



(51) International Patent Classification:

C07K 19/00 (2006.01) A61K 47/42 (2017.01)

A61K 39/395 (2006.01) A61P 35/00 (2006.01)

(21) International Application Number:

PCT/CN2021/091103

(22) International Filing Date:

29 April 2021 (29.04.2021)

(25) Filing Language:

English

(26) Publication Language:

English

(30) Priority Data:

PCT/CN2020/088226

30 April 2020 (30.04.2020) CN

(71) Applicant (for CN only): **I-MAB BIOPHARMA CO., LTD.** [CN/CN]; Suite 802, West Tower, OmniVision Tech Park, 88 Shangke Road, Shanghai 201210 (CN).

(71) Applicant (for all designated States except CN): **I-MAB BIOPHARMA US LIMITED** [US/US]; 9801 Washington Blvd., Suite 710, Gaithersburg, MD 20878 (US).

(72) Inventors: **CAO, Wei**; Suite 802, West Tower, OmniVision Tech Park, 88 Shangke Road, Shanghai 201210 (CN). **YE, Chuanyan**; Suite 802, West Tower, OmniVision Tech Park, 88 Shangke Road, Shanghai 201210 (CN). **WANG, Zhengyi**; Suite 802, West Tower, OmniVision Tech Park, 88 Shangke Road, Shanghai 201210 (CN). **GUO, Bingshi**; Suite 802, West Tower, OmniVision Tech Park, 88 Shangke Road, Shanghai 201210 (CN).

(74) Agent: **BEIJING CELUE LAW FIRM**; 601 Room, Beijing Raffles Center Office Building, Dongzhimen South Rd., No. 1, Dongcheng District, Beijing 100007 (CN).

(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, IT, 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,

(54) Title: PHARMACEUTICAL COMPOSITIONS CONTAINING ANTI-CD47 ANTIBODIES

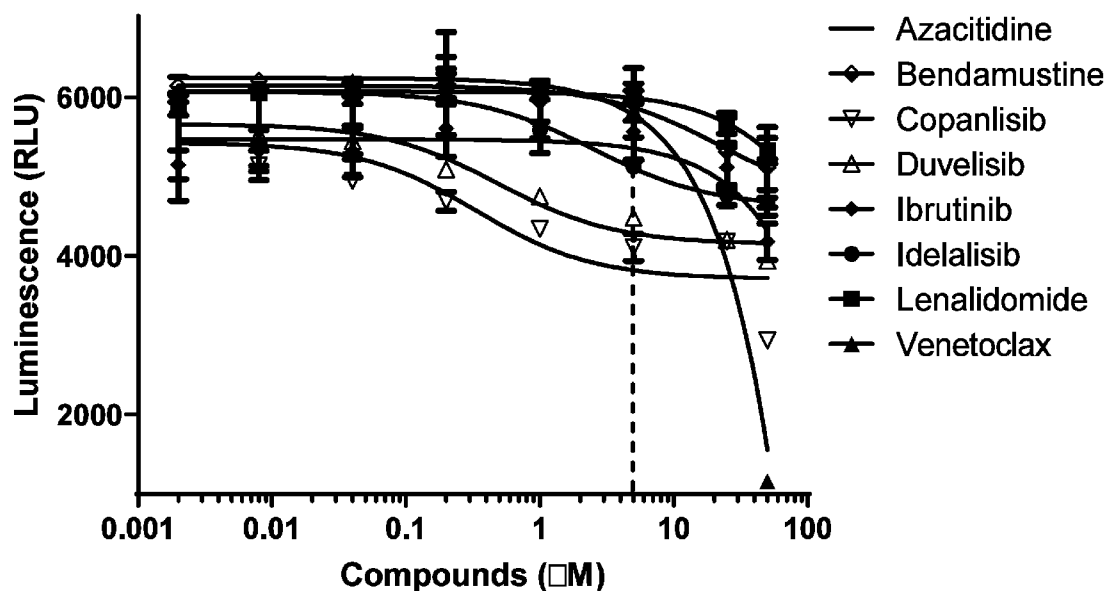


Fig. 1

(57) Abstract: Provided is a pharmaceutical composition comprising an isolated anti-CD47 antibody or an immunologically active fragment thereof, a therapeutic agent and a pharmaceutically acceptable carrier. These pharmaceutical compositions are synergistic by enhancing phagocytosis of cancer cells as compared to the use of single agents. In addition, also provided is a method for treating a disease in a human subject in need thereof by administering the pharmaceutical composition.



SA, SC, SD, SE, SG, SK, SL, ST, SV, SY, TH, TJ, TM, TN,
TR, TT, TZ, UA, UG, US, UZ, VC, VN, WS, 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, 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))
- with sequence listing part of description (Rule 5.2(a))

PHARMACEUTICAL COMPOSITIONS CONTAINING ANTI-CD47 ANTIBODIES**Cross-Reference to Related Application**

[01] This application claims priority to international application number PCT/CN2020/088226, filed on April 30, 2020, the contents of which are incorporated herein by reference in their entirety.

Background of the Invention

[02] CD47 (Cluster of Differentiation 47) was first identified as a tumor antigen on human ovarian cancer in the 1980s. Since then, CD47 has been found to be expressed on multiple human tumor types including acute myeloid leukemia (AML), chronic myeloid leukemia, acute lymphoblastic leukemia (ALL), non-Hodgkin's lymphoma (NHL), multiple myeloma (MM), bladder cancer, and other solid tumors. High levels of CD47 allow cancer cells to avoid phagocytosis despite having a higher level of calreticulin - the dominant pro-phagocytic signal.

[03] Also known as integrin-associated protein (IAP), ovarian cancer antigen OA3, Rh-related antigen and MER6, CD47 is a multi-spanning transmembrane protein belonging to the immunoglobulin (Ig) superfamily that is universally expressed on mammalian cells and tissues. Through its interactions with signal-regulatory proteins alpha (SIRP α), an inhibitory protein expressed on macrophages, CD47 triggers tyrosine phosphorylation in the SIRP α cytoplasmic immunoreceptor tyrosine-based inhibitory motifs (ITIMs) and recruitment of protein tyrosine phosphatases SHP-1/SHP-2, which further mediates negative signaling events that inhibit macrophage phagocytosis. For this, CD47 acts as a "self" marker and a "do not eat me" signal, preventing macrophage engulfment of host cells. The interactions between CD47 and SIRP α play a critical role in restraining macrophages.

[04] For this reason, blood cells, such as red blood cells, platelets, and lymphocytes, express CD47 on their surface to protect themselves from rapid elimination by splenic macrophages. However, CD47 is expressed at even higher levels on leukemia stem cells (LSCs) than their normal counterparts. Higher expression levels of CD47 on human LSCs contribute to pathogenesis by inhibiting their phagocytosis through the interaction of CD47 with SIRP α . Accumulating evidence suggests that CD47 expression on human solid tumor cells is a common mechanism through which these cancer cells protect themselves from

phagocytosis, allowing tumor cell proliferation and metastasis (See: Frontiers in immunology, April 2017, Volume 8, Article 404).

[05] Therefore, interruption of the CD47-SIRP α pathway by anti-CD47 antibodies might have a therapeutic effect to enhance cancer cell phagocytic uptake. As disclosed in the International Application No. PCT/US2017/057535, we have provided novel anti-CD47 antibodies or an immunologically active fragments thereof that binds human CD47, thereby preventing human CD47 from interacting with SIRP α and promoting macrophage-mediated phagocytosis of a CD47-expressing cell. Very significantly, it does not cause a significant level of hemagglutination or depletion of red blood cells.

[06] By contrast to the antiphagocytic (don't-eat-me) signal CD47 on tumor cells, accumulated evidences indicate that cell surface calreticulin is considered as an "eat-me" signal and facilitates phagocytic uptake of cancer cells by immune system. Clarke and Smyth demonstrated that drug treatments (anthracyclines) caused tumor cell to expose a surface pro-phagocytic protein, calreticulin, which induced immunogenic cell death (See: Nature Biotechnology. 2007;25(2):192–193). Therefore, calreticulin-mediated immune mechanisms might be an important strategy for developing new anticancer therapy.

[07] Therapeutic monoclonal antibodies have proven clinically important in the treatment of cancer, however, there still remains considerable needs in manipulating and promoting phagocytosis of tumor cell. The present invention satisfies these, and other, needs.

Brief Summary of the Invention

[08] Methods and pharmaceutical compositions are provided for disease treatment in this invention. In this pharmaceutical composition, one essential component is a novel CD47 antibody (disclosed in PCT/US2017/057535) that blocks CD47 on the cell surface and prevents interactions between CD47 and SIRP α . Another essential component is a second therapeutic agent that can be a small molecule therapeutic agent for increasing the expression level of calreticulin and/or inhibiting the expression levels of CD47, or a large molecule (e.g., a second antibody such as CD20 antibody) with synergistic effect. In some embodiments, the pharmaceutical composition comprises three essential components, i.e., a novel CD47 antibody, a small molecule therapeutic agent and a second antibody with synergistic effect (such as CD20 antibody). The pharmaceutical compositions are synergistic

in promoting phagocytosis of cancer cells as compared to the use of any single agent. The combination of agents is particularly useful in the treatment of cancer, a fibrotic disease, a disease related to inhibition of phagocytosis, or a disease related to platelet aggregation.

[009] In some embodiments, this invention provides a pharmaceutical composition, comprising a novel CD47 antibody or an immunologically active fragment, a therapeutic agent and a pharmaceutically acceptable carrier or excipient, in which the isolated antibody or an immunologically active fragment comprises a heavy chain variable region (VH) having amino acid sequence that is at least 95% identical to an amino acid sequence selected from the group consisting of SEQ ID NO: 1, SEQ ID NO: 3, SEQ ID NO: 5, SEQ ID NO: 7, SEQ ID NO: 9, SEQ ID NO: 11, SEQ ID NO: 13, SEQ ID NO: 15, SEQ ID NO: 17, SEQ ID NO: 19, SEQ ID NO: 21, SEQ ID NO: 23, SEQ ID NO: 25, SEQ ID NO: 27, SEQ ID NO: 29, SEQ ID NO: 31, SEQ ID NO: 33, SEQ ID NO: 35, SEQ ID NO: 37, SEQ ID NO: 39, SEQ ID NO: 41, SEQ ID NO: 43, SEQ ID NO: 45, SEQ ID NO: 47, SEQ ID NO: 49, SEQ ID NO: 51, SEQ ID NO: 53, SEQ ID NO: 55, SEQ ID NO: 57, SEQ ID NO: 59, SEQ ID NO: 61, SEQ ID NO: 63, SEQ ID NO: 65, SEQ ID NO: 67, SEQ ID NO: 69, SEQ ID NO: 71, SEQ ID NO: 73, SEQ ID NO: 75, SEQ ID NO: 77, SEQ ID NO: 79, and SEQ ID NO: 81; and a light chain variable region (VL) having amino acid sequence that is at least 95% identical to an amino acid sequence selected from the group consisting of SEQ ID NO: 2, SEQ ID NO: 4, SEQ ID NO: 6, SEQ ID NO: 8, SEQ ID NO: 10, SEQ ID NO: 12, SEQ ID NO: 14, SEQ ID NO: 16, SEQ ID NO: 18, SEQ ID NO: 20, SEQ ID NO: 22, SEQ ID NO: 24, SEQ ID NO: 26, SEQ ID NO: 28, SEQ ID NO: 30, SEQ ID NO: 32, SEQ ID NO: 34, SEQ ID NO: 36, SEQ ID NO: 38, SEQ ID NO: 40, SEQ ID NO: 42, SEQ ID NO: 44, SEQ ID NO: 46, SEQ ID NO: 48, SEQ ID NO: 50, SEQ ID NO: 52, SEQ ID NO: 54, SEQ ID NO: 56, SEQ ID NO: 58, SEQ ID NO: 60, SEQ ID NO: 62, SEQ ID NO: 64, SEQ ID NO: 66, SEQ ID NO: 68, SEQ ID NO: 70, SEQ ID NO: 72, SEQ ID NO: 74, SEQ ID NO: 76, SEQ ID NO: 78, SEQ ID NO: 80, and SEQ ID NO: 82.

[010] In some other embodiments, the isolated antibody or an immunologically active fragment thereof comprises a VH/VL pair, wherein the VH/VL pair comprises VH and VL chain sequences that are respectively at least 95% identical to amino acid sequences selected from the group consisting of SEQ ID NO: 1 and SEQ ID NO: 2 (i.e., 1A1), SEQ ID NO: 3 and SEQ ID NO: 4 (i.e., 1F8), SEQ ID NO: 5 and SEQ ID NO: 6 (i.e., 2A11), SEQ ID NO: 7 and SEQ ID NO: 8 (i.e., 2C2), SEQ ID NO: 9 and SEQ ID NO: 10 (i.e., 2D7), SEQ ID NO: 11 and SEQ ID NO: 12 (i.e., 2G4), SEQ ID NO: 13 and SEQ ID NO: 14 (i.e., 2G11), SEQ ID NO: 15 and SEQ ID NO: 16 (i.e., 6F4), SEQ ID NO: 17 and SEQ ID NO: 18 (i.e., 5H1), SEQ ID NO: 19 and SEQ ID NO:

20 (i.e., 5F6), SEQ ID NO: 21 and SEQ ID NO: 22 (i.e., 1F3), SEQ ID NO: 23 and SEQ ID NO: 24 (i.e., 2A4), SEQ ID NO: 25 and SEQ ID NO: 26 (i.e., 2B12), SEQ ID NO: 27 and SEQ ID NO: 28 (i.e., 13A11), SEQ ID NO: 29 and SEQ ID NO: 30 (i.e., 15E1), SEQ ID NO: 31 and SEQ ID NO: 32 (i.e., 13H3), SEQ ID NO: 33 and SEQ ID NO: 34 (i.e., 14A8), SEQ ID NO: 35 and SEQ ID NO: 36 (i.e., 16H3), SEQ ID NO: 37 and SEQ ID NO: 38 (i.e., 1A1), SEQ ID NO: 39 and SEQ ID NO: 40 (i.e., 1A1-A), SEQ ID NO: 41 and SEQ ID NO: 42 (i.e., 1A1-Q), SEQ ID NO: 43 and SEQ ID NO: 44 (i.e., 1A2), SEQ ID NO: 45 and SEQ ID NO: 46 (i.e., 1A8), SEQ ID NO: 47 and SEQ ID NO: 48 (i.e., 1B1), SEQ ID NO: 49 and SEQ ID NO: 50 (i.e., 1B2), SEQ ID NO: 51 and SEQ ID NO: 52 (i.e., 1H3), SEQ ID NO: 53 and SEQ ID NO: 54 (i.e., 1H3-Q), SEQ ID NO: 55 and SEQ ID NO: 56 (i.e., 1H3-A), SEQ ID NO: 57 and SEQ ID NO: 58 (i.e., 2A2), SEQ ID NO: 59 and SEQ ID NO: 60 (i.e., 2A3), SEQ ID NO: 61 and SEQ ID NO: 62 (i.e., 2A6), SEQ ID NO: 63 and SEQ ID NO: 64 (i.e., 2A10), SEQ ID NO: 65 and SEQ ID NO: 66 (i.e., 2B1), SEQ ID NO: 67 and SEQ ID NO: 68 (i.e., 2C6), SEQ ID NO: 69 and SEQ ID NO: 70 (i.e., 2E7), SEQ ID NO: 71 and SEQ ID NO: 72 (i.e., 2E9), SEQ ID NO: 73 and SEQ ID NO: 74 (i.e., 2F1), SEQ ID NO: 75 and SEQ ID NO: 76 (i.e., 2F3), SEQ ID NO: 77 and SEQ ID NO: 78 (i.e., 34C5), SEQ ID NO: 79 and SEQ ID NO: 80 (i.e., A1A), and SEQ ID NO: 81 and SEQ ID NO: 82 (i.e., T4J).

[011] In some other embodiments, the isolated antibody or an immunologically active fragment that specifically binds human CD47 comprises a variable heavy (VH) chain sequence that is at least 95% identical to an amino acid sequence selected from the group consisting of SEQ ID NO: 3, SEQ ID NO: 31, SEQ ID NO: 79, and SEQ ID NO: 81; and a variable light (VL) chain sequence that is at least 95% identical to an amino acid sequence selected from the group consisting of SEQ ID NO: 4, SEQ ID NO: 32, SEQ ID NO: 80, and SEQ ID NO: 82.

[012] In another aspect, the present invention provides a pharmaceutical composition, comprising a novel CD47 antibody or an immunologically active fragment, a therapeutic agent and a pharmaceutically acceptable carrier or excipient, in which the isolated antibody or an immunologically active fragment comprises a VH chain having VH CDR1, VH CDR2 and VH CDR3 of the sequences shown below, and a VL chain having VL CDR1, VL CDR2, and VL CDR3 of the sequences shown below:

VH CDR1: NAWMN (SEQ ID NO: 85) or RAWMN (SEQ ID NO: 86)

VH CDR2: RIKRKTDGETTDYAAPVKG (SEQ ID NO: 87)

VH CDR3: SNRAFDI (SEQ ID NO: 88)

VL CDR1: KSSQSVLYSSNNRNYLA (SEQ ID NO: 89) or KSSQSVLYAGNNRNYLA (SEQ ID NO: 90)

VL CDR2: QASTRAS (SEQ ID NO: 91)

VL CDR3: QQYYTPPLA (SEQ ID NO: 92)

[013] In some other embodiments, the isolated antibody or an immunologically active fragment that specifically binds human CD47 comprises a VH/VL pair, in which the VH/VL pair comprises VH and VL chain sequences that are respectively at least 95% identical to amino acid sequences selected from the group consisting of SEQ ID NO: 3 and SEQ ID NO: 4 (i.e., 1F8), SEQ ID NO: 31 and SEQ ID NO: 32 (i.e., 13H3), SEQ ID NO: 79 and SEQ ID NO: 80 (i.e., A1A); and SEQ ID NO: 81 and SEQ ID NO: 82 (i.e., T4J). Antibodies 13H3 and A1A are affinity matured clones of antibody 1F8. Amino acid sequences of the three antibodies are highly similar.

[014] In further embodiments, the isolated antibody or an immunologically active fragment thereof that specifically binds human CD47 includes a heavy chain of SEQ ID NO: 81 and a light chain of SEQ ID NO: 82.

[015] The isolated antibody or an immunologically active fragment can be chimeric or humanized. And the isolated antibody can be a monoclonal antibody, a bispecific antibody or a fusion antibody that binds human CD47. They can prevent or significantly reduce human CD47 from interacting with SIRP α , or promotes macrophage-mediated phagocytosis of a CD47-expressing cell. The CD47 antibodies of this invention do not cause a significant or noticeable level of hemagglutination or depletion of red blood cells, and in many cases, they do not cause hemagglutination or depletion of red blood cells at all.

[016] In one aspect, the isolated bispecific antibody comprises a first arm and a second arm. The first arm comprises the antibody or an immunologically active fragment thereof that binds human CD47, and the second arm comprises a second monoclonal antibody that does not bind human CD47.

[017] In another aspect, the second arm of the isolated bispecific antibody binds to cancer cell.

[018] In yet another aspect, the fusion antibody is the isolated antibody or an immunologically active fragment thereof conjugated with an additional protein, a small-molecule agent or a marker.

[019] In yet another aspect, the additional protein is an antibody or a cytokine. The small molecule agent is an anti-cancer or anti-inflammation agent. And the marker is a biomarker or fluorescent marker.

[020] In some embodiments, the therapeutic agent in the pharmaceutical composition is a small molecule chemotherapeutic agent, which does not cause substantial toxicity to macrophages. As used herein, the term “substantial toxicity” means toxicity of considerable concern because of (a) the seriousness of the toxicity effect, and (b) the fact or probability of its occurrence. These therapeutic agents in the pharmaceutical composition can synergize with the anti-CD47 antibodies to increase the macrophage phagocytosis effect by increasing the expression level of calreticulin and/or decrease the expression level of CD47, or provide additive effect to anti-CD47 antibody.

[021] In one aspect, the therapeutic agent increases expression level of Calreticulin. In another aspect, the therapeutic agent inhibits expression level of CD47.

[022] In some embodiments, the therapeutic agent in the pharmaceutical composition is a chemotherapeutic agent. Such a chemotherapeutic agent can be a small molecule drug and can be Azacitidine, Venetoclax or Copanlisib. In some other embodiments, the therapeutic agent is Azacitidine or Venetoclax. In some other embodiments, the therapeutic agent is Azacitidine.

[023] In yet another embodiment, the therapeutic agent in the pharmaceutical composition is a second antibody or an immunologically active fragment thereof. In some further embodiments, this second antibody selectively binds CD20 (thereby called “CD20 antibody” or “anti-CD20 antibody”) and can promote phagocytic elimination of cancer cell. In a further embodiment, the CD47 antibody or an immunologically active fragment synergize the CD20 antibody promoting phagocytic elimination of cancer cell.

[024] In yet another embodiment, the additional/second antibody that selectively binds CD20 is Rituximab (of SEQ ID NO: 83 and SEQ ID NO: 84) or its biosimilar, and the second antibody, such as Rituximab (of SEQ ID NO: 83 and SEQ ID NO: 84), can synergize with a CD47 antibody to facilitate the phagocytosis of tumor cell.

[025] In yet another embodiment, the therapeutic agent in the pharmaceutical composition comprises both the small molecular chemotherapeutic agent and the second antibody that binds CD20. In some further embodiments, the therapeutic agent comprises

both Azacitidine and Rituximab as synergistic agents. In another further embodiments, the therapeutic agent comprises Venetoclax and Rituximab as synergistic agents.

[026] The present invention also provides a method for treating diseases in a subject using the pharmaceutical composition. Examples of diseases include, but are not limited to, cancer, a fibrotic disease, a disease related to inhibition of phagocytosis, or a disease related to platelet aggregation.

[027] Examples of cancer are, but not limited to, ovarian cancer, colon cancer, breast cancer, lung cancer, head and neck cancer, bladder cancer, colorectal cancer, pancreatic cancer, non-Hodgkin's lymphoma, acute lymphocytic leukemia, chronic lymphocytic leukemia, acute myeloid leukemia, chronic myelogenous leukemia, hairy cell leukemia (HCL), T-cell prolymphocytic leukemia (T-PLL), large granular lymphocytic leukemia, adult T-cell leukemia, multiple myeloma, melanoma, leiomyoma, leiomyosarcoma, glioma, glioblastoma, myelomas, monocytic leukemias, B-cell derived leukemias, T-cell derived leukemias, B-cell derived lymphomas, T-cell derived lymphomas, endometrial cancer, kidney cancer, melanoma, prostate cancer, thyroid cancer, cervical cancer, gastric cancer, liver cancer, and solid tumors; the fibrotic disease is selected from the group consisting of: myocardial infarction, angina, osteoarthritis, pulmonary fibrosis, asthma, cystic fibrosis, bronchitis, and asthma; the disease related to inhibition of phagocytosis is a cardiovascular disease; the disease related to platelet aggregation is Glanzmann Thrombasthenia, prolonged bleeding time, immune thrombocytopenia (ITP), von Willebrand disease (vWD).

[028] Examples of cardiovascular disease are, but not limited to, atherosclerosis, stroke, hypertensive heart disease, rheumatic heart disease, cardiomyopathy, heart arrhythmia, congenital heart disease, valvular heart disease, carditis, aortic aneurysms, peripheral artery disease, and venous thrombosis.

[029] The present invention further provides uses of the pharmaceutical composition for the manufacture of a medicament for treatment of diseases.

Brief Descriptions of the Drawings

[030] Fig. 1 shows the viability of macrophages after being treated with selected small molecule therapeutic agents for 16 hours.

[031] Fig. 2 shows the expression levels of CD47 and Calreticulin. In this assay, Toledo cells were treated with selected small molecule therapeutic agents for 12 hours and then the CD47(left) and Calreticulin(right) expression level were assessed by FACS assay.

[032] Fig. 3 shows the phagocytic ability of macrophages after being co-cultured with tumor cells in the presence of the isolated anti-CD47 antibody, Rituximab and selected small molecule therapeutic agents for 2-6 hours. The phagocytosis was analyzed by FACS assay.

[033] Fig. 4 shows tumor growth over time in four different treatment groups.

[034] Fig. 5 shows changes of tumor body weight in four different treatment groups.

[035] Fig. 6 shows tumor body weight changes in different treatment groups using female NOG mice bearing subcutaneous HL-60 xenograft model. Fig. 6a shows tumor body weight changes and Fig. 6b shows the change in percentage (%).

[036] Fig. 7 shows tumor growth curves in different treatment groups using female NOG mice bearing subcutaneous HL-60 xenograft model.

Detailed Description of the Invention

[037] Before the present invention is further described, it is to be understood that this invention is not limited to particular embodiments described, as such may, of course, vary. It is also to be understood that the terminology used herein is for the purpose of describing particular embodiments only, and is not intended to be limiting, since the scope of the present invention will be limited only by the appended claims.

[038] Unless defined otherwise, all technical and scientific terms used herein have the same meaning as commonly understood by one of ordinary skill in the art to which this invention belongs.

[039] As used herein, the term "antibody" is used in the broadest sense and specifically covers monoclonal antibodies (including full length monoclonal antibodies), polyclonal antibodies, multi-specific antibodies (e.g., bispecific antibodies), and antibody fragments so long as they exhibit the desired biological activity. "Antibodies" (or "Abs") and "immunoglobulins" (or "Igs") are glycoproteins having the same structural characteristics. While antibodies exhibit binding specificity to a specific antigen, immunoglobulins include both antibodies and other antibody-like molecules which lack antigen specificity.

[040] As used herein, the term "immunologically active fragment", and all grammatical variants thereof, are defined as a portion of an intact antibody comprising the antigen

binding site or variable region of the intact antibody, wherein the portion is free of the constant heavy chain domains (i.e. CH2, CH3, and CH4, depending on antibody isotype) of the Fc region of the intact antibody. Examples of antibody fragments include Fab, Fab', Fab'-SH, F(ab')₂, and Fv fragments; diabodies; any antibody fragment that is a polypeptide having a primary structure consisting of one uninterrupted sequence of contiguous amino acid residues (referred to herein as a "single-chain antibody fragment" or "single chain polypeptide"), including without limitation (1) single-chain Fv (scFv) molecules, (2) single chain polypeptides containing only one light chain variable domain, or a fragment thereof that contains the three CDRs of the light chain variable domain, without an associated heavy chain moiety, and (3) single chain polypeptides containing only one heavy chain variable region, or a fragment thereof containing the three CDRs of the heavy chain variable region, without an associated light chain moiety; and multi-specific or multivalent structures formed from antibody fragments. In an antibody fragment comprising one or more heavy chains, the heavy chain(s) can contain any constant domain sequence (e.g. CH1 in the IgG isotype) found in a non-Fc region of an intact antibody, and/or can contain any hinge region sequence found in an intact antibody, and/or can contain a leucine zipper sequence fused to or situated in the hinge region sequence or the constant domain sequence of the heavy chain(s).

[041] As used herein, the term "monoclonal antibody" (mAb) 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. Monoclonal antibodies are highly specific, being directed against a single antigenic site. Each mAb is directed against a single determinant on the antigen. In addition to their specificity, the monoclonal antibodies are advantageous in that they can be synthesized by hybridoma culture, uncontaminated by other immunoglobulins. The modifier "monoclonal" indicates the character of the antibody as being obtained from a substantially homogeneous population of antibodies, and is not to be construed as requiring production of the antibody by any particular method. For example, the monoclonal antibodies to be used in accordance with the present invention may be made in an immortalized B cell or hybridoma thereof, or may be made by recombinant DNA methods.

[042] As used herein, the term "an immunologically active fragment" of an antibody refers to a fragment of an antibody that exhibits immunologically active effect similar to that of the entire antibody. It is also referred to as "an antigen-binding fragment" of an antibody.

[043] The monoclonal antibodies herein include hybrid and recombinant antibodies produced by splicing a variable (including hypervariable) domain of an CD47 antibody with a constant domain (e.g. "humanized" antibodies), or a light chain with a heavy chain, or a chain from one species with a chain from another species, or fusions with heterologous proteins, regardless of species of origin or immunoglobulin class or subclass designation, as well as antibody fragments (e.g., Fab, F(ab')₂, and Fv), so long as they exhibit the desired biological activity.

[044] The monoclonal antibodies herein specifically include "chimeric" antibodies(immunoglobulins) in which a portion of the heavy and/or light chain is identical with or homologous to corresponding sequences in antibodies derived from a particular species or belonging to a particular antibody class or subclass, while the remainder of the chain(s) is identical with or homologous to corresponding sequences in antibodies derived from another species or belonging to another antibody class or subclass, as well as fragments of such antibodies, so long as they exhibit the desired biological activity.

[045] As used herein, an "isolated" antibody is one which has been identified and separated and/or recovered from a component of its natural environment. Contaminant components of its natural environment are materials which would interfere with diagnostic or therapeutic uses for the antibody, and may include enzymes, hormones, and other proteinaceous or nonproteinaceous solutes. In some embodiments, the antibody will be purified (1) to greater than 75% by weight of antibody as determined by the Lowry method, and most preferably more than 80%, 90% or 99% by weight, or (2) to homogeneity by SDS-PAGE under reducing or nonreducing conditions using Coomassie blue or, preferably, silver stain. Isolated antibody includes the antibody in situ within recombinant cells since at least one component of the antibody's natural environment will not be present. Ordinarily, however, isolated antibody will be prepared by at least one purification step.

[046] As used herein, the term "treatment" or "treating" refers to both therapeutic treatment and prophylactic or preventative measures of a disease (such as cancer or a fibrotic disease). Those in need of treatment include those already with the disease as well as those in which the disease is to be prevented.

[047] Examples of cancer include, but are not limited to, ovarian cancer, colon cancer, breast cancer, lung cancer, head and neck cancer, bladder cancer, colorectal cancer, pancreatic cancer, non-Hodgkin's lymphoma, acute lymphocytic leukemia, chronic lymphocytic leukemia, acute myeloid leukemia, chronic myelogenous leukemia, hairy cell leukemia (HCL), T-cell prolymphocytic leukemia (T-PLL), large granular lymphocytic leukemia, adult T-cell leukemia, multiple myeloma, melanoma, leiomyoma, leiomyosarcoma, glioma, glioblastoma, myelomas, monocytic leukemias, B-cell derived leukemias, T-cell derived leukemias, B-cell derived lymphomas, T-cell derived lymphomas, endometrial cancer, kidney cancer, melanoma, prostate cancer, thyroid cancer, cervical cancer, gastric cancer, liver cancer, and solid tumors. The fibrotic disease can be myocardial infarction, angina, osteoarthritis, pulmonary fibrosis, asthma, cystic fibrosis, bronchitis, and asthma. The disease related to inhibition of phagocytosis can be a cardiovascular disease; and the disease related to platelet aggregation can be Glanzmann Thrombasthenia, prolonged bleeding time, immune thrombocytopenia (ITP), von Willebrand disease (vWD).

[048] As used herein, the term "pharmaceutically acceptable carrier or excipient" refers to a carrier or an excipient that is useful for preparing a pharmaceutical composition or formulation that is generally safe, non-toxic, and neither biologically nor otherwise undesirable. A carrier or excipient employed is typically one suitable for administration to human subjects or other mammals. In making the compositions, the active ingredient is usually mixed with, diluted by, or enclosed with a carrier or excipient. When the carrier or excipient serves as a diluent, it can be a solid, semi-solid, or liquid material, which acts as a vehicle, carrier, or medium for the active ingredient of the antibody.

[049] The CD47 antibodies of the invention can be bound to many different carriers and used to detect the presence of CD47 expressing cells. Examples of well-known carriers include glass, polystyrene, polypropylene, polyethylene, dextran, nylon, amylases, natural and modified celluloses, polyacrylamides, agaroses and magnetite. The nature of the carrier can be either soluble or insoluble for purposes of the invention. Those skilled in the art will know of other suitable carriers for binding monoclonal antibodies, or will be able to ascertain such, using routine experimentation.

[050] The terms "pharmaceutically acceptable", "physiologically tolerable" and grammatical variations thereof, as they refer to compositions, carriers, diluents and reagents, are used interchangeably and represent that the materials are capable of

administration to or upon a human without the production of undesirable physiological effects to a degree that would prohibit administration of the composition.

[051] Unless specifically indicated to the contrary, the term "conjugate" and "conjugated" used herein is defined as a heterogeneous molecule formed by the covalent attachment of one or more antibody fragment(s) to one or more polymer molecule(s), wherein the heterogeneous molecule is water soluble, i.e. soluble in physiological fluids such as blood, and wherein the heterogeneous molecule is free of any structured aggregate.

[052] The term "chemotherapeutic agent" is a broad one covering many chemotherapeutic agents having different mechanisms of action. The chemotherapeutic agents that can be administered in combination with an anti-CD47 agent include, without limitation, azacitidine, idelalisib, duvelisib, venetoclax, copanlisib, lbrutinib, bendamustine, and lenalidomide.

[053] The additional monoclonal antibodies that can be included in the present pharmaceutical composition is an antibody selectively binds CD20, which may include, without limitation, Rituximab which has a heavy chain of the following sequence:

QVQLQQPGAELVKPGASVKMSCKASGYFTSYNMHWVKQTPGRGLEWIGAIYPGNGDTSYNQKF
KGKATLTADKSSSTAYMQLSSLTSEDSAVYYCARSTYYGGDWYFNVWGAGTTVTVSAASTKGPSVF
PLAPSSKSTSGGTAALGCLVKDYFPEPVTVSWNSGALTSGVHTFPAVLQSSGLYSLSSVVTVPSSSLGT
QTYICNVNHKPSNTKVDKKAEPKSCDKHTCPPCPAPELLGGPSVFLFPPKPKDTLMISRTPEVTCVV
VDVSHEDPEVKFNWYVDGVEVHNAKTKPREEQYNSTYRVVSVLTVLHQDWLNGKEYKCKVSNKAL
PAPIEKTISKAKGQPREPQVYTLPPSRDELTKNQVSLTCLVKGFYPSDIAVEWESNGQPENNYKTTTP
VLDSGDGSFFLYSKLTVDKSRWQQGNVFSCSVMHEALHNHYTQKSLSLSPGK (SEQ ID NO: 83)

and a light chain of the following sequence:

QIVLSQSPAILSASPGEKVTMTCRASSSVSYIHWFQQKPGSSPKPWYATSNLASGVPVRFSGSGSGT
SYSLTISRVEAEDAATYYCQQWTSNPPTFGGGTKLEIKRTVAAPSVFIFPPSDEQLKSGTASVVCLLNN
FYPREAKVQWKVDNALQSGNSQESVTEQDSKDYSLSTLTLSKADYEKHKVYACEVTHQGLSSPV
TKSFNRGEC (SEQ ID NO: 84)

[054] The following examples are put forth so as to provide those of ordinary skill in the art with a complete disclosure and description of how to make and use the present invention, and are not intended to limit the scope of what the inventors regard as their invention nor are they intended to represent that the experiments below are all or the only experiments performed. Efforts have been made to ensure accuracy with respect to numbers used (e.g.

amounts, temperature, etc.) but some experimental errors and deviations should be accounted for. Unless indicated otherwise, parts are parts by weight, molecular weight is weight average molecular weight, temperature is in degrees Centigrade, and pressure is at or near atmospheric.

[055] Although any methods and materials similar or equivalent to those described herein can also be used in the practice or testing of the present invention, the preferred methods are described in the Examples and the materials are now described below.

[056] Materials used in the following examples included:

CellTraceTMViolet: Thermo Fisher Scientific, Catalog No.: C34557

CellTraceTMFar Red: Thermo Fisher Scientific, Catalog No.: 34564

CellTiter-GloTM Luminescent Cell Viability Assay Kit: Promega, Catalog No.: G7573

Anti-calreticulin antibody: Abcam, Catalog No.: ab83220

Alexa Fluor[®] 647 Mouse Anti-Human CD47: BD biosciences, Catalog No.: 561249

Ficoll-Paque Plus: Axis-Shield, Catalog No.: AS1114547

MACS system: Miltenyi Biotech, Catalog No.: 130-045-201.

Tumor cell line, Toledo, purchased from ATCC.

[057] As examples, a CD47 antibody suitable for the compositions of this invention would include (a) a variable heavy chain (VH) sequence that is selected from the group consisting of SEQ ID NO: 1, SEQ ID NO: 3, SEQ ID NO: 5, SEQ ID NO: 7, SEQ ID NO: 9, SEQ ID NO: 11, SEQ ID NO: 13, SEQ ID NO: 15, SEQ ID NO: 17, SEQ ID NO: 19, SEQ ID NO: 21, SEQ ID NO: 23, SEQ ID NO: 25, SEQ ID NO: 27, SEQ ID NO: 29, SEQ ID NO: 31, SEQ ID NO: 33, SEQ ID NO: 35, SEQ ID NO: 37, SEQ ID NO: 39, SEQ ID NO: 41, SEQ ID NO: 43, SEQ ID NO: 45, SEQ ID NO: 47, SEQ ID NO: 49, SEQ ID NO: 51, SEQ ID NO: 53, SEQ ID NO: 55, SEQ ID NO: 57, SEQ ID NO: 59, SEQ ID NO: 61, SEQ ID NO: 63, SEQ ID NO: 65, SEQ ID NO: 67, SEQ ID NO: 69, SEQ ID NO: 71, SEQ ID NO: 73, SEQ ID NO: 75, SEQ ID NO: 77, SEQ ID NO: 79, and SEQ ID NO: 81; and (b) a variable light (VL) chain sequence that is selected from the group consisting of SEQ ID NO: 2, SEQ ID NO: 4, SEQ ID NO: 6, SEQ ID NO: 8, SEQ ID NO: 10, SEQ ID NO: 12, SEQ ID NO: 14, SEQ ID NO: 16, SEQ ID NO: 18, SEQ ID NO: 20, SEQ ID NO: 22, SEQ ID NO: 24, SEQ ID NO: 26, SEQ ID NO: 28, SEQ ID NO: 30, SEQ ID NO: 32, SEQ ID NO: 34, SEQ ID NO: 36, SEQ ID NO: 38, SEQ ID NO: 40, SEQ ID NO: 42, SEQ ID NO: 44, SEQ ID NO: 46, SEQ ID NO: 48, SEQ ID NO: 50, SEQ ID NO: 52, SEQ ID NO: 54, SEQ ID NO: 56, SEQ ID NO: 58, SEQ ID NO: 60, SEQ ID NO: 62, SEQ

ID NO: 64, SEQ ID NO: 66, SEQ ID NO: 68, SEQ ID NO: 70, SEQ ID NO: 72, SEQ ID NO: 74, SEQ ID NO: 76, SEQ ID NO: 78, SEQ ID NO: 80, and SEQ ID NO: 82.

[058] In some instance, the isolated antibody or an immunologically active fragment that specifically binds human CD47 comprises a variable heavy (VH) chain sequence that is at least 95% identical to an amino acid sequence selected from the group consisting of SEQ ID NO: 3, SEQ ID NO: 31, SEQ ID NO: 79, and SEQ ID NO: 81; and a variable light (VL) chain sequence that is at least 95% identical to an amino acid sequence selected from the group consisting of SEQ ID NO: 4, SEQ ID NO: 32, SEQ ID NO: 80, and SEQ ID NO: 82; wherein the isolated monoclonal antibody or an immunologically active fragment thereof comprising a VH CDR1, VH CDR2 and VH CDR3 of the VH sequence shown in SEQ ID NO: 3, SEQ ID NO: 31, SEQ ID NO: 79, or SEQ ID NO: 81, and a VL CDR1, VL CDR2 and VL CDR3 of the VL sequence shown in SEQ ID NO: 4, SEQ ID NO: 32, SEQ ID NO: 80, or SEQ ID NO: 82; wherein the VH CDR1 having the amino acid sequence of NAWMN (SEQ ID NO: 85) or RAWMN (SEQ ID NO: 86), the VH CDR2 having the amino acid sequence of RIKRKTDGETTDYAAPVKG (SEQ ID NO: 87), and the VH CDR3 having the amino acid sequence of SNRAFDI (SEQ ID NO: 88); wherein the VL CDR1 having the amino acid sequence of KSSQSVLYSSNNRNYLA (SEQ ID NO: 89) or KSSQSVLYAGNNRNYLA (SEQ ID NO: 90), the VL CDR2 having the amino acid sequence of QASTRAS (SEQ ID NO: 91), and the VL CDR3 having the amino acid sequence of QQYYTPPLA (SEQ ID NO: 92).

[059] In some further instance, a CD47 antibody suitable for the compositions of this invention would include a combined VH/VL chain sequence that is selected from the group consisting of SEQ ID NO: 1 and SEQ ID NO: 2 (i.e., 1A1), SEQ ID NO: 3 and SEQ ID NO: 4 (i.e., 1F8), SEQ ID NO: 5 and SEQ ID NO: 6 (i.e., 2A11), SEQ ID NO: 7 and SEQ ID NO: 8 (i.e., 2C2), SEQ ID NO: 9 and SEQ ID NO: 10 (i.e., 2D7), SEQ ID NO: 11 and SEQ ID NO: 12 (i.e., 2G4), SEQ ID NO: 13 and SEQ ID NO: 14 (i.e., 2G11), SEQ ID NO: 15 and SEQ ID NO: 16 (i.e., 6F4), SEQ ID NO: 17 and SEQ ID NO: 18 (i.e., 5H1), SEQ ID NO: 19 and SEQ ID NO: 20 (i.e., 5F6), SEQ ID NO: 21 and SEQ ID NO: 22 (i.e., 1F3), SEQ ID NO: 23 and SEQ ID NO: 24 (i.e., 2A4), SEQ ID NO: 25 and SEQ ID NO: 26 (i.e., 2B12), SEQ ID NO: 27 and SEQ ID NO: 28 (i.e., 13A11), SEQ ID NO: 29 and SEQ ID NO: 30 (i.e., 15E1), SEQ ID NO: 31 and SEQ ID NO: 32 (i.e., 13H3), SEQ ID NO: 33 and SEQ ID NO: 34 (i.e., 14A8), SEQ ID NO: 35 and SEQ ID NO: 36 (i.e., 16H3), SEQ ID NO: 37 and SEQ ID NO: 38 (i.e., 1A1), SEQ ID NO: 39 and SEQ ID NO: 40 (i.e., 1A1-A), SEQ ID NO: 41 and SEQ ID NO: 42 (i.e., 1A1-Q), SEQ ID NO: 43 and SEQ ID NO: 44 (i.e., 1A2), SEQ ID NO: 45

and SEQ ID NO: 46 (i.e., 1A8), SEQ ID NO: 47 and SEQ ID NO: 48 (i.e., 1B1), SEQ ID NO: 49 and SEQ ID NO: 50 (i.e., 1B2), SEQ ID NO: 51 and SEQ ID NO: 52 (i.e., 1H3), SEQ ID NO: 53 and SEQ ID NO: 54 (i.e., 1H3-Q), SEQ ID NO: 55 and SEQ ID NO: 56 (i.e., 1H3-A), SEQ ID NO: 57 and SEQ ID NO: 58 (i.e., 2A2), SEQ ID NO: 59 and SEQ ID NO: 60 (i.e., 2A3), SEQ ID NO: 61 and SEQ ID NO: 62 (i.e., 2A6), SEQ ID NO: 63 and SEQ ID NO: 64 (i.e., 2A10), SEQ ID NO: 65 and SEQ ID NO: 66 (i.e., 2B1), SEQ ID NO: 67 and SEQ ID NO: 68 (i.e., 2C6), SEQ ID NO: 69 and SEQ ID NO: 70 (i.e., 2E7), SEQ ID NO: 71 and SEQ ID NO: 72 (i.e., 2E9), SEQ ID NO: 73 and SEQ ID NO: 74 (i.e., 2F1), SEQ ID NO: 75 and SEQ ID NO: 76 (i.e., 2F3), SEQ ID NO: 77 and SEQ ID NO: 78 (i.e., 34C5), SEQ ID NO: 79 and SEQ ID NO: 80 (i.e., A1A), and SEQ ID NO: 81 and SEQ ID NO: 82 (i.e., T4J).

[060] In some particular examples, a CD47 antibody suitable for the compositions of this invention would include a combined VH/VL chain sequence that is selected from the group consisting of SEQ ID NO: 3 and SEQ ID NO: 4 (i.e., 1F8), SEQ ID NO: 31 and SEQ ID NO: 32 (i.e., 13H3), or SEQ ID NO: 79 and SEQ ID NO: 80 (i.e., A1A). Antibodies 13H3 and A1A are the affinity matured clones of antibody 1F8. And the amino acid sequences of the three antibodies are highly similar.

Example 1: Isolation of mononuclear cells from human peripheral blood

[061] Human peripheral blood mononuclear cells (PBMCs) were isolated by Ficoll-Hypaque density gradient centrifugation. All experiments with human blood were conducted under Institutional Review Board (IRB) approved protocols.

[062] Fresh blood sample was diluted with phosphate-buffered saline (PBS), and then carefully layered on top of Ficoll-Paque Plus density gradient centrifugation media. The sample was then centrifuged at 2000 rpm for 20 min with breaks off. After density gradient centrifugation, differential migration of cells during centrifugation resulted in the formation of layers containing different cell types. PBMCs can be found together with other low-density slowly sedimenting particles (e.g., platelets) at the interface between the plasma and the Ficoll-Paque layer. The PBMCs were harvested by pouring the top layer, transferred to a new tube and then washed with PBS. Through centrifugation at 1500 rpm for 10 min, freshly isolated PBMCs were collected and re-suspended in PBS for further T cell subset isolation.

Example 2: Generation of macrophages from human peripheral blood CD14⁺ monocytes

[063] Human peripheral blood CD14⁺ monocytes were isolated by a positive selection method using a magnetic activated cell sorting (MACS) system according to the manufacturer's protocol.

[064] Then, the monocytes isolated by MACS were subsequently cultured in fresh complete medium supplemented with recombinant human granulocyte-macrophage colony-stimulating factor GM-CSF (50 ng/ml) and human recombinant IL-4 (35 ng/ml) to activate differentiated macrophages. After 6 days of culture (on day 7), cells were harvested, pooled together and counted for next use.

Example 3. Macrophage viability assay

[065] The cells from Example 2 were plated into a 96-well flat bottom plate at a density of 0.05×10^6 cells/well. After 2 hours of incubation, the macrophages were well attached to the plate. Then selected small molecule therapeutic agents were added to the well and cocultured for 16 hours. On day 8, macrophage viability was assessed by CellTiter-Glo[®] Luminescent Cell Viability Assay, according to the manufacturer's instruction.

Table 1. Selected small molecule therapeutic agents (Vendors are provided)

Therapeutic agents	Mechanism of Action	Vendor & Catalog Number
Lenalidomide	Immunomodulatory, po	MedChemExpress (HY-A0003)
Bendamustine	Alkylating agent, iv	MedChemExpress (HY-B0077)
Ibrutinib	BTK inhibitor, po	MedChemExpress (HY-10997)
Venetoclax	Bcl-2 inhibitor, po	MedChemExpress (HY-15531)
Copanlisib	PI3K inhibitor, po	MedChemExpress (HY-15346)
Duvelisib	PI3K inhibitor, po	MedChemExpress (HY-17044)
Idelalisib	PI3K inhibitor, po	MedChemExpress (HY-13026)
Azacitidine	Nucleoside antimetabolite, DNA Methyltransferase	MedChemExpress (HY-10586)

[066] Results showed that therapeutic agents Copanlisib, Duvelisib and Idelalisib present obvious toxicity to macrophages at a dose of 0.1-50 μ M. Azacytidine, Bendamustine, Ibrutinib, Lenalidomide and Venetoclax showed little toxic to macrophages when dose is

lower than 10 μM , although it showed toxicity to macrophages when dose is as high as 50 μM (see Figure 1, Table 2).

Table 2: Results of Macrophage Viability Assay

Cpd(uM) Cpd	50	25	5	1	0.2	0.04	0.008	0.002
Azacitidine	4580	4560	4768	5140	5160	4792	4648	4076
	5420	5520	6132	6108	6136	5892	6100	6020
	4932	5324	6032	6256	6440	6380	6132	5860
Bendamustine	4112	4340	5064	5436	5768	5788	6028	5908
	5500	5884	6424	6220	6320	6248	6248	6292
	5736	5724	6308	6200	6664	6124	6356	6220
Copanlisib	2748	4108	3780	4156	4572	4856	5272	5856
	3032	4176	4352	4492	4572	5120	4772	5928
	3028	4248	4196	4368	4928	4872	5388	6000
Duvelisib	3972	4176	4464	4592	5028	5228	5484	5688
	3992	4276	4584	4776	4984	5348	5444	5876
	3860	4156	4404	4880	5264	5760	5664	6184
Ibrutinib	3732	4680	4864	5056	4932	4688	4880	4948
	4296	5100	5828	5624	5736	5488	5316	4988
	4508	5572	6004	6224	6152	5780	5780	5520
Idelalisib	4404	4508	5092	5376	6040	6144	6164	5888
	4648	4884	5220	5640	6048	6304	6056	5908
	4964	4908	5060	5744	6220	5916	5948	5980
Lenalidomide	5208	5432	5840	6016	6216	6152	5992	5972
	5124	5752	6236	6108	6088	6132	5996	5872
	5644	5744	6108	6088	6456	5892	6180	5776
Venetoclax	1240	4696	5760	6052	7260	6288	6148	5968
	1132	4596	5660	6020	6072	6072	6172	6024
	1108	4776	6120	6088	5756	6264	5912	5856

[067] Example4: Fluorescence-activated cell sorting (FACS) analysis of expression levels of CD47 and calreticulin on tumor cellsurface For FACS analysis, on the day of analysis (day 1), Toledo cells were seeded into 96 well plate and cultured at a density of 0.2×10^6 cells/well. Then the cells were treated with the selected small molecule therapeutic agents for 12 hours.

[068] On day 2, cells were harvested, washed twice and then re-suspended in 100 μL FACS buffer subject to FACS analysis. For detection of the expression of calreticulin and CD47, cells were incubated with anti-calreticulin antibody and commercially available anti-CD47 antibody (BD Biosciences, Catalog number: 561249) in dark at 4°C for 30 mins.

[069] Then, the cells were subject to FACS analysis. Data was acquired and analyzed.

[070] In this assay, we studied the effects of the selected small molecule therapeutic agents on the expression level of calreticulin and CD47, to see if the therapeutic agents had the capability to affect the phagocytic ability of macrophage. Results showed that Azacytidine and Venecoclax can obviously inhibit CD47 expression level by inhibiting almost 50% of CD47 expression at a dose of 5 μ M, while Copanlisib only slightly inhibited CD47 expression level. Results also showed that Azacytidine and Venecoclax can obviously increase the expression level of calreticulin to about 20 folds and 120 folds at a dose of 5 μ M, respectively, while Copanlisib only slightly increased the expression level of Calreticulin (See Figure 2, Table 3).

Table 3: The expression level of CD47 and calreticulin on tumor cell analyzed by FACS

	Mean Fluorescence Intensity (MFI) CD47						Mean Fluorescence Intensity (MFI) Calreticulin					
	0	0.008 μ M	0.04 μ M	0.2 μ M	1 μ M	5 μ M	0	0.008 μ M	0.04 μ M	0.2 μ M	1 μ M	5 μ M
Azacytidine	8590	8204	8062	8460	6638	5229	66.8	65.8	71.9	88.4	174	491
Bendamustine	9138	9132	8684	8687	8319	9192	70.2	68.6	70.1	70.2	76.3	86.2
Copanlisib	9158	8024	8243	7844	7097	6803	69.2	69.8	77	79.4	106	142
Duvelisib	9124	8368	8376	8471	8432	8482	65.7	71.4	71.2	71.4	75.7	75.9
Ibrutinib	8544	8453	8490	8415	8345	8345	65.2	70.6	73.1	74.5	69.9	79
Idelalisib	9310	8342	8538	8246	8465	8263	69.3	69.4	72.9	70.6	77.6	75.8
Lenalidomide	9360	8706	8881	8730	8985	8443	69	69.3	70.1	73.4	75.6	75.2
Venetoclax	9512	7873	6201	5206	4232	3540	70	117	258	471	1123	2533

Example 5: Analysis of phagocytic ability of macrophages by FACS

[071] FACS-based phagocytosis assays were performed to evaluate the phagocytic abilities of macrophages.

[072] Toledo Cells (a tumor cell line, as a target) were labelled with CellTrace™ Far Red and plated into 96-well plate at a density of 0.15×10^6 cells/well for 12 hours. The Macrophages were prepared as discussed above and then labelled with CellTrace Violet. Next, the macrophages were added into the plate as an effector at an effector:target (Toledo) ratio of 1:3, giving a final density of macrophages at 0.05×10^6 cells/well. Macrophages and tumor cells were cocultured for 2-6h at 37 °C in the presence of the indicated treatments (small molecule therapeutic agents, CD47 antibody, CD20 antibody or combinations). The incubation time depending on the phagocytosis rate, expecting phagocytosis of 5-20% cells.

[073] Then, cells were acquired and data were analyzed.

[074] Results demonstrated that the isolated anti-CD47 antibody (i.e.,T4J)or Rituximab itself can promote macrophage phagocytosis,moreover, combination of the isolated anti-CD47 antibody (i.e., T4J) and Rituximab enhances the phagocyticability of macrophages even higher (See Table 4).

[075] Further,Axacytidineor Venetoclaxitself can also enhance the phagocytic ability of macrophages, moreover, the triple combination(isolated anti-CD47 antibody (i.e.,T4J), Rituximab and Axacytidine or Venetoclax) enhances the phagocytic ability of macrophages much higher.On the other hands, Bendamustine or Lenalidomide does not show the synergistic effects (see Figure 3, Table 4).

Table 4: Treatment effects of triple combinationtherapy on macrophage phagocytosis

Cpd (uM)	Azacitidine				Venetoclax			
	T4J=0		T4J=0.1 ug/ml		T4J=0		T4J=0.1 ug/ml	
	R=0	R=0.01 ug/ml	R=0	R=0.01 ug/ml	R=0	R=0.01 ug/ml	R=0	R=0.01 ug/ml
0	1.00	1.57	2.41	3.00	1.00	1.38	2.30	2.89
0.0016	1.09	1.63	2.66	3.26	1.00	1.45	2.62	3.23
0.008	1.07	1.73	2.69	3.18	1.10	1.54	2.59	3.30
0.04	1.12	1.71	2.76	3.29	1.17	1.82	2.64	3.37
0.2	1.16	1.69	2.57	3.13	1.41	1.93	2.81	3.64
1	1.30	1.94	2.70	3.45	1.56	2.03	2.91	3.65

Cpd (uM)	Bendamustine				Lenalidomide			
	T4J=0		T4J=0.1 ug/ml		T4J=0		T4J=0.1 ug/ml	
	R=0	R=0.01 ug/ml	R=0	R=0.01 ug/ml	R=0	R=0.01 ug/ml	R=0	R=0.01 ug/ml
0	1.00	1.36	2.20	3.03	1.00	1.31	2.20	2.86
0.0016	1.06	1.58	2.53	3.29	1.02	1.46	2.48	3.10
0.008	1.00	1.52	2.67	3.26	1.03	1.47	2.57	3.04
0.04	0.98	1.53	2.57	3.19	1.08	1.46	2.56	3.06
0.2	0.96	1.41	2.51	3.10	0.96	1.51	2.61	3.04
1	1.03	1.46	2.47	3.05	1.01	1.47	2.62	3.07

Example 6 In vivo test of the anti-tumor efficacy of anti-CD47 antibody in combination with anti-CD20 antibodyin a tumor xenograft model

[076] All animal experimental procedures were approved by the Institutional Animal Care and Use Committee (IACUC). Anti-CD47 antibody (i.e.T4J) and anti-CD20 antibody (i.e. rituximab) were used in this study.

[077] To establishthe xenograft model, human diffuse large B Cell lymphoma (WSU-DLCL2 cells) were subcutaneously (s.c.) injected into6- to 7-week-old NOD/SCID mice(Shanghai

Lingchang Biotechnology Co., Ltd, Shanghai, China) for tumor development. When tumor volume reaches a mean value of approximately 94 mm³, 32 mice were divided into 4 groups of 8 equally and injected intravenously (i.v.) with PBS (control group), anti-CD47 antibody (i.e. T4J), rituximab, or anti-CD47 antibody T4J+ rituximab at a dose of 5 mg/kg, respectively (See, Table 5). The anti-tumor efficacy studies were performed using a twice per week dosing schedule (5 mg/kg) for 4 weeks.

Table 5. Description of the treatment groups and control group

Group No.	No. of Mice	Treatment	Dosage (mg/kg)	Dose Vol. (ml/kg)	Route of administration	Dosing Schedule
1	8	PBS(control)	0	5	<i>i.v.</i>	BIW x 4 weeks
2	8	Rituximab	5	5	<i>i.v.</i>	BIW x 4 weeks
3	8	T4J	5	5	<i>i.v.</i>	BIW x 4 weeks
4	8	Rituximab	5	5	<i>i.v.</i>	BIW x 4 weeks
		T4J	5	5	<i>i.v.</i>	BIW x 4 weeks

[078] The first day of antibody administration was designated as day 0. Mice were then monitored for tumor development and progression, and observation continued until day 43. Tumor volumes were measured twice a week in two dimensions (length and width) using a caliper, and the volumes were calculated using the following formula: Volume (V) = (L x W x W)/2, where L is tumor length (the longest tumor dimension) and W is tumor width (the longest tumor dimension perpendicular to L). The percentage of tumor growth inhibition (TGI) was calculated as follows: $100\% \times (1 - [(V_{\text{treated}}^{\text{(final day)}} - V_{\text{treated}}^{\text{(initial day)}}) / (V_{\text{control}}^{\text{(final day)}} - V_{\text{control}}^{\text{(initial day)}})])$, where V is the tumor volume. Tumor body weight was measured twice per week after randomization and at day 43. Antibody treatment was then stopped after 43 days of study and mice were euthanized and necropsied for evidence of tumors.

[079] Group comparisons were carried out using one-way analysis of variance (ANOVA). Data analysis was performed using IBM SPSS software version 18.0 (IBM, Armonk, NY, U.S.). Values of tumor volume and tumor body weight were expressed as mean $\pm$ standard error of the mean (SEM). A P value of less than 0.05 ($p < 0.05$) was considered statistically significant.

[080] After four weeks of dosing, the group treated with T4J showed no anti-tumor efficacy (1% TGI, $p=1.000$) compared with the control group. The group treated with a combination

of T4J (5 mg/kg) and Rituximab (5 mg/kg) showed significant anti-tumor efficacy compared with the control group (63% TGI, $p=0.004$), and demonstrated significantly improved anti-tumor efficacy ($p=0.002$) compared with the group treated with T4J, and showed improved but not significant anti-tumor efficacy ($p=0.086$) compared with the group treated with rituximab (Table 6). No deaths or adverse effect occurred in all groups during the period of study. Therefore, results indicate that rituximab can increase the therapeutic effects of T4J in the tumor xenograft model.

Table 6. Anti-tumor efficacy of antibody

Group	Treatment	Tumor Volume (mm ³) on day 43 (Mean $\pm$ SEM)	TGI (%)	T/C (%)	<i>P</i> value (compared with control)	<i>P</i> value (compared with group 4)
1	PBS (Control)	2161.32 $\pm$ 252.88	-	-	-	
2	Rituximab 5mg/kg	1578.37 $\pm$ 241.53	27	73	0.376	0.086
3	T4J 5mg/kg	2148.89 $\pm$ 227.87	1	99	1.000	0.002
4	Rituximab+ T4J 5mg/kg+5mg/kg	794.9 $\pm$ 174.05	63	37	0.004	

[081] Analysis of tumor growth showed that the group treated with T4J combined with rituximab showed significant tumor regression at day 43 (See Figures 4-5, Table 7). In summary, the group treated with a combination of T4J and rituximab improved therapeutic efficacy compared with the group treated with any of single agents, either T4J or rituximab itself (See Figures 4-5, Table 7).

Table 7. Analysis of Tumor Body Weight

Group	Treatment	Tumor Body Weight (mg) on day 43 (Mean $\pm$ SEM)	TGI (%)	T/C (%)	<i>P</i> value (compared with control)	<i>P</i> value (compared with group 4)
1	PBS (Control)	2520.7 $\pm$ 354.9	-	-	-	
2	Rituximab 5mg/kg	1673.7 $\pm$ 269.0	34	66	0.174	0.365
3	T4J 5mg/kg	2426.0 $\pm$ 283.0	4	96	0.995	0.007
4	Rituximab+T4J 5mg/kg+5mg/kg	1009.0 $\pm$ 207.7	60	40	0.004	

Example 7 In vivo test of anti-tumor efficacy of anti-CD47 antibody in combination with Azacitidine(AZA) in a tumor xenograft model

[082] All animal experimental procedures were approved by the Institutional Animal Care and Use Committee (IACUC). In this study, therapeutic efficacy of anti-CD47 antibody (i.e. T4J) and AZA alone or jointly in the treatment of the HL-60 xenograft model was evaluated.

Cell Culture

[083] HL-60 cells (a human promyelocytic leukemia cell line, ATCC® cat# CCL-240) were maintained in suspension culture in complete cell growth medium at 37°C in an atmosphere with 5% CO₂. The cells were split twice a week to maintain an exponential growth. After culturing, cells were harvested and counted for tumor inoculation.

Tumor Inoculation and Animal Grouping

[084] To establish the xenograft model, HL-60 cells (10×10^6) were suspended in 0.2 mL of PBS with the same volume of Matrigel and subcutaneously injected into the right flank of each mouse. When tumor volume reaches a mean value of approximately 72 mm³, i.e., on day 6 after inoculation, mice were divided into several groups and treated with PBS (control group), anti-CD47 antibody (i.e., T4J), AZA, or a combination of T4J and AZA, respectively.

Observations

[085] At the time of routine monitoring, the animals were daily checked for any effects of tumor growth and treatments on normal behavior such as mobility, food and water consumption (by looking only), body weight gain/loss (body weights were measured twice a week), eye/hair matting and any other abnormal effects. Death and observed clinical signs were recorded.

Tumor Measurements

[086] Tumor size was measured twice a week in two dimensions using a caliper, and the volume was calculated using the formula: Volume (V) = (L x W x W)/2, where L is tumor length (the longest tumor dimension) and W is tumor width (the longest tumor dimension perpendicular to L). The tumor volume was then used for calculating T/C values. The T/C value (in percentage) is an indicator of antitumor effectiveness, in which T and C are the mean volumes of the treatment group and the control groups, respectively.

[087] The percentage of tumor growth inhibition (TGI) was calculated for each group as follows: $100\% \times (1 - [(V_{\text{treated}}^{\text{final day}} - V_{\text{treated}}^{\text{initial day}}) / (V_{\text{control}}^{\text{final day}} - V_{\text{control}}^{\text{initial day}})])$, where V is the tumor volume.

[088] Tumor body weight was measured at the end of the study. T/Cweight value (in percentage) was calculated using the formula: T/Cweight % = Tweight / Cweight x 100 %

where Tweight and Cweight were the mean tumor body weight of the treatment group and the control group (vehicle group), respectively.

[089] Treatment efficacy was evaluated using Jin's formula:

$$Q = \frac{TGI(A+B)}{(TGI(A) + TGI(B) - TGI(A) \times TGI(B))}$$

[090] According to Jin's formula, $Q < 0.85$ indicates antagonistic effect, $0.85 \leq Q < 1.15$ indicates additive effect, and $Q \geq 1.15$ indicates synergistic effect.

Statistical Analysis

[091] T-test was performed to compare tumor body weight among groups. One-way ANOVA was performed to compare tumor volume among groups, and when a significant F-statistics (a ratio of treatment variance to the error variance) was obtained, comparisons between groups were carried out with Games-Howell test. All data were analyzed using IBM SPSS software. A P value of less than 0.05 ($p < 0.05$) was considered statistically significant.

Results:

[092] Tumor body weight was monitored regularly as an indirect measure of toxicity. No deaths or adverse effect occurred in all groups during the period of study. Tumor body weight changes in different treatment groups are shown in Figs 6a and 6b.

[093] Tumor volume over time was shown in Table 8. Tumor growth curve was shown in Fig. 7.

Table 8. Tumor volume over time

Days	Tumor volume (mm ³) ^a					
	Vehicle	T4J 3mg/kg	AZA 1mg/kg	AZA 2mg/kg	T4J+ AZA 3+1mg/kg	T4J+ AZA 3+2mg/kg
0 ^b	70 ± 4	70 ± 7	70 ± 7	70 ± 7	70 ± 6	70 ± 7
3	355 ± 31	180 ± 10	189 ± 11	161 ± 20	127 ± 20	184 ± 29
7	1166 ± 90	473 ± 36	589 ± 41	498 ± 82	186 ± 77	289 ± 85
10	2033 ± 166	760 ± 52	1142 ± 86	773 ± 145	262 ± 147	434 ± 140
12	2964 ± 248	1235 ± 89	2081 ± 177	1452 ± 253	431 ± 254	850 ± 258

^a. Mean ± SEM; ^b. days after first dosing

Conclusion

[094] As summarized in Table 9, the group treated with 1mg/kg AZA showed no obvious antitumor activity with a mean volume of 2081 ± 177 mm³ by comparing with the control group (2964 ± 248 mm³). The group treated with 3 mg/kg TJC4 and 1 mg/kg AZA for 12 days

showed a significant decrease in tumor volume ($431 \pm 254 \text{ mm}^3$) comparing to the control group ($2964 \pm 248 \text{ mm}^3$), indicating a significant anti-tumor effect.

[095] The group treated with 2mg/ml AZA showed certain anti-tumor activity with a mean volume of $1452 \pm 253 \text{ mm}^3$. The group treated with 3mg/kg TJC4 and 2 mg/kg AZA for 12 days showed a significant decrease in tumor volume ($850 \pm 258 \text{ mm}^3$) comparing to the control group ($2964 \pm 248 \text{ mm}^3$), indicating a significant antitumor effect.

[096] Statistical evaluation of two combination treatment groups was conducted based on the JIN's Formulation. Results showed that combination treatment with 1mg/kg AZA and 3mg/kg TJC4 resulted in Q value of 1.22, demonstrating a synergistic effect between AZA and CD47 antibody; and treatment with 2mg/kg AZA and 3mg/kg TJC4 resulted in Q value of 0.90, demonstrating an additive effect between AZA and CD47 antibody.

Table 9. Antitumor efficacy of antibody T4J in combination with AZA at day 12

Treatment	Tumor Size (mm^3) ^a at day 12	T/C (%)	TGI (%)	<i>p</i> value ^b	<i>Q</i> value	Drug combination
Vehicle	2964 ± 248	--	--	--	--	--
T4J(3mg/kg)	1235 ± 89	41.68	59.74	<0.001	--	--
AZA (1mg/kg)	2081 ± 177	70.21	30.52	0.032	--	--
AZA (2mg/kg)	1452 ± 253	48.98	52.27	<0.001	--	--
AZA+ T4J(1+3mg/kg)	431 ± 254	14.53	87.55	<0.001	1.22	synergistic effect
AZA+ T4J(2+3mg/kg)	850 ± 258	28.68	73.04	<0.001	0.90	Additive Effect

a. Mean $\pm$ SEM.

b. *p* value is calculated based on tumor size.

WHAT IS CLAIMED IS:

1. A pharmaceutical composition comprising an isolated antibody or an immunologically active fragment thereof that specifically binds human CD47, a therapeutic agent, and a pharmaceutically acceptable carrier; wherein the isolated antibody or an immunologically active fragment thereof that specifically binds human CD47 comprises a heavy chain variable region (VH) having amino acid sequence that is at least 95% identical to an amino acid sequence selected from the group consisting of SEQ ID NO: 1, SEQ ID NO: 3, SEQ ID NO: 5, SEQ ID NO: 7, SEQ ID NO: 9, SEQ ID NO: 11, SEQ ID NO: 13, SEQ ID NO: 15, SEQ ID NO: 17, SEQ ID NO: 19, SEQ ID NO: 21, SEQ ID NO: 23, SEQ ID NO: 25, SEQ ID NO: 27, SEQ ID NO: 29, SEQ ID NO: 31, SEQ ID NO: 33, SEQ ID NO: 35, SEQ ID NO: 37, SEQ ID NO: 39, SEQ ID NO: 41, SEQ ID NO: 43, SEQ ID NO: 45, SEQ ID NO: 47, SEQ ID NO: 49, SEQ ID NO: 51, SEQ ID NO: 53, SEQ ID NO: 55, SEQ ID NO: 57, SEQ ID NO: 59, SEQ ID NO: 61, SEQ ID NO: 63, SEQ ID NO: 65, SEQ ID NO: 67, SEQ ID NO: 69, SEQ ID NO: 71, SEQ ID NO: 73, SEQ ID NO: 75, SEQ ID NO: 77, SEQ ID NO: 79, and SEQ ID NO: 81; and a light chain variable region (VL) having amino acid sequence that is at least 95% identical to an amino acid sequence selected from the group consisting of SEQ ID NO: 2, SEQ ID NO: 4, SEQ ID NO: 6, SEQ ID NO: 8, SEQ ID NO: 10, SEQ ID NO: 12, SEQ ID NO: 14, SEQ ID NO: 16, SEQ ID NO: 18, SEQ ID NO: 20, SEQ ID NO: 22, SEQ ID NO: 24, SEQ ID NO: 26, SEQ ID NO: 28, SEQ ID NO: 30, SEQ ID NO: 32, SEQ ID NO: 34, SEQ ID NO: 36, SEQ ID NO: 38, SEQ ID NO: 40, SEQ ID NO: 42, SEQ ID NO: 44, SEQ ID NO: 46, SEQ ID NO: 48, SEQ ID NO: 50, SEQ ID NO: 52, SEQ ID NO: 54, SEQ ID NO: 56, SEQ ID NO: 58, SEQ ID NO: 60, SEQ ID NO: 62, SEQ ID NO: 64, SEQ ID NO: 66, SEQ ID NO: 68, SEQ ID NO: 70, SEQ ID NO: 72, SEQ ID NO: 74, SEQ ID NO: 76, SEQ ID NO: 78, SEQ ID NO: 80, and SEQ ID NO: 82.
2. The pharmaceutical composition of claim 1, wherein the isolated antibody or an immunologically active fragment thereof that specifically binds human CD47 comprises a VH/VL pair, wherein the VH/VL pair comprises VH and VL chain sequences selected from the group consisting of SEQ ID NO: 1 and SEQ ID NO: 2, SEQ ID NO: 3 and SEQ ID NO: 4, SEQ ID NO: 5 and SEQ ID NO: 6, SEQ ID NO: 7 and SEQ ID NO: 8, SEQ ID NO: 9 and SEQ ID NO: 10, SEQ ID NO: 11 and SEQ ID NO: 12, SEQ ID NO: 13 and SEQ ID NO: 14, SEQ ID NO: 15 and SEQ ID NO: 16, SEQ ID NO: 17 and SEQ ID NO: 18, SEQ ID NO: 19 and SEQ ID NO: 20, SEQ ID NO: 21 and SEQ ID NO: 22, SEQ ID NO: 23 and SEQ ID NO: 24, SEQ ID NO: 25

and SEQ ID NO: 26, SEQ ID NO: 27 and SEQ ID NO: 28, SEQ ID NO: 29 and SEQ ID NO: 30, SEQ ID NO: 31 and SEQ ID NO: 32, SEQ ID NO: 33 and SEQ ID NO: 34, SEQ ID NO: 35 and SEQ ID NO: 36, SEQ ID NO: 37 and SEQ ID NO: 38, SEQ ID NO: 39 and SEQ ID NO: 40, SEQ ID NO: 41 and SEQ ID NO: 42, SEQ ID NO: 43 and SEQ ID NO: 44, SEQ ID NO: 45 and SEQ ID NO: 46, SEQ ID NO: 47 and SEQ ID NO: 48, SEQ ID NO: 49 and SEQ ID NO: 50, SEQ ID NO: 51 and SEQ ID NO: 52, SEQ ID NO: 53 and SEQ ID NO: 54, SEQ ID NO: 55 and SEQ ID NO: 56, SEQ ID NO: 57 and SEQ ID NO: 58, SEQ ID NO: 59 and SEQ ID NO: 60, SEQ ID NO: 61 and SEQ ID NO: 62, SEQ ID NO: 63 and SEQ ID NO: 64, SEQ ID NO: 65 and SEQ ID NO: 66, SEQ ID NO: 67 and SEQ ID NO: 68, SEQ ID NO: 69 and SEQ ID NO: 70, SEQ ID NO: 71 and SEQ ID NO: 72, SEQ ID NO: 73 and SEQ ID NO: 74, SEQ ID NO: 75 and SEQ ID NO: 76, SEQ ID NO: 79 and SEQ ID NO: 80, and SEQ ID NO: 81 and SEQ ID NO: 82 (i.e., T4J).

3. The pharmaceutical composition of claim 1, wherein the isolated antibody or an immunologically active fragment that specifically binds human CD47 comprises a variable heavy (VH) chain sequence that is at least 95% identical to an amino acid sequence selected from the group consisting of SEQ ID NO: 3, SEQ ID NO: 31, SEQ ID NO: 79, and SEQ ID NO: 81; and a variable light (VL) chain sequence that is at least 95% identical to an amino acid sequence selected from the group consisting of SEQ ID NO: 4, SEQ ID NO: 32, SEQ ID NO: 80 and SEQ ID NO: 82.
4. The pharmaceutical composition of any one of claims 1-3, wherein the isolated antibody or an immunologically active fragment that specifically binds human CD47 comprises a VH/VL pair, wherein the VH/VL pair comprises VH and VL chain sequences that are respectively at least 95% identical to amino acid sequences selected from the group consisting of SEQ ID NO: 3 and SEQ ID NO: 4, SEQ ID NO: 31 and SEQ ID NO: 32, SEQ ID NO: 79 and SEQ ID NO: 80, and SEQ ID NO: 81 and SEQ ID NO: 82.
5. The pharmaceutical composition of any one of claims 1-4, wherein the isolated antibody or an immunologically active fragment thereof that specifically binds human CD47 comprises a heavy chain of SEQ ID NO: 81 and a light chain of SEQ ID NO: 82.
6. A pharmaceutical composition comprising an isolated antibody or an immunologically active fragment thereof that specifically binds human CD47, a therapeutic agent, and a pharmaceutically acceptable carrier, wherein the isolated antibody or an immunologically active fragment thereof that specifically binds human CD47 comprises

a heavy chain variable region (VH) having CDR1 of the amino acid sequence of NAWMN (SEQ ID NO: 85) or RAWMN (SEQ ID NO: 86), CDR2 having the amino acid sequence of RIKRKTDGETTDYAAPVKG (SEQ ID NO: 87), and CDR3 having the amino acid sequence of SNRAFDI (SEQ ID NO: 88), and

a light chain variable region (VL) having CDR1 of the amino acid sequence of KSSQSVLYSSNNRNYLA (SEQ ID NO: 89) or KSSQSVLYAGNNRNYLA (SEQ ID NO: 90), CDR2 of the amino acid sequence of QASTRAS (SEQ ID NO: 91), and CDR3 of the amino acid sequence of QQYYTPPLA (SEQ ID NO: 92).

7. The pharmaceutical composition of claim 6, wherein the isolated antibody or an immunologically active fragment that specifically binds human CD47 comprises a VH/VL pair, wherein the VH/VL pair comprises VH and VL chain sequences that are respectively at least 95% identical to amino acid sequences selected from the group consisting of SEQ ID NO: 3 and SEQ ID NO: 4, SEQ ID NO: 31 and SEQ ID NO: 32, SEQ ID NO: 79 and SEQ ID NO: 80, and SEQ ID NO: 81 and SEQ ID NO: 82.
8. The pharmaceutical composition of claim 6 or 7, where the isolated antibody or an immunologically active fragment thereof that specifically binds human CD47 comprises a heavy chain of SEQ ID NO: 81 and a light chain of SEQ ID NO: 82.
9. The pharmaceutical composition of any one of claims 1-8, wherein the isolated antibody or an immunologically active fragment thereof that specifically binds human CD47 is chimeric or humanized.
10. The pharmaceutical composition of any one of claims 1-9, wherein the isolated antibody or an immunologically active fragment thereof that specifically binds human CD47 is a monoclonal antibody, a bispecific antibody or a fusion antibody.
11. The pharmaceutical composition of claim 10, wherein the isolated bispecific antibody comprises a first arm and a second arm, wherein the first arm comprises the antibody or an immunologically active fragment thereof of any one of claims 1-4 which specifically binds human CD47, and the second arm comprises a second monoclonal antibody that does not bind human CD47.
12. The pharmaceutical composition of claim 11, wherein the second arm of the isolated bispecific antibody binds to a cancer cell.

13. The pharmaceutical composition of claim 10, wherein the fusion antibody is the isolated antibody or an immunologically active fragment thereof of claim 1 conjugated with an additional protein, a small-molecule agent or a marker.
14. The pharmaceutical composition of claim 13, wherein the additional protein in the fusion protein is an antibody or a cytokine; wherein the small molecule agent in the fusion protein is an anti-cancer or anti-inflammation agent; wherein the marker is a biomarker or fluorescent marker.
15. The pharmaceutical composition of any one of claims 1-14, wherein the isolated antibody or an immunologically active fragment thereof prevents human CD47 from interacting with signal-regulatory-protein α (SIRP α).
16. The pharmaceutical composition of any one of claims 1-12, wherein the isolated antibody or an immunologically active fragment thereof promotes macrophage-mediated phagocytosis of a CD47-expressing cell.
17. The pharmaceutical composition of any one of claims 1-16, wherein the isolated antibody or an immunologically active fragment thereof does not cause a significant level of hemagglutination or depletion of red blood cells.
18. The pharmaceutical composition of any one of claims 1-17, wherein the therapeutic agent synergizes the effect of the isolated antibody or an immunologically active fragment thereof that specifically binds human CD47 to promote phagocytosis.
19. The pharmaceutical composition of any one of claims 1-18, wherein the pharmaceutical composition is a synergistic combination, and the therapeutic agent enhances the therapeutic effect of the isolated antibody or immunologically active fragment thereof on phagocytic elimination of cancer cell.
20. The pharmaceutical composition of any one of claims 1-19, wherein the therapeutic agent increases expression level of Calreticulin.
21. The pharmaceutical composition of any one of claims 1-20, wherein the therapeutic agent inhibits expression level of CD47.
22. The pharmaceutical composition of any one of claims 1-21, wherein the therapeutic agent does not cause substantial toxicity to macrophages.
23. The pharmaceutical composition of any one of claims 1-22, wherein the therapeutic agent is a chemotherapeutic agent.

24. The pharmaceutical composition of claim 23, wherein the chemotherapeutic agent comprises Azacitidine, Venetoclax, or Copanlisib.
25. The pharmaceutical composition of claim 23 or 24, wherein the chemotherapeutic agent comprises Azacitidine or Venetoclax.
26. The pharmaceutical composition of any one of claims 23-25, wherein the chemotherapeutic agent comprises Azacitidine.
27. The pharmaceutical composition of any one of claims 1-22, wherein the therapeutic agent is an antibody or an immunologically active fragment thereof that selectively binds CD20 and promotes phagocytic elimination of cancer cell.
28. The pharmaceutical composition of claim 27, wherein the antibody or an immunologically active fragment thereof that selectively binds CD20 is Rituximab or a biosimilar thereof.
29. The pharmaceutical composition of any one of claims 1-22, wherein the therapeutic agent comprises a chemotherapeutic agent and an antibody or an immunologically active fragment thereof that selectively binds CD20.
30. The pharmaceutical composition of any one of claims 27-29, wherein the isolated antibody or an immunologically active fragment that binds CD47 synergizes with the antibody selectively binding CD20 and promotes phagocytic elimination of cancer cell.
31. The pharmaceutical composition of claim 30, wherein the therapeutic agent comprises Azacitidine and Rituximab, or Venetoclax and Rituximab.
32. A method for treating a disease in a human subject in need thereof, comprising administering to the subject a pharmaceutical composition of any one of claims 1-31 in a therapeutically effective amount, wherein the disease is cancer, a fibrotic disease, a disease related to inhibition of phagocytosis, or a disease related to platelet aggregation.
33. The method of claim 32, wherein the cancer is selected from the group consisting of ovarian cancer, colon cancer, breast cancer, lung cancer, head and neck cancer, bladder cancer, colorectal cancer, pancreatic cancer, non-Hodgkin's lymphoma, acute lymphocytic leukemia, chronic lymphocytic leukemia, acute myeloid leukemia, chronic myelogenous leukemia, hairy cell leukemia (HCL), T-cell prolymphocytic leukemia (T-PLL), large granular lymphocytic leukemia, adult T-cell leukemia, multiple myeloma, melanoma, leiomyoma, leiomyosarcoma, glioma, glioblastoma, myelomas, monocytic leukemias, B-cell derived leukemias, T-cell derived leukemias, B-cell derived lymphomas,

T-cell derived lymphomas, endometrial cancer, kidney cancer, melanoma, prostate cancer, thyroid cancer, cervical cancer, gastric cancer, liver cancer, and solid tumors; the fibrotic disease is selected from the group consisting of: myocardial infarction, angina, osteoarthritis, pulmonary fibrosis, asthma, cystic fibrosis, bronchitis, and asthma; the disease related to inhibition of phagocytosis is a cardiovascular disease; the disease related to platelet aggregation is Glanzmann Thrombasthenia, prolonged bleeding time, immune thrombocytopenia (ITP), von Willebrand disease (vWD).

34. The method of claim 32 or 33, wherein the cardiovascular disease is selected from the group consisting of atherosclerosis, stroke, hypertensive heart disease, rheumatic heart disease, cardiomyopathy, heart arrhythmia, congenital heart disease, valvular heart disease, carditis, aortic aneurysms, peripheral artery disease, and venous thrombosis.
35. Use of a pharmaceutical composition of any one of claims 1-31 for the manufacture of a medicament for treatment of a disease, wherein the disease is cancer, a fibrotic disease, a disease related to inhibition of phagocytosis, or a disease related to platelet aggregation.

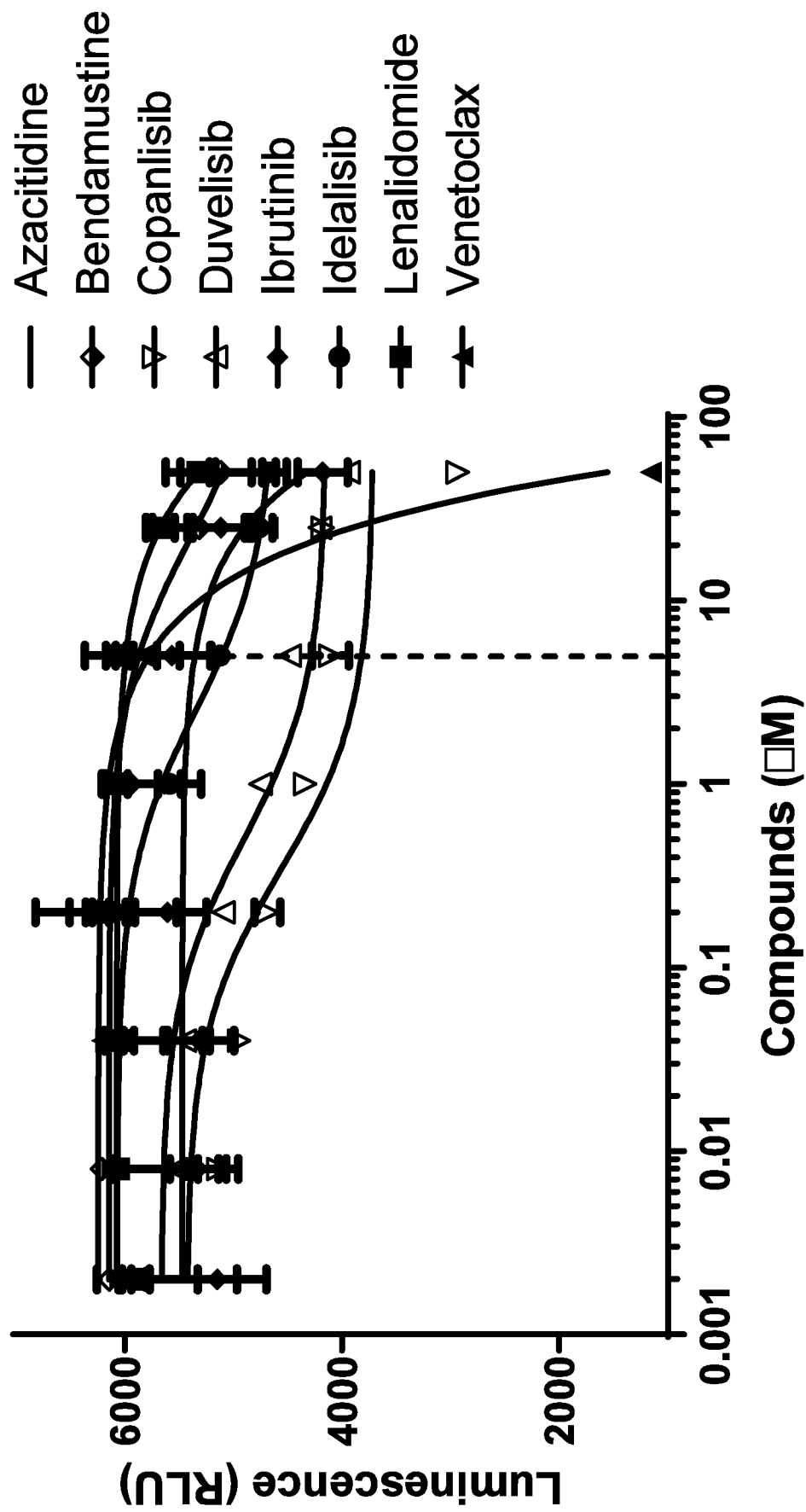


Fig. 1

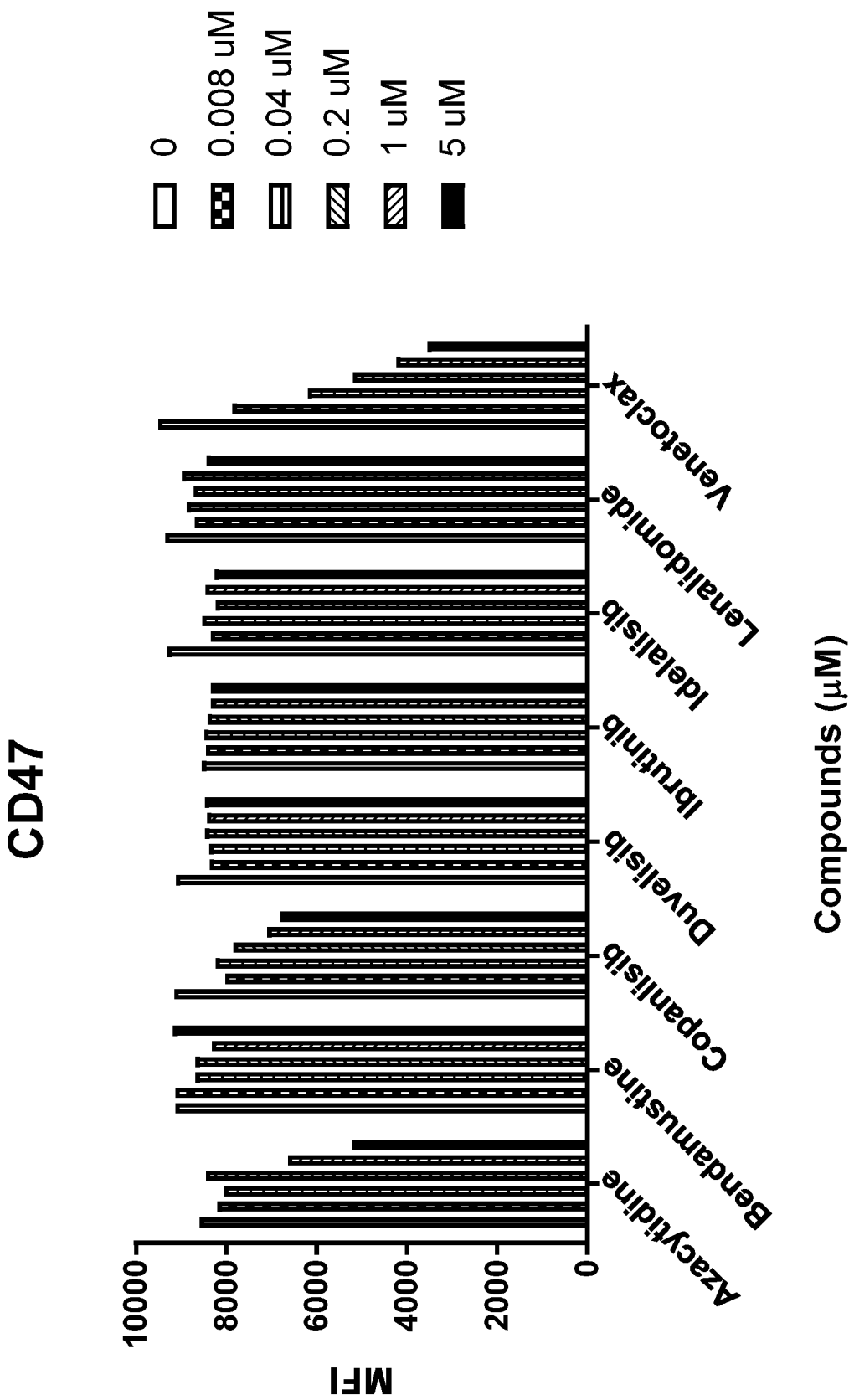


Fig. 2A

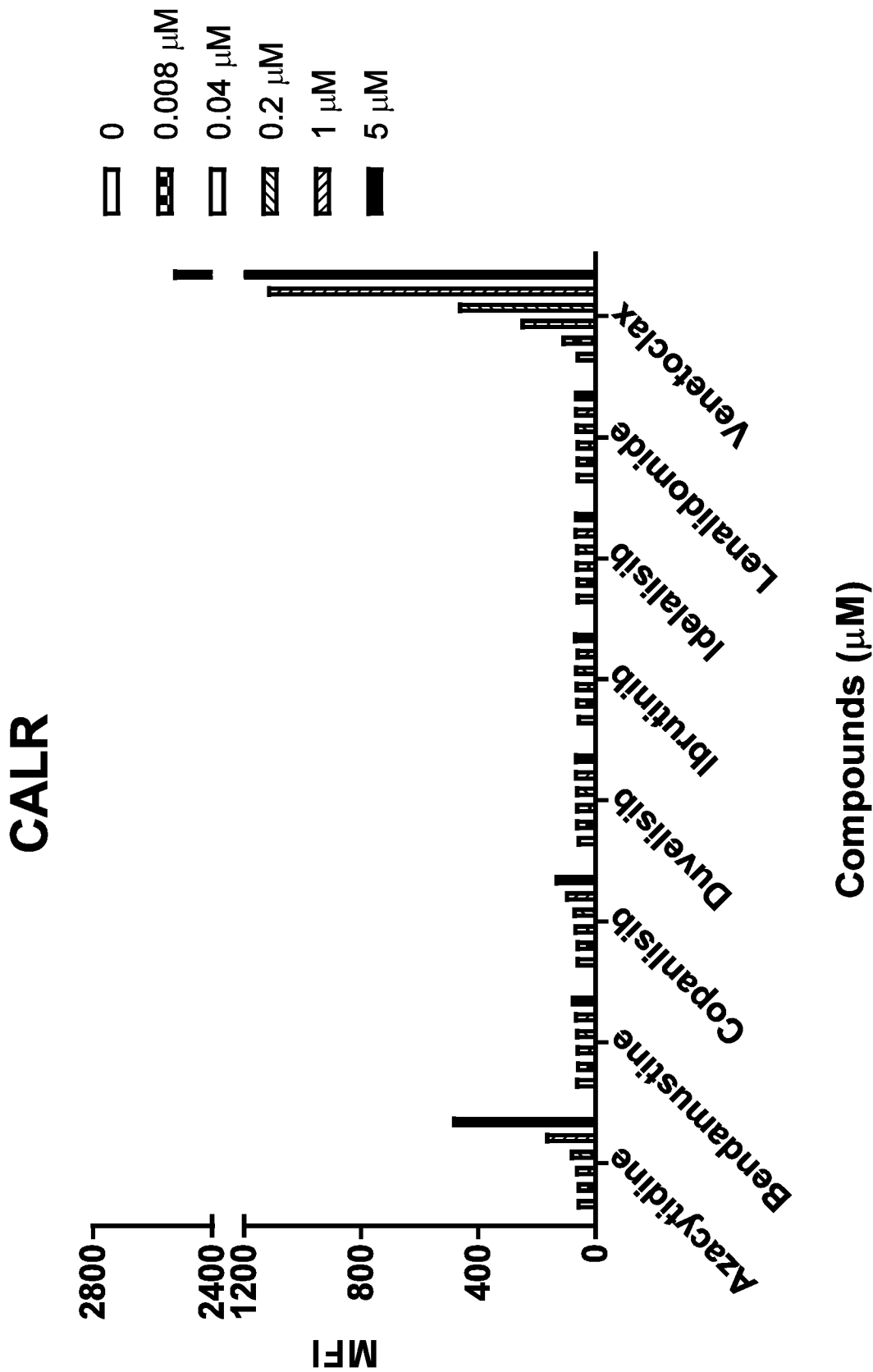


Fig. 2B

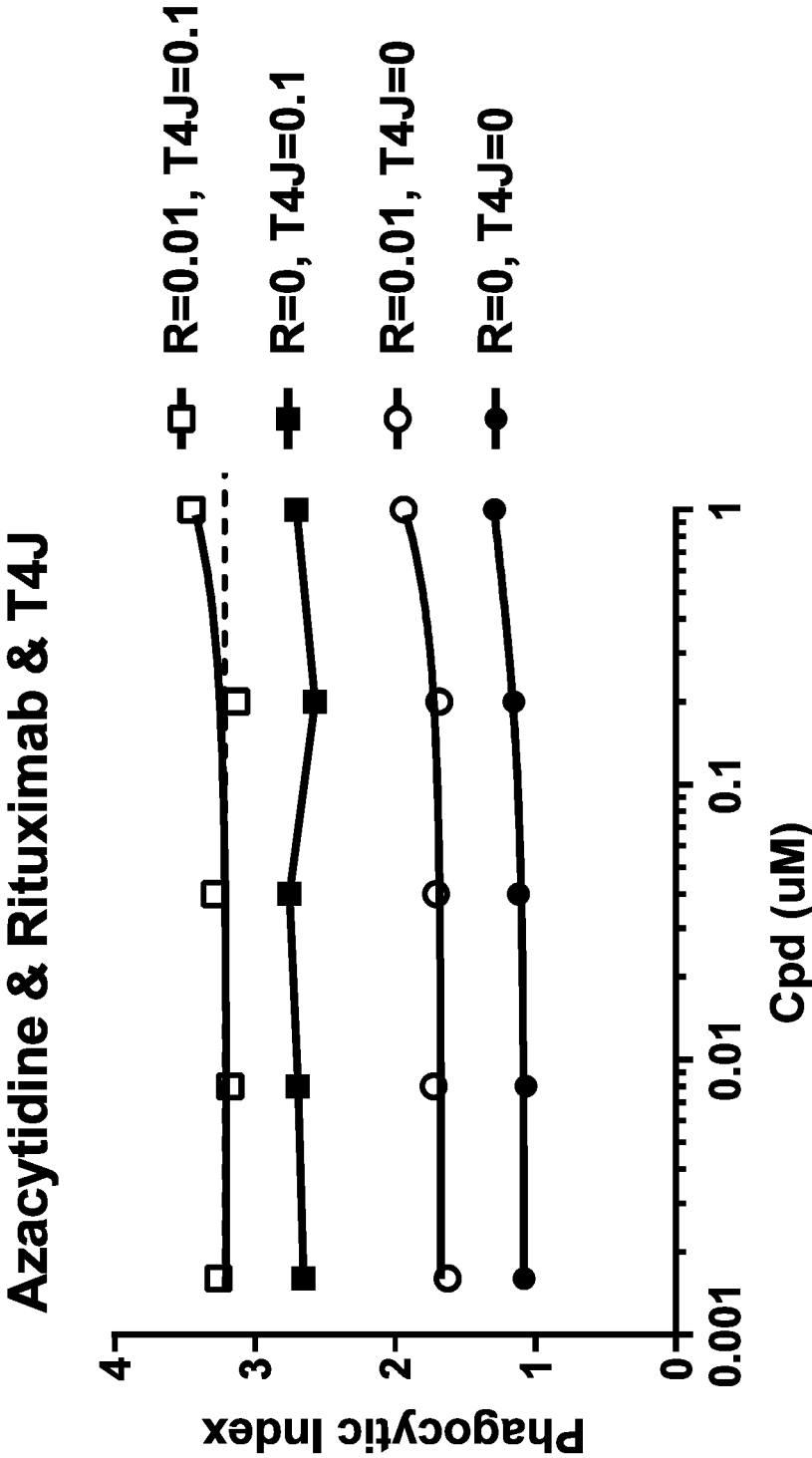


Fig. 3A

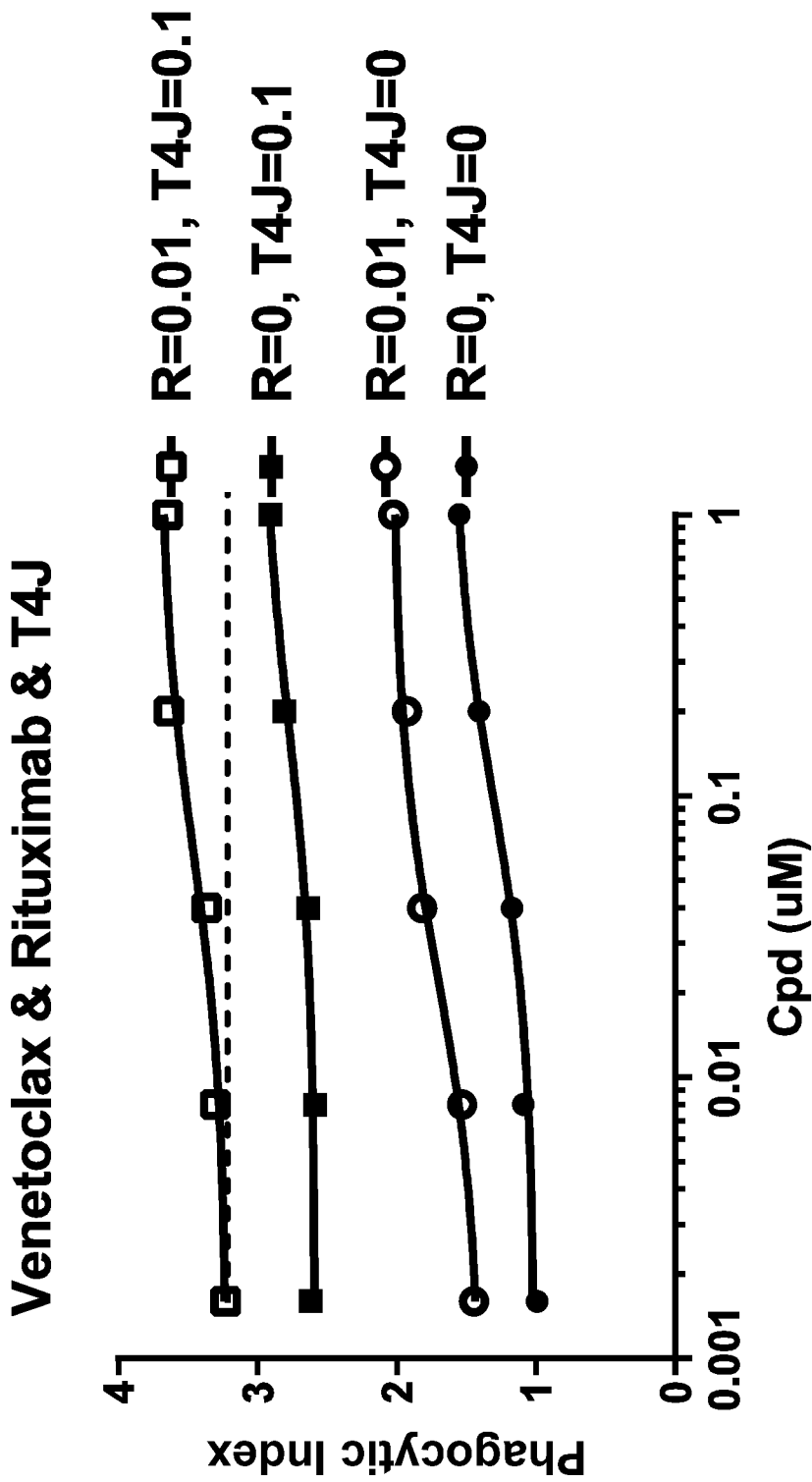


Fig. 3B

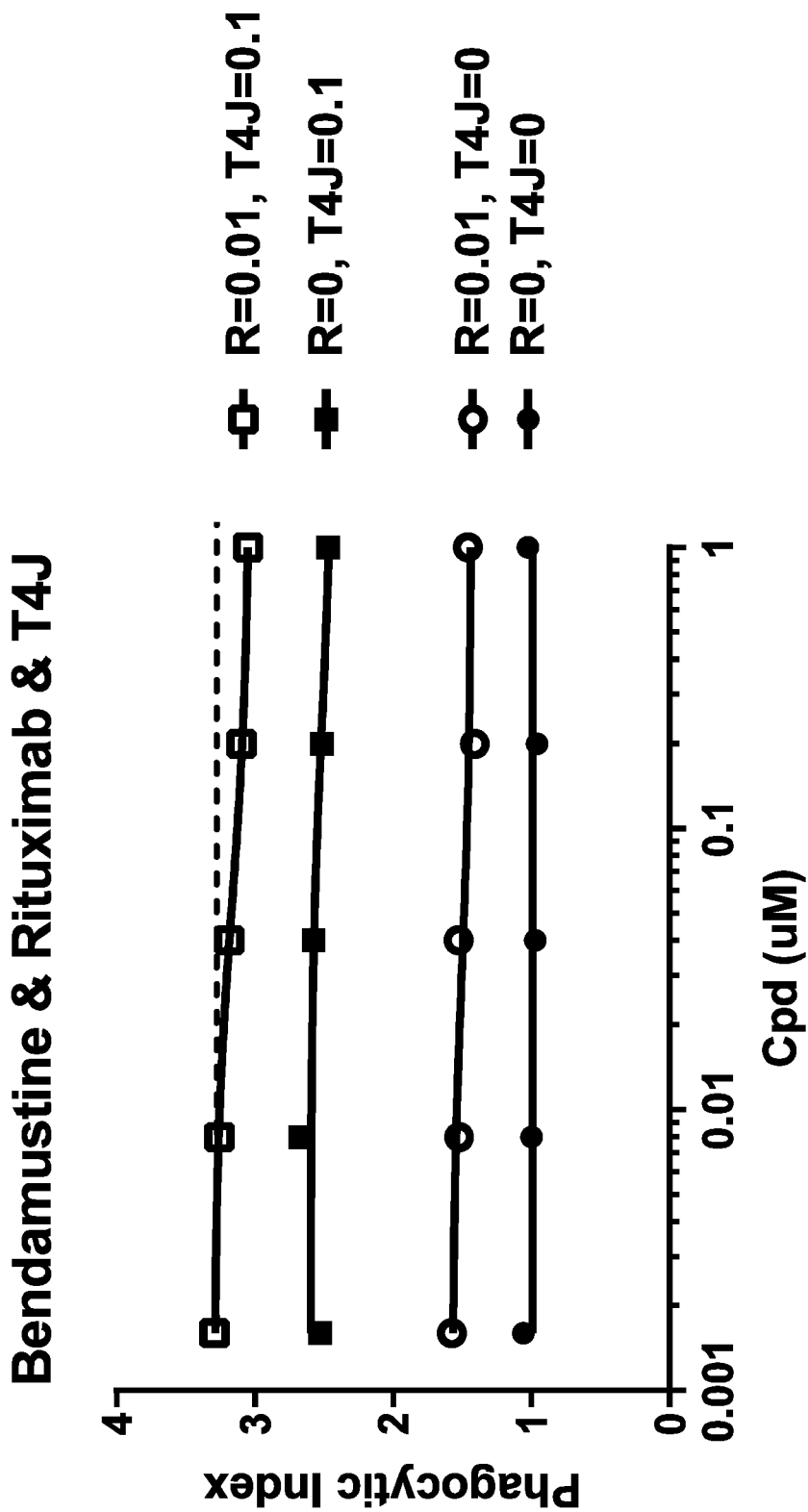


Fig. 3C

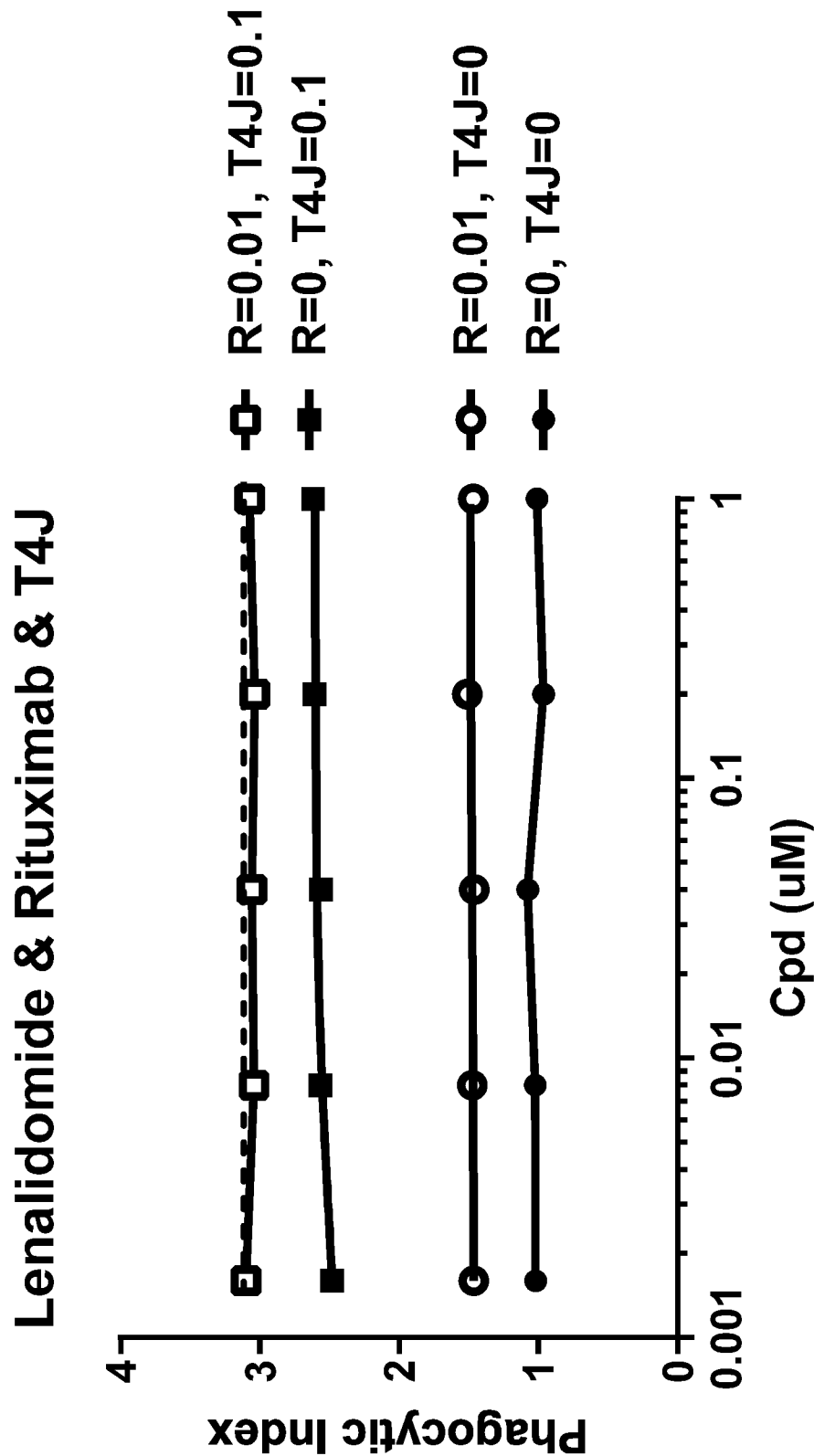


Fig. 3D

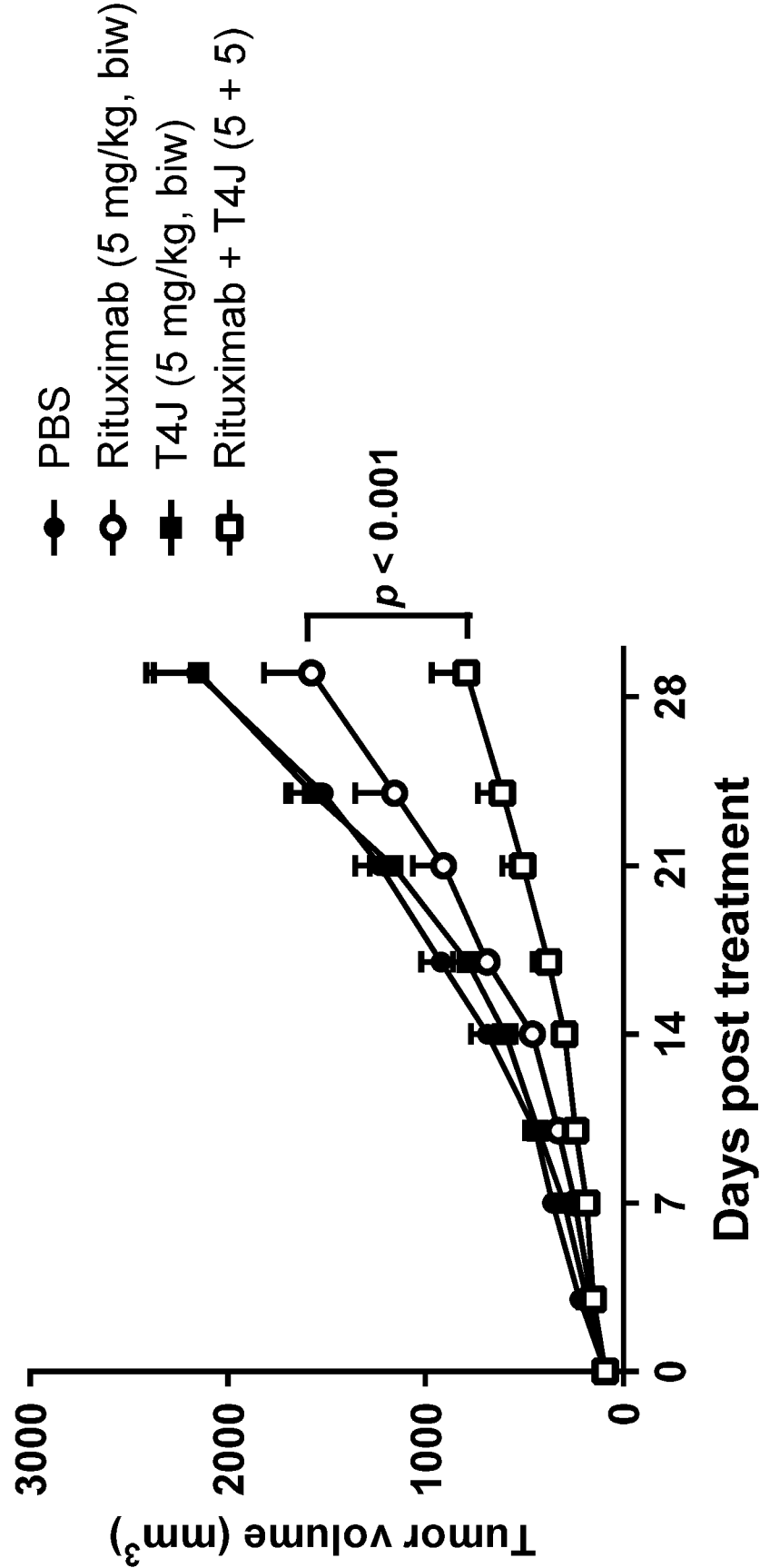


Fig. 4

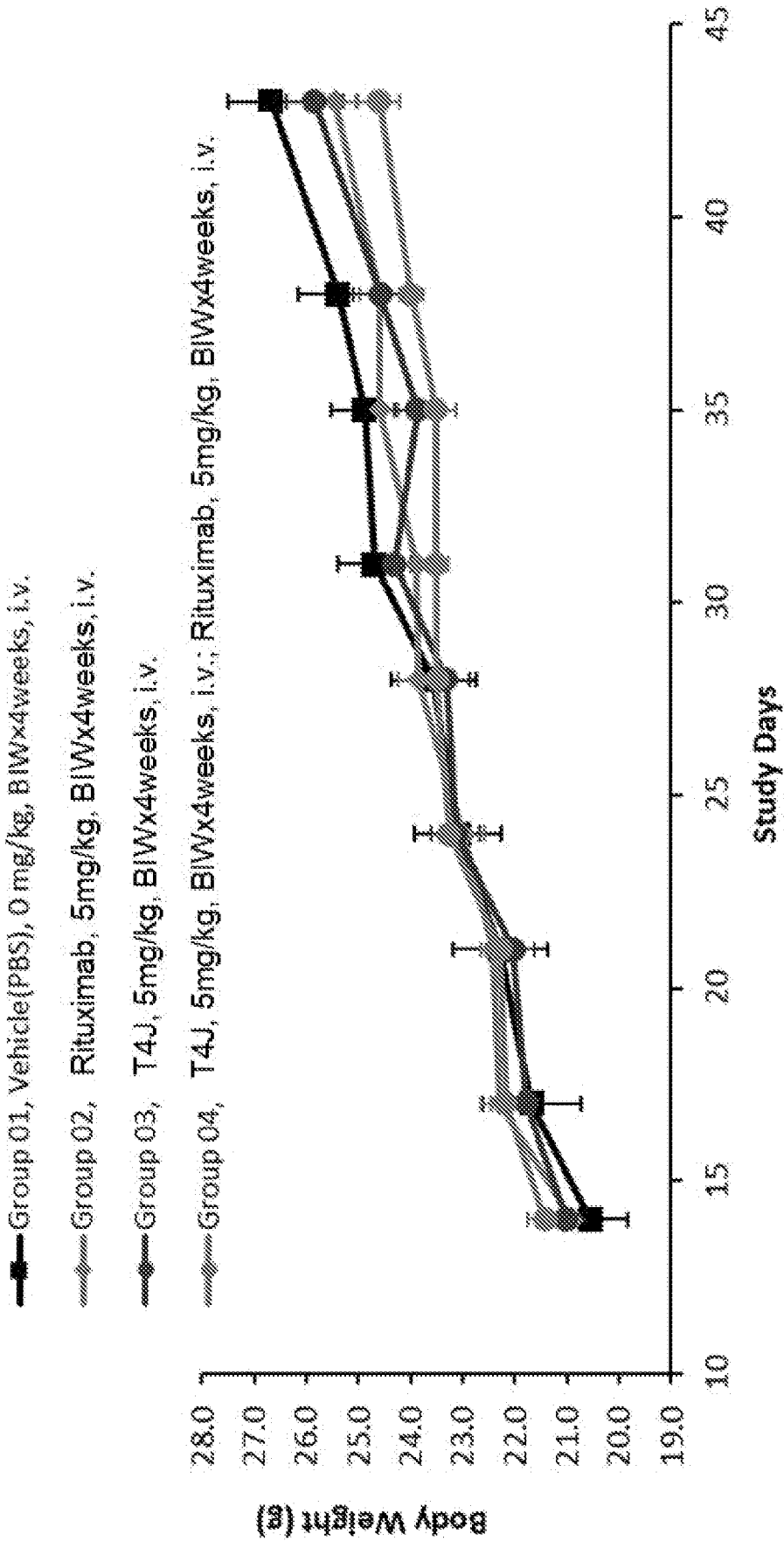


Fig. 5

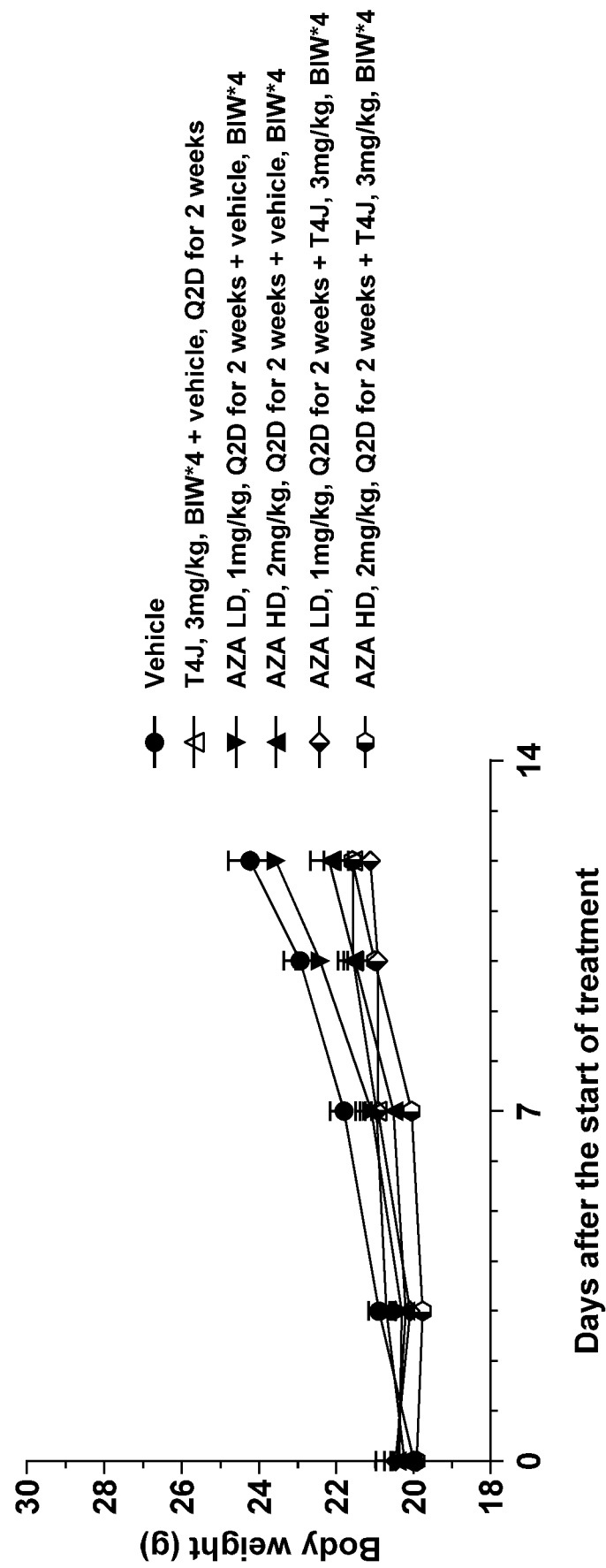


Fig 6a.

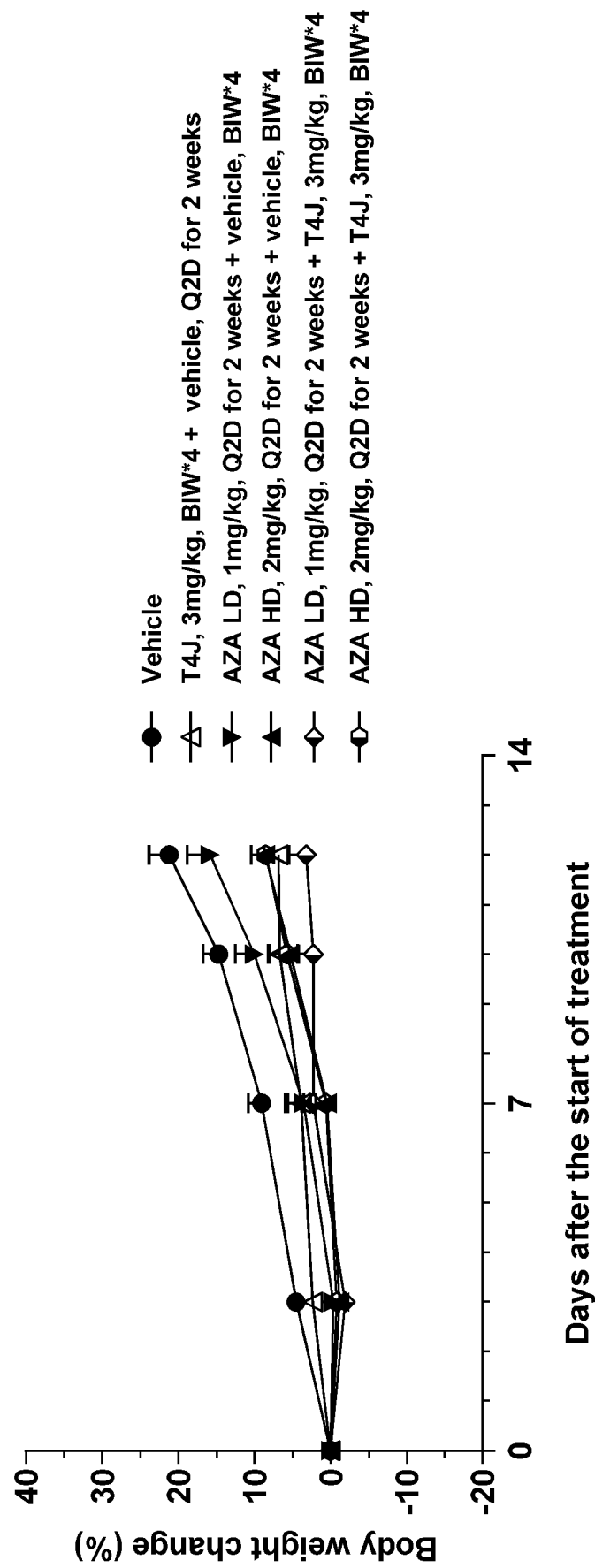


Fig.6b

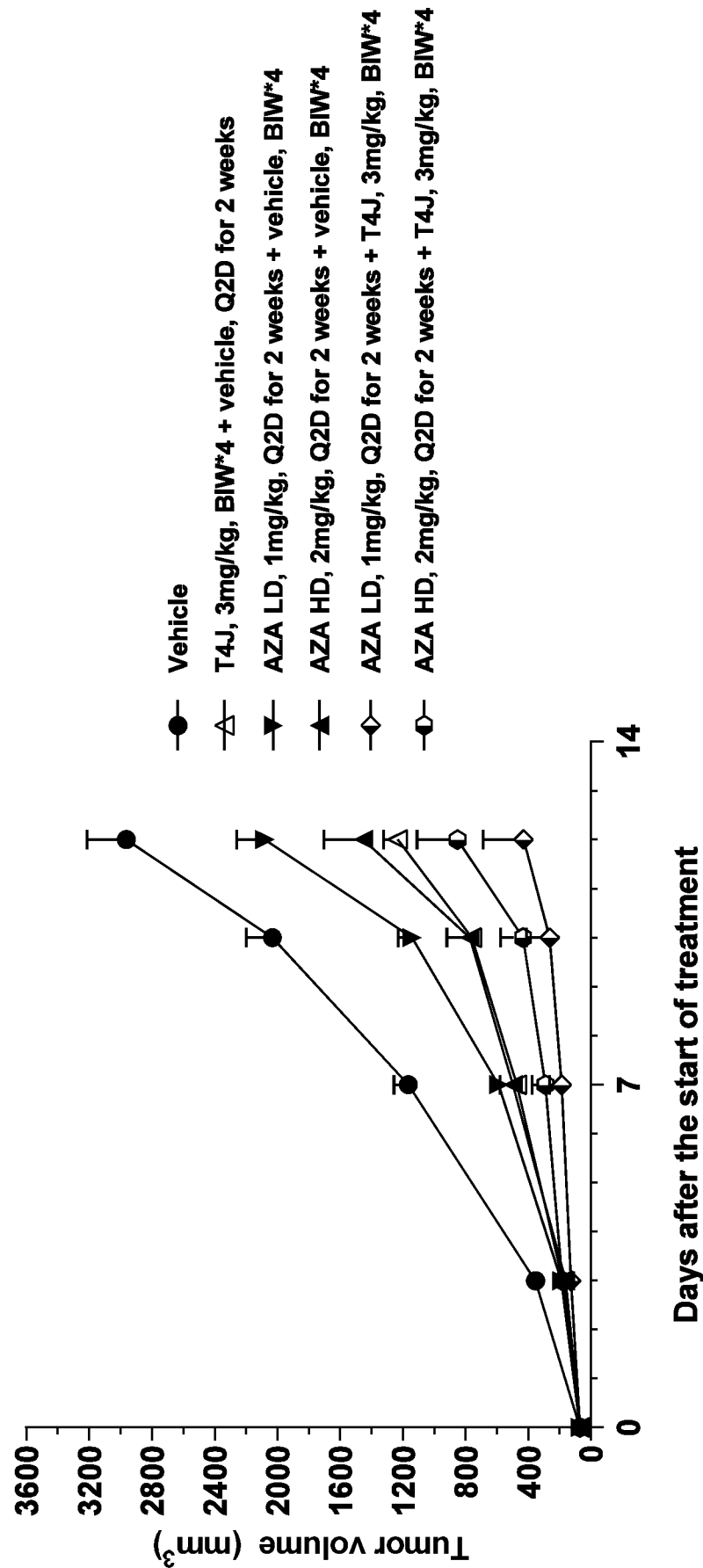


Fig.7

INTERNATIONAL SEARCH REPORT

International application No.

PCT/CN2021/091103

A. CLASSIFICATION OF SUBJECT MATTER

C07K 19/00(2006.01)i; A61K 39/395(2006.01)i; A61K 47/42(2017.01)i; A61P 35/00(2006.01)i

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

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)

C07K; A61K; A61P

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

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

DWPI, CNABS, CNTXT, SIPOABS, CNKI, PUMED, EMBL, NCBI, PUMED, NATURE :I-MAB BIOPHARMA, SEQ ID NO : 1-82, CD47, T4J, IG4, ANTIBODY, EPITOPE, PI3K, FACS, PHAGOCYTIC, CARLRETICULIN, ANTI-CD20, RITUXIMAB

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	CN 110582515 A (I-MAB BIOPHARMA CO., LTD.) 17 December 2019 (2019-12-17) claims 1-23, description paragraphs 23-38	1-4, 32-35
Y	CN 110582515 A (I-MAB BIOPHARMA CO., LTD.) 17 December 2019 (2019-12-17) claims 1-23, description paragraphs 23-38	1-35
Y	WO 9957151 A3 (BOEHRINGER INGELHEIM INTERNATIONAL GMBH) 16 March 2000 (2000-03-16) abstract, claims 1-22	1-35
Y	EP 2641918 A2 (AMGEN INC.) 25 September 2013 (2013-09-25) abstract, description paragraphs 327, 453-454, fig. 1	1-35
Y	WO 2019157432 A1 (FORTY SEVEN, INC.) 15 August 2019 (2019-08-15) abstract, claims 1-3	6-35
Y	CHAO, M.P. "Anti-CD47 antibody synergizes with rituximab to promote phagocytosis and eradicate non-Hodgkin lymphoma" CELL, Vol. 142, No. 5, 03 September 2010 (2010-09-03), abstract, pages 700-703	6-35
A	WO 2019185029 A1 (I-MAB) 03 October 2019 (2019-10-03) the whole document	1-35



Further documents are listed in the continuation of Box C.



See patent family annex.

* Special categories of cited documents:

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

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

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

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

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

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

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

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

"&" document member of the same patent family

Date of the actual completion of the international search

08 July 2021

Date of mailing of the international search report

26 July 2021

Name and mailing address of the ISA/CN

National Intellectual Property Administration, PRC
6, Xitucheng Rd., Jimen Bridge, Haidian District, Beijing
100088
China

Authorized officer

HUANG, Rui

Facsimile No. (86-10)62019451

Telephone No. 86-(10)-53961958

INTERNATIONAL SEARCH REPORT

International application No.

PCT/CN2021/091103**C. DOCUMENTS CONSIDERED TO BE RELEVANT**

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
A	WO 2018075857 A1 (I-MAB) 26 April 2018 (2018-04-26) the whole document	1-35
A	WO 2019154415 A1 (I-MAB) 15 August 2019 (2019-08-15) the whole document	1-35
A	PICCIONE, E.C. et al. "A bispecific antibody targeting CD47 and CD20 selectively binds and eliminates dual antigen expressing lymphoma cells" <i>MABS</i> , Vol. 7, No. 5, 31 October 2015 (2015-10-31), pages 946-956	1-35

INTERNATIONAL SEARCH REPORT

International application No.

PCT/CN2021/091103

Box No. I **Nucleotide and/or amino acid sequence(s) (Continuation of item 1.c of the first sheet)**

1. With regard to any nucleotide and/or amino acid sequence disclosed in the international application, the international search was carried out on the basis of a sequence listing:
 - a. ☒ forming part of the international application as filed:
 - ☒ in the form of an Annex C/ST.25 text file.
 - ☐ on paper or in the form of an image file.
 - b. ☐ furnished together with the international application under PCT Rule 13*ter*.1(a) for the purposes of international search only in the form of an Annex C/ST.25 text file.
 - c. ☐ furnished subsequent to the international filing date for the purposes of international search only:
 - ☐ in the form of an Annex C/ST.25 text file (Rule 13*ter*.1(a)).
 - ☐ on paper or in the form of an image file (Rule 13*ter*.1(b) and Administrative Instructions, Section 713).
2. ☐ In addition, in the case that more than one version or copy of a sequence listing has been filed or furnished, the required statements that the information in the subsequent or additional copies is identical to that forming part of the application as filed or does not go beyond the application as filed, as appropriate, were furnished.
3. Additional comments:

INTERNATIONAL SEARCH REPORT

International application No.

PCT/CN2021/091103

Box No. II Observations where certain claims were found unsearchable (Continuation of item 2 of first sheet)

This international search report has not been established in respect of certain claims under Article 17(2)(a) for the following reasons:

1. ☒ Claims Nos.: **32-34**
because they relate to subject matter not required to be searched by this Authority, namely:
 - [1] Claims 32-34 relate to a method for treating a disease, therefore do not warrant an international search.
The search is carried out on the basis of the composition in the manufacture of a medicament for treating diseases.
2. ☐ Claims Nos.:
because they relate to parts of the international application that do not comply with the prescribed requirements to such an extent that no meaningful international search can be carried out, specifically:
3. ☐ Claims Nos.:
because they are dependent claims and are not drafted in accordance with the second and third sentences of Rule 6.4(a).

INTERNATIONAL SEARCH REPORT
Information on patent family members

International application No.

PCT/CN2021/091103

Patent document cited in search report			Publication date (day/month/year)	Patent family member(s)			Publication date (day/month/year)
CN	110582515	A	17 December 2019	CA	3097443	A1	22 May 2020
WO	9957151	A3	16 March 2000	JP	2002513556	A	14 May 2002
				JP	4421779	B2	24 February 2010
				DE	69935516	D1	26 April 2007
				WO	9957151	A2	11 November 1999
				PL	358087	A1	09 August 2004
				SK	16192000	A3	09 April 2001
				NO	20005412	D0	27 October 2000
				IL	138701	D0	31 October 2001
				EP	1098979	B1	14 March 2007
				EP	0953639	A1	03 November 1999
				AU	4032299	A	23 November 1999
				YU	66300	A	12 March 2004
				ZA	200005506	B	14 May 2002
				NZ	508456	A	26 March 2004
				EA	005401	B1	24 February 2005
				AU	760305	B2	15 May 2003
				EE	200000642	A	15 April 2002
				UA	73276	C2	15 July 2005
				BG	65034	B1	29 December 2006
				ZA	200005506	A	14 May 2002
				AR	016243	A1	20 June 2001
				ES	2283114	T3	16 October 2007
				AT	356873	T	15 April 2007
				EP	1098979	A2	16 May 2001
				DE	69935516	T2	31 January 2008
				BG	104828	A	28 September 2001
				HU	0101501	A3	28 January 2004
				KR	100580936	B1	17 May 2006
				CN	1303430	A	11 July 2001
				CA	2327586	C	26 February 2013
				HU	0101501	A2	28 August 2001
				CO	5050255	A1	27 June 2001
				TR	200003181	T2	20 April 2001
				NO	20005412	L	06 December 2000
				BR	9910577	A	11 September 2001
				EA	200001023	A1	25 June 2001
				NO	20005412	A	06 December 2000
				ID	26555	A	18 January 2001
				KR	20010042826	A	25 May 2001
				CA	2327586	A1	11 November 1999
EP	2641918	A2	25 September 2013	ES	2808660	T3	01 March 2021
				EP	2641917	A2	25 September 2013
				EP	2641917	A3	19 February 2014
				EP	2641918	A3	05 March 2014
				EP	2641917	B1	06 May 2020
WO	2019157432	A1	15 August 2019	KR	20200119834	A	20 October 2020
				US	2019248915	A1	15 August 2019
				CA	3090489	A1	15 August 2019
				AU	2019218271	A1	10 September 2020

INTERNATIONAL SEARCH REPORT
Information on patent family members

International application No.

PCT/CN2021/091103

Patent document cited in search report				Publication date (day/month/year)		Patent family member(s)	Publication date (day/month/year)
WO	2019185029	A1	03 October 2019	EP	3752190	A1	23 December 2020
				CN	111699005	A	22 September 2020
				WO	2019157432	A8	25 June 2020
				KR	20200130747	A	19 November 2020
				AU	2019241339	A1	14 November 2019
				US	2020157222	A1	21 May 2020
				KR	102177931	B1	13 November 2020
				BR	112020019827	A2	05 January 2021
				JP	2020518233	A	25 June 2020
				SG	11202006008W	A	29 July 2020
				CA	3086434	A1	03 October 2019
				IL	275734	D0	31 August 2020
				EP	3589660	A1	08 January 2020
				KR	20190128716	A	18 November 2019
				EA	202091590	A1	21 January 2021
				EP	3589660	A4	07 April 2021
				CL	2020002223	A1	29 January 2021
				CN	110891975	A	17 March 2020
WO	2018075857	A1	26 April 2018	CN	108738313	A	02 November 2018
				PH	12019500862	A1	19 August 2019
				CO	2019005033	A2	31 July 2019
				KR	102136742	B1	23 July 2020
				CL	2019001088	A1	09 August 2019
				IL	258352	D0	28 June 2018
				BR	112019008010	A2	09 July 2019
				PE	20190975	A1	09 July 2019
				KR	20180056682	A	29 May 2018
				JP	2019537547	A	26 December 2019
				AU	2017332960	B2	12 September 2019
				US	2021054093	A1	25 February 2021
				EP	3411071	A4	28 August 2019
				US	2020377611	A1	03 December 2020
				AU	2019222870	A1	26 September 2019
				WO	2018075857	A8	08 November 2018
				AU	2020217349	A1	03 September 2020
				US	2020140565	A1	07 May 2020
				AU	2017332960	A1	10 May 2018
				KR	20200088523	A	22 July 2020
				MX	2019004691	A	09 December 2019
				CA	2999058	A1	20 April 2018
				SG	11201903514V	A	30 May 2019
				EP	3411071	A1	12 December 2018
WO	2019154415	A1	15 August 2019	EP	3568415	A1	20 November 2019
				US	2021015858	A1	21 January 2021
				KR	102224556	B1	09 March 2021
				EP	3568415	A4	06 January 2021
				SG	11202004521Q	A	29 June 2020
				AU	2019218516	A1	21 November 2019
				KR	20200054133	A	19 May 2020
				CN	110352200	B	11 December 2020

INTERNATIONAL SEARCH REPORT
Information on patent family members

International application No.

PCT/CN2021/091103

Patent document cited in search report	Publication date (day/month/year)	Patent family member(s)	Publication date (day/month/year)
		CA 3086936 A1	15 August 2019
		IL 276257 D0	30 September 2020
		CN 110352200 A	18 October 2019
		KR 20210006530 A	18 January 2021