereof is chimeric and/or human or humanized.

5. The isolated monoclonal antibody or antigen-binding fragment of any one of claims 1-4, wherein the antibody or antigen-binding fragment thereof is capable of blocking binding of CD47 to signal regulatory protein alpha (SIRPα), inducing macrophage-mediated phagocytosis of cancer cells, and/or binding cancer cells with minimal to undetectable binding to red blood cells.

6. A bispecific antibody comprising the monoclonal antibody or antigen-binding fragment thereof of any one of claims 1-5.

7. An isolated nucleic acid encoding the monoclonal antibody or antigen-binding fragment thereof of any of claims 1-5.

8. A vector comprising the isolated nucleic acid of claim 7.

9. An isolated host cell comprising the vector of claim 8.

10. A pharmaceutical composition, comprising the isolated monoclonal antibody or antigen-binding fragment thereof of any one of claims 1-5 and a pharmaceutically acceptable carrier.

11. A method of blocking binding of CD47 to signal regulatory protein α (SIRPα) comprising administering to the subject the pharmaceutical composition of claim 10.

12. A method of treating a CD47 expressing disease and/or condition selected from the group consisting of a cancer, an inflammatory disease, an infectious disease, atherosclerosis, a cardiovascular disease, a metabolic disease, a radiation-induced injury, and an autoimmune disease in a subject in need thereof, comprising administering to the subject the pharmaceutical composition of claim 10.

13. A method of determining a level of CD47 in a subject, the method comprising:

a. obtaining a sample from the subject;

b. contacting the sample with an antibody or antigen-binding fragment thereof of any one of claims 1-5;

c. determining a level of CD47 in the subject.

14. The method of claim 13, wherein the sample is a tissue sample or a blood sample, optionally wherein the tissue sample is a cancer tissue sample.

15. A method of producing the monoclonal antibody or antigen-binding fragment thereof of any one of claims 1-5, comprising culturing a cell comprising a nucleic acid encoding the monoclonal antibody or antigen-binding fragment thereof under conditions to produce the monoclonal antibody or antigen-binding fragment thereof, and recovering the antibody or antigen-binding fragment thereof from the cell or culture.

16. A method of producing a pharmaceutical composition comprising the monoclonal antibody or antigen-binding fragment thereof of any one of claims 1-5, comprising combining the monoclonal antibody or antigen-binding fragment thereof with a pharmaceutically acceptable carrier to obtain the pharmaceutical composition.

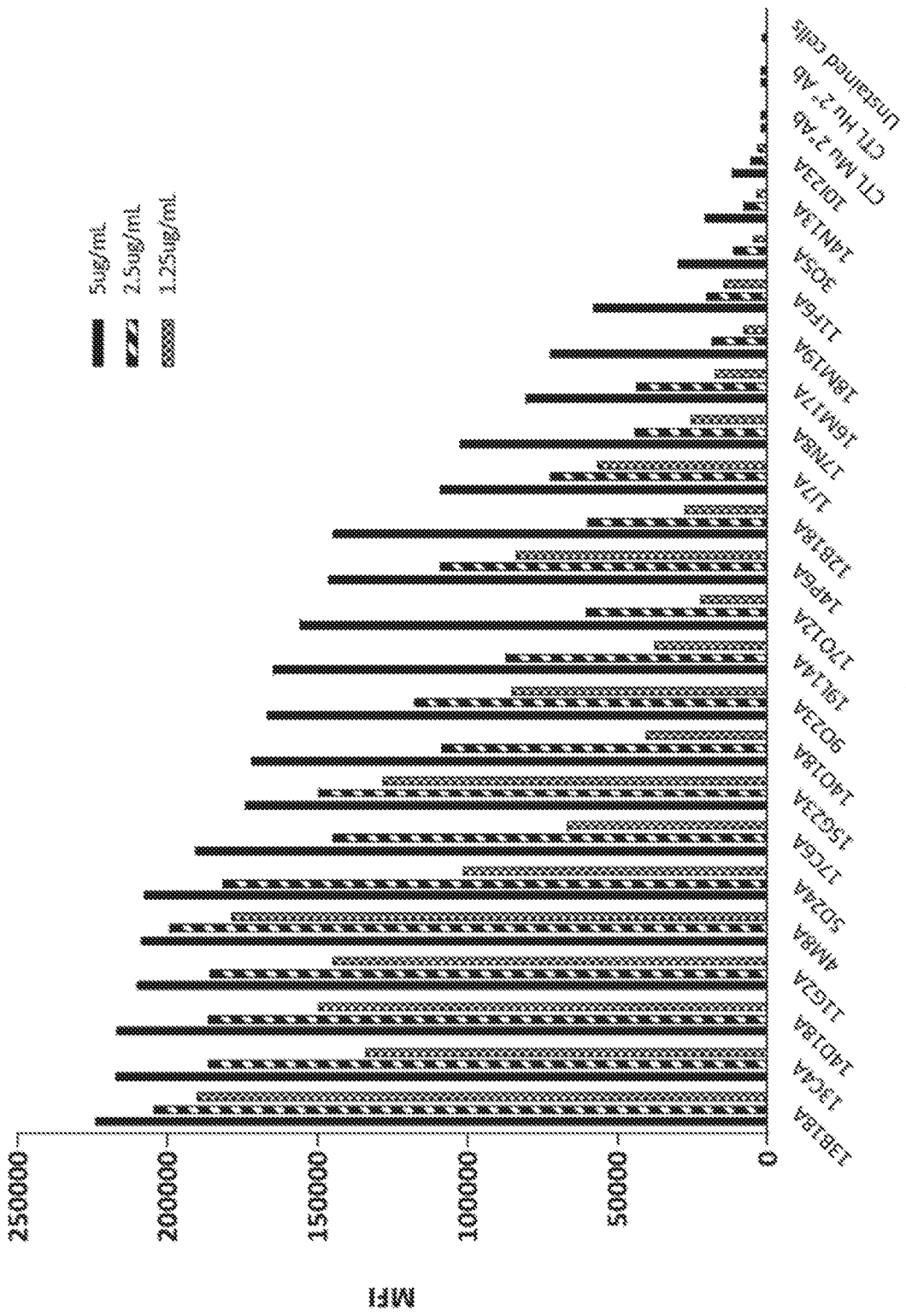
5ug/mL
2.5ug/mL
1.25ug/mL
MFI
250000
200000
150000
100000
50000
0
13B18A
13C4A
14D1A
14D18A
11EG2A
4M8A
5D2AA
17C6A
15G23A
14D18A
9D23A
19C14A
17D12A
14F6A
12B13A
LI7A
17N8A
16M17A
18M19A
11E6A
3D5A
14M13A
10D23A
CTL Mu 2° Ab
CTL Mu 2° Ab
Unstained cells

FIG. 1

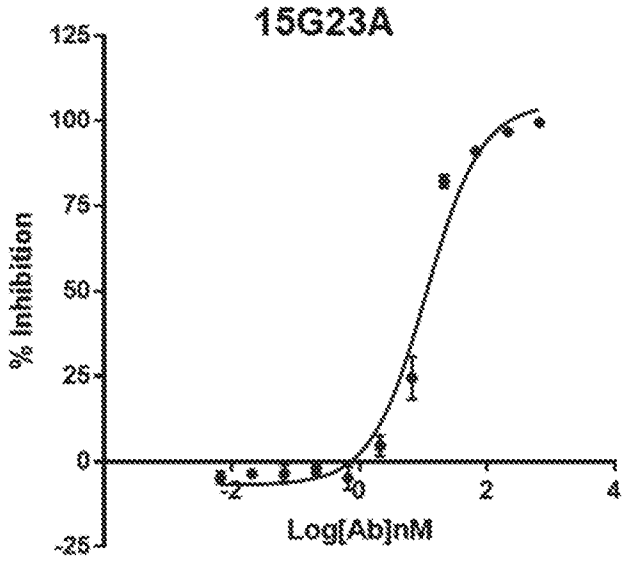
15G23A
% Inhibition
125
100
75
50
25
0
-25
-2
0
2
4
Log[Ab]nM

FIG. 2A

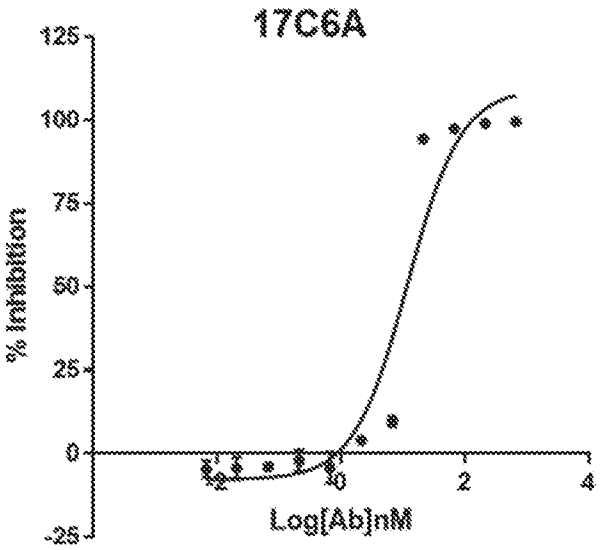
17C6A
% Inhibition
125
100
75
50
25
0
-25
-2
0
2
4
Log[Ab]nM

FIG. 2B

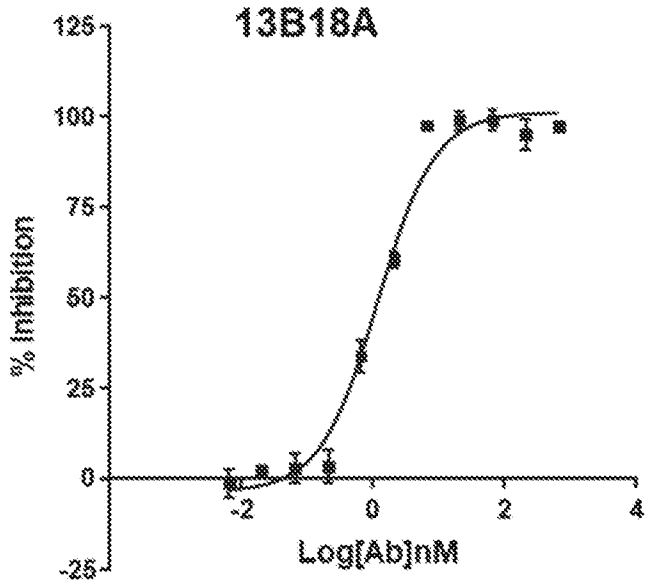
13B18A
% Inhibition
125
100
75
50
25
0
-25
-2
0
2
4
Log[Ab]nM

FIG. 2C

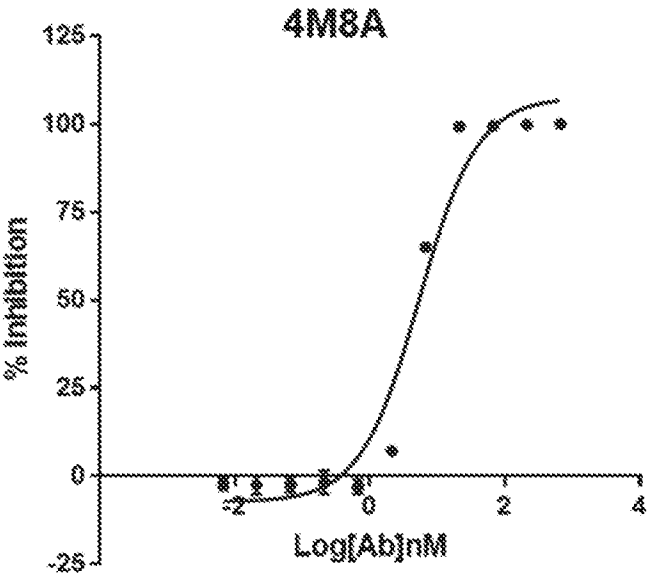
4M8A
% Inhibition
125
100
75
50
25
0
-25
-2
0
2
4
Log[Ab]nM

FIG. 2D

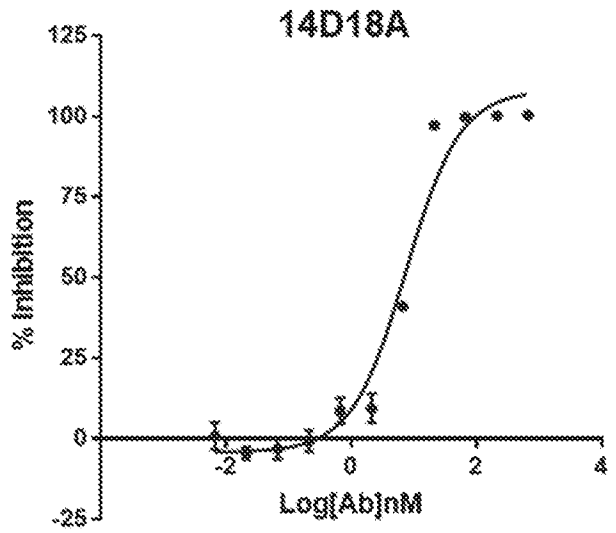
14D18A
% Inhibition
125
100
75
50
25
0
-25
-2
0
2
4
Log[Ab]nM

FIG. 2E

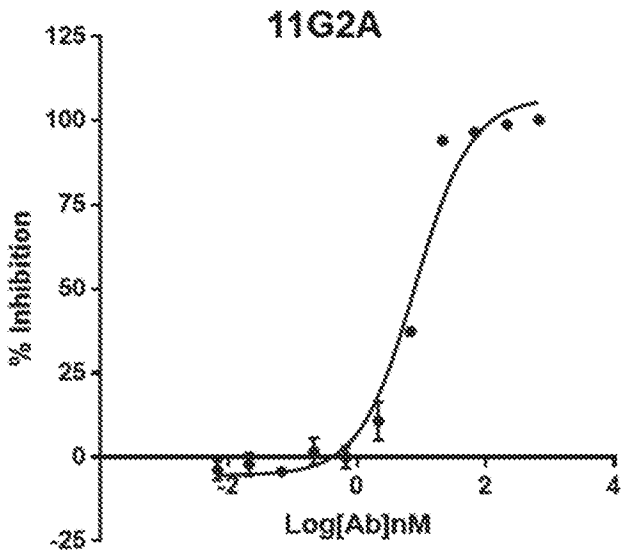
11G2A
% Inhibition
125
100
75
50
25
0
-25
-2
0
2
4
Log[Ab]nM

FIG. 2F

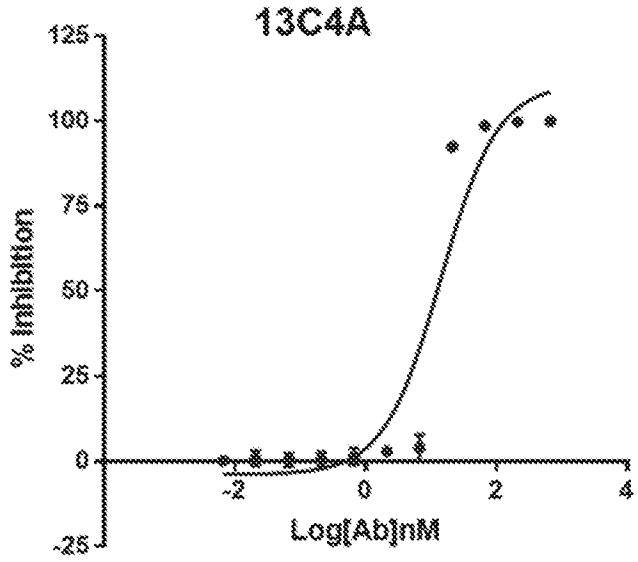
13C4A
% Inhibition
125
100
75
50
25
0
-25
-2
0
2
4
Log[Ab]nM

FIG. 2G

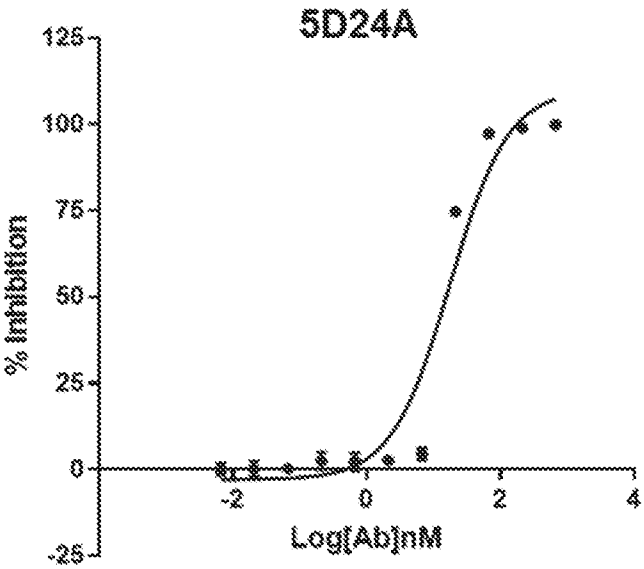
5D24A
% Inhibition
125
100
75
50
25
0
-25
-2
0
2
4
Log[Ab]nM

FIG. 2H

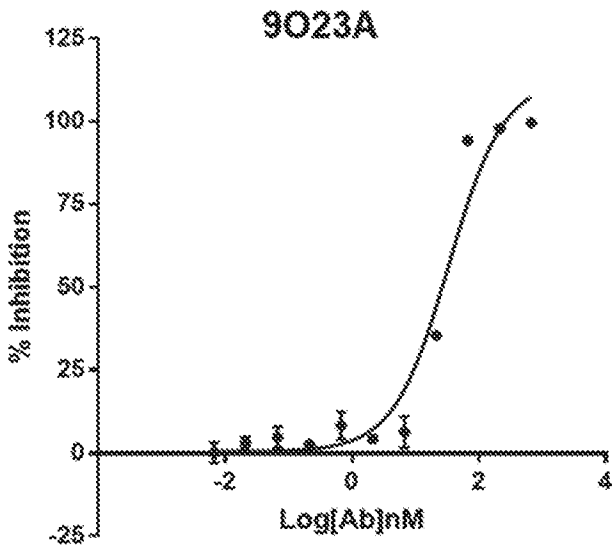
9O23A
% Inhibition
125
100
75
50
25
0
-25
-2
0
2
4
Log[Ab]nM

FIG. 2I

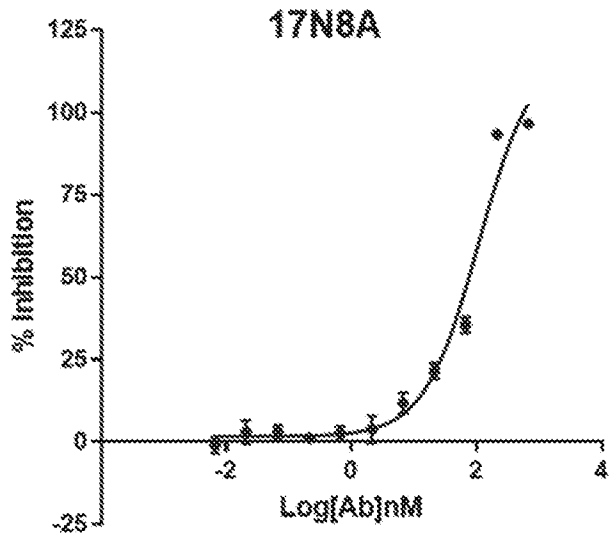
17N8A
% Inhibition
125
100
75
50
25
0
-25
-2
0
2
4
Log[Ab]nM

FIG. 2J

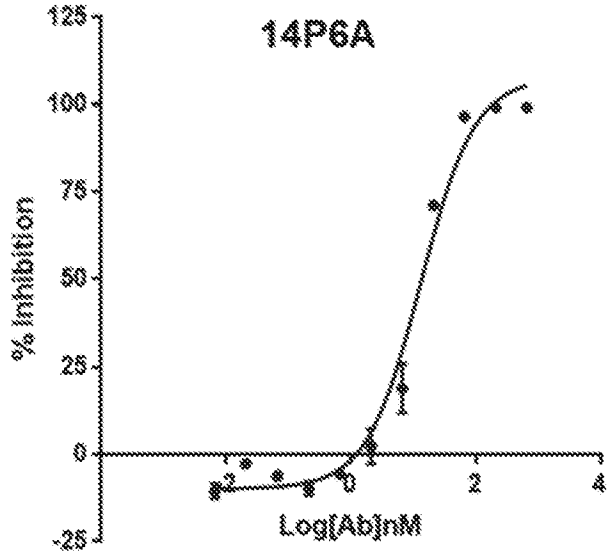
14P6A
% Inhibition
125
100
75
50
25
0
-25
-2
0
2
4
Log[Ab]nM

FIG. 2K

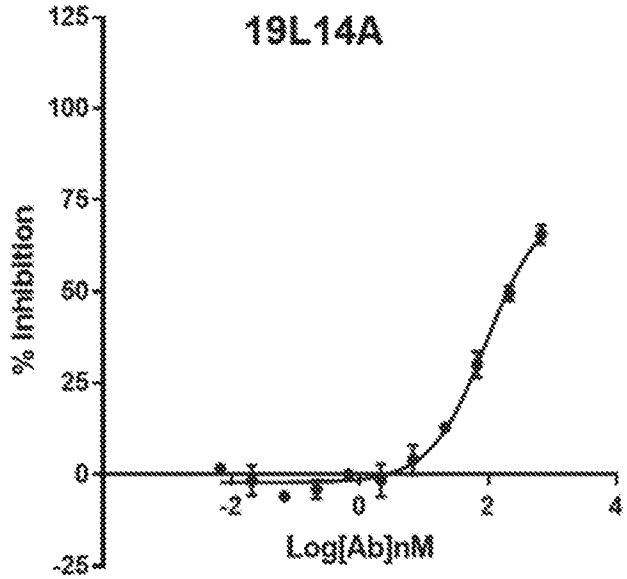
19L14A
% Inhibition
125
100
75
50
25
0
-25
-2
0
2
4
Log[Ab]nM

FIG. 2L

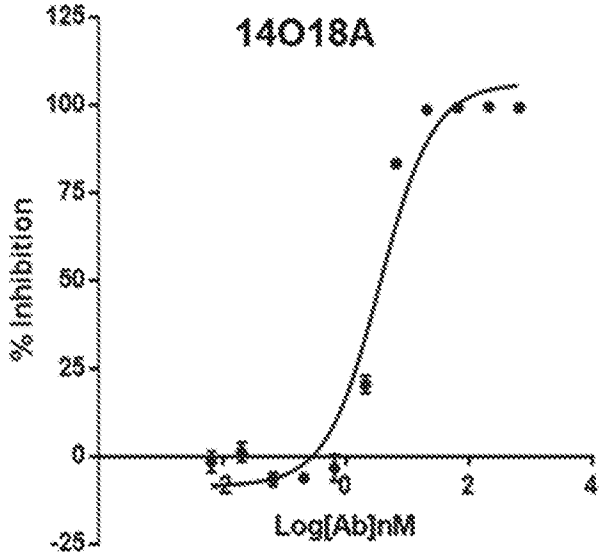
14O18A
% Inhibition
125
100
75
50
25
0
-25
-2
0
2
4
Log[Ab]nM

FIG. 2M

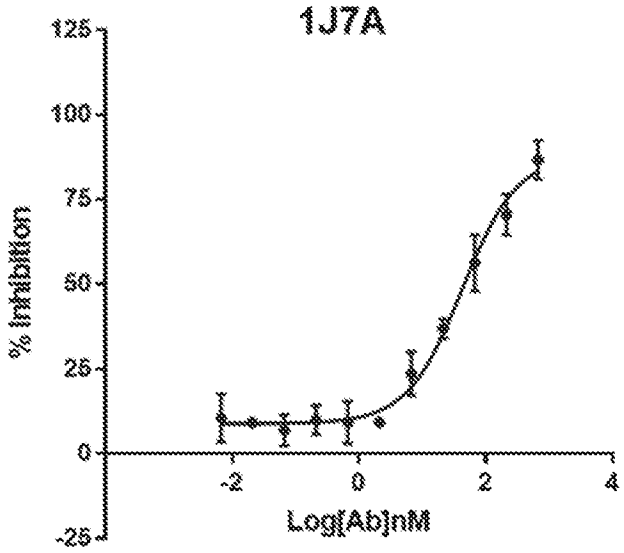
1J7A
% Inhibition
125
100
75
50
25
0
-25
-2
0
2
4
Log[Ab]nM

FIG. 2N

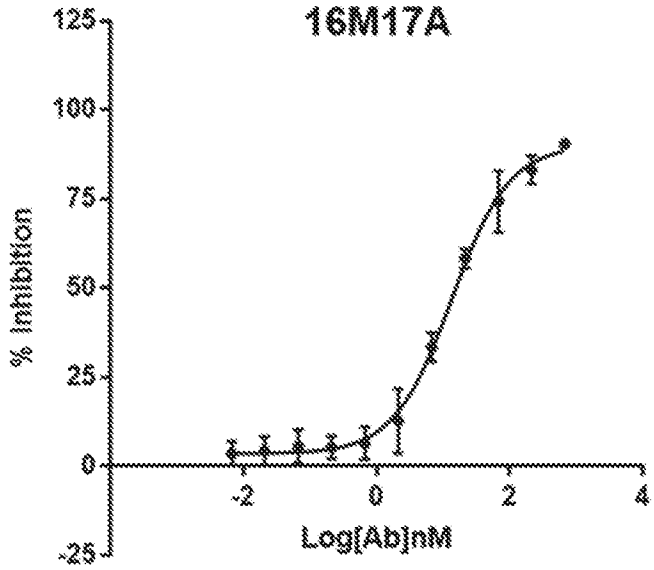
16M17A
% Inhibition
125
100
75
50
25
0
-25
-2
0
2
4
Log[Ab]nM

FIG. 2O

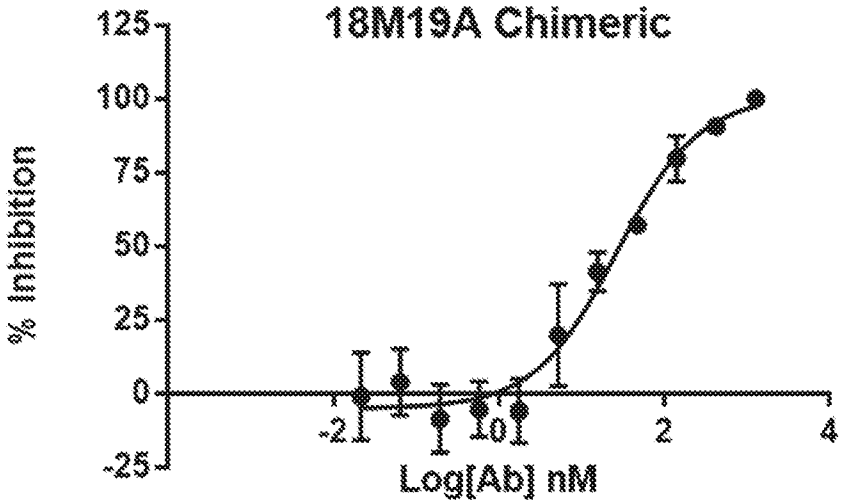
18M19A Chimeric
% Inhibition
125
100
75
50
25
0
-25
-2
0
2
4
Log[Ab] nM

FIG. 2P

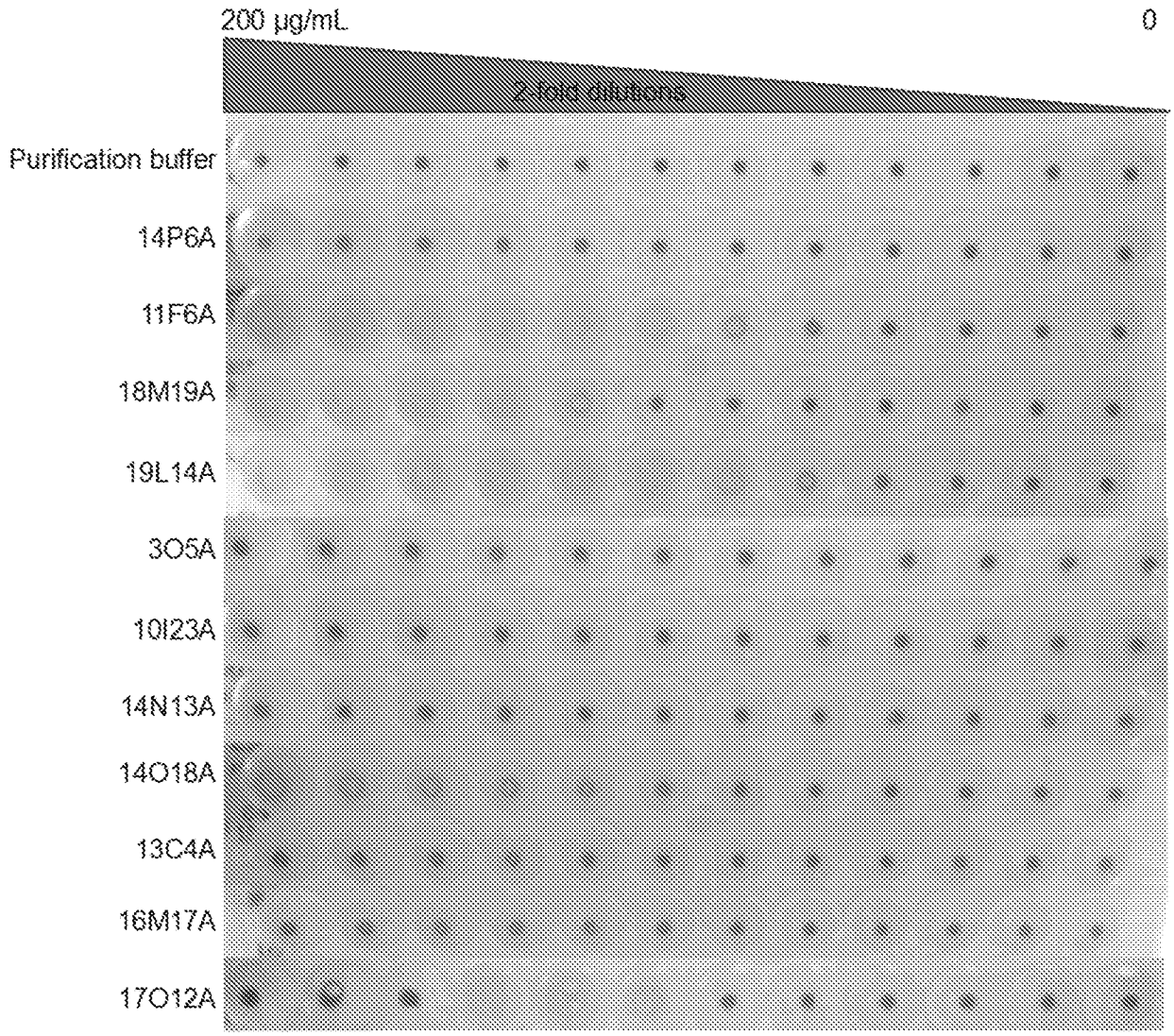
200 µg/mL
0
2-fold dilutions
Purification buffer
14P6A
11F6A
18M19A
19L14A
3O5A
10I23A
14N13A
14O18A
13C4A
16M17A
17O12A

FIG. 3A

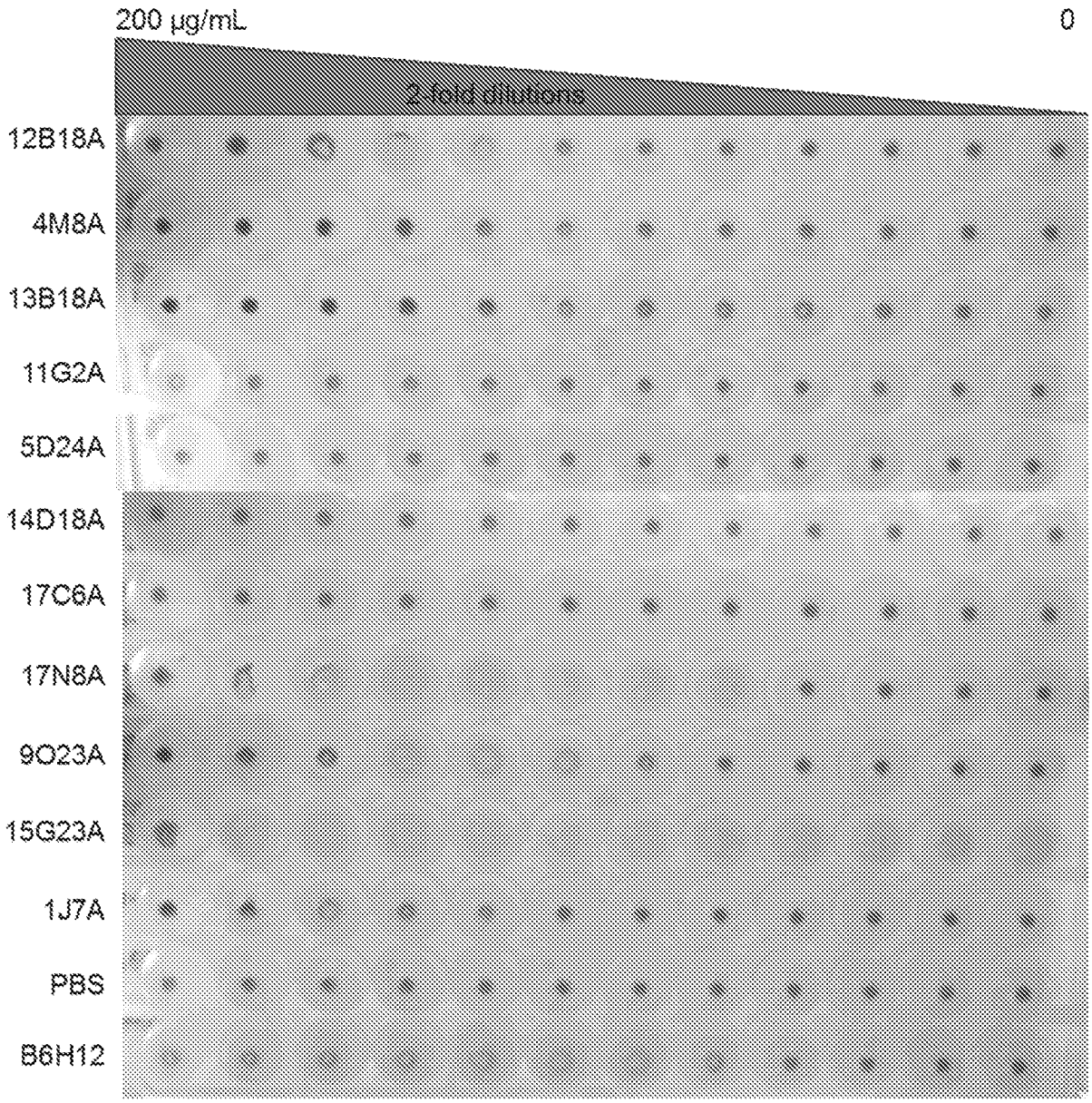
200 µg/mL
0
2-fold dilutions
12B18A
4M8A
13B18A
11G2A
5D24A
14D18A
17C6A
17N8A
9O23A
15G23A
1J7A
PBS
B6H12

FIG. 3B

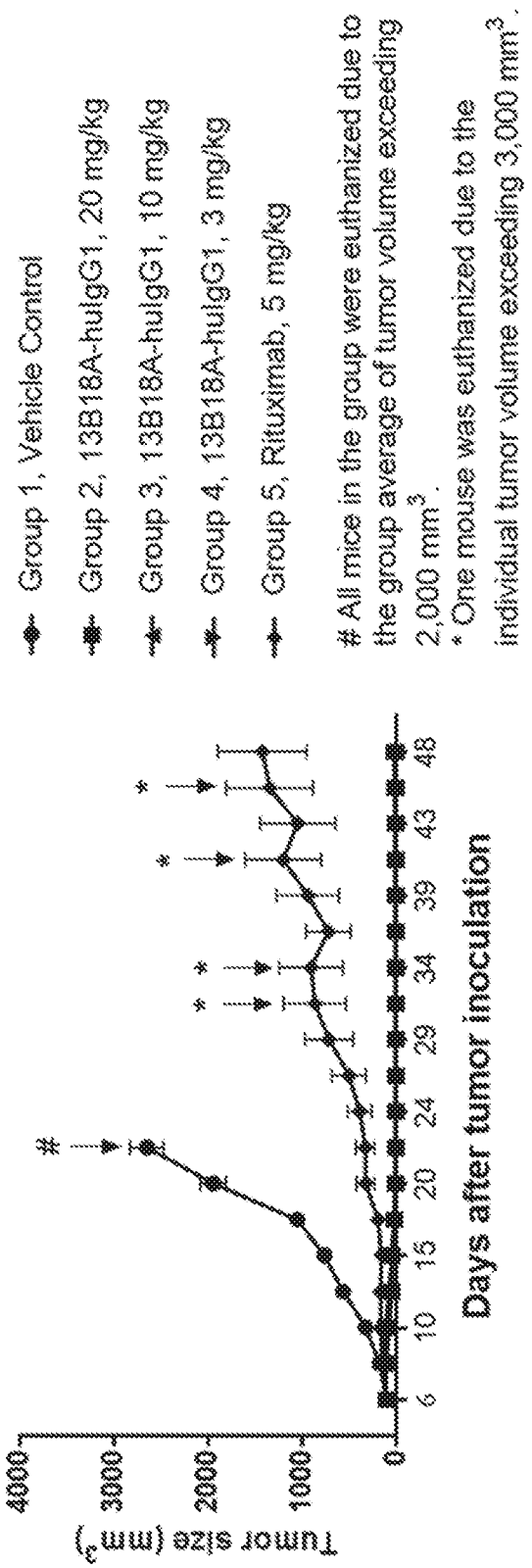
Group 1, Vehicle Control
Group 2, 13B18A-hulgG1, 20 mg/kg
Group 3, 13B18A-hulgG1, 10 mg/kg
Group 4, 13B18A-hulgG1, 3 mg/kg
Group 5, Rituximab, 5 mg/kg
All mice in the group were euthanized due to the group average of tumor volume exceeding 2,000 mm3.
* One mouse was euthanized due to the individual tumor volume exceeding 3,000 mm3.
Tumor size (mm3)
4000
3000
2000
1000
0
6
10
15
20
24
29
34
39
43
48
Days after tumor inoculation

FIG. 4A

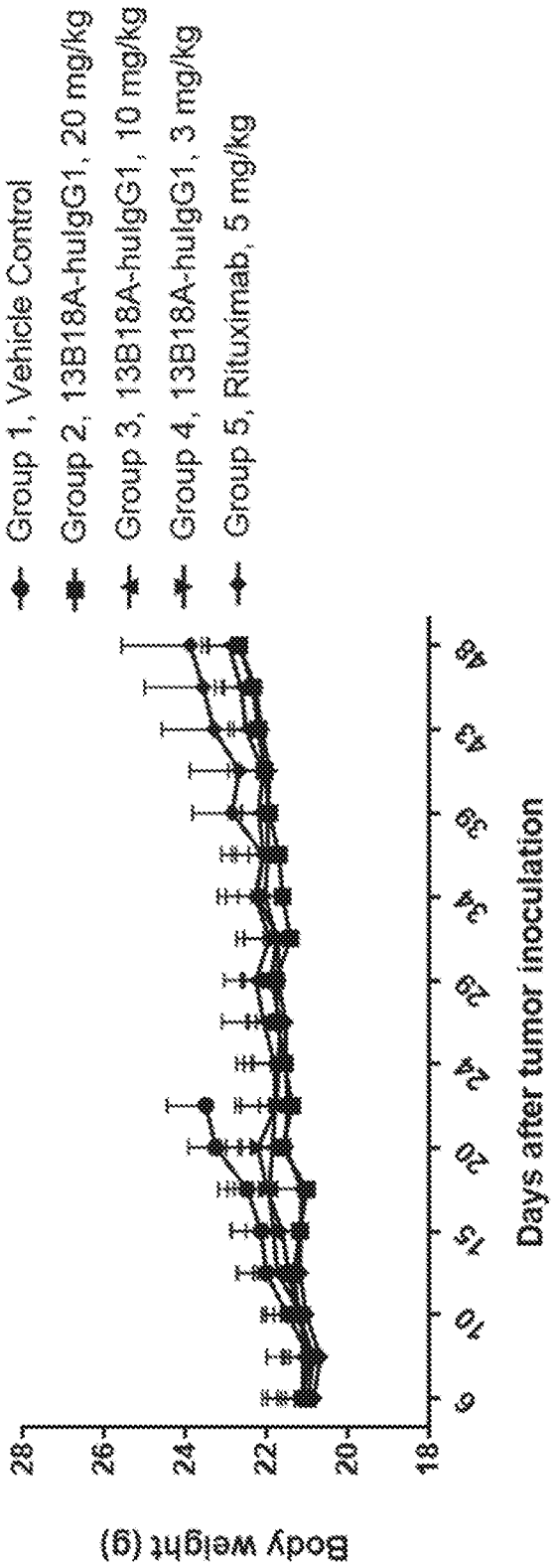
Group 1, Vehicle Control
Group 2, 13B18A-hulgG1, 20 mg/kg
Group 3, 13B18A-hulgG1, 10 mg/kg
Group 4, 13B18A-hulgG1, 3 mg/kg
Group 5, Rituximab, 5 mg/kg
Body weight (g)
28
26
24
22
20
18
0
10
15
20
25
30
35
39
43
49
Days after tumor inoculation

FIG. 4B

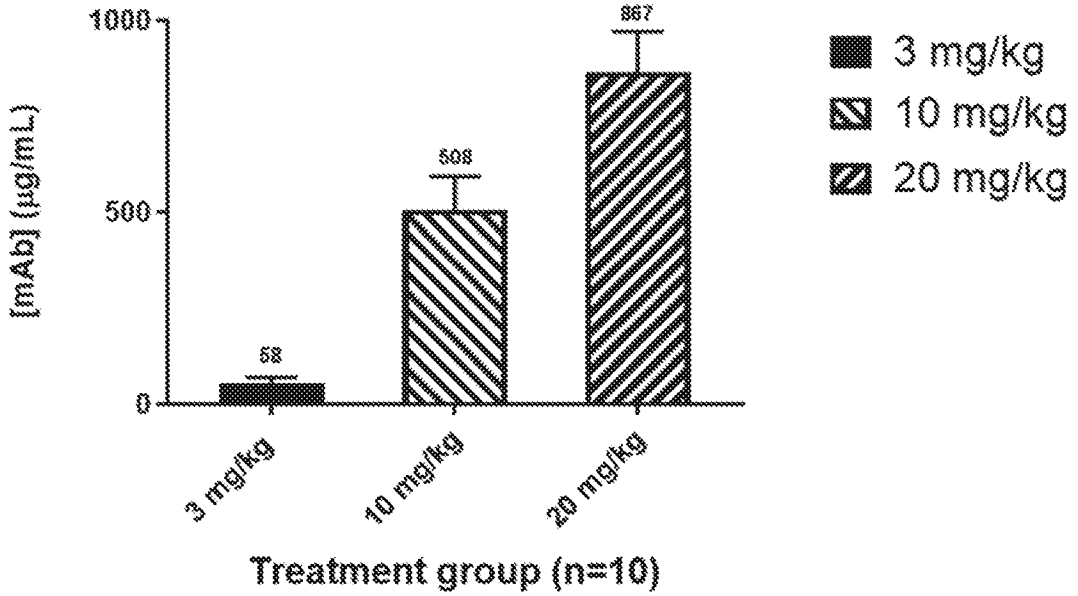
1000
500
0
[mAb] (µg/mL)
58
508
867
3 mg/kg
10 mg/kg
20 mg/kg
3 mg/kg
10 mg/kg
20 mg/kg
Treatment group (n=10)

FIG. 4C

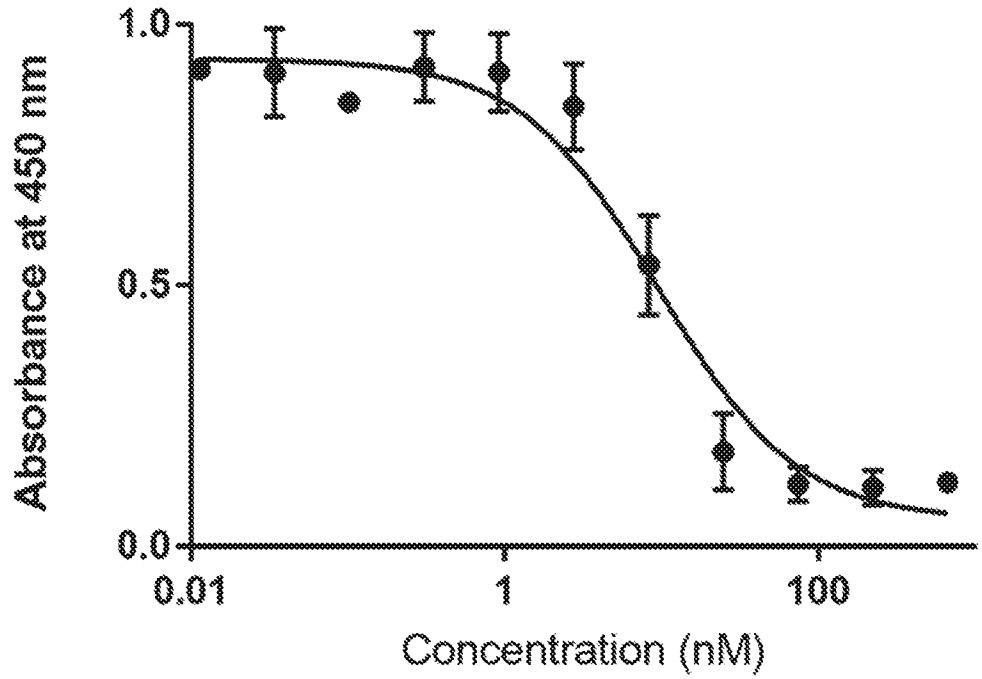
1.0
0.5
0.0
Absorbance at 450 nm
0.01
1
100
Concentration (nM)

FIG. 5A

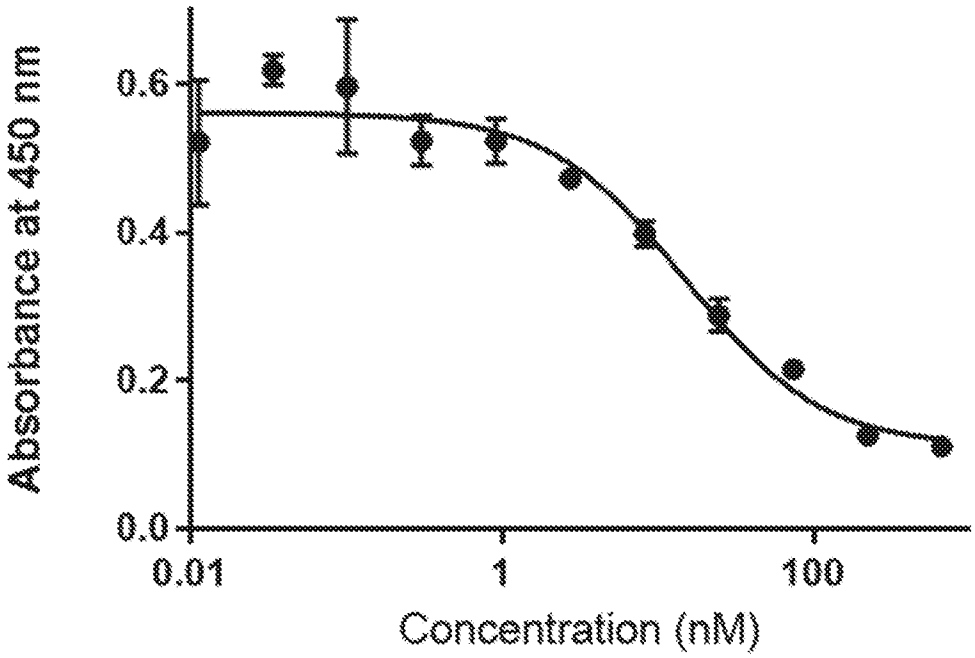

FIG. 5B

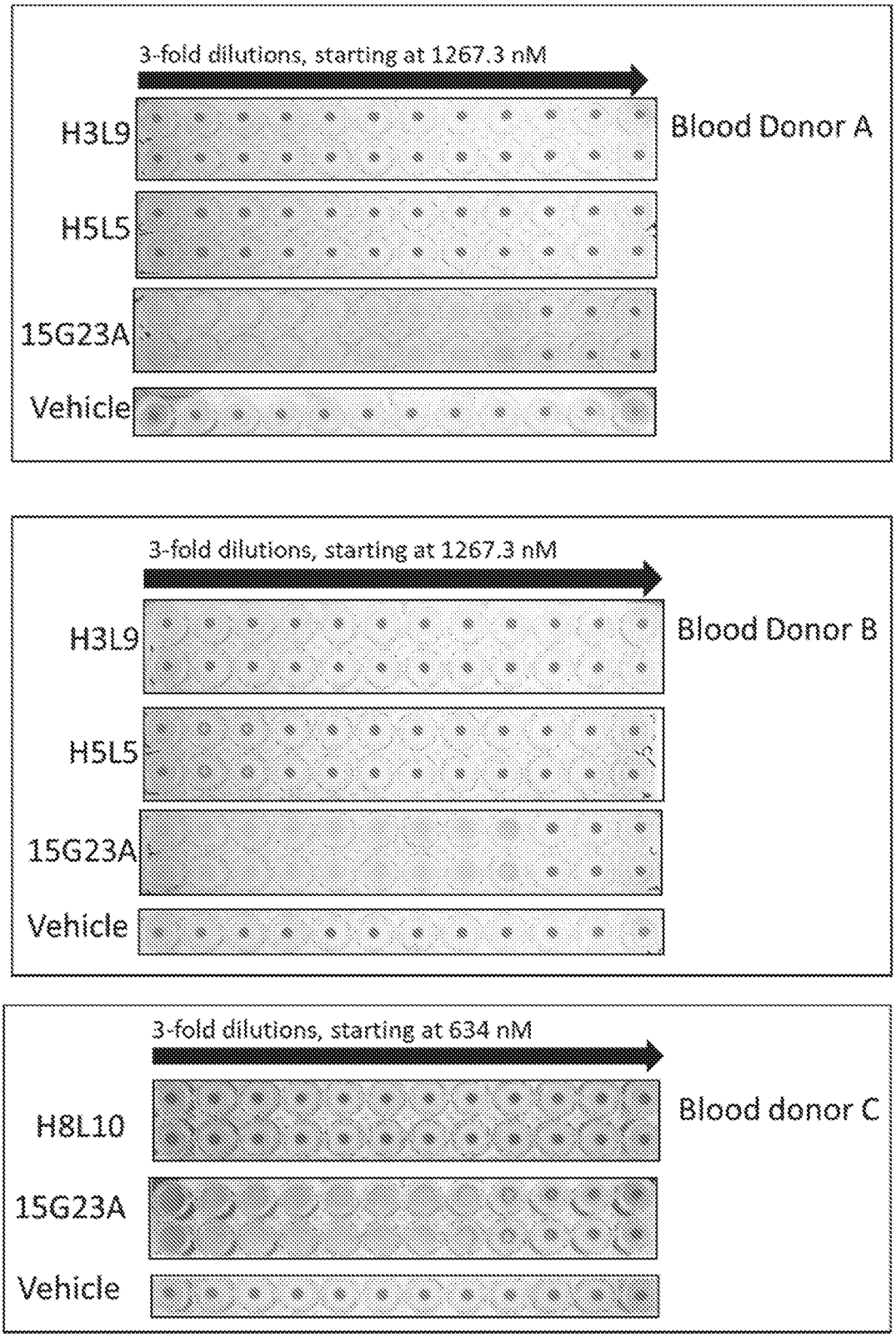
3-fold dilutions, starting at 1267.3 nM
H3L9
H5L5
15G23A
Vehicle
Blood Donor A
3-fold dilutions, starting at 1267.3 nM
H3L9
H5L5
15G23A
Vehicle
Blood Donor B
3-fold dilutions, starting at 634 nM
H8L10
15G23A
Vehicle
Blood donor C

FIG. 6

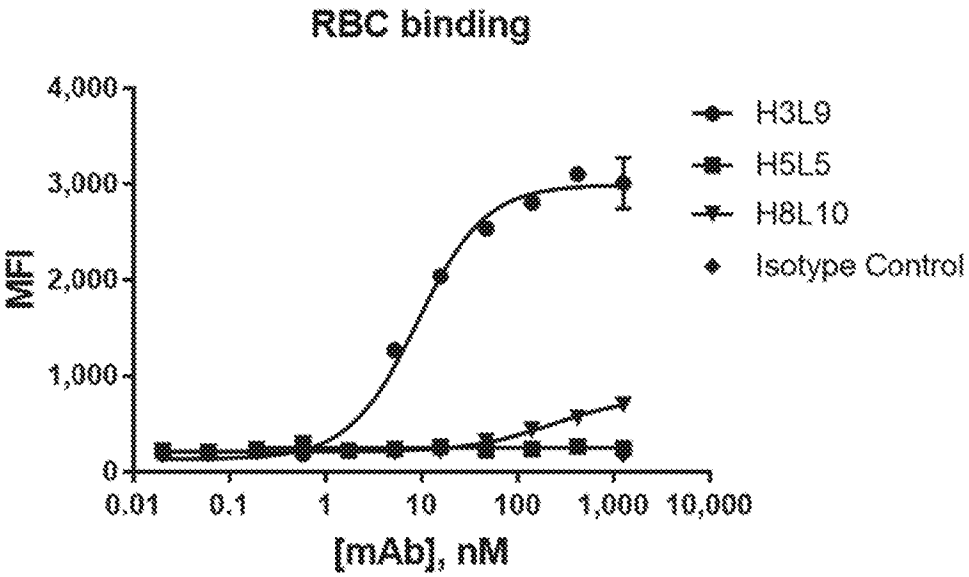
RBC binding
4,000
3,000
2,000
1,000
0
MFI
0.01
0.1
1
10
100
1,000
10,000
[mAb], nM
H3L9
H5L5
H8L10
Isotype Control

FIG. 7A

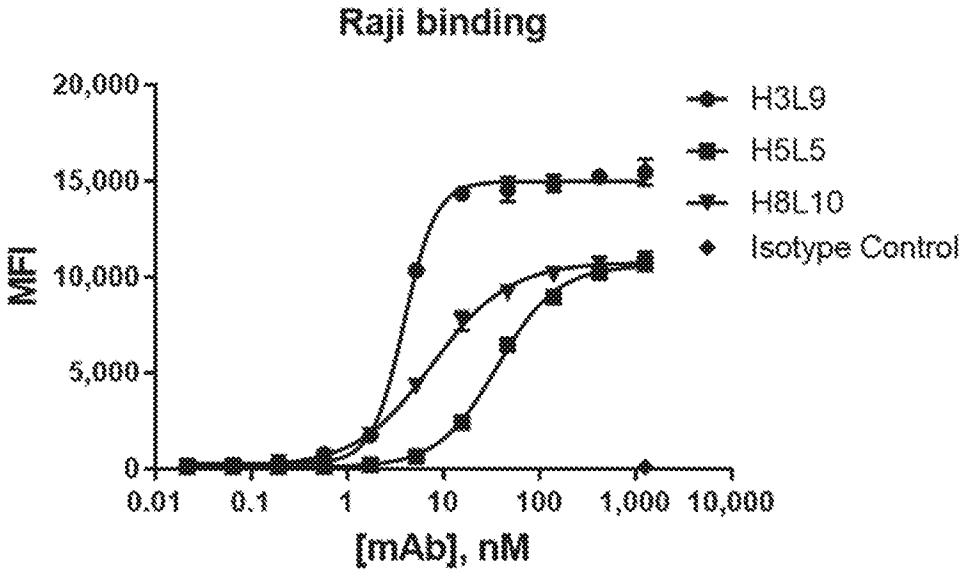
Raji binding
20,000
15,000
10,000
5,000
0
MFI
0.01
0.1
1
10
100
1,000
10,000
[mAb], nM
H3L9
H5L5
H8L10
Isotype Control

FIG. 7B

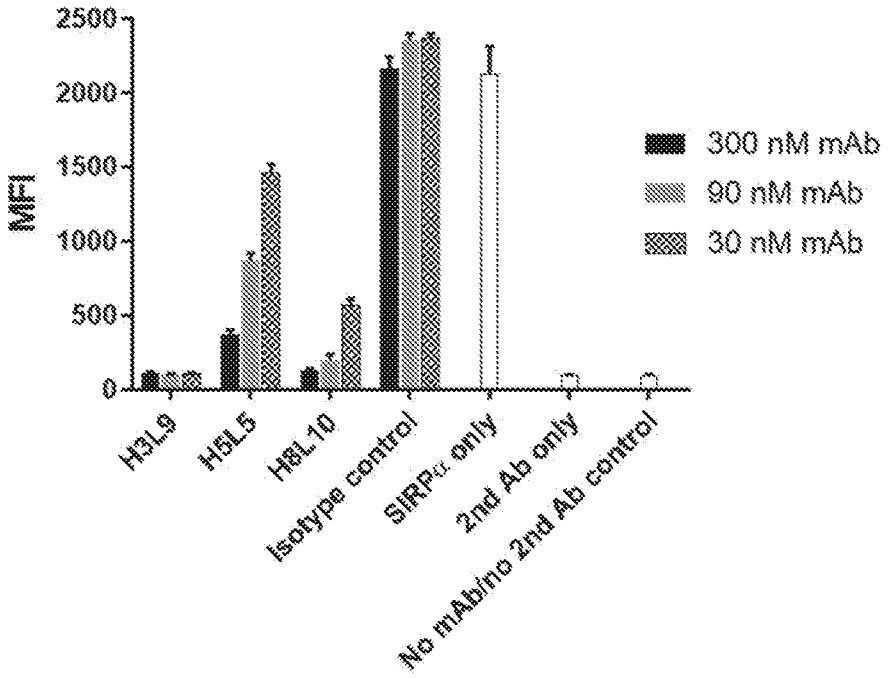
MFI
2500
2000
1500
1000
500
0
300 nM mAb
90 nM mAb
30 nM mAb
H3L9
H5L5
H8L10
Isotype control
SIRPα only
2nd Ab only
No mAb/no 2nd Ab control

FIG. 8

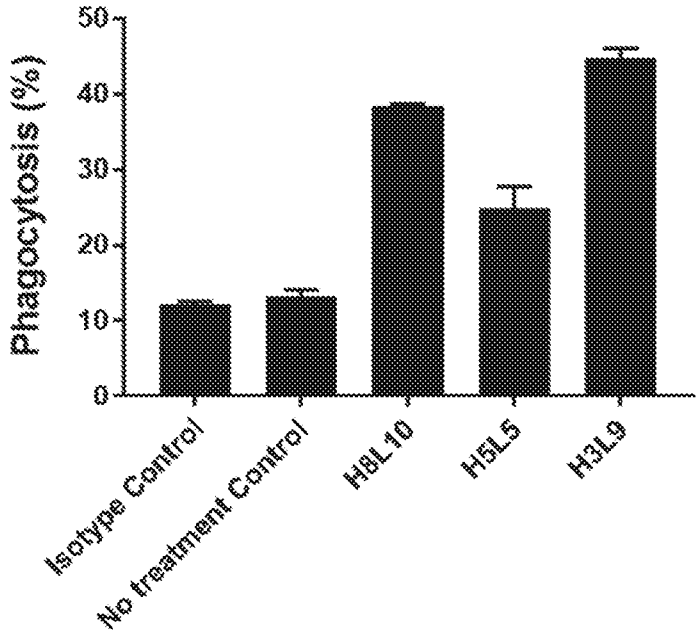
Phagocytosis (%)
50
40
30
20
10
0
Isotype Control
No treatment Control
H8L10
H5L5
H3L9

FIG. 9