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(54) Title: ANTI-CD47/ANTI-PD-L1 MULTIPLE ANTIGEN BINDING PROTEINS AND METHODS OF USE THEREOF

(57) Abstract: The present application provides multiple antigen binding proteins containing a first antigen binding portion that specifically binds an epitope on CD47, preferably human CD47, and a second antigen binding portion that specifically binds an epitope on programmed death-ligand 1 (PD-L1). Also provided are nucleic acids encoding the multiple antigen binding proteins, vectors comprising the nucleic acids, host cells comprising the vectors, and pharmaceutical compositions comprising the multiple antigen binding proteins, as well as methods for treating cancer using the multiple antigen binding proteins and pharmaceutical compositions.



ANTI-CD47/ANTI-PD-L1 MULTIPLE ANTIGEN BINDING PROTEINS AND METHODS OF USE THEREOF

CROSS REFERENCE TO RELATED APPLICATION

[1] This application claims priority to PCT/CN2019/087364, filed on May 17, 2019, the disclosure of which is incorporated herein by reference in its entirety.

SUBMISSION OF SEQUENCE LISTING ON ASCII TEXT FILE

[2] This application contains a sequence listing, which is submitted electronically via EFS-Web as an ASCII formatted sequence listing with a file name "065782.9US1 Sequence Listing" and a creation date of April 5, 2020 and having a size of 124 kB. The sequence listing is submitted via EFS-Web is part of the specification and is herein incorporated by reference in its entirety.

FIELD OF THE INVENTION

[3] The present invention relates to multiple antigen binding proteins, especially bispecific antibodies, comprising a first antigen binding moiety that specifically binds an epitope of human CD47 and a second antigen binding moiety that specifically binds an epitope of programmed death-ligand 1 (PD-L1) and methods of making and using thereof.

BACKGROUND OF THE INVENTION

[4] The mammalian immune system is a host defense system to fight off pathogens and to protect against disease. This pervasive and complex system is comprised of numerous immune cells, tissues and organs coordinated in a spatiotemporal manner. When it functions properly, the abnormal cells are identified and distinguished from the body's own healthy cells followed by elimination. So, the intact human immune system is essential for our survival. On the contrary, disorder of this system will lead to autoimmune diseases, inflammatory diseases and cancer. The immune system can be broadly sorted into humoral immunity and cell-mediated immunity. The humoral immunity is mediated by macromolecules like antibodies. The cell-mediated immunity, by contrast, involves the activation of macrophages, natural killer cells (NK), and antigen-specific cytotoxic T-lymphocytes.

[5] The activation and inhibition of immune response is largely mediated by two independent signaling pathways. The first signal is antigen-specific provided by the binding of specific T cell receptor (TCR) to antigenic peptide complexed with major histocompatibility complex (MHC) on the membrane of antigen presenting cells (APC). The second signal is antigen nonspecific through engagement of co-

stimulatory molecules expressed on the membrane of APC and the T cells. Activation of T cells without co-stimulation results in T cell unresponsiveness or anergy.

[6] The lymphocyte response to antigen-receptor engagement is modulated by a series of co-stimulatory and co-inhibitory receptors involved in the second signaling pathway balancing the positive and negative signals to maximize immune responses against invaders while maintaining self-tolerance.

[7] CD28, a member of the CD28 family, is a major T cell co-stimulatory receptor constitutively expressed on naive CD4⁺ and CD8⁺ T cells. Cytotoxic T lymphocyte-associated antigen 4 (CTLA-4), also one member of the CD28 receptor family, is a co-inhibitory receptor constitutively expressed on regulatory T cells (Treg) or induced following T-cell activation via CD28. Either CD28 or CTLA-4 can bind to the B7.1 (CD80) or B7.2 (CD86) ligands, which transmits activation or inhibition signal in T cells, respectively.

[8] Ligands for the CD28 receptor include CD80, CD86, the programmed death-1 ligand (PD-L1), the programmed death-2 ligand (PD-L2) and others. In particular, PD-L1 is a transmembrane protein that binds to inhibitory checkpoint molecule of PD-1 leading to suppression of adaptive immune response by transmitting an inhibitory “don't find me” signal. The PD-1/PD-L1 signaling pathway plays an essential role in the development of immune tolerance by preventing over-reactivity of the immune system, and, thus, avoiding the development of autoimmune diseases. However, this is often deregulated during cancer progression, allowing tumor cells to bypass safeguarding mechanisms by masquerading as healthy tissues. Tumor cells that highly express PD-L1 can evade T-cell-mediated death and dampen anti-tumor adaptive immune response through activation of the PD-1/PD-L1 signaling pathway. PD-1 overexpression in human tumor-associated macrophages (TAMs) has also been proven to inhibit phagocytosis and tumor immunity. Currently, anti-PD-1 or anti-PD-L1 monoclonal antibodies (mAbs) that interrupt the PD-1/PD-L1 interaction have shown exciting improvement in cancer treatment. Despite the FDA approval of Keytruda, Opdivo, and Tecentriq for the treatment of advanced cancers, more effective approaches are needed for immunotherapy improvement because these antitumor agents generate only a partial response.

[9] CD47 (Cluster of Differentiation 47), also known as integrin associated protein (IAP), is a ubiquitous innate immune checkpoint receptor that serves as a “don't eat me” signal. It binds to its receptor SIRPα that is primarily expressed on phagocytic macrophages and dendritic cells, and, therefore, leads to inhibition of phagocytosis. Cancer cells have evolved to evade immune surveillance by upregulating CD47 expression to prevent phagocytosis. CD47 is broadly overexpressed on hematological and solid tumors, which highly correlates with poorer prognosis. Thus, blocking the CD47-SIRPα interaction with anti-CD47 antibodies or high-affinity SIRPα variant has become a potential strategy to promote macrophage-dependent destruction of tumors. However, given the ubiquitous expression of CD47, anti-CD47 antibodies have high potential to bind to healthy cells, especially red blood cells,

increasing the risk of hematologic toxicity. Meanwhile, studies have revealed that a CD47 blockade alone is not sufficient to generate antitumor immunity in immunocompetent hosts. Also, SIRP α treatment interfering with the CD47/SIRP α pathway did not induce phagocytosis reported by Sockolosky et al. (Sockolosky et al., PNAS 113:E2646-2654 (2016)). So, it is challenging for cancer treatment by anti-CD47 antibodies or high-affinity SIRP α when considering the efficacy and safety.

[10] Despite an inappropriate immune response by single CD47 blockade, the synergistic activity could be achieved after a combinatorial blockade of PD-L1, which was reported by Sockolosky et al. (Sockolosky et al., PNAS 113:E2646-2654 (2016)). As both CD47 and PD-L1 are overexpressed on some tumor cells, particularly, co-expression of these two proteins was identified in melanoma patients, it is reasonable to maximize cancer treatment by simultaneously blocking CD47 and PD-L1. More importantly, utilizing dual-targeting bispecific antibodies could further improve CD47 specificity of tumor targeting and consequently minimize side effects.

[11] The disclosures of all publications, patents, patent applications and published patent applications referred to herein are hereby incorporated herein by reference in their entirety.

BRIEF SUMMARY OF THE INVENTION

[12] The present invention relates to constructs comprising a multiple antigen binding protein comprising a first antigen binding moiety that specifically binds an epitope on human CD47 and a second antigen binding moiety that specifically binds an epitope on programmed death-ligand 1 (PD-L1), and methods of making and using thereof.

[13] Provided herein are isolated anti-CD47/anti-PD-L1 multiple antigen binding proteins or antigen binding fragments thereof. The isolated anti-CD47/anti-PD-L1 multiple antigen binding protein or antigen binding fragment thereof comprises (a) a first antigen binding portion comprising a heavy chain variable domain (V_H) and a light chain variable domain (V_L), wherein the V_H and V_L together form an antigen-binding site that specifically binds CD47, and wherein the V_H comprises a heavy chain complementarity determining region 1 (HCDR1), HCDR2, and HCDR3 comprising the amino acid sequences of SEQ ID NO:19, SEQ ID NO:20, and SEQ ID NO:21, respectively, and the V_L comprises a light chain complementarity determining region 1 (LCDR1), LCDR2, and LCDR3 comprising the amino acid sequences of SEQ ID NO:22, SEQ ID NO:23, and SEQ ID NO:24, respectively; and (b) a second antigen binding portion comprising a single-domain antibody that specifically binds PD-L1; wherein the first antigen binding portion and the second antigen binding portion are fused to each other.

[14] In certain embodiments, the second antigen binding portion comprises a single-domain antibody that comprises a complementarity determining region 1 (CDR1), CDR2, and CDR3 comprising the amino acid sequences of: (i) SEQ ID NO:25, SEQ ID NO:26, and SEQ ID NO:27, respectively, (ii)

SEQ ID NO:28, SEQ ID NO:29, and SEQ ID NO:30, respectively, or (iii) SEQ ID NO:31, SEQ ID NO:32, and SEQ ID NO:33, respectively.

[15] In certain embodiments, the first antigen binding portion is a full-length antibody comprising two heavy chains and two light chains. The first antigen binding portion can, for example, be an antibody fragment comprising a heavy chain comprising the V_H and a light chain comprising the V_L .

[16] In certain embodiments, the second antigen binding portion comprises a single polypeptide chain. In certain embodiments, the first antigen binding portion and the second antigen binding portion are fused. The carboxy (C)-terminus of the second antigen binding portion can, for example, be fused to the amino (N)-terminus of at least one heavy chain of the first antigen binding portion or the amino (N)-terminus of at least one light chain of the first antigen binding portion. The amino (N)-terminus of the second antigen binding portion can, for example, be fused to the carboxy (C)-terminus of at least one heavy chain of the first antigen binding portion or the carboxy (C)-terminus of at least one light chain of the first antigen binding portion. In certain embodiments, the first antigen binding portion and the second antigen binding portion are fused to each other via a peptide bond or a peptide linker. In certain embodiments, the peptide linker is a GS linker or a mutated human IgG1 hinge. The peptide linker can, for example, comprise an amino acid sequence selected from SEQ ID NO:8, SEQ ID NO:10, or SEQ ID NO:69.

[17] In certain embodiments, the heavy chain of the first antigen binding portion comprises an amino acid sequence at least 95% identical to SEQ ID NO:4, and the light chain of the first antigen binding portion comprises an amino acid sequence at least 95% identical to SEQ ID NO:6. The heavy chain of the first antigen binding portion can comprise the amino acid sequence of SEQ ID NO:4, and the light chain of the first antigen portion can comprise the amino acid sequence of SEQ ID NO:6.

[18] In certain embodiments, the second antigen binding portion comprises an amino acid sequence at least 95% identical to an amino acid sequence selected from SEQ ID NO:14, SEQ ID NO:16, or SEQ ID NO:18. The second antigen binding portion can comprise the amino acid sequence selected from SEQ ID NO:14, SEQ ID NO:16, or SEQ ID NO:18.

[19] In certain embodiments, the first antigen binding portion comprises a human, humanized, or chimeric antibody or antigen binding fragment thereof. In certain embodiments, the second antigen binding portion comprising a single-domain antibody that specifically binds PD-L1 is camelid, chimeric, human, partially humanized, or fully humanized.

[20] In certain embodiments, the isolated anti-CD47/anti-PD-L1 multiple antigen binding protein or antigen binding fragment thereof comprises an anti-CD47 full-length antibody and an anti-PD-L1 single-domain antibody, wherein:

- (a) the N-terminus of the anti-PD-L1 sdAb is fused to the C-terminus of both heavy chains of the anti-CD47 full-length antibody, and wherein the heavy chain fusion polypeptide comprises the amino acid sequence of SEQ ID NOs: 36, 38, 40, or 48 and the light chain polypeptide comprises the amino acid sequence of SEQ ID NO:6;
- (b) the C-terminus of the anti-PD-L1 sdAb is fused to the N-terminus of both heavy chains of the anti-CD47 full-length antibody, and wherein the heavy chain fusion polypeptide comprises the amino acid sequence of SEQ ID NOs: 42, 44, 46, or 50 and the light chain polypeptide comprises the amino acid sequence of SEQ ID NO:6;
- (c) the N-terminus of the anti-PD-L1 sdAb is fused to the C-terminus of both light chains of the anti-CD47 full-length antibody, and wherein the light chain fusion polypeptide comprises the amino acid sequence of SEQ ID NOs: 52, 54, 56, 64, or 68 and the heavy chain polypeptide comprises the amino acid sequence of SEQ ID NO:4; or
- (d) the C-terminus of the anti-PD-L1 sdAb is fused to the N-terminus of both light chains of the anti-CD47 full-length antibody, and wherein the light chain fusion polypeptide comprises the amino acid sequence of SEQ ID NOs: 58, 60, 62, or 66 and the heavy chain polypeptide comprises the amino acid sequence of SEQ ID NO:4.

[21] Also provided is an isolated nucleic acid encoding an isolated anti-CD47/anti-PD-L1 multiple antigen binding protein or antigen binding fragment thereof as described herein.

[22] Also provided is a vector comprising an isolated nucleic acid encoding an isolated anti-CD47/anti-PD-L1 multiple antigen binding protein or antigen binding fragment thereof as described herein.

[23] Also provided is a host cell comprising an isolated nucleic acid or an isolated vector as described herein.

[24] Further provided is a pharmaceutical composition comprising an isolated anti-CD47/anti-PD-L1 multiple antigen binding protein or antigen binding fragment as described herein, and a pharmaceutical acceptable carrier.

[25] Another aspect of the present application provides a method of treating a subject having or at risk of having a CD47 and/or PD-L1-related disease, comprising administering to the individual an effective amount of any one of the pharmaceutical compositions as described herein. In some embodiments, the CD47 and/or PD-L1 related disease is cancer. In some embodiments, the cancer is a solid tumor, such as a colon cancer. In some embodiments, the cancer is a hematological cancer, such as a leukemia, lymphoma, or myeloma. The leukemia can, for example, be selected from the group consisting of acute lymphocytic leukemia (ALL), acute myeloid leukemia (AML), chronic lymphocytic leukemia (CLL), chronic myelogenous leukemia (CML), Myeloproliferative disorder/neoplasm (MPDS), and

myelodysplasia syndrome. The lymphoma can, for example, be selected from the group consisting of a Hodgkin's lymphoma, both indolent and aggressive non-Hodgkin's lymphoma, Burkitt's lymphoma, and follicular lymphoma (small cell and large cell). The myeloma can, for example, be selected from the group consisting of multiple myeloma (MM), giant cell myeloma, heavy-chain myeloma, and light chain or Bence-Jones myeloma.

[26] In some embodiments, the method further comprises administering to the individual an additional cancer therapy, such as surgery, radiation, chemotherapy, immunotherapy, hormone therapy, or a combination thereof.

[27] In some embodiments, the PD-L1 related disease is a pathogenic infection.

[28] In some embodiments, the pharmaceutical composition is administered systemically, such as intravenously (i.v.). In some embodiments, the pharmaceutical composition is administered locally, such as intratumorally. In some embodiments, the individual is a human.

[29] Another aspect of the present application provides a method of producing any one of isolated anti-CD47/anti-PD-L1 multiple antigen binding proteins or antigen binding fragments described herein, comprising culturing a host cell comprising any one of the isolated nucleic acids or vectors described herein, or culturing any one of the isolated host cells described above, under conditions effective to express the encoded anti-CD47/anti-PD-L1 multiple antigen binding protein or antigen binding fragment thereof; and obtaining the expressed anti-CD47/anti-PD-L1 multiple antigen binding protein or antigen binding fragment thereof from said host cell. In some embodiments, the method further comprises producing a host cell comprising any one of the isolated nucleic acids or vectors described herein.

BRIEF DESCRIPTION OF THE DRAWINGS

[30] The foregoing summary, as well as the following detailed description of the invention, will be better understood when read in conjunction with the appended drawings. For the purpose of illustrating the invention there are shown in the drawings embodiments which are presently preferred. It should be understood, however, that the invention is not limited to the precise arrangements and instrumentalities shown. In the drawings:

[31] FIG. 1 depicts the results of a FACS binding assay using CHO-K1 cells expressing CD47.

[32] FIG. 2 depicts the results of a FACS binding assay using CHO-K1 cells expressing CD47.

[33] FIG. 3 depicts the results of a FACS binding assay using CHO-K1 cells expressing PD-L1.

[34] FIG. 4 depicts the results of a FACS binding assay using CHO-K1 cells expressing PD-L1.

[35] FIG. 5 depicts the results of a PD-1/PD-L1 blockade assay.

[36] FIG. 6 depicts the results of a PD-1/PD-L1 blockade assay.

[37] FIG. 7 depicts the results of a PD-1/PD-L1 blockade assay.

- [38] FIG. 8 depicts the results of a PD-1/PD-L1 blockade assay.
- [39] FIG. 9 depicts the results of a PD-1/PD-L1 blockade assay.
- [40] FIG. 10 depicts the results of a PD-1/PD-L1 blockade assay.
- [41] FIG. 11 depicts the results of a bioassay of anti-CD47 antibody mediated phagocytosis.
- [42] FIG. 12 depicts the results of a bioassay of anti-CD47 antibody mediated phagocytosis.
- [43] FIG. 13 depicts the results of a bioassay of anti-CD47 antibody mediated phagocytosis.
- [44] FIG. 14 depicts the results of a bioassay of anti-CD47 antibody mediated phagocytosis.
- [45] FIG. 15 depicts the results of a bioassay of anti-CD47 antibody mediated phagocytosis.
- [46] FIG. 16 depicts the results of a bioassay of anti-CD47 antibody mediated phagocytosis.
- [47] FIG. 17 depicts the results of a bioassay of anti-CD47 antibody mediated phagocytosis.
- [48] FIG. 18 depicts the results of a PD-1/PD-L1 blockade assay

DETAILED DESCRIPTION OF THE INVENTION

[49] The present invention provides an isolated anti-CD47/anti-PD-L1 multiple antigen binding protein or antigen-binding fragment thereof. The isolated anti-CD47/anti-PD-L1 multiple antigen binding protein or antigen-binding fragment thereof comprises a first antigen binding portion comprising a heavy chain variable domain (V_H) and a light chain variable domain (V_L), wherein the V_H and V_L together form an antigen-binding site that specifically binds CD47, and a second antigen binding portion comprising a single-domain antibody (sdAb) that specifically binds PD-L1, and its antibody variants. As a building block in an anti-CD47/anti-PD-L1 multiple antigen binding protein or antigen binding fragment thereof, the anti-PD-L1 sdAb has several advantages over other antigen binding fragments, such as Fab and scFv used in currently known multispecific formats. The advantages can include, but are not limited to, small size, high solubility and stability, and weak immunogenicity in humans. Thus, the anti-CD47/anti-PD-L1 multiple antigen binding protein can have similar molecular weight and pharmacokinetic properties as compared to those of the full-length antibody or antigen binding fragment component.

[50] Also provided are compositions (such as pharmaceutical compositions), kits, and articles of manufacture comprising the anti-CD47/anti-PD-L1 multiple antigen binding proteins or antigen-binding fragments thereof, methods of making the anti-CD47/anti-PD-L1 multiple antigen binding proteins or antigen-binding fragments thereof, and methods of treating CD47 and/or PD-L1 related diseases (such as cancer) using the anti-CD47/anti-PD-L1 multiple antigen binding proteins or antigen binding fragments thereof.

I. Definitions

[51] The practice of the present disclosure will employ, unless indicated specifically to the contrary, conventional methods of virology, immunology, microbiology, molecular biology and recombinant DNA

techniques within the skill in the art, many of which are described below for the purpose of illustration. Such techniques are explained fully in the literature, see, e.g., *Current Protocols in Molecular Biology* or *Current Protocols in Immunology*, John Wiley & Sons, New York, N.Y. (2009); Ausubel et al., *Short Protocols in Molecule Biology*, 3rd Ed., Wiley & Sons, 1995; Sambrook and Russell, *Molecule Cloning: A Laboratory Manual* (3rd Edition, 2001); Maniatis et al., *Molecule Cloning: A Laboratory Manual* (1982); *DNA Cloning: A Practical Approach*, Vol. I & II (D. Glover, ed.); *Oligonucleotide Synthesis* (N. Gait, ed., 1984); *Nucleic Acid Hybridization* (B. Hames & S. Higgins, eds., 1985); *Transcription and Translation* (B. Hames & S. Higgins, eds., 1984); *Animal Cell Culture* (R. Freshney, ed., 1986); Perbal, *A Practical Guide to Molecular Cloning* (1984) and other like references.

[52] The terms “CD47,” “integrin-associated protein (IAP),” “ovarian cancer antigen OA3,” “Rh-related antigen and MER6,” are used interchangeably, and include variants, isoforms, species homologs of human CD47, and analogs having at least one common epitope with CD47. Accordingly, the anti-CD47/anti-PD-L1 multiple antigen binding protein or antigen binding fragment thereof of the invention can, in certain cases, cross-react with CD47 from species other than human, or other proteins which are structurally related to human CD47 (e.g., human CD47 homologs). In other cases, the anti-CD47/anti-PD-L1 multiple antigen binding protein or antigen binding fragment thereof can be completely specific for human CD47 and not exhibit species or other types of cross-reactivity.

[53] The term “CD47” is a multi-spanning transmembrane receptor belonging to the immunoglobulin superfamily. CD47 expression and/or activity has been implicated in a number of diseases and disorders, e.g., cancer. CD47 interacts with SIRP α (signal-regulatory-protein α) on macrophages and thereby inhibits phagocytosis. This is a discovered mechanism of tumor immune avoidance, and therapeutically targeting CD47 has widespread application in numerous cancers. The expression of CD47 correlates with worse clinical outcomes in many distinct malignancies, including Acute Lymphocytic Leukemia (ALL), Non-Hodgkin Lymphoma (NHL), Acute Myelogenous Leukemia (AML), glioma, ovarian cancer, glioblastoma, etc. In addition, CD47 has been identified as a cancer stem cell marker in both leukemias and solid tumors (Jaiswal *et al.*, 2009 *Cell*, 138(2): 271-85; Chan *et al.*, 2010 *Curr Opin Urol*, 20(5): 393- 7; Majeti R *et al.*, 2011 *Oncogene*, 30(9): 1009-19).

[54] The terms “Programmed cell death 1 ligand 1,” “PD-L1,” “B7 homolog 1 (B7-H1),” “PD-L1 antigen,” “PDCD1 ligand 1” and “CD274” (see, e.g., Chemnitz (2004) *J. Immunol.* 173:945-954) are used interchangeably, and include variants, isoforms, species homologs of human PD-L1, and analogs having at least one common epitope with PD-L1 (see, e.g., Butte (2008) *Mol Immunol.* 45:3567-3572). Accordingly, the anti-CD47/anti-PD-L1 multiple antigen binding protein or antigen binding fragment thereof of the invention can, in certain cases, cross-react with PD-L1 from species other than human, or other proteins which are structurally related to human PD-L1 (e.g., human PD-L1 homologs). In other

cases, the anti-CD47/anti-PD-L1 multiple antigen binding protein or antigen binding fragment thereof can be completely specific for human PD-L1 and not exhibit species or other types of cross-reactivity.

[55] The term “human PD-L1” refers to human sequence PD-L1, such as the complete amino acid sequence of human PD-L1 having Genbank Accession Number Q9NZQ7. The human PD-L1 sequence can differ from human PD-L1 of Genbank Accession Number Q9NZQ7 by having, for example, conserved mutations or mutations in non-conserved regions and the PD-L1 has substantially the same biological function as the human PD-L1 of Genbank Accession Number Q9NZQ7. For example, a biological function of human PD-L1 is having an epitope in the extracellular domain of PD-L1 that is specifically bound by an anti-CD47/anti-PD-L1 multiple antigen binding protein or antigen binding fragment thereof of the instant disclosure or a biological function of human PD-L1 is modulation of T cell activity.

[56] The term “epitope” means a protein determinant capable of specific binding to an antibody. Epitopes usually consist of chemically active surface groupings of molecules such as amino acids or sugar side chains and usually have specific three dimensional structural characteristics, as well as specific charge characteristics. Conformational and nonconformational epitopes are distinguished in that the binding to the former, but not the latter, is lost in the presence of denaturing solvents.

[57] The term “Programmed cell death 1 (PD-1)” as used herein is intended to refer to a cell surface receptor that belongs to the immunoglobulin superfamily and is expressed on T cells and pro-B cells. The amino acid sequences of human B7-1 (CD80) are disclosed at Genbank Accession Numbers NP_005009.

[58] As used herein, “treatment” or “treating” is an approach for obtaining beneficial or desired results including clinical results. For purposes of this invention, beneficial or desired clinical results include, but are not limited to, one or more of the following: alleviating one or more symptoms resulting from the disease, diminishing the extent of the disease, stabilizing the disease (*e.g.*, preventing or delaying the worsening of the disease), preventing or delaying the spread (*e.g.*, metastasis) of the disease, preventing or delaying the recurrence of the disease, delay or slowing the progression of the disease, ameliorating the disease state, providing a remission (partial or total) of the disease, decreasing the dose of one or more other medications required to treat the disease, delaying the progression of the disease, increasing the quality of life, and/or prolonging survival. Also encompassed by “treatment” is a reduction of pathological consequence of cancer. The methods of the invention contemplate any one or more of these aspects of treatment.

[59] The term “effective amount” used herein refers to an amount of an agent or a combination of agents, sufficient to treat a specified disorder, condition or disease such as ameliorate, palliate, lessen, and/or delay one or more of its symptoms. In reference to cancer, an effective amount comprises an

amount sufficient to cause a tumor to shrink and/or to decrease the growth rate of the tumor (such as to suppress tumor growth) or to prevent or delay other unwanted cell proliferation. In some embodiments, an effective amount is an amount sufficient to delay development. In some embodiments, an effective amount is an amount sufficient to prevent or delay recurrence. An effective amount can be administered in one or more administrations. The effective amount of the drug or composition may: (i) reduce the number of cancer cells; (ii) reduce tumor size; (iii) inhibit, retard, slow to some extent and preferably stop cancer cell infiltration into peripheral organs; (iv) inhibit (*i.e.*, slow to some extent and preferably stop) tumor metastasis; (v) inhibit tumor growth; (vi) prevent or delay occurrence and/or recurrence of tumor; and/or (vii) relieve to some extent one or more of the symptoms associated with the cancer.

[60] As used herein, an “individual” or a “subject” refers to a mammal, including, but not limited to, human, bovine, horse, feline, canine, rodent, or primate. In certain embodiments, the subject is a human.

[61] The term “antibody” or “antibody moiety” is used in the broadest sense and encompasses various antibody structures, including but not limited to monoclonal antibodies, polyclonal antibodies, multispecific antibodies (*e.g.*, bispecific antibodies), full-length antibodies and antigen-binding fragments thereof, so long as they exhibit the desired antigen-binding activity.

[62] The basic 4-chain antibody unit is a heterotetrameric glycoprotein composed of two identical light (L) chains and two identical heavy (H) chains. An IgM antibody consists of 5 of the basic heterotetramer units along with an additional polypeptide called a J chain, and contains 10 antigen-binding sites, while IgA antibodies comprise from 2-5 of the basic 4-chain units which can polymerize to form polyvalent assemblages in combination with the J chain. In the case of IgGs, the 4-chain unit is generally about 150,000 Daltons. Each L chain is linked to an H chain by one covalent disulfide bond, while the two H chains are linked to each other by one or more disulfide bonds depending on the H chain isotype. Each H and L chain also has regularly spaced intrachain disulfide bridges. Each H chain has at the N-terminus, a variable domain (V_H) followed by three constant domains (C_H) for each of the α and γ chains and four C_H domains for μ and ϵ isotypes. Each L chain has at the N-terminus, a variable domain (V_L) followed by a constant domain at its other end. The V_L is aligned with the V_H and the C_L is aligned with the first constant domain of the heavy chain (C_{H1}). Particular amino acid residues are believed to form an interface between the light chain and heavy chain variable domains. The pairing of a V_H and V_L together forms a single antigen-binding site. For the structure and properties of the different classes of antibodies, see *e.g.*, *Basic and Clinical Immunology*, 8th Edition, Daniel P. Sties, Abba I. Terr and Tristram G. Parslow (eds), Appleton & Lange, Norwalk, Conn., 1994, page 71 and Chapter 6. The L chain from any vertebrate species can be assigned to one of two clearly distinct types, called kappa and lambda, based on the amino acid sequences of their constant domains. Depending on the amino acid sequence of the constant domain of their heavy chains (C_H), immunoglobulins can be assigned to different

classes or isotypes. There are five classes of immunoglobulins: IgA, IgD, IgE, IgG and IgM, having heavy chains designated α , δ , ϵ , γ and μ , respectively. The γ and α classes are further divided into subclasses on the basis of relatively minor differences in the C_H sequence and function, *e.g.*, humans express the following subclasses: IgG1, IgG2A, IgG2B, IgG3, IgG4, IgA1 and IgA2.

[63] An “isolated” antibody (or construct) is one that has been identified, separated, and/or recovered from a component of its production environment (*e.g.*, natural or recombinant). Preferably, the isolated polypeptide is free of association with all other components from its production environment. Contaminant components of its production environment, such as that resulting from recombinant transfected cells, are materials that would typically interfere with research, diagnostic, or therapeutic uses for the antibody, and may include enzymes, hormones, and other proteinaceous or non-proteinaceous solutes. In preferred embodiments, the polypeptide will be purified: (1) to greater than 95% by weight of antibody as determined by, for example, the Lowry method, and in some embodiments, to greater than 99% by weight; (2) to a degree sufficient to obtain at least 15 residues of N-terminal or internal amino acid sequence by use of a spinning cup sequenator; or (3) to homogeneity by SDS-PAGE under non-reducing or reducing conditions using Coomassie Blue or, preferably, silver stain. An 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, an isolated polypeptide, antibody, or construct will be prepared by at least one purification step.

[64] The “variable region” or “variable domain” of an antibody refers to the amino-terminal domains of the heavy or light chain of the antibody. The variable domains of the heavy chain and light chain may be referred to as “V_H” and “V_L”, respectively. These domains are generally the most variable parts of the antibody (relative to other antibodies of the same class) and contain the antigen binding sites. Heavy-chain only antibodies from the *Camelid* species have a single heavy chain variable region, which is referred to as “V_HH.” V_HH is thus a special type of V_H.

[65] The term “variable” refers to the fact that certain segments of the variable domains differ extensively in sequence among antibodies. The V domain mediates antigen binding and defines the specificity of a particular antibody for its particular antigen. However, the variability is not evenly distributed across the entire span of the variable domains. Instead, it is concentrated in three segments called complementary determining regions (CDRs) or hypervariable regions (HVRs) both in the light-chain and the heavy chain variable domains. The more highly conserved portions of variable domains are called the framework regions (FR). The variable domains of native heavy and light chains each comprise four FR regions, largely adopting a beta-sheet configuration, connected by three CDRs, which form loops connecting, and in some cases forming part of, the beta-sheet structure. The CDRs in each chain are held together in close proximity by the FR regions and, with the CDRs from the other chain, contribute to the

formation of the antigen binding site of antibodies (see Kabat *et al.*, *Sequences of Immunological Interest*, Fifth Edition, National Institute of Health, Bethesda, Md. (1991)). The constant domains are not involved directly in the binding of antibody to an antigen, but exhibit various effector functions, such as participation of the antibody in antibody-dependent cellular toxicity.

[66] The term “monoclonal antibody” as used herein 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 and/or post-translation modifications (*e.g.*, isomerizations, amidations) that may be present in minor amounts. Monoclonal antibodies are highly specific, being directed against a single antigenic site. In contrast to polyclonal antibody preparations which typically include different antibodies directed against different determinants (epitopes), each monoclonal antibody is directed against a single determinant on the antigen. In addition to their specificity, the monoclonal antibodies are advantageous in that they are synthesized by the 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 by a variety of techniques, including, for example, the hybridoma method (*e.g.*, Kohler and Milstein., *Nature*, 256:495-97 (1975); Hongo *et al.*, *Hybridoma*, 14 (3): 253-260 (1995), Harlow *et al.*, *Antibodies: A Laboratory Manual*, (Cold Spring Harbor Laboratory Press, 2nd ed. 1988); Hammerling *et al.*, in: *Monoclonal Antibodies and T-Cell Hybridomas* 563-681 (Elsevier, N.Y., 1981)), recombinant DNA methods (see, *e.g.*, U.S. Pat. No. 4,816,567), phage-display technologies (see, *e.g.*, Clackson *et al.*, *Nature*, 352: 624-628 (1991); Marks *et al.*, *J. Mol. Biol.* 222: 581-597 (1992); Sidhu *et al.*, *J. Mol. Biol.* 338(2): 299-310 (2004); Lee *et al.*, *J. Mol. Biol.* 340(5): 1073-1093 (2004); Fellouse, *Proc. Natl. Acad. Sci. USA* 101(34): 12467-12472 (2004); and Lee *et al.*, *J. Immunol. Methods* 284(1-2): 119-132 (2004), and technologies for producing human or human-like antibodies in animals that have parts or all of the human immunoglobulin loci or genes encoding human immunoglobulin sequences (see, *e.g.*, WO 1998/24893; WO 1996/34096; WO 1996/33735; WO 1991/10741; Jakobovits *et al.*, *Proc. Natl. Acad. Sci. USA* 90: 2551 (1993); Jakobovits *et al.*, *Nature* 362: 255-258 (1993); Bruggemann *et al.*, *Year in Immunol.* 7:33 (1993); U.S. Pat. Nos. 5,545,807; 5,545,806; 5,569,825; 5,625,126; 5,633,425; and 5,661,016; Marks *et al.*, *Bio/Technology* 10: 779-783 (1992); Lonberg *et al.*, *Nature* 368: 856-859 (1994); Morrison, *Nature* 368: 812-813 (1994); Fishwild *et al.*, *Nature Biotechnol.* 14: 845-851 (1996); Neuberger, *Nature Biotechnol.* 14: 826 (1996); and Lonberg and Huszar, *Intern. Rev. Immunol.* 13: 65-93 (1995).

[67] The terms “full-length antibody,” “intact antibody,” or “whole antibody” are used interchangeably to refer to an antibody in its substantially intact form, as opposed to an antibody fragment.

Specifically, full-length 4-chain antibodies include those with heavy and light chains including an Fc region. Full-length heavy-chain only antibodies include the heavy chain (such as V_HH) and an Fc region. The constant domains may be native sequence constant domains (*e.g.*, human native sequence constant domains) or amino acid sequence variants thereof. In some cases, the intact antibody may have one or more effector functions.

[68] The term “constant domain” refers to the portion of an immunoglobulin molecule having a more conserved amino acid sequence relative to the other portion of the immunoglobulin, the variable domain, which contains the antigen-binding site. The constant domain contains the C_H1, C_H2 and C_H3 domains (collectively, CH) of the heavy chain and the CHL (or CL) domain of the light chain.

[69] The “light chains” of antibodies (immunoglobulins) from any mammalian species can be assigned to one of two clearly distinct types, called kappa (“κ”) and lambda (“λ”), based on the amino acid sequences of their constant domains.

[70] An “antibody fragment” comprises a portion of an intact antibody, preferably the antigen binding and/or the variable region of the intact antibody. Examples of antibody fragments include, but are not limited to Fab, Fab', F(ab')₂ and Fv fragments; diabodies; linear antibodies (see U.S. Pat. No. 5,641,870, Example 2; Zapata *et al.*, *Protein Eng.* 8(10): 1057-1062 [1995]); single-chain antibody molecules; single-domain antibodies (such as V_HH), and multispecific antibodies formed from antibody fragments. Papain digestion of antibodies produced two identical antigen-binding fragments, called “Fab” fragments, and a residual “Fc” fragment, a designation reflecting the ability to crystallize readily. The Fab fragment consists of an entire L chain along with the variable region domain of the H chain (V_H), and the first constant domain of one heavy chain (C_H1). Each Fab fragment is monovalent with respect to antigen binding, *i.e.*, it has a single antigen-binding site. Pepsin treatment of an antibody yields a single large F(ab')₂ fragment which roughly corresponds to two disulfide linked Fab fragments having different antigen-binding activity and is still capable of cross-linking antigen. Fab' fragments differ from Fab fragments by having a few additional residues at the carboxy-terminus of the C_H1 domain including one or more cysteines from the antibody hinge region. Fab'-SH is the designation herein for Fab' in which the cysteine residue(s) of the constant domains bear a free thiol group. F(ab')₂ antibody fragments originally were produced as pairs of Fab' fragments which have hinge cysteines between them. Other chemical couplings of antibody fragments are also known.

[71] The Fc fragment comprises the carboxy-terminal portions of both H chains held together by disulfides. The effector functions of antibodies are determined by sequences in the Fc region, the region which is also recognized by Fc receptors (FcR) found on certain types of cells.

[72] “Fv” is the minimum antibody fragment which contains a complete antigen-recognition and -binding site. This fragment consists of a dimer of one heavy- and one light-chain variable region domain

in tight, non-covalent association. From the folding of these two domains emanate six hypervariable loops (3 loops each from the H and L chain) that contribute the amino acid residues for antigen binding and confer antigen binding specificity to the antibody. However, even a single variable domain (or half of an Fv comprising only three CDRs specific for an antigen) has the ability to recognize and bind antigen, although at a lower affinity than the entire binding site.

[73] “Single-chain Fv” also abbreviated as “sFv” or “scFv” are antibody fragments that comprise the V_H and V_L antibody domains connected into a single polypeptide chain. Preferably, the sFv polypeptide further comprises a polypeptide linker between the V_H and V_L domains which enables the sFv to form the desired structure for antigen binding. For a review of the sFv, see Pluckthun in *The Pharmacology of Monoclonal Antibodies*, vol. 113, Rosenberg and Moore eds., Springer-Verlag, New York, pp. 269-315 (1994).

[74] “Functional fragments” of the antibodies described herein comprise a portion of an intact antibody, generally including the antigen binding or variable region of the intact antibody or the Fc region of an antibody which retains or has modified FcR binding capability. Examples of antibody fragments include linear antibody, single-chain antibody molecules, and multispecific antibodies formed from antibody fragments.

[75] The term “heavy chain-only antibody” or “HCAb” refers to a functional antibody, which comprises heavy chains, but lacks the light chains usually found in 4-chain antibodies. Camelid animals (such as camels, llamas, or alpacas) are known to produce HCAs.

[76] The term “single-domain antibody” or “sdAb” refers to a single antigen-binding polypeptide having three complementary determining regions (CDRs). The sdAb alone is capable of binding to the antigen without pairing with a corresponding CDR-containing polypeptide. In some cases, single-domain antibodies are engineered from camelid HCAs, and their heavy chain variable domains are referred to herein as “V_HHs” (Variable domain of the heavy chain of the Heavy chain antibody). Some V_HHs can also be known as nanobodies. Camelid sdAb is one of the smallest known antigen-binding antibody fragments (see, *e.g.*, Hamers-Casterman *et al.*, *Nature* 363:446-8 (1993); Greenberg *et al.*, *Nature* 374:168-73 (1995); Hassanzadeh-Ghassabeh *et al.*, *Nanomedicine (Lond)*, 8:1013-26 (2013)). A basic V_HH has the following structure from the N-terminus to the C-terminus: FR1-CDR1-FR2-CDR2-FR3-CDR3-FR4, in which FR1 to FR4 refer to framework regions 1 to 4, respectively, and in which CDR1 to CDR3 refer to the complementarity determining regions 1 to 3.

[77] 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(are) 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 (U.S. Pat. No. 4,816,567; Morrison *et al.*, *Proc. Natl. Acad. Sci. USA*, 81:6851-6855 (1984)). “Humanized antibody” is used as a subset of “chimeric antibodies”.

[78] “Humanized” forms of non-human (*e.g.*, llama, camelid, and/or murine) antibodies are antibodies that contain minimal sequence derived from non-human immunoglobulin. In some embodiments, a humanized antibody is a human immunoglobulin (recipient antibody) in which residues from a CDR (hereinafter defined) of the recipient are replaced by residues from a CDR of a non-human species (donor antibody) such as mouse, rat, rabbit, camel, llama, alpaca, or non-human primate having the desired specificity, affinity, and/or capacity. In some instances, framework (“FR”) residues of the human immunoglobulin are replaced by corresponding non-human residues. Furthermore, humanized antibodies can comprise residues that are not found in the recipient antibody or in the donor antibody. These modifications can be made to further refine antibody performance, such as binding affinity. In general, a humanized antibody will comprise substantially all of at least one, and typically two, variable domains, in which all or substantially all of the hypervariable loops correspond to those of a non-human immunoglobulin sequence, and all or substantially all of the FR regions are those of a human immunoglobulin sequence, although the FR regions may include one or more individual FR residue substitutions that improve antibody performance, such as binding affinity, isomerization, immunogenicity, *etc.* The number of these amino acid substitutions in the FR is typically no more than 6 in the H chain, and in the L chain, no more than 3. The humanized antibody optionally will also comprise at least a portion of an immunoglobulin constant region (Fc), typically that of a human immunoglobulin. For further details, see, *e.g.*, Jones *et al.*, *Nature* 321:522-525 (1986); Riechmann *et al.*, *Nature* 332:323-329 (1988); and Presta, *Curr. Op. Struct. Biol.* 2:593-596 (1992). See also, for example, Vaswani and Hamilton, *Ann. Allergy, Asthma & Immunol.* 1:105-115 (1998); Harris, *Biochem. Soc. Transactions* 23:1035-1038 (1995); Hurle and Gross, *Curr. Op. Biotech.* 5:428-433 (1994); and U.S. Pat. Nos. 6,982,321 and 7,087,409.

[79] A “human antibody” is an antibody that possesses an amino-acid sequence corresponding to that of an antibody produced by a human and/or has been made using any of the techniques for making human antibodies as disclosed herein. This definition of a human antibody specifically excludes a humanized antibody comprising non-human antigen-binding residues. Human antibodies can be produced using various techniques known in the art, including phage-display libraries. Hoogenboom and Winter, *J. Mol. Biol.*, 227:381 (1991); Marks *et al.*, *J. Mol. Biol.*, 222:581 (1991). Also available for the preparation of human monoclonal antibodies are methods described in Cole *et al.*, *Monoclonal Antibodies and Cancer Therapy*, Alan R. Liss, p. 77 (1985); Boerner *et al.*, *J. Immunol.*, 147(1):86-95 (1991). See also

van Dijk and van de Winkel, *Curr. Opin. Pharmacol.*, 5: 368-74 (2001). Human antibodies can be prepared by administering the antigen to a transgenic animal that has been modified to produce such antibodies in response to antigenic challenge, but whose endogenous loci have been disabled, *e.g.*, immunized xenomice (see, *e.g.*, U.S. Pat. Nos. 6,075,181 and 6,150,584 regarding XENOMOUSE™ technology). See also, for example, Li *et al.*, *Proc. Natl. Acad. Sci. USA*, 103:3557-3562 (2006) regarding human antibodies generated via a human B-cell hybridoma technology.

[80] The term “hypervariable region,” “HVR,” or “HV,” when used herein refers to the regions of an antibody variable domain which are hypervariable in sequence and/or form structurally defined loops. Generally, 4-chain antibodies comprise six HVRs; three in the V_H (H1, H2, H3), and three in the V_L (L1, L2, L3). Single-domain antibodies comprise three HVRs (or CDRs): HVR1 (or CDR1), HVR2 (or CDR2), and HVR3 (or CDR3). In native 4-chain antibodies, H3 and L3 display the most diversity of the six HVRs, and in single-domain antibodies, HVR3 (or CDR3), display the most diversity of the three HVRs. H3, L3, and HVR3 are believed to play a unique role in conferring fine specificity to antibodies. See, *e.g.*, Hamers-Casterman *et al.*, *Nature* 363:446-448 (1993); Sheriff *et al.*, *Nature Struct. Biol.* 3:733-736 (1996); Xu *et al.*, *Immunity* 13:37-45 (2000); Johnson and Wu, *Methods in Molecular Biology* 248:1-25 (Lo, ed., Human Press, Totowa, NJ, 2003).

[81] The terms “Complementarity Determining Region” or “CDR” are used to refer to hypervariable regions as defined by the Kabat system. See Kabat *et al.*, *Sequences of Proteins of Immunological Interest*, 5th Ed. Public Health Service, National Institutes of Health, Bethesda, Md. (1991).

[82] A number of HVR delineations are in use and are encompassed herein. The Kabat Complementarity Determining Regions (CDRs) are based on sequence variability and are the most commonly used (Kabat *et al.*, *Sequences of Proteins of Immunological Interest*, 5th Ed. Public Health Service, National Institutes of Health, Bethesda, Md. (1991)). Chothia refers instead to the location of the structural loops (Chothia and Lesk, *J. Mol. Biol.* 196:901-917 (1987)). The AbM HVRs represent a compromise between the Kabat HVRs and Chothia structural loops and are used by Oxford Molecular’s AbM antibody modeling software. The “contact” HVRs are based on an analysis of the available complex crystal structures. The residues from each of these HVRs are noted below in Table 1.

Table 1. HVR delineations.

Loop	Kabat	AbM	Chothia	Contact
L1	L24-L34	L24-L34	L26-L32	L30-L36
L2	L50-L56	L50-L56	L50-L52	L46-L55
L3	L89-L97	L89-L97	L91-L96	L89-L96
H1	H31-H35B	H26-H35B	H26-H32	H30-H35B

Loop	Kabat	AbM	Chothia	Contact
(Kabat Numbering)				
H1	H31-H35	H26-H35	H26-H32	H30-H35
(Chothia Numbering)				
H2	H50-H65	H50-H58	H53-H55	H47-H58
H3	H95-H102	H95-H102	H96-H101	H93-H101

[83] HVRs may comprise “extended HVRs” as follows: 24-36 or 24-34 (L1), 46-56 or 50-56 (L2) and 89-97 or 89-96 (L3) in the V_L and 26-35 (H1), 50-65 or 49-65 (H2) and 93-102, 94-102, or 95-102 (H3) in the V_H . The variable domain residues are numbered according to Kabat *et al.*, supra, for each of these definitions.

[84] The amino acid residues of a single-domain antibody (such as V_HH) are numbered according to the general numbering for VH domains given by Kabat *et al.* (“Sequence of proteins of immunological interest,” US Public Health Services, NIH Bethesda, Md., Publication No. 91), as applied to V_HH domains from Camelids in the article of Riechmann and Muyldermans, *J. Immunol. Methods* 2000 Jun. 23; 240 (1-2): 185-195. According to this numbering, FR1 of a V_HH comprises the amino acid residues at positions 1-30, CDR1 of a V_HH comprises the amino acid residues at positions 31-35, FR2 of a V_HH comprises the amino acids at positions 36-49, CDR2 of a V_HH comprises the amino acid residues at positions 50-65, FR3 of a V_HH comprises the amino acid residues at positions 66-94, CDR3 of a V_HH comprises the amino acid residues at positions 95-102, and FR4 of a V_HH comprises the amino acid residues at positions 103-113. In this respect, it should be noted that—as is well known in the art for V_H domains and for V_HH domains—the total number of amino acid residues in each of the CDRs may vary and may not correspond to the total number of amino acid residues indicated by the Kabat numbering (that is, one or more positions according to the Kabat numbering may not be occupied in the actual sequence, or the actual sequence may contain more amino acid residues than the number allowed for by the Kabat numbering).

[85] The expression “variable-domain residue-numbering as in Kabat” or “amino-acid-position numbering as in Kabat,” and variations thereof, refers to the numbering system used for heavy-chain variable domains or light-chain variable domains of the compilation of antibodies in Kabat *et al.*, supra. Using this numbering system, the actual linear amino acid sequence may contain fewer or additional amino acids corresponding to a shortening of, or insertion into, a FR or HVR of the variable domain. For example, a heavy-chain variable domain may include a single amino acid insert (residue 52a according to Kabat) after residue 52 of H2 and inserted residues (*e.g.* residues 82a, 82b, and 82c, etc. according to Kabat) after heavy-chain FR residue 82. The Kabat numbering of residues may be determined for a given

antibody by alignment at regions of homology of the sequence of the antibody with a “standard” Kabat numbered sequence.

[86] Unless indicated otherwise herein, the numbering of the residues in an immunoglobulin heavy chain is that of the EU index as in Kabat *et al.*, supra. The “EU index as in Kabat” refers to the residue numbering of the human IgG1 EU antibody.

[87] “Framework” or “FR” residues are those variable-domain residues other than the HVR residues as herein defined.

[88] A “human consensus framework” or “acceptor human framework” is a framework that represents the most commonly occurring amino acid residues in a selection of human immunoglobulin V_L or V_H framework sequences. Generally, the selection of human immunoglobulin V_L or V_H sequences is from a subgroup of variable domain sequences. Generally, the subgroup of sequences is a subgroup as in Kabat *et al.*, *Sequences of Proteins of Immunological Interest*, 5th Ed. Public Health Service, National Institutes of Health, Bethesda, Md. (1991). Examples include for the V_L, the subgroup may be subgroup kappa I, kappa II, kappa III or kappa IV as in Kabat *et al.*, supra. Additionally, for the V_H, the subgroup may be subgroup I, subgroup II, or subgroup III as in Kabat *et al.* Alternatively, a human consensus framework can be derived from the above in which particular residues, such as when a human framework residue is selected based on its homology to the donor framework by aligning the donor framework sequence with a collection of various human framework sequences. An acceptor human framework “derived from” a human immunoglobulin framework or a human consensus framework may comprise the same amino acid sequence thereof, or it may contain pre-existing amino acid sequence changes. In some embodiments, the number of pre-existing amino acid changes are 10 or less, 9 or less, 8 or less, 7 or less, 6 or less, 5 or less, 4 or less, 3 or less, or 2 or less.

[89] An “affinity-matured” antibody is one with one or more alterations in one or more CDRs thereof that result in an improvement in the affinity of the antibody for antigen, compared to a parent antibody that does not possess those alteration(s). In some embodiments, an affinity-matured antibody has nanomolar or even picomolar affinities for the target antigen. Affinity-matured antibodies are produced by procedures known in the art. For example, Marks *et al.*, *Bio/Technology* 10:779-783 (1992) describes affinity maturation by V_H- and V_L-domain shuffling. Random mutagenesis of CDR and/or framework residues is described by, for example: Barbas *et al.* *Proc Nat. Acad. Sci. USA* 91:3809-3813 (1994); Schier *et al.* *Gene* 169:147-155 (1995); Yelton *et al.* *J. Immunol.* 155:1994-2004 (1995); Jackson *et al.*, *J. Immunol.* 154(7):3310-9 (1995); and Hawkins *et al.*, *J. Mol. Biol.* 226:889-896 (1992).

[90] As use herein, the term “specifically binds,” “specifically recognizes,” or is “specific for” refers to measurable and reproducible interactions such as binding between a target and an antibody, which is determinative of the presence of the target in the presence of a heterogeneous population of molecules

including biological molecules. For example, an antibody that specifically binds a target (which can be an epitope) is an antibody that binds this target with greater affinity, avidity, more readily, and/or with greater duration than it binds other targets. In some embodiments, the extent of binding of an antibody to an unrelated target is less than about 10% of the binding of the antibody to the target as measured, *e.g.*, by a radioimmunoassay (RIA). In some embodiments, an antibody that specifically binds a target has a dissociation constant (K_d) of $\leq 10^{-5}$ M, $\leq 10^{-6}$ M, $\leq 10^{-7}$ M, $\leq 10^{-8}$ M, $\leq 10^{-9}$ M, $\leq 10^{-10}$ M, $\leq 10^{-11}$ M, or $\leq 10^{-12}$ M. In some embodiments, an antibody specifically binds an epitope on a protein that is conserved among the protein from different species. In some embodiments, specific binding can include, but does not require exclusive binding.

[91] The term “specificity” refers to selective recognition of an antigen binding protein or antibody for a particular epitope of an antigen. Natural antibodies, for example, are monospecific. The term “multispecific” as used herein denotes that an antigen binding protein or antibody has polyepitopic specificity (*i.e.*, is capable of specifically binding to two, three, or more, different epitopes on one biological molecule or is capable of specifically binding to epitopes on two, three, or more, different biological molecules). “Bispecific” as used herein denotes that an antigen binding protein or antibody has two different antigen-binding specificities. Unless otherwise indicated, the order in which the antigens bound by a multiple antigen binding protein listed is arbitrary. That is, for example, the terms “anti-CD47/anti-PD-L1,” “anti-PD-L1/anti-CD47,” “CD47×PD-L1,” “PD-L1×CD47,” “CD47-PD-L1,” and “PD-L1-CD47” may be used interchangeably to refer to multiple antigen binding proteins that specifically bind to both CD47 and PD-L1. The term “monospecific” as used herein denotes an antibody that has one or more binding sites each of which bind the same epitope of the same antigen.

[92] The term “valent” as used herein denotes the presence of a specified number of binding sites in an antigen binding protein or antibody. A natural antibody for example or a full length antibody has two binding sites and is bivalent. As such, the terms “trivalent,” “tetravalent,” “pentavalent,” and “hexavalent” denote the presence of three binding sites, four binding sites, five binding sites, and six binding sites, respectively, in an antigen binding protein or antibody. The bispecific antibodies of the present application are at least “bivalent.”

[93] A “blocking” antibody or an “antagonist” antibody is one that inhibits or reduces a biological activity of the antigen it binds. In certain embodiments, blocking antibodies or antagonist antibodies substantially or completely inhibit the biological activity of the antigen.

[94] An “agonist” or activating antibody is one that enhances or initiates signaling by the antigen to which it binds. In certain embodiments, agonist antibodies cause or activate signaling without the presence of the natural ligand.

[95] “Antibody effector functions” refer to those biological activities attributable to the Fc region (a native sequence Fc region or amino acid sequence variant Fc region) of an antibody and vary with the antibody isotype. Examples of antibody effector functions include: C1q binding and complement dependent cytotoxicity; Fc receptor binding; antibody-dependent cell-mediated cytotoxicity (ADCC); phagocytosis; down regulation of cell surface receptors (*e.g.*, B cell receptors); and B cell activation. “Reduced or minimized” antibody effector function means that which is reduced by at least 50% (alternatively 60%, 65%, 70%, 75%, 80%, 85%, 90%, 95%, 96%, 97%, 98%, 99%) from the wild type or unmodified antibody. The determination of antibody effector function is readily determinable and measurable by one of ordinary skill in the art. In a preferred embodiment, the antibody effector functions of complement binding, complement dependent cytotoxicity and antibody dependent cytotoxicity are affected. In some embodiments, effector function is eliminated through a mutation in the constant region that eliminated glycosylation, *e.g.*, “effector-less mutation.” In one aspect, the effector-less mutation is an N297A or DANA mutation (D265A+N297A) in the C_H2 region. Shields *et al.*, *J. Biol. Chem.* 276 (9): 6591-6604 (2001). Alternatively, additional mutations resulting in reduced or eliminated effector function include: K322A and L234A/L235A (LALA). Alternatively, effector function can be reduced or eliminated through production techniques, such as expression in host cells that do not glycosylate (*e.g.*, *E. coli.*) or in which result in an altered glycosylation pattern that is ineffective or less effective at promoting effector function (*e.g.*, Shinkawa *et al.*, *J. Biol. Chem.* 278(5): 3466-3473 (2003).

[96] “Antibody-dependent cell-mediated cytotoxicity” or ADCC refers to a form of cytotoxicity in which secreted Ig bound onto Fc receptors (FcRs) present on certain cytotoxic cells (*e.g.*, natural killer (NK) cells, neutrophils and macrophages) enable these cytotoxic effector cells to bind specifically to an antigen-bearing target cell and subsequently kill the target cell with cytotoxins. The antibodies “arm” and the cytotoxic cells are required for killing of the target cell by this mechanism. The primary cells for mediating ADCC, NK cells, express FcγRIII only, whereas monocytes express FcγRI, FcγRII and FcγRIII. Fc expression on hematopoietic cells is summarized in Table 3 on page 464 of Ravetch and Kinet, *Annu. Rev. Immunol.* 9: 457-92 (1991). To assess ADCC activity of a molecule of interest, an *in vitro* ADCC assay, such as that described in U.S. Pat. No. 5,500,362 or 5,821,337 may be performed. Useful effector cells for such assays include peripheral blood mononuclear cells (PBMC) and natural killer (NK) cells. Alternatively, or additionally, ADCC activity of the molecule of interest may be assessed *in vivo*, *e.g.*, in an animal model such as that disclosed in Clynes *et al.*, *PNAS USA* 95:652-656 (1998).

[97] The term “Fc region” herein is used to define a C-terminal region of an immunoglobulin heavy chain, including native-sequence Fc regions and variant Fc regions. Although the boundaries of the Fc region of an immunoglobulin heavy chain might vary, the human IgG heavy-chain Fc region is usually

defined to stretch from an amino acid residue at position Cys226, or from Pro230, to the carboxyl-terminus thereof. The C-terminal lysine (residue 447 according to the EU numbering system) of the Fc region may be removed, for example, during production or purification of the antibody, or by recombinantly engineering the nucleic acid encoding a heavy chain of the antibody. Accordingly, a composition of intact antibodies may comprise antibody populations with all K447 residues removed, antibody populations with no K447 residues removed, and antibody populations having a mixture of antibodies with and without the K447 residue. Suitable native-sequence Fc regions for use in the antibodies described herein include human IgG1, IgG2 (IgG2A, IgG2B), IgG3 and IgG4.

[98] “Fc receptor” or “FcR” describes a receptor that binds the Fc region of an antibody. The preferred FcR is a native sequence human FcR. Moreover, a preferred FcR is one which binds an IgG antibody (a gamma receptor) and includes receptors of the FcγRI, FcγRII, and FcγRIII subclasses, including allelic variants and alternatively spliced forms of these receptors, FcγRII receptors include FcγRIIA (an “activating receptor”) and FcγRIIB (an “inhibiting receptor”), which have similar amino acid sequences that differ primarily in the cytoplasmic domains thereof. Activating receptor FcγRIIA contains an immunoreceptor tyrosine-based activation motif (ITAM) in its cytoplasmic domain. Inhibiting receptor FcγRIIB contains an immunoreceptor tyrosine-based inhibition motif (ITIM) in its cytoplasmic domain. (see M. Daëron, *Annu. Rev. Immunol.* 15:203-234 (1997). FcRs are reviewed in Ravetch and Kinet, *Annu. Rev. Immunol.* 9: 457-92 (1991); Capel *et al.*, *Immunomethods* 4: 25-34 (1994); and de Haas *et al.*, *J. Lab. Clin. Med.* 126: 330-41 (1995). Other FcRs, including those to be identified in the future, are encompassed by the term “FcR” herein.

[99] The term “Fc receptor” or “FcR” also includes the neonatal receptor, FcRn, which is responsible for the transfer of maternal IgGs to the fetus. Guyer *et al.*, *J. Immunol.* 117: 587 (1976) and Kim *et al.*, *J. Immunol.* 24: 249 (1994). Methods of measuring binding to FcRn are known (see, *e.g.*, Ghetie and Ward, *Immunol. Today* 18: (12): 592-8 (1997); Ghetie *et al.*, *Nature Biotechnology* 15 (7): 637-40 (1997); Hinton *et al.*, *J. Biol. Chem.* 279 (8): 6213-6 (2004); WO 2004/92219 (Hinton *et al.*). Binding to FcRn *in vivo* and serum half-life of human FcRn high-affinity binding polypeptides can be assayed, *e.g.*, in transgenic mice or transfected human cell lines expressing human FcRn, or in primates to which the polypeptides having a variant Fc region are administered. WO 2004/42072 (Presta) describes antibody variants which improved or diminished binding to FcRs. See also, *e.g.*, Shields *et al.*, *J. Biol. Chem.* 9(2): 6591-6604 (2001).

[100] “Effector cells” are leukocytes which express one or more FcRs and perform effector functions. In one aspect, the effector cells express at least FcγRIII and perform ADCC effector function. Examples of human leukocytes which mediate ADCC include peripheral blood mononuclear cells (PBMC), natural killer (NK) cells, monocytes, cytotoxic T cells and neutrophils. The effector cells may be isolated from a

native source, e.g., blood. Effector cells generally are lymphocytes associated with the effector phase, and function to produce cytokines (helper T cells), killing cells infected with pathogens (cytotoxic T cells) or secreting antibodies (differentiated B cells).

[101] “Complement dependent cytotoxicity” or “CDC” refers to the lysis of a target cell in the presence of complement. Activation of the classical complement pathway is initiated by the binding of the first component of the complement system (C1q) to antibodies (of the appropriate subclass) which are bound to their cognate antigen. To assess complement activation, a CDC assay, e.g., as described in Gazzano-Santoro *et al.*, *J. Immunol. Methods* 202: 163 (1996), may be performed. Antibody variants with altered Fc region amino acid sequences and increased or decreased C1q binding capability are described in U.S. Pat. No. 6,194,551B1 and WO99/51642. The contents of those patent publications are specifically incorporated herein by reference. See, also, Idusogie *et al.* *J. Immunol.* 164: 4178-4184 (2000).

[102] “Binding affinity” generally refers to the strength of the sum total of non-covalent interactions between a single binding site of a molecule (e.g., an antibody) and its binding partner (e.g., an antigen). Unless indicated otherwise, as used herein, “binding affinity” refers to intrinsic binding affinity that reflects a 1:1 interaction between members of a binding pair (e.g., antibody and antigen). The affinity of a molecule X for its partner Y can generally be represented by the dissociation constant (K_d). Affinity can be measured by common methods known in the art, including those described herein. Low-affinity antibodies generally bind antigen slowly and tend to dissociate readily, wherein high-affinity antibodies generally bind antigen faster and tend to remain bound longer. A variety of methods of measuring binding affinity are known in the art, any of which can be used for purposes of the present application. Specific illustrative and exemplary embodiments for measuring binding affinity are described in the following.

[103] “Binding specificity of the antibody or antigen-binding domain” can be determined experimentally by methods known in the art. Such methods comprise, but are not limited to Western blots, ELISA-, RIA-, ECL-, IRMA-, EIA-, BIAcore-tests and peptide scans.

[104] “Half maximal inhibitory concentration (IC₅₀)” is a measure of the effectiveness of a substance (such as an antibody) in inhibiting a specific biological or biochemical function. It indicates how much of a particular drug or other substance (inhibitor, such as an antibody) is needed to inhibit a given biological process (e.g., the binding between PD-L1 and B7-1, the binding between CD47 and SIRPα, or component of a process, i.e. an enzyme, cell, cell receptor or microorganism) by half. The values are typically expressed as molar concentration. IC₅₀ is comparable to an EC₅₀ for agonist drug or other substance (such as an antibody). EC₅₀ also represents the plasma concentration required for obtaining 50% of a maximum effect *in vivo*. As used herein, an “IC₅₀” is used to indicate the effective concentration of an antibody (such as an anti-CD/47/anti-PD-L1 multiple antigen binding protein) needed to neutralize 50% of the

antigen bioactivity (such as CD47 and/or PD-L1 bioactivity) *in vitro*. IC₅₀ or EC₅₀ can be measured by bioassays such as inhibition of ligand binding by FACS analysis (competition binding assay), cell based cytokine release assay, or amplified luminescent proximity homogeneous assay (AlphaLISA).

[105] “Percent (%) amino acid sequence identity” and “homology” with respect to a peptide, polypeptide or antibody sequence are defined as the percentage of amino acid residues in a candidate sequence that are identical with the amino acid residues in the specific peptide or polypeptide sequence, after aligning the sequences and introducing gaps, if necessary, to achieve the maximum percent sequence identity, and not considering any conservative substitutions as part of the sequence identity. Alignment for purposes of determining percent amino acid sequence identity can be achieved in various ways that are within the skill in the art, for instance, using publicly available computer software such as BLAST, BLAST-2, ALIGN or MEGALIGN™ (DNASTAR) software. Those skilled in the art can determine appropriate parameters for measuring alignment, including any algorithms needed to achieve maximal alignment over the full length of the sequences being compared.

[106] An “isolated” nucleic acid molecule encoding an antibody or antigen-binding fragment thereof described herein is a nucleic acid molecule that is identified and separated from at least one contaminant nucleic acid molecule with which it is ordinarily associated in the environment in which it was produced. Preferably, the isolated nucleic acid is free of association with all components associated with the production environment. The isolated nucleic acid molecules encoding the polypeptides and antibodies described herein is in a form other than in the form or setting in which it is found in nature. Isolated nucleic acid molecules therefore are distinguished from nucleic acid encoding the polypeptides and antibodies described herein existing naturally in cells. An isolated nucleic acid includes a nucleic acid molecule contained in cells that ordinarily contain the nucleic acid molecule, but the nucleic acid molecule is present extrachromosomally or at a chromosomal location that is different from its natural chromosomal location.

[107] Nucleic acid is “operably linked” when it is placed into a functional relationship with another nucleic acid sequence. For example, DNA for a presequence or secretory leader is operably linked to DNA for a polypeptide if it is expressed as a preprotein that participates in the secretion of the polypeptide; a promoter or enhancer is operably linked to a coding sequence if it affects the transcription of the sequence; or a ribosome binding site is operably linked to a coding sequence if it is positioned so as to facilitate translation. Generally, “operably linked” means that the DNA sequences being linked are contiguous, and, in the case of a secretory leader, contiguous and in reading phase. However, enhancers do not have to be contiguous. Linking is accomplished by ligation at convenient restriction sites. If such sites do not exist, the synthetic oligonucleotide adaptors or linkers are used in accordance with conventional practice.

[108] The term “vector,” as used herein, refers to a nucleic acid molecule capable of propagating another nucleic acid to which it is linked. The term includes the vector as a self-replicating nucleic acid structure as well as the vector incorporated into the genome of a host cell into which it has been introduced. Certain vectors are capable of directing the expression of nucleic acids to which they are operatively linked. Such vectors are referred to herein as “expression vectors.”

[109] The term “transfected” or “transformed” or “transduced” as used herein refers to a process by which exogenous nucleic acid is transferred or introduced into the host cell. A “transfected” or “transformed” or “transduced” cell is one which has been transfected, transformed or transduced with exogenous nucleic acid. The cell includes the primary subject cell and its progeny.

[110] The terms “host cell,” “host cell line,” and “host cell culture” are used interchangeably and refer to cells into which exogenous nucleic acid has been introduced, including the progeny of such cells. Host cells include “transformants” and “transformed cells,” which include the primary transformed cell and progeny derived therefrom without regard to the number of passages. Progeny may not be completely identical in nucleic acid content to a parent cell, but may contain mutations. Mutant progeny that have the same function or biological activity as screened or selected for in the originally transformed cell are included herein.

[111] “Adjuvant setting” refers to a clinical setting in which an individual has had a history of cancer, and generally (but not necessarily) been responsive to therapy, which includes, but is not limited to, surgery (*e.g.*, surgery resection), radiotherapy, and chemotherapy. However, because of their history of cancer, these individuals are considered at risk of development of the disease. Treatment or administration in the “adjuvant setting” refers to a subsequent mode of treatment. The degree of risk (*e.g.*, when an individual in the adjuvant setting is considered as “high risk” or “low risk”) depends upon several factors, most usually the extent of disease when first treated.

[112] “Neoadjuvant setting” refers to a clinical setting in which the method is carried out before the primary/definitive therapy.

[113] The term “pharmaceutical formulation” or “pharmaceutical composition” refers to a preparation that is in such form as to permit the biological activity of the active ingredient to be effective, and that contains no additional components that are unacceptably toxic to a subject to which the formulation would be administered. Such formulations are sterile. A “sterile” formulation is aseptic or free from all living microorganisms and their spores.

[114] It is understood that embodiments of the invention described herein include “consisting” and/or “consisting essentially of” embodiments.

[115] Reference to “about” a value or parameter herein includes (and describes) variations that are directed to that value or parameter *per se*. For example, description referring to “about X” includes description of “X”.

[116] As used herein, reference to “not” a value or parameter generally means and describes “other than” a value or parameter. For example, the method is not used to treat cancer of type X means the method is used to treat cancer of types other than X.

[117] The term “about X-Y” used herein has the same meaning as “about X to about Y.”

[118] As used herein and in the appended claims, the singular forms “a,” “or,” and “the” include plural referents unless the context clearly dictates otherwise.

II. Anti-CD47/anti-PD-L1 multiple antigen binding protein or antigen-binding fragment thereof

[119] One aspect of the present application provides a multispecific antigen binding protein (MABP), e.g., an isolated anti-CD47/anti-PD-L1 multiple antigen binding protein or antigen-binding fragment thereof. The isolated anti-CD47/anti-PD-L1 multiple antigen binding proteins or antigen binding fragments thereof can, for example, comprise (a) a first antigen binding portion comprising a heavy chain variable domain (V_H) and a light chain variable domain (V_L), wherein the V_H and V_L together form an antigen-binding site that specifically binds CD47, and wherein the V_H comprises a heavy chain complementarity determining region 1 (HCDR1), HCDR2, and HCDR3 comprising the amino acid sequences of SEQ ID NO:19, SEQ ID NO:20, and SEQ ID NO:21, respectively, and the V_L comprises a light chain complementarity determining region 1 (LCDR1), LCDR2, and LCDR3 comprising the amino acid sequences of SEQ ID NO:22, SEQ ID NO:23, and SEQ ID NO:24, respectively; and (b) a second antigen binding portion comprising a single-domain antibody that specifically binds PD-L1; wherein the first antigen binding portion and the second antigen binding portion are fused to each other.

[120] The isolated anti-CD47/anti-PD-L1 multiple antigen binding proteins or antigen binding fragments thereof can, for example, comprise (a) a first antigen binding portion comprising a heavy chain variable domain (V_H) and a light chain variable domain (V_L), wherein the V_H and V_L together form an antigen-binding site that specifically binds CD47, and wherein the V_H comprises a heavy chain complementarity determining region 1 (HCDR1), HCDR2, and HCDR3 comprising the amino acid sequences of SEQ ID NO:19, SEQ ID NO:20, and SEQ ID NO:21, respectively, and the V_L comprises a light chain complementarity determining region 1 (LCDR1), LCDR2, and LCDR3 comprising the amino acid sequences of SEQ ID NO:22, SEQ ID NO:23, and SEQ ID NO:24, respectively; and (b) a second antigen binding portion comprising a single-domain antibody that specifically binds PD-L1, wherein the single-domain antibody comprises a complementarity determining region 1 (CDR1), CDR2, and CDR3 comprising the amino acid sequences of: (i) SEQ ID NO:25, SEQ ID NO:26, and SEQ ID NO:27,

respectively, (ii) SEQ ID NO:28, SEQ ID NO:29, and SEQ ID NO:30, respectively, or (iii) SEQ ID NO:31, SEQ ID NO:32, and SEQ ID NO:33, respectively; wherein the first antigen binding portion and the second antigen binding portion are fused to each other.

[121] In certain aspects of the present application, the first antigen binding portion is a full-length antibody comprising two heavy chains and two light chains. The first antigen binding portion can, for example, be an antibody fragment comprising a heavy chain comprising the V_H and a light chain comprising the V_L . In certain aspects of the present application, the second antigen binding portion comprises a single polypeptide chain.

[122] In certain aspects of the present application, the first antigen binding portion of the anti-CD47/anti-PD-L1 multiple antigen binding protein or antigen binding portion thereof comprises a heavy chain variable domain (V_H) with a heavy chain CDR1 comprising the amino acid sequence of SEQ ID NO:19 or a variant thereof comprising up to about 3 (such as about any of 1, 2, or 3) amino acid substitutions; a heavy chain CDR2 comprising the amino acid sequence of SEQ ID NO:20 or a variant thereof comprising up to about 3 (such as about any of 1, 2, or 3) amino acid substitutions; and a heavy chain CDR3 comprising the amino acid sequence of SEQ ID NO:21 or a variant thereof comprising up to about 3 (such as about any of 1, 2, or 3) amino acid substitutions; and a light chain variable domain (V_L) with a light chain CDR1 comprising the amino acid sequence of SEQ ID NO:22 or a variant thereof comprising up to about 3 (such as about any of 1, 2, or 3) amino acid substitutions; a light chain CDR2 comprising the amino acid sequence of SEQ ID NO:23 or a variant thereof comprising up to about 3 (such as about any of 1, 2, or 3) amino acid substitutions; and a light chain CDR3 comprising the amino acid sequence of SEQ ID NO:24 or a variant thereof comprising up to about 3 (such as about any of 1, 2, or 3) amino acid substitutions. In certain embodiments, there is provided an anti-CD47 monoclonal antibody moiety comprising a heavy chain variable domain (V_H) with a heavy chain CDR1 comprising the amino acid sequence of SEQ ID NO:19; a heavy chain CDR2 comprising the amino acid sequence of SEQ ID NO:20; and a heavy chain CDR3 comprising the amino acid sequence of SEQ ID NO:21; and a light chain variable domain (V_L) with a light chain CDR1 comprising the amino acid sequence of SEQ ID NO:22; a light chain CDR2 comprising the amino acid sequence of SEQ ID NO:23; and a light chain CDR3 comprising the amino acid sequence of SEQ ID NO:24.

[123] In certain aspects of the present application, the first antigen binding portion of the anti-CD47/anti-PD-L1 multiple antigen binding protein or antigen binding fragment thereof comprises a variable heavy chain comprising the amino acid sequence of SEQ ID NO:4 and a variable light chain comprising an amino acid sequence of SEQ ID NO:6. In certain embodiments, the anti-CD47/anti-PD-L1 multiple antigen binding protein or antigen binding fragment thereof comprises a variable heavy chain that is at least 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99% or more identical to the sequence

set forth in SEQ ID NO:4, and a variable light chain that is at least 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99% or more identical to the sequence set forth in SEQ ID NO:6.

[124] In certain aspects of the present application, the second antigen binding portion of the anti-CD47/anti-PD-L1 multiple antigen binding protein or antigen binding portion thereof is an anti-PD-L1 sdAb moiety comprising a CDR1 comprising the amino acid sequence of any one of SEQ ID NOs: 25, 28, or 31, or a variant thereof comprising up to about 3 (such as about any of 1, 2, or 3) amino acid substitutions; a CDR2 comprising the amino acid sequence of any one of SEQ ID NOs: 26, 29, or 32, or a variant thereof comprising up to about 3 (such as about any of 1, 2, or 3) amino acid substitutions; and a CDR3 comprising the amino acid sequence of any one of SEQ ID NOs: 27, 30, or 33, or a variant thereof comprising up to about 3 (such as about any of 1, 2, or 3) amino acid substitutions.

[125] In certain aspects of the present application, the second antigen binding portion of the anti-CD47/anti-PD-L1 multiple antigen binding protein or antigen binding portion thereof is an anti-PD-L1 sdAb moiety comprising a CDR1 comprising the amino acid sequence of any one of SEQ ID NOs: 25, 28, or 31; a CDR2 comprising the amino acid sequence of any one of SEQ ID NOs: 26, 29, or 32; and a CDR3 comprising the amino acid sequence of any one of SEQ ID NOs: 27, 30, or 33. The CDRs can be combined in various pair-wise combinations to generate a number of second antigen binding portions of the anti-CD47/anti-PD-L1 multiple antigen binding protein or antigen binding portion thereof.

[126] In certain aspects of the present application, the second antigen binding portion of the anti-CD47/anti-PD-L1 multiple antigen binding protein or antigen binding portion thereof is an anti-PD-L1 sdAb moiety comprising a V_HH domain comprising the amino acid sequence of any one of SEQ ID NOs: 14, 16, or 18, or a variant thereof having at least about 80% (such as at least about any of 80%, 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, or 99%) sequence identity to any one of SEQ ID NOs: 14, 16, or 18. In some embodiments, the anti-PD-L1 sdAb moiety comprising a V_HH domain comprising the amino acid sequence of any one of SEQ ID NOs: 14, 16, or 18, or a variant thereof comprising up to about 3 (such as about any of 1, 2, or 3) amino acid substitutions in the V_HH domain. In some embodiments, the anti-PD-L1 sdAb moiety comprising the V_HH domain comprising the amino acid sequence of any one of SEQ ID NOs: 14, 16, or 18 or a variant thereof comprises amino acid substitutions in CDRs, such as the CDR1, and/or the CDR2, and/or the CDR3. In some embodiments, the anti-PD-L1 sdAb moiety comprising the V_HH domain comprising the amino acid sequence of any one of SEQ ID NOs: 14, 16, or 18 or a variant thereof comprises CDR1, CDR2, and CDR3 of any one of SEQ ID NOs: 14, 16, or 18, and the amino acid substitutions are in FRs, such as the FR1, and/or the FR2, and/or the FR3, and/or the FR4.

[127] In certain aspects of the present application, the first antigen binding portion and the second antigen binding portion are fused. The carboxy (C)-terminus of the second antigen binding portion can,

for example, be fused to the amino (N)-terminus of at least one heavy chain of the first antigen binding portion or the amino (N)-terminus of at least one light chain of the first antigen binding portion. The amino (N)-terminus of the second antigen binding portion can, for example, be fused to the carboxy (C)-terminus of at least one heavy chain of the first antigen binding portion or the carboxy (C)-terminus of at least one light chain of the first antigen binding portion. In certain embodiments, the first antigen binding portion and the second antigen binding portion are fused to each other via a peptide bond or a peptide linker. The peptide linker can, for example, comprise an amino acid sequence selected from SEQ ID NO:8, SEQ ID NO:10, or SEQ ID NO:69.

[128] In certain aspects of the present application, the first antigen binding portion comprises a human, humanized, or chimeric antibody or antigen binding fragment thereof. In certain embodiments, the second antigen binding portion comprising a single-domain antibody that specifically binds PD-L1 is camelid, chimeric, human, partially humanized, or fully humanized.

[129] In certain aspects of the present application, the first antigen binding portion comprises an Fc region. In certain embodiments, the second antigen binding portion is fused to the N-terminus of the Fc region. The Fc region can, for example, be an IgG1 Fc. The Fc region can, for example, be an IgG4 Fc having an S228P mutation and/or an L235E mutation.

[130] In some embodiments, there is provided an isolated anti-CD47/anti-PD-L1 bispecific antibody or antigen binding fragment thereof comprising an anti-CD47 full-length antibody and an anti-PD-L1 single-domain antibody, wherein the N-terminus of the anti-PD-L1 sdAb is fused to the C-terminus of both heavy chains of the anti-CD47 full-length antibody, and wherein the heavy chain fusion polypeptide comprises the amino acid sequence of SEQ ID NOs: 36, 38, 40, or 48 and the light chain polypeptide comprises the amino acid sequence of SEQ ID NO:6. In some embodiments, the isolated anti-CD47/anti-PD-L1 bispecific antibody or antigen binding fragment thereof comprising an anti-CD47 full-length antibody and an anti-PD-L1 single-domain antibody, wherein the N-terminus of the anti-PD-L1 sdAb is fused to the C-terminus of both heavy chains of the anti-CD47 full-length antibody, and wherein the heavy chain fusion polypeptide comprises the amino acid sequence of SEQ ID NOs: 36 or 48 and the light chain polypeptide comprises the amino acid sequence of SEQ ID NO:6 (hereinafter denoted as “PDL1a-E-HC” or “PDL1a-G15-HC”). In some embodiments, the isolated anti-CD47/anti-PD-L1 bispecific antibody or antigen binding fragment thereof comprising an anti-CD47 full-length antibody and an anti-PD-L1 single-domain antibody, wherein the N-terminus of the anti-PD-L1 sdAb is fused to the C-terminus of both heavy chains of the anti-CD47 full-length antibody, and wherein the heavy chain fusion polypeptide comprises the amino acid sequence of SEQ ID NO: 38 and the light chain polypeptide comprises the amino acid sequence of SEQ ID NO:6 (hereinafter denoted as “PDL1b-E-HC”). In some embodiments, the isolated anti-CD47/anti-PD-L1 bispecific antibody or antigen binding fragment thereof

comprising an anti-CD47 full-length antibody and an anti-PD-L1 single-domain antibody, wherein the N-terminus of the anti-PD-L1 sdAb is fused to the C-terminus of both heavy chains of the anti-CD47 full-length antibody, and wherein the heavy chain fusion polypeptide comprises the amino acid sequence of SEQ ID NO: 40 and the light chain polypeptide comprises the amino acid sequence of SEQ ID NO:6 (hereinafter denoted as “PDL1c-E-HC”).

[131] In some embodiments, there is provided an isolated anti-CD47/anti-PD-L1 bispecific antibody or antigen binding fragment thereof comprising an anti-CD47 full-length antibody and an anti-PD-L1 single-domain antibody, wherein the C-terminus of the anti-PD-L1 sdAb is fused to the N-terminus of both heavy chains of the anti-CD47 full-length antibody, and wherein the heavy chain fusion polypeptide comprises the amino acid sequence of SEQ ID NOs: 42, 44, 46, or 50 and the light chain polypeptide comprises the amino acid sequence of SEQ ID NO:6. In some embodiments, the isolated anti-CD47/anti-PD-L1 bispecific antibody or antigen binding fragment thereof comprising an anti-CD47 full-length antibody and an anti-PD-L1 single-domain antibody, wherein the C-terminus of the anti-PD-L1 sdAb is fused to the N-terminus of both heavy chains of the anti-CD47 full-length antibody, and wherein the heavy chain fusion polypeptide comprises the amino acid sequence of SEQ ID NOs: 42 or 50 and the light chain polypeptide comprises the amino acid sequence of SEQ ID NO:6 (hereinafter denoted as “PDL1a-E-HN” or “PDL1a-G15-HN”). In some embodiments, the isolated anti-CD47/anti-PD-L1 bispecific antibody or antigen binding fragment thereof comprising an anti-CD47 full-length antibody and an anti-PD-L1 single-domain antibody, wherein the C-terminus of the anti-PD-L1 sdAb is fused to the N-terminus of both heavy chains of the anti-CD47 full-length antibody, and wherein the heavy chain fusion polypeptide comprises the amino acid sequence of SEQ ID NO: 44 and the light chain polypeptide comprises the amino acid sequence of SEQ ID NO:6 (hereinafter denoted as “PDL1b-E-HN”). In some embodiments, the isolated anti-CD47/anti-PD-L1 bispecific antibody or antigen binding fragment thereof comprising an anti-CD47 full-length antibody and an anti-PD-L1 single-domain antibody, wherein the C-terminus of the anti-PD-L1 sdAb is fused to the N-terminus of both heavy chains of the anti-CD47 full-length antibody, and wherein the heavy chain fusion polypeptide comprises the amino acid sequence of SEQ ID NO: 46 and the light chain polypeptide comprises the amino acid sequence of SEQ ID NO:6 (hereinafter denoted as “PDL1c-E-HN”).

[132] In some embodiments, there is provided an isolated anti-CD47/anti-PD-L1 bispecific antibody or antigen binding fragment thereof comprising an anti-CD47 full-length antibody and an anti-PD-L1 single-domain antibody, wherein the N-terminus of the anti-PD-L1 sdAb is fused to the C-terminus of both light chains of the anti-CD47 full-length antibody, and wherein the light chain fusion polypeptide comprises the amino acid sequence of SEQ ID NOs: 52, 54, 56, 64, or 68 and the heavy chain polypeptide comprises the amino acid sequence of SEQ ID NO:4. In some embodiments, the isolated anti-CD47/anti-

PD-L1 bispecific antibody or antigen binding fragment thereof comprising an anti-CD47 full-length antibody and an anti-PD-L1 single-domain antibody, wherein the N-terminus of the anti-PD-L1 sdAb is fused to the C-terminus of both light chains of the anti-CD47 full-length antibody, and wherein the light chain fusion polypeptide comprises the amino acid sequence of SEQ ID NOs: 52, 64, or 68 and the heavy chain polypeptide comprises the amino acid sequence of SEQ ID NO:4 (hereinafter denoted as “PDL1a-E-LC,” “PDL1a-G15-LC,” or “PDL1a-E4-LC”). In some embodiments, the isolated anti-CD47/anti-PD-L1 bispecific antibody or antigen binding fragment thereof comprising an anti-CD47 full-length antibody and an anti-PD-L1 single-domain antibody, wherein the N-terminus of the anti-PD-L1 sdAb is fused to the C-terminus of both light chains of the anti-CD47 full-length antibody, and wherein the light chain fusion polypeptide comprises the amino acid sequence of SEQ ID NOs: 54 and the heavy chain polypeptide comprises the amino acid sequence of SEQ ID NO:4 (hereinafter denoted as “PDL1b-E-LC”). In some embodiments, the isolated anti-CD47/anti-PD-L1 bispecific antibody or antigen binding fragment thereof comprising an anti-CD47 full-length antibody and an anti-PD-L1 single-domain antibody, wherein the N-terminus of the anti-PD-L1 sdAb is fused to the C-terminus of both light chains of the anti-CD47 full-length antibody, and wherein the light chain fusion polypeptide comprises the amino acid sequence of SEQ ID NOs: 56 and the heavy chain polypeptide comprises the amino acid sequence of SEQ ID NO:4 (hereinafter denoted as “PDL1c-E-LC”).

[133] In some embodiments, there is provided an isolated anti-CD47/anti-PD-L1 bispecific antibody or antigen binding fragment thereof comprising an anti-CD47 full-length antibody and an anti-PD-L1 single-domain antibody, wherein the C-terminus of the anti-PD-L1 sdAb is fused to the N-terminus of both light chains of the anti-CD47 full-length antibody, and wherein the light chain fusion polypeptide comprises the amino acid sequence of SEQ ID NOs: 58, 60, 62, or 66 and the heavy chain polypeptide comprises the amino acid sequence of SEQ ID NO:4. In some embodiments, the isolated anti-CD47/anti-PD-L1 bispecific antibody or antigen binding fragment thereof comprising an anti-CD47 full-length antibody and an anti-PD-L1 single-domain antibody, wherein the C-terminus of the anti-PD-L1 sdAb is fused to the N-terminus of both light chains of the anti-CD47 full-length antibody, and wherein the light chain fusion polypeptide comprises the amino acid sequence of SEQ ID NOs: 58 or 66 and the heavy chain polypeptide comprises the amino acid sequence of SEQ ID NO:4 (hereinafter denoted as “PDL1a-E-LN” or “PDL1a-G15-LN”). In some embodiments, the isolated anti-CD47/anti-PD-L1 bispecific antibody or antigen binding fragment thereof comprising an anti-CD47 full-length antibody and an anti-PD-L1 single-domain antibody, wherein the C-terminus of the anti-PD-L1 sdAb is fused to the N-terminus of both light chains of the anti-CD47 full-length antibody, and wherein the light chain fusion polypeptide comprises the amino acid sequence of SEQ ID NOs: 60 and the heavy chain polypeptide comprises the amino acid sequence of SEQ ID NO:4 (hereinafter denoted as “PDL1b-E-LN”). In some

embodiments, the isolated anti-CD47/anti-PD-L1 bispecific antibody or antigen binding fragment thereof comprising an anti-CD47 full-length antibody and an anti-PD-L1 single-domain antibody, wherein the C-terminus of the anti-PD-L1 sdAb is fused to the N-terminus of both light chains of the anti-CD47 full-length antibody, and wherein the light chain fusion polypeptide comprises the amino acid sequence of SEQ ID NOs: 62 and the heavy chain polypeptide comprises the amino acid sequence of SEQ ID NO:4 (hereinafter denoted as “PDL1c-E-LN”).

[134] In another general aspect, the present application relates to an isolated nucleic acid encoding an anti-CD47/anti-PD-L1 multiple antigen binding protein or antigen-binding fragment thereof described herein. 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 antibodies or antigen-binding fragments thereof of the invention can be altered without changing the amino acid sequences of the proteins.

[135] In another general aspect, the present application relates to a vector comprising an isolated nucleic acid encoding an anti-CD47/anti-PD-L1 multiple antigen binding protein or antigen-binding fragment thereof described herein. 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. Such techniques are well known to those skilled in the art in view of the present disclosure.

[136] In another general aspect, the present application relates to a host cell comprising an isolated nucleic acid encoding an anti-CD47/anti-PD-L1 multiple antigen binding protein or antigen-binding fragment thereof described herein. 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., a 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.

[137] In another general aspect, the present application relates to a method of producing an anti-CD47/anti-PD-L1 multiple antigen binding protein or antigen-binding fragment thereof described herein, comprising culturing a cell comprising a nucleic acid encoding the multiple antigen binding protein or antigen-binding fragment thereof under conditions to produce a multiple antigen binding protein or antigen-binding fragment thereof of the invention, and recovering the multiple antigen binding protein or antigen-binding fragment thereof from the cell or cell culture (e.g., from the supernatant). Expressed multiple antigen binding proteins 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.

Anti-CD47 monoclonal antibody moiety

[138] The isolated anti-CD47/anti-PD-L1 multiple antigen binding protein or antigen-binding fragment thereof described herein comprises a first antigen-binding portion comprising a heavy chain variable domain (V_H) and a light chain variable domain (V_L), wherein the V_H and V_L together form an antigen-binding site that specifically binds CD47. The V_H and V_L can, for example be from an anti-CD47 monoclonal antibody. Anti-CD47 monoclonal antibodies or antigen-binding fragments there are described in PCT/CN2019/072929, filed on January 24, 2019 and published as International Publication No. WO2019/144895 on August 1, 2019, which is incorporated by reference herein in its entirety.

[139] Anti-CD47 antibodies can, for example, include monoclonal antibodies, human antibodies, chimeric antibodies, humanized antibodies, primatized antibodies, bi-specific antibody (e.g., an anti-CD47/anti-PD-L1 multiple antigen binding protein), conjugated antibodies, a Small Modular ImmunoPharmaceuticals, single chain antibodies, camelid antibodies, CDR-grafted antibodies, and functional variants of an anti-CD47 antibody (such as, for example, a fusion protein), and fragments and derivatives thereof. These antibodies recognize and bind to CD47 protein, particularly human CD47. These antibodies can modulate, e.g., inhibit, block, antagonize, neutralize or otherwise interfere with CD47 expression, activity and/or signaling; and these antibodies do not cause a significant level of agglutination of cells (also referred to as cell agglutination), including hemagglutination of red blood cells. These antibodies can modulate, e.g., inhibit, block, antagonize, neutralize or otherwise interfere with the interaction between CD47 and SIRP α (signal-regulatory-protein α) (for example, human CD47 and human SIRP α). These antibodies, including fragments, functional variants, and derivatives thereof, may be referred to collectively as “anti-CD47 antibodies of this disclosure,” “disclosed anti-CD47 antibodies,” “disclosed antibodies,” “CD47 antibodies of this disclosure,” and the like.

[140] It is possible to quantitate, without undue experimentation, the level of agglutination, e.g., the level of hemagglutination of RBCs. For example, those skilled in the art will recognize that the level of

hemagglutination is ascertained by measuring the area of an RBC dot after performing a hemagglutination assay in the presence of the anti-CD47 monoclonal antibody moiety, as described in PCT Application No. PCT/CN2019/072929, filed on January 24, 2019. In certain embodiments, the area of the RBC dot in the presence of the anti-CD47 monoclonal antibody moiety is compared to the area of the RBC dot in the absence of an anti-CD47 antibody, *i.e.*, in the presence of zero hemagglutination. In this manner, hemagglutination is quantified relative to a baseline control. A larger RBC dot area corresponds to a higher level of hemagglutination. A large RBC red dot area may appear as a haze. Alternatively, densitometry of the RBC dot may also be utilized to quantitate hemagglutination. The comparison may also be done between an anti-CD47 monoclonal antibody moiety and a prior art antibody, such as B6H12.

[141] In certain embodiments, there is provided an anti-CD47 monoclonal antibody moiety comprising a heavy chain variable domain (V_H) with a heavy chain CDR1 comprising the amino acid sequence of SEQ ID NO:19 or a variant thereof comprising up to about 3 (such as about any of 1, 2, or 3) amino acid substitutions; a heavy chain CDR2 comprising the amino acid sequence of SEQ ID NO:20 or a variant thereof comprising up to about 3 (such as about any of 1, 2, or 3) amino acid substitutions; and a heavy chain CDR3 comprising the amino acid sequence of SEQ ID NO:21 or a variant thereof comprising up to about 3 (such as about any of 1, 2, or 3) amino acid substitutions; and a light chain variable domain (V_L) with a light chain CDR1 comprising the amino acid sequence of SEQ ID NO:22 or a variant thereof comprising up to about 3 (such as about any of 1, 2, or 3) amino acid substitutions; a light chain CDR2 comprising the amino acid sequence of SEQ ID NO:23 or a variant thereof comprising up to about 3 (such as about any of 1, 2, or 3) amino acid substitutions; and a light chain CDR3 comprising the amino acid sequence of SEQ ID NO:24 or a variant thereof comprising up to about 3 (such as about any of 1, 2, or 3) amino acid substitutions. In certain embodiments, there is provided an anti-CD47 monoclonal antibody moiety comprising a heavy chain variable domain (V_H) with a heavy chain CDR1 comprising the amino acid sequence of SEQ ID NO:19; a heavy chain CDR2 comprising the amino acid sequence of SEQ ID NO:20; and a heavy chain CDR3 comprising the amino acid sequence of SEQ ID NO:21; and a light chain variable domain (V_L) with a light chain CDR1 comprising the amino acid sequence of SEQ ID NO:22; a light chain CDR2 comprising the amino acid sequence of SEQ ID NO:23; and a light chain CDR3 comprising the amino acid sequence of SEQ ID NO:24.

[142] In certain embodiments, this disclosure provides an anti-CD47 monoclonal antibody moiety comprising a variable heavy chain comprising the amino acid sequence of SEQ ID NO:4 and a variable light chain comprising an amino acid sequence of SEQ ID NO:6. In certain embodiments, the anti-CD47 monoclonal antibody moiety comprises a variable heavy chain that is at least 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99% or more identical to the sequence set forth in SEQ ID NO:4, and a variable

light chain that is at least 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99% or more identical to the sequence set forth in SEQ ID NO:6.

[143] The anti-CD47 monoclonal antibody moiety provided herein exhibits inhibitory activity, for example, by inhibiting CD47 expression (e.g., inhibiting cell surface expression of CD47), activity, and/or signaling, or by interfering with the interaction between CD47 and SIRP α . The anti-CD47 monoclonal antibody moiety provided herein completely or partially reduces or otherwise modulates CD47 expression or activity upon binding to, or otherwise interacting with, CD47, e.g., a human CD47. The reduction or modulation of a biological function of CD47 is complete, significant, or partial upon interaction between the anti-CD47 monoclonal antibody moiety and the human CD47 polypeptide and/or peptide.

[144] The anti-CD47 monoclonal antibody moiety is considered to completely inhibit CD47 expression or activity when the level of CD47 expression or activity in the presence of the antibody is decreased by at least 95%, e.g., by 96%, 97%, 98%, 99% or 100% as compared to the level of CD47 expression or activity in the absence of interaction, e.g., binding, with the antibody moiety described herein.

[145] The anti-CD47 monoclonal antibody moiety is considered to significantly inhibit CD47 expression or activity when the level of CD47 expression or activity in the presence of the anti-CD47 monoclonal antibody moiety is decreased by at least 50%, e.g., 55%, 60%, 75%, 80%, 85% or 90% as compared to the level of CD47 expression or activity in the absence of binding with an anti-CD47 monoclonal antibody moiety described herein. The anti-CD47 monoclonal antibody moiety is considered to partially inhibit CD47 expression or activity when the level of CD47 expression or activity in the presence of the antibody moiety is decreased by less than 95%, e.g., 10%, 20%, 25%, 30%, 40%, 50%, 60%, 75%, 80%, 85% or 90% as compared to the level of CD47 expression or activity in the absence of interaction, e.g., binding, with an antibody moiety described herein.

Anti-PD-L1 single-domain antibody moiety

[146] The isolated anti-CD47/anti-PD-L1 multiple antigen binding protein or antigen-binding fragment thereof described herein comprises a second antigen-binding portion comprising a single-domain antibody (sdAb) moiety that specifically recognizes PD-L1 (or “anti-PD-L1 sdAb”). Anti-PD-L1 single-domain antibodies are described in PCT/CN2018/115213, filed on November 13, 2018 and published as International Publication No. WO2019/096121 on May 23, 2019, which is incorporated by reference herein in its entirety.

Single-domain antibody (sdAb) moiety

[147] Exemplary sdAb moieties include, but are not limited to, heavy chain variable domains from heavy-chain only antibodies (e.g., V_HH (Variable domain of the heavy chain of the Heavy chain antibody))

in *Camelidae* or V_{NAR} (Variable domain of the shark New Antigen Receptor) in cartilaginous fish), binding molecules naturally devoid of light chains, single domains (such as V_{H} or V_{L}) derived from conventional 4-chain antibodies, humanized heavy-chain only antibodies, human single-domain antibodies produced by transgenic mice or rats expressing human heavy chain segments, and engineered domains and single domain scaffolds other than those derived from antibodies. The sdAb moieties may be derived from any species including, but not limited to mouse, rat, human, camel, llama, lamprey, fish, shark, goat, rabbit, and bovine. The sdAb moieties contemplated herein also include naturally occurring single-domain antibody molecules from species other than *Camelidae* and sharks.

[148] In some embodiments, the sdAb moiety is derived from a naturally occurring single-domain antigen binding molecule known as heavy chain antibody devoid of light chains (also referred herein as “heavy chain-only antibodies”, or “HCAb”). Such single domain molecules are disclosed in WO 94/04678 and Hamers-Casterman, C. *et al.* (1993) *Nature* 363:446-448, for example. For clarity reasons, the variable domain derived from a heavy chain molecule naturally devoid of light chain is known herein as a V_{HH} to distinguish it from the conventional V_{H} of four chain immunoglobulins. Such a V_{HH} molecule can be derived from antibodies raised in *Camelidae* species, for example, camel, llama, vicuna, dromedary, alpaca and guanaco. Other species besides *Camelidae* may produce heavy chain molecules naturally devoid of light chain, and such V_{HH} s are within the scope of the present application.

[149] In some embodiments, the sdAb moiety is recombinant, CDR-grafted, humanized, camelized, de-immunized and/or *in vitro* generated (*e.g.*, selected by phage display). In some embodiments, the amino acid sequence of the framework regions may be altered by “camelization” of specific amino acid residues in the framework regions. Camelization refers to the replacing or substitution of one or more amino acid residues in the amino acid sequence of a (naturally occurring) V_{H} domain from a conventional 4-chain antibody by one or more of the amino acid residues that occur at the corresponding position(s) in a V_{HH} domain of a heavy chain antibody. This can be performed in a manner known per se, which will be clear to the skilled person, for example on the basis of the further description herein. Such “camelizing” substitutions are preferably inserted at amino acid positions that form and/or are present at the V_{H} - V_{L} interface, and/or at the so-called *Camelidae* hallmark residues, as defined herein (see for example WO 94/04678, Davies and Riechmann *FEBS Letters* 339: 285-290, 1994; Davies and Riechmann *Protein Engineering* 9 (6): 531-537, 1996; Riechmann *J. Mol. Biol.* 259: 957-969, 1996; and Riechmann and Muyldermans *J. Immunol. Meth.* 231: 25-38, 1999).

[150] In some embodiments, the sdAb moiety is a human sdAb moiety produced by transgenic mice or rats expressing human heavy chain segments. See, *e.g.*, US20090307787A1, U.S. Pat. No. 8,754,287, US20150289489A1, US20100122358A1, and WO2004049794. In some embodiments, the sdAb moiety is affinity matured.

[151] In some embodiments, naturally occurring V_HH domains against a particular antigen or target, can be obtained from (naïve or immune) libraries of Camelid V_HH sequences. Such methods may or may not involve screening such a library using said antigen or target, or at least one part, fragment, antigenic determinant or epitope thereof using one or more screening techniques known per se. Such libraries and techniques are for example described in WO 99/37681, WO 01/90190, WO 03/025020 and WO 03/035694. Alternatively, improved synthetic or semi-synthetic libraries derived from (naïve or immune) V_HH libraries may be used, such as V_HH libraries obtained from (naïve or immune) V_HH libraries by techniques such as random mutagenesis and/or CDR shuffling, as for example described in WO 00/43507.

[152] In some embodiments, the sdAb moieties are generated from conventional four-chain antibodies. See, for example, EP 0 368 684, Ward et al. (Nature 1989 Oct. 12; 341 (6242): 544-6), Holt et al., Trends Biotechnol., 2003, 21(11):484-490; WO 06/030220; and WO 06/003388.

[153] In some embodiments, there is provided an anti-PD-L1 sdAb moiety comprising a CDR1 comprising the amino acid sequence of any one of SEQ ID NOs: 25, 28, or 31, or a variant thereof comprising up to about 3 (such as about any of 1, 2, or 3) amino acid substitutions; a CDR2 comprising the amino acid sequence of any one of SEQ ID NOs: 26, 29, or 32, or a variant thereof comprising up to about 3 (such as about any of 1, 2, or 3) amino acid substitutions; and a CDR3 comprising the amino acid sequence of any one of SEQ ID NOs: 27, 30, or 33, or a variant thereof comprising up to about 3 (such as about any of 1, 2, or 3) amino acid substitutions.

[154] In some embodiments, there is provided an anti-PD-L1 sdAb moiety comprising a CDR1 comprising the amino acid sequence of any one of SEQ ID NOs: 25, 28, or 31; a CDR2 comprising the amino acid sequence of any one of SEQ ID NOs: 26, 29, or 32; and a CDR3 comprising the amino acid sequence of any one of SEQ ID NOs: 27, 30, or 33.

[155] The CDRs can be combined in various pair-wise combinations to generate a number of anti-PD-L1 sdAb moieties.

[156] In some embodiments, there is provided an anti-PD-L1 sdAb moiety comprising a V_HH domain comprising the amino acid sequence of any one of SEQ ID NOs: 14, 16, or 18, or a variant thereof having at least about 80% (such as at least about any of 80%, 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, or 99%) sequence identity to any one of SEQ ID NOs: 14, 16, or 18. In some embodiments, there is provided an anti-PD-L1 sdAb moiety comprising a V_HH domain comprising the amino acid sequence of any one of SEQ ID NOs: 14, 16, or 18, or a variant thereof comprising up to about 3 (such as about any of 1, 2, or 3) amino acid substitutions in the V_HH domain. In some embodiments, the anti-PD-L1 sdAb moiety comprising the V_HH domain comprising the amino acid sequence of any one of SEQ ID NOs: 14, 16, or 18 or a variant thereof comprises amino acid substitutions in CDRs, such as the CDR1, and/or the CDR2, and/or the CDR3. In some embodiments, the anti-PD-L1 sdAb moiety comprising the V_HH

domain comprising the amino acid sequence of any one of SEQ ID NOs: 14, 16, or 18 or a variant thereof comprises CDR1, CDR2, and CDR3 of any one of SEQ ID NOs: 14, 16, or 18, and the amino acid substitutions are in FRs, such as the FR1, and/or the FR2, and/or the FR3, and/or the FR4.

Biological activities

[157] The biological activity of anti-PD-L1 sdAb moiety described herein can be determined by measuring its half maximal inhibitory concentration (IC_{50}), which is a measure of the effectiveness of an antibody in inhibiting a specific biological or biochemical function (such as inhibiting the binding between PD-L1 and its receptor PD-1). For example, here IC_{50} can be used to indicate the effective concentration of anti-PD-L1 sdAb needed to neutralize 50% of PD-L1 bioactivity *in vitro*. IC_{50} is comparable to an EC_{50} for agonist drug or other substance (such as an antibody). EC_{50} also represents the plasma concentration required for obtaining 50% of a maximum effect *in vivo*. IC_{50} or EC_{50} can be measured by assays known in the art, for example, bioassays such as inhibition of ligand binding by FACS analysis (competition binding assay), cell based cytokine release assay, or amplified luminescent proximity homogeneous assay (AlphaLISA).

[158] In some embodiments, the anti-PD-L1 sdAb moiety blocks or antagonizes signals transduced by the PD-L1 ligand. In some embodiments, the anti-PD-L1 sdAb moiety can bind to an epitope on PD-L1 so as to inhibit PD-L1 from interacting with a PD-1. In some embodiments, the anti-PD-L1 sdAb moiety can reduce the binding of PD-L1 to its receptor PD-1 by at least about any of 5%, 10%, 20%, 25%, 30%, 40%, 50%, 60%, 70%, 75%, 80%, 90%, 95%, 99% or 99.9% under conditions in which the ratio of antibody combining site to PD-L1 ligand binding site is greater than 1:1 and the concentration of antibody is greater than 10^{-8} M.

Peptide linkers

[159] In some embodiments, the first and second antigen binding portions within the anti-CD-47/anti-PD-L1 multiple antigen binding protein or antigen-binding fragment thereof can be optionally connected by a peptide linker. The length, the degree of flexibility and/or other properties of the peptide linker(s) used in the anti-CD47/anti-PD-L1 multiple antigen binding protein or antigen binding fragment thereof may have some influence on properties, including but not limited to the affinity, specificity, or avidity for one or more particular antigens or epitopes. For example, longer peptide linkers may be selected to ensure that two adjacent domains do not sterically interfere with one another. In some embodiment, a peptide linker comprises flexible residues (such as glycine and serine) so that the adjacent domains are free to move relative to each other. For example, a glycine-serine doublet can be a suitable peptide linker.

[160] The peptide linker can be of any suitable length. In some embodiments, the peptide linker is at least about any of 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 25, 30, 35, 40, 50, 75, 100 or more amino acids long. In some embodiments, the peptide linker is no more than about any of 100,

75, 50, 40, 35, 30, 25, 20, 19, 18, 17, 16, 15, 14, 13, 12, 11, 10, 9, 8, 7, 6, 5 or fewer amino acids long. In some embodiments, the length of the peptide linker is any of about 1 amino acid to about 10 amino acids, about 1 amino acid to about 20 amino acids, about 1 amino acid to about 30 amino acids, about 5 amino acids to about 15 amino acids, about 10 amino acids to about 25 amino acids, about 5 amino acids to about 30 amino acids, about 10 amino acids to about 30 amino acids long, about 30 amino acids to about 50 amino acids, about 50 amino acids to about 100 amino acids, or about 1 amino acid to about 100 amino acids.

[161] The peptide linker may have a naturally occurring sequence, or a non-naturally occurring sequence. For example, a sequence derived from the hinge region of heavy chain only antibodies may be used as the linker. *See*, for example, WO1996/34103. In some embodiments, the peptide linker is a mutated human IgG1 hinge. In some embodiments, the peptide linker is a flexible linker. Exemplary flexible linkers include glycine polymers (G)_n, glycine-serine polymers (including, for example, (GS)_n, (GSGGS)_n, (GGGS)_n, and (GGGGS)_n, where n is an integer of at least one), glycine-alanine polymers, alanine-serine polymers, and other flexible linkers known in the art. In some embodiments, the peptide linker comprises the amino acid sequence of SEQ ID NO: 10 (GGGGSGGGGSGGGGS). In some embodiments, the peptide linker comprises the amino acid sequence of SEQ ID NO: 8 (EPKSSDKTHTSPSP). In some embodiments, the peptide linker comprises the amino acid sequence of SEQ ID NO: 69 (ESKYGPPSPSP).

Chimeric or humanized anti-CD47/anti-PD-L1 multiple antigen binding protein or antigen binding fragment thereof

[162] In some embodiments, the anti-CD47/anti-PD-L1 multiple antigen binding protein provided herein is a chimeric antibody. Certain chimeric antibodies are described, *e.g.*, in U.S. Patent No. 4,816,567; and Morrison et al., *Proc. Natl. Acad. Sci. USA*, 81:6851-6855 (1984)). In one example, a chimeric antibody comprises a non-human variable region (*e.g.*, a variable region derived from a camelid species, such as llama) and a human constant region. In a further example, a chimeric antibody is a “class switched” antibody in which the class or subclass has been changed from that of the parent antibody. Chimeric antibodies include antigen-binding fragments thereof.

[163] In some embodiments, a chimeric antibody is a humanized antibody. Typically, a non-human antibody is humanized to reduce immunogenicity to humans, while retaining the specificity and affinity of the parental non-human antibody. Generally, a humanized antibody comprises one or more variable domains in which HVRs, *e.g.*, CDRs, (or portions thereof) are derived from a non-human antibody, and FRs (or portions thereof) are derived from human antibody sequences. A humanized antibody optionally will also comprise at least a portion of a human constant region. In some embodiments, some FR residues in a humanized antibody are substituted with corresponding residues from a non-human antibody

(e.g., the antibody from which the HVR residues are derived), e.g., to restore or improve antibody specificity or affinity.

[164] Humanized antibodies and methods of making them are reviewed, e.g., in Almagro and Fransson, *Front. Biosci.* 13:1619-1633 (2008), and are further described, e.g., in Riechmann et al., *Nature* 332:323-329 (1988); Queen et al., *Proc. Nat'l Acad. Sci. USA* 86:10029-10033 (1989); US Patent Nos. 5,821,337, 7,527,791, 6,982,321, and 7,087,409; Kashmiri et al., *Methods* 36:25-34 (2005) (describing SDR (a-CDR) grafting); Padlan, *Mol. Immunol.* 28:489-498 (1991) (describing "resurfacing"); Dall'Acqua et al., *Methods* 36:43-60 (2005) (describing "FR shuffling"); and Osbourn et al., *Methods* 36:61-68 (2005) and Klimka et al., *Br. J. Cancer*, 83:252-260 (2000) (describing the "guided selection" approach to FR shuffling).

[165] Human framework regions that may be used for humanization include but are not limited to: framework regions selected using the "best-fit" method (see, e.g., Sims et al. *J. Immunol.* 151:2296 (1993)); framework regions derived from the consensus sequence of human antibodies of a particular subgroup of light or heavy chain variable regions (see, e.g., Carter et al. *Proc. Natl. Acad. Sci. USA*, 89:4285 (1992); and Presta et al. *J. Immunol.*, 151:2623 (1993)); human mature (somatically mutated) framework regions or human germline framework regions (see, e.g., Almagro and Fransson, *Front. Biosci.* 13:1619-1633 (2008)); and framework regions derived from screening FR libraries (see, e.g., Baca et al., *J. Biol. Chem.* 272:10678-10684 (1997) and Rosok et al., *J. Biol. Chem.* 271:22611-22618 (1996)).

[166] In some embodiments, the second antigen binding portion of the anti-CD47/anti-PD-L1 multiple antigen binding protein or antigen binding fragment thereof is modified, such as humanized, without diminishing the native affinity of the domain for antigen and while reducing its immunogenicity with respect to a heterologous species. For example, the amino acid residues of the antibody variable domain ($V_{\text{H}}\text{H}$) of an llama antibody can be determined, and one or more of the Camelid amino acids, for example, in the framework regions, are replaced by their human counterpart as found in the human consensus sequence, without that polypeptide losing its typical character, *i.e.* the humanization does not significantly affect the antigen binding capacity of the resulting polypeptide. Humanization of Camelid single-domain antibodies requires the introduction and mutagenesis of a limited amount of amino acids in a single polypeptide chain. This is in contrast to humanization of scFv, Fab', (Fab')₂ and IgG, which requires the introduction of amino acid changes in two chains, the light and the heavy chain and the preservation of the assembly of both chains.

[167] Single-domain antibodies comprising a $V_{\text{H}}\text{H}$ domain can be humanized to have human-like sequences. In some embodiments, the FR regions of the $V_{\text{H}}\text{H}$ domain used herein comprise at least about any one of 50%, 60%, 70%, 80%, 90%, 95% or more of amino acid sequence homology to human V_{H} framework regions. One exemplary class of humanized $V_{\text{H}}\text{H}$ domains is characterized in that the $V_{\text{H}}\text{H}$ s

carry an amino acid from the group consisting of glycine, alanine, valine, leucine, isoleucine, proline, phenylalanine, tyrosine, tryptophan, methionine, serine, threonine, asparagine, or glutamine at position 45, such as, for example, L45 and a tryptophan at position 103, according to the Kabat numbering. As such, polypeptides belonging to this class show a high amino acid sequence homology to human V_H framework regions and said polypeptides might be administered to a human directly without expectation of an unwanted immune response therefrom, and without the burden of further humanization.

[168] Another exemplary class of humanized Camelid single-domain antibodies has been described in WO 03/035694 and contains hydrophobic FR2 residues typically found in conventional antibodies of human origin or from other species, but compensating this loss in hydrophilicity by the charged arginine residue on position 103 that substitutes the conserved tryptophan residue present in V_H from double-chain antibodies. As such, peptides belonging to these two classes show a high amino acid sequence homology to human V_H framework regions and said peptides might be administered to a human directly without expectation of an unwanted immune response therefrom, and without the burden of further humanization.

Human antibodies

[169] In some embodiments, the anti-CD47/anti-PD-L1 multiple antigen binding protein or antigen binding fragment thereof provided herein is a human antibody. Human antibodies can be produced using various techniques known in the art. Human antibodies are described generally in van Dijk and van de Winkel, *Curr. Opin. Pharmacol.* 5: 368-74 (2001) and Lonberg, *Curr. Opin. Immunol.* 20:450-459 (2008). Transgenic mice or rats capable of producing fully human single-domain antibodies are known in the art. See, e.g., US20090307787A1, U.S. Pat. No. 8,754,287, US20150289489A1, US20100122358A1, and WO2004049794.

[170] Human antibodies may be prepared by administering an immunogen to a transgenic animal that has been modified to produce intact human antibodies or intact antibodies with human variable regions in response to antigenic challenge. Such animals typically contain all or a portion of the human immunoglobulin loci, which replace the endogenous immunoglobulin loci, or which are present extrachromosomally or integrated randomly into the animal's chromosomes. In such transgenic mice, the endogenous immunoglobulin loci have generally been inactivated. For review of methods for obtaining human antibodies from transgenic animals, see Lonberg, *Nat. Biotech.* 23:1117-1125 (2005). See also, e.g., U.S. Patent Nos. 6,075,181 and 6,150,584 describing XENOMOUSETM technology; U.S. Patent No. 5,770,429 describing HUMAB® technology; U.S. Patent No. 7,041,870 describing K-M MOUSE® technology, and U.S. Patent Application Publication No. US 2007/0061900, describing VELOCIMOUSE® technology). Human variable regions from intact antibodies generated by such animals may be further modified, e.g., by combining with a different human constant region.

[171] Human antibodies can also be made by hybridoma-based methods. Human myeloma and mouse-human heteromyeloma cell lines for the production of human monoclonal antibodies have been described. (See, e.g., Kozbor *J. Immunol.*, 133: 3001 (1984); Brodeur et al., *Monoclonal Antibody Production Techniques and Applications*, pp. 51-63 (Marcel Dekker, Inc., New York, 1987); and Boerner et al., *J. Immunol.*, 147: 86 (1991).) Human antibodies generated via human B-cell hybridoma technology are also described in Li et al., *Proc. Natl. Acad. Sci. USA*, 103:3557-3562 (2006). Additional methods include those described, for example, in U.S. Patent No. 7,189,826 (describing production of monoclonal human IgM antibodies from hybridoma cell lines) and Ni, *Xiandai Mianyixue*, 26(4):265-268 (2006) (describing human-human hybridomas). Human hybridoma technology (Trioma technology) is also described in Vollmers and Brandlein, *Histology and Histopathology*, 20(3):927-937 (2005) and Vollmers and Brandlein, *Methods and Findings in Experimental and Clinical Pharmacology*, 27(3):185-91 (2005).

[172] Human antibodies may also be generated by isolating Fv clone variable domain sequences selected from human-derived phage display libraries. Such variable domain sequences may then be combined with a desired human constant domain. Techniques for selecting human antibodies from antibody libraries are described below.

[173] One technique for obtaining V_{HH} sequences directed against a particular antigen or target involves suitably immunizing a transgenic mammal that is capable of expressing heavy chain antibodies (i.e. so as to raise an immune response and/or heavy chain antibodies directed against said antigen or target), obtaining a suitable biological sample from said transgenic mammal that contains (nucleic acid sequences encoding) said V_{HH} sequences (such as a blood sample, serum sample or sample of B-cells), and then generating V_{HH} sequences directed against said antigen or target, starting from said sample, using any suitable technique known per se (such as any of the methods described herein or a hybridoma technique). For example, for this purpose, the heavy chain antibody-expressing mice and the further methods and techniques described in WO 02/085945, WO 04/049794 and WO 06/008548 and Janssens et al., *Proc. Natl. Acad. Sci. USA*, 2006 Oct. 10; 103(41):15130-5 can be used. For example, such heavy chain antibody expressing mice can express heavy chain antibodies with any suitable (single) variable domain, such as (single) variable domains from natural sources (e.g. human (single) variable domains, Camelid (single) variable domains or shark (single) variable domains), as well as for example synthetic or semi-synthetic (single) variable domains.

Library-derived antibodies

[174] Antibodies of the present application may be isolated by screening combinatorial libraries for antibodies with the desired activity or activities. For example, a variety of methods are known in the art for generating phage display libraries and screening such libraries for antibodies possessing the desired

binding characteristics. Such methods are reviewed, *e.g.*, in Hoogenboom et al. in *Methods in Molecular Biology* 178:1-37 (O'Brien et al., ed., Human Press, Totowa, NJ, 2001) and further described, *e.g.*, in the McCafferty et al., *Nature* 348:552-554; Clackson et al., *Nature* 352: 624-628 (1991); Marks et al., *J. Mol. Biol.* 222: 581-597 (1992); Marks and Bradbury, in *Methods in Molecular Biology* 248:161-175 (Lo, ed., Human Press, Totowa, NJ, 2003); Sidhu et al., *J. Mol. Biol.* 338(2): 299-310 (2004); Lee et al., *J. Mol. Biol.* 340(5): 1073-1093 (2004); Fellouse, *Proc. Natl. Acad. Sci. USA* 101(34): 12467-12472 (2004); and Lee et al., *J. Immunol. Methods* 284(1-2): 119-132(2004). Methods for constructing single-domain antibody libraries have been described, for example, see U.S. Pat. No. 7,371,849.

[175] In certain phage display methods, repertoires of V_H and V_L genes are separately cloned by polymerase chain reaction (PCR) and recombined randomly in phage libraries, which can then be screened for antigen-binding phage as described in Winter et al., *Ann. Rev. Immunol.*, 12: 433-455 (1994). Phage typically display antibody fragments, either as single-chain Fv (scFv) fragments or as Fab fragments. Libraries from immunized sources provide high-affinity antibodies to the immunogen without the requirement of constructing hybridomas. Alternatively, the naive repertoire can be cloned (*e.g.*, from human) to provide a single source of antibodies to a wide range of non-self and also self-antigens without any immunization as described by Griffiths et al., *EMBO J*, 12: 725-734 (1993). Finally, naive libraries can also be made synthetically by cloning unrearranged V-gene segments from stem cells, and using PCR primers containing random sequence to encode the highly variable CDR3 regions and to accomplish rearrangement *in vitro*, as described by Hoogenboom and Winter, *J. Mol. Biol.*, 227: 381-388 (1992). Patent publications describing human antibody phage libraries include, for example: US Patent No. 5,750,373, and US Patent Publication Nos. 2005/0079574, 2005/0119455, 2005/0266000, 2007/0117126, 2007/0160598, 2007/0237764, 2007/0292936, and 2009/0002360.

[176] Antibodies or antibody fragments isolated from human antibody libraries are considered human antibodies or human antibody fragments herein.

Anti-CD47/anti-PD-L1 multiple antigen binding protein or antigen binding fragment thereof variants

[177] In some embodiments, amino acid sequence variants of the antibodies provided herein are contemplated. For example, it may be desirable to improve the binding affinity and/or other biological properties of the antibody. Amino acid sequence variants of an antibody may be prepared by introducing appropriate modifications into the nucleic acid sequence encoding the antibody, or by peptide synthesis. Such modifications include, for example, deletions from, and/or insertions into and/or substitutions of residues within the amino acid sequences of the antibody. Any combination of deletion, insertion, and substitution can be made to arrive at the final construct, provided that the final construct possesses the desired characteristics, *e.g.*, antigen-binding.

a) Substitution, insertion, deletion and variants

[178] In some embodiments, antibody variants having one or more amino acid substitutions are provided. Sites of interest for substitutional mutagenesis include the HVRs and FRs. Conservative substitutions are shown in Table 2 under the heading of “Preferred substitutions.” More substantial changes are provided in Table 2 under the heading of “exemplary substitutions,” and as further described below in reference to amino acid side chain classes. Amino acid substitutions may be introduced into an antibody of interest and the products screened for a desired activity, *e.g.*, retained/improved antigen binding, decreased immunogenicity, or improved ADCC or CDC.

Table 2. Amino acid substitutions

Original Residue	Exemplary Substitutions	Preferred Substitutions
Ala (A)	Val; Leu; Ile	Val
Arg (R)	Lys; Gln; Asn	Lys
Asn (N)	Gln; His; Asp, Lys; Arg	Gln
Asp (D)	Glu; Asn	Glu
Cys (C)	Ser; Ala	Ser
Gln (Q)	Asn; Glu	Asn
Glu (E)	Asp; Gln	Asp
Gly (G)	Ala	Ala
His (H)	Asn; Gln; Lys; Arg	Arg
Ile (I)	Leu; Val; Met; Ala; Phe; Norleucine	Leu
Leu (L)	Norleucine; Ile; Val; Met; Ala; Phe	Ile
Lys (K)	Arg; Gln; Asn	Arg
Met (M)	Leu; Phe; Ile	Leu
Phe (F)	Trp; Leu; Val; Ile; Ala; Tyr	Tyr
Pro (P)	Ala	Ala
Ser (S)	Thr	Thr
Thr (T)	Val; Ser	Ser
Trp (W)	Tyr; Phe	Tyr
Tyr (Y)	Trp; Phe; Thr; Ser	Phe
Val (V)	Ile; Leu; Met; Phe; Ala; Norleucine	Leu

[179] Amino acids may be grouped according to common side-chain properties:

- (1) hydrophobic: Norleucine, Met, Ala, Val, Leu, Ile;
- (2) neutral hydrophilic: Cys, Ser, Thr, Asn, Gln;
- (3) acidic: Asp, Glu;
- (4) basic: His, Lys, Arg;
- (5) residues that influence chain orientation: Gly, Pro;

(6) aromatic: Trp, Tyr, Phe.

[180] Non-conservative substitutions will entail exchanging a member of one of these classes for another class.

[181] One type of substitutional variant involves substituting one or more hypervariable region residues of a parent antibody (*e.g.*, a humanized or human antibody). Generally, the resulting variant(s) selected for further study will have modifications (*e.g.*, improvements) in certain biological properties (*e.g.*, increased affinity, reduced immunogenicity) relative to the parent antibody and/or will have substantially retained certain biological properties of the parent antibody. An exemplary substitutional variant is an affinity matured antibody, which may be conveniently generated, *e.g.*, using phage display-based affinity maturation techniques such as those described herein. Briefly, one or more HVR residues are mutated and the variant antibodies displayed on phage and screened for a particular biological activity (*e.g.* binding affinity).

[182] Alterations (*e.g.*, substitutions) may be made in HVRs, *e.g.*, to improve antibody affinity. Such alterations may be made in HVR “hotspots,” *i.e.*, residues encoded by codons that undergo mutation at high frequency during the somatic maturation process (*see, e.g.*, Chowdhury, *Methods Mol. Biol.* 207:179-196 (2008)), and/or SDRs (a-CDRs), with the resulting variant VH or VL being tested for binding affinity. Affinity maturation by constructing and reselecting from secondary libraries has been described, *e.g.*, in Hoogenboom et al. in *Methods in Molecular Biology* 178:1-37 (O’Brien et al., ed., Human Press, Totowa, NJ, (2001)). In some embodiments of affinity maturation, diversity is introduced into the variable genes chosen for maturation by any of a variety of methods (*e.g.*, error-prone PCR, chain shuffling, or oligonucleotide-directed mutagenesis). A secondary library is then created. The library is then screened to identify any antibody variants with the desired affinity. Another method to introduce diversity involves HVR-directed approaches, in which several HVR residues (*e.g.*, 4-6 residues at a time) are randomized. HVR residues involved in antigen binding may be specifically identified, *e.g.*, using alanine scanning mutagenesis or modeling. CDR-H3 and CDR-L3 in particular are often targeted.

[183] In some embodiments, substitutions, insertions, or deletions may occur within one or more HVRs so long as such alterations do not substantially reduce the ability of the antibody to bind antigen. For example, conservative alterations (*e.g.*, conservative substitutions as provided herein) that do not substantially reduce binding affinity may be made in HVRs. Such alterations may be outside of HVR “hotspots” or CDRs. In some embodiments of the variant V_HH sequences provided above, each HVR either is unaltered, or contains no more than one, two or three amino acid substitutions.

[184] A useful method for identification of residues or regions of an antibody that may be targeted for mutagenesis is called “alanine scanning mutagenesis” as described by Cunningham and Wells (1989) *Science*, 244:1081-1085. In this method, a residue or group of target residues (*e.g.*, charged residues such

as Arg, Asp, His, Lys, and Glu) are identified and replaced by a neutral or negatively charged amino acid (*e.g.*, alanine or polyalanine) to determine whether the interaction of the antibody with antigen is affected. Further substitutions may be introduced at the amino acid locations demonstrating functional sensitivity to the initial substitutions. Alternatively, or additionally, a crystal structure of an antigen-antibody complex to identify contact points between the antibody and antigen. Such contact residues and neighboring residues may be targeted or eliminated as candidates for substitution. Variants may be screened to determine whether they contain the desired properties.

[185] Amino acid sequence insertions include amino- and/or carboxyl-terminal fusions ranging in length from one residue to polypeptides containing a hundred or more residues, as well as intrasequence insertions of single or multiple amino acid residues. Examples of terminal insertions include an antibody with an N-terminal methionyl residue. Other insertional variants of the antibody molecule include the fusion to the N- or C-terminus of the antibody to an enzyme (*e.g.*, for ADEPT) or a polypeptide which increases the serum half-life of the antibody.

b) Glycosylation variants

[186] In some embodiments, an anti-CD47/anti-PD-L1 multiple antigen binding protein or antigen binding fragment thereof provided herein is altered to increase or decrease the extent to which the construct is glycosylated. Addition or deletion of glycosylation sites to an antibody may be conveniently accomplished by altering the amino acid sequence such that one or more glycosylation sites is created or removed.

[187] Where the anti-CD47/anti-PD-L1 multiple antigen binding protein or antigen binding fragment thereof comprises an Fc region, the carbohydrate attached thereto may be altered. Native antibodies produced by mammalian cells typically comprise a branched, biantennary oligosaccharide that is generally attached by an N-linkage to Asn297 of the CH2 domain of the Fc region. *See, e.g.*, Wright et al. *TIBTECH* 15:26-32 (1997). The oligosaccharide may include various carbohydrates, *e.g.*, mannose, N-acetyl glucosamine (GlcNAc), galactose, and sialic acid, as well as a fucose attached to a GlcNAc in the “stem” of the biantennary oligosaccharide structure. In some embodiments, modifications of the oligosaccharide in an anti-CD47/anti-PD-L1 multiple antigen binding protein or antigen binding fragment thereof of the present application may be made in order to create antibody variants with certain improved properties.

[188] In some embodiments, antibody variants are provided having a carbohydrate structure that lacks fucose attached (directly or indirectly) to an Fc region. For example, the amount of fucose in such antibody may be from 1% to 80%, from 1% to 65%, from 5% to 65% or from 20% to 40%. The amount of fucose is determined by calculating the average amount of fucose within the sugar chain at Asn297, relative to the sum of all glycostructures attached to Asn 297 (*e.g.*, complex, hybrid and high mannose

structures) as measured by MALDI-TOF mass spectrometry, as described in WO 2008/077546, for example. Asn297 refers to the asparagine residue located at about position 297 in the Fc region (EU numbering of Fc region residues); however, Asn297 may also be located about ± 3 amino acids upstream or downstream of position 297, *i.e.*, between positions 294 and 300, due to minor sequence variations in antibodies. Such fucosylation variants may have improved ADCC function. *See, e.g.*, US Patent Publication Nos. US 2003/0157108 (Presta, L.); US 2004/0093621 (Kyowa Hakko Kogyo Co., Ltd). Examples of publications related to “defucosylated” or “fucose-deficient” antibody variants include: US 2003/0157108; WO 2000/61739; WO 2001/29246; US 2003/0115614; US 2002/0164328; US 2004/0093621; US 2004/0132140; US 2004/0110704; US 2004/0110282; US 2004/0109865; WO 2003/085119; WO 2003/084570; WO 2005/035586; WO 2005/035778; WO2005/053742; WO2002/031140; Okazaki et al. *J. Mol. Biol.* 336:1239-1249 (2004); Yamane-Ohnuki et al. *Biotech. Bioeng.* 87: 614 (2004). Examples of cell lines capable of producing defucosylated antibodies include Lec13 CHO cells deficient in protein fucosylation (Ripka et al. *Arch. Biochem. Biophys.* 249:533-545 (1986); US Patent Application No. US 2003/0157108 A1, Presta, L; and WO 2004/056312 A1, Adams *et al.*, especially at Example 11), and knockout cell lines, such as alpha-1,6-fucosyltransferase gene, *FUT8*, knockout CHO cells (*see, e.g.*, Yamane-Ohnuki et al. *Biotech. Bioeng.* 87: 614 (2004); Kanda, Y. et al., *Biotechnol. Bioeng.*, 94(4):680-688 (2006); and WO2003/085107).

[189] Anti-CD47/anti-PD-L1 multiple antigen binding protein variants are further provided with bisected oligosaccharides, *e.g.*, in which a biantennary oligosaccharide attached to the Fc region of the antibody is bisected by GlcNAc. Such antibody variants may have reduced fucosylation and/or improved ADCC function. Examples of such antibody variants are described, *e.g.*, in WO 2003/011878 (Jean-Mairet et al.); US Patent No. 6,602,684 (Umana et al.); and US 2005/0123546 (Umana *et al.*). Antibody variants with at least one galactose residue in the oligosaccharide attached to the Fc region are also provided. Such antibody variants may have improved CDC function. Such antibody variants are described, *e.g.*, in WO 1997/30087 (Patel et al.); WO 1998/58964 (Raju, S.); and WO 1999/22764 (Raju, S.).

c) Fc region variants

[190] In some embodiments, one or more amino acid modifications may be introduced into the Fc region of the anti-CD47/anti-PD-L1 multiple antigen binding protein or antigen binding fragment thereof provided herein, thereby generating an Fc region variant. The Fc region variant may comprise a human Fc region sequence (*e.g.*, a human IgG1, IgG2, IgG3 or IgG4 Fc region) comprising an amino acid modification (*e.g.* a substitution) at one or more amino acid positions.

[191] In some embodiments, the present application contemplates an anti-CD47/anti-PD-L1 multiple antigen binding protein variant that possesses some but not all effector functions, which make it a desirable candidate for applications in which the half-life of the anti-CD47/anti-PD-L1 multiple antigen

binding protein or antigen binding fragment thereof *in vivo* is important yet certain effector functions (such as complement and ADCC) are unnecessary or deleterious. *In vitro* and/or *in vivo* cytotoxicity assays can be conducted to confirm the reduction/depletion of CDC and/or ADCC activities. For example, Fc receptor (FcR) binding assays can be conducted to ensure that the antibody lacks Fc γ R binding (hence likely lacking ADCC activity), but retains Fc γ Rn binding ability. The primary cells for mediating ADCC, NK cells, express Fc γ RIII only, whereas monocytes express Fc γ RI, Fc γ RII and Fc γ RIII. FcR expression on hematopoietic cells is summarized in Table 3 on page 464 of Ravetch and Kinet, *Annu. Rev. Immunol.* 9:457-492 (1991). Non-limiting examples of *in vitro* assays to assess ADCC activity of a molecule of interest is described in U.S. Patent No. 5,500,362 (*see, e.g.* Hellstrom, I. et al. *Proc. Nat'l Acad. Sci. USA* 83:7059-7063 (1986)) and Hellstrom, I et al., *Proc. Nat'l Acad. Sci. USA* 82:1499-1502 (1985); 5,821,337 (*see* Bruggemann, M. et al., *J. Exp. Med.* 166:1351-1361 (1987)). Alternatively, non-radioactive assays methods may be employed (*see, for example*, ACTI™ non-radioactive cytotoxicity assay for flow cytometry (Cell Technology, Inc. Mountain View, CA; and CytoTox 96® non-radioactive cytotoxicity assay (Promega, Madison, WI). Useful effector cells for such assays include peripheral blood mononuclear cells (PBMC) and Natural Killer (NK) cells. Alternatively, or additionally, ADCC activity of the molecule of interest may be assessed *in vivo, e.g.,* in an animal model such as that disclosed in Clynes et al. *Proc. Nat'l Acad. Sci. USA* 95:652-656 (1998). C1q binding assays may also be carried out to confirm that the antibody is unable to bind C1q and hence lacks CDC activity. *See, e.g.,* C1q and C3c binding ELISA in WO 2006/029879 and WO 2005/100402. To assess complement activation, a CDC assay may be performed (*see, for example*, Gazzano-Santoro et al., *J. Immunol. Methods* 202:163 (1996); Cragg, M.S. et al., *Blood* 101:1045-1052 (2003); and Cragg, M.S. and M.J. Glennie, *Blood* 103:2738-2743 (2004)). Fc γ Rn binding and *in vivo* clearance/half-life determinations can also be performed using methods known in the art (*see, e.g.,* Petkova, S.B. et al., *Int'l. Immunol.* 18(12):1759-1769 (2006)).

[192] Antibodies with reduced effector function include those with substitution of one or more of Fc region residues 238, 265, 269, 270, 297, 327 and 329 (U.S. Patent No. 6,737,056). Such Fc mutants include Fc mutants with substitutions at two or more of amino acid positions 265, 269, 270, 297 and 327, including the so-called “DANA” Fc mutant with substitution of residues 265 and 297 to alanine (US Patent No. 7,332,581).

[193] Certain antibody variants with improved or diminished binding to FcRs are described. (*See, e.g.,* U.S. Patent No. 6,737,056; WO 2004/056312, and Shields et al., *J. Biol. Chem.* 9(2): 6591-6604 (2001)).

[194] In some embodiments, an anti-CD47/anti-PD-L1 multiple antigen binding protein variant comprises an Fc region with one or more amino acid substitutions which improve ADCC, *e.g.,* substitutions at positions 298, 333, and/or 334 of the Fc region (EU numbering of residues).

[195] In some embodiments, alterations are made in the Fc region that result in altered (*i.e.*, either improved or diminished) C1q binding and/or Complement Dependent Cytotoxicity (CDC), *e.g.*, as described in US Patent No. 6,194,551, WO 99/51642, and Idusogie et al. *J. Immunol.* 164: 4178-4184 (2000).

[196] In some embodiments, there is provided an anti-CD47/anti-PD-L1 multiple antigen binding protein (*e.g.*, a HCAb) variant comprising a variant Fc region comprising one or more amino acid substitutions which increase half-life and/or improve binding to the neonatal Fc receptor (FcRn). Antibodies with increased half-lives and improved binding to the neonatal Fc receptor (FcRn), which is responsible for the transfer of maternal IgGs to the fetus (Guyer et al., *J. Immunol.* 117:587 (1976) and Kim et al., *J. Immunol.* 24:249 (1994)), are described in US2005/0014934A1 (Hinton et al.). Those antibodies comprise an Fc region with one or more substitutions therein which improve binding of the Fc region to FcRn. Such Fc variants include those with substitutions at one or more of Fc region residues: 238, 256, 265, 272, 286, 303, 305, 307, 311, 312, 317, 340, 356, 360, 362, 376, 378, 380, 382, 413, 424 or 434, *e.g.*, substitution of Fc region residue 434 (US Patent No. 7,371,826).

[197] See also Duncan & Winter, *Nature* 322:738-40 (1988); U.S. Patent No. 5,648,260; U.S. Patent No. 5,624,821; and WO 94/29351 concerning other examples of Fc region variants.

d) Cysteine engineered antibody variants

[198] In some embodiments, it may be desirable to create cysteine engineered anti-CD47/anti-PD-L1 multiple antigen binding proteins or antigen binding fragments thereof, *e.g.*, “thioMAbs,” in which one or more residues of an antibody are substituted with cysteine residues. In particular embodiments, the substituted residues occur at accessible sites of the antibody. By substituting those residues with cysteine, reactive thiol groups are thereby positioned at accessible sites of the antibody and may be used to conjugate the antibody to other moieties, such as drug moieties or linker-drug moieties, to create an immunoconjugate, as described further herein. In some embodiments, any one or more of the following residues may be substituted with cysteine: A118 (EU numbering) of the heavy chain; and S400 (EU numbering) of the heavy chain Fc region. Cysteine engineered anti-PD-L1 constructs may be generated as described, *e.g.*, in U.S. Patent No. 7,521,541.

e) Antibody derivatives

[199] In some embodiments, an anti-CD47/anti-PD-L1 multiple antigen binding protein or antigen binding fragment thereof provided herein may be further modified to contain additional nonproteinaceous moieties that are known in the art and readily available. The moieties suitable for derivatization of the antibody include but are not limited to water soluble polymers. Non-limiting examples of water soluble polymers include, but are not limited to, polyethylene glycol (PEG), copolymers of ethylene glycol/propylene glycol, carboxymethylcellulose, dextran, polyvinyl alcohol, polyvinyl pyrrolidone, poly-

1, 3-dioxolane, poly-1,3,6-trioxane, ethylene/maleic anhydride copolymer, polyaminoacids (either homopolymers or random copolymers), and dextran or poly(n-vinyl pyrrolidone)polyethylene glycol, propylene glycol homopolymers, polypropylene oxide/ethylene oxide co-polymers, polyoxyethylated polyols (*e.g.*, glycerol), polyvinyl alcohol, and mixtures thereof. Polyethylene glycol propionaldehyde may have advantages in manufacturing due to its stability in water. The polymer may be of any molecular weight, and may be branched or unbranched. The number of polymers attached to the antibody may vary, and if more than one polymer are attached, they can be the same or different molecules. In general, the number and/or type of polymers used for derivatization can be determined based on considerations including, but not limited to, the particular properties or functions of the antibody to be improved, whether the antibody derivative will be used in a therapy under defined conditions, *etc.*

[200] In some embodiments, conjugates of an anti-CD47/anti-PD-L1 multiple antigen binding protein or antigen binding fragment thereof and nonproteinaceous moiety that may be selectively heated by exposure to radiation are provided. In some embodiments, the nonproteinaceous moiety is a carbon nanotube (Kam et al., *Proc. Natl. Acad. Sci. USA* 102: 11600-11605 (2005)). The radiation may be of any wavelength, and includes, but is not limited to, wavelengths that do not harm ordinary cells, but which heat the nonproteinaceous moiety to a temperature at which cells proximal to the antibody-nonproteinaceous moiety are killed.

[201] In some embodiments, an anti-CD47/anti-PD-L1 multiple antigen binding protein or antigen binding fragment thereof provided herein may be further modified to contain one or more biologically active proteins, polypeptides, or fragments thereof. "Bioactive" or "biologically active" as used herein means showing biological activity in the body to carry out a specific function. For example, it may mean the combination with a particular biomolecule such as protein, DNA, *etc.*, and then promotion or inhibition of the activity of such biomolecule. In some embodiments, the bioactive protein or fragments thereof have immunostimulatory/immunoregulatory, membrane transport, or enzymatic activities.

[202] In some embodiments, the bioactive protein or fragments thereof that can be fused with the anti-CD47/anti-PD-L1 multiple antigen binding protein or antigen binding fragment thereof described herein is a ligand, such as a lymphokine and/or a cellular factor which interacts with a specific cellular receptor. Lymphokines are low molecular weight proteins which are secreted by T cells when antigens or lectins stimulate T cell growth. Examples of lymphokines include, but are not limited to, interferon- α , interferon- γ , interleukin-1 (IL-1), interleukin-2 (IL-2), interleukin-3 (IL-3), tumor necrosis factor (TNF), a colony stimulating factor (*e.g.* CSF-1, G-CSF or GM-CSF), a chemotaxin, macrophage migration inhibitory factor (MIF), macrophage-activating factor (MAF), NK cell activating factor, T cell replacing factor, leukocyte-inhibitory factor (LIF), a lymphotoxin, osteoclast-activating factor (OAF), soluble immune response suppressor (SIRS), growth-stimulating factor, monocyte growth factor, *etc.* Cellular

factors which may be incorporated into the anti-CD47/anti-PD-L1 multiple antigen binding proteins or antigen binding fragments thereof include but are not limited to tumor necrosis factor α (TNF α), interferons (IFNs), and nerve growth factor (NGF), etc.

III. Pharmaceutical compositions

[203] Further provided by the present application are pharmaceutical compositions comprising the anti-CD47/anti-PD-L1 multiple antigen binding proteins or antigen binding fragments thereof, and optionally a pharmaceutically acceptable carrier. Pharmaceutical compositions can be prepared by mixing an anti-CD47/anti-PD-L1 multiple antigen binding protein or antigen binding fragment thereof having the desired degree of purity with optional pharmaceutically acceptable carriers, excipients or stabilizers (Remington's Pharmaceutical Sciences 16th edition, Osol, A. Ed. (1980)), in the form of lyophilized formulations or aqueous solutions.

[204] Preferably, the pharmaceutical composition is stable, in which the anti-CD47/anti-PD-L1 multiple antigen binding protein or antigen binding fragment thereof described here essentially retains its physical and chemical stability and integrity upon storage. Various analytical techniques for measuring protein stability are available in the art and are reviewed in *Peptide and Protein Drug Delivery*, 247-301, Vincent Lee Ed., Marcel Dekker, Inc., New York, N.Y., Pubs. (1991) and Jones, A. *Adv. Drug Delivery Rev.* 10: 29-90 (1993). Stability can be measured at a selected temperature for a selected time period. For rapid screening, the formulation may be kept at 40°C for 2 weeks to 1 month, at which time stability is measured. Where the formulation is to be stored at 2-8°C, generally the formulation should be stable at 30°C or 40°C for at least 1 month, and/or stable at 2-8°C for at least 2 years. Where the formulation is to be stored at 30°C, generally the formulation should be stable for at least 2 years at 30°C, and/or stable at 40°C for at least 6 months. For example, the extent of aggregation during storage can be used as an indicator of protein stability. In some embodiments, the stable formulation of an anti-CD47/anti-PD-L1 multiple antigen binding protein or antigen binding fragment thereof described herein may comprise less than about 10% (preferably less than about 5%) of the anti-CD47/anti-PD-L1 multiple antigen binding protein or antigen binding fragment thereof present as an aggregate in the formulation.

[205] Acceptable carriers, excipients, or stabilizers are nontoxic to recipients at the dosages and concentrations employed, and include buffers, antioxidants including ascorbic acid, methionine, Vitamin E, sodium metabisulfite; preservatives, isotonicifiers (e.g. sodium chloride), stabilizers, metal complexes (e.g. Zn-protein complexes); chelating agents such as EDTA and/or non-ionic surfactants.

[206] In order for the pharmaceutical compositions to be used for *in vivo* administration, they must be sterile. The pharmaceutical composition may be rendered sterile by filtration through sterile filtration membranes. The pharmaceutical compositions herein generally are placed into a container having a sterile

access port, for example, an intravenous solution bag or vial having a stopper pierceable by a hypodermic injection needle.

[207] The route of administration is in accordance with known and accepted methods, such as by single or multiple bolus or infusion over a long period of time in a suitable manner, *e.g.*, injection or infusion by subcutaneous, intravenous, intraperitoneal, intramuscular, intra-arterial, intralesional or intraarticular routes, topical administration, inhalation or by sustained release or extended-release means. In some embodiments, the pharmaceutical composition is administered locally, such as intratumorally.

[208] Sustained-release preparations may be prepared. Suitable examples of sustained-release preparations include semi-permeable matrices of solid hydrophobic polymers containing the antagonist, which matrices are in the form of shaped articles, *e.g.* films, or microcapsules.

[209] The pharmaceutical compositions herein may also contain more than one active compound as necessary for the particular indication being treated, preferably those with complementary activities that do not adversely affect each other. Alternatively, or in addition, the composition may comprise a cytotoxic agent, chemotherapeutic agent, cytokine, immunosuppressive agent, or growth inhibitory agent. Such molecules are suitably present in combination in amounts that are effective for the purpose intended.

[210] The active ingredients may also be entrapped in microcapsules prepared, for example, by coacervation techniques or by interfacial polymerization, for example, hydroxymethylcellulose or gelatin-microcapsules and poly-(methylmethacrylate) microcapsules, respectively, in colloidal drug delivery systems (for example, liposomes, albumin microspheres, microemulsions, nano-particles and nanocapsules) or in macroemulsions. Such techniques are disclosed in *Remington's Pharmaceutical Sciences* 18th edition.

[211] In some embodiments, the pharmaceutical composition is contained in a single-use vial, such as a single-use sealed vial. In some embodiments, the pharmaceutical composition is contained in a multi-use vial. In some embodiments, the pharmaceutical composition is contained in bulk in a container. In some embodiments, the pharmaceutical composition is cryopreserved.

IV. Methods of use

[212] The anti-CD47/anti-PD-L1 multiple antigen binding protein or antigen binding fragment thereof described herein, and the compositions (such as pharmaceutical compositions) thereof are useful for a variety of applications, such as in diagnosis, molecular assays, and therapy.

[213] One aspect of the invention provides a method of treating a CD47 and/or a PD-L1 related disease or a condition in a subject in need thereof, comprising administering to the subject an effective amount of a pharmaceutical composition comprising the anti-CD47/anti-PD-L1 multiple antigen binding protein or antigen binding fragment thereof described herein. In some embodiments, the CD47 and/or PD-

L1 related disease is cancer. In some embodiments, the PD-L1 related disease is a pathogenic infection, such as viral infection.

[214] The present invention contemplates, in part, protein constructs (such as an anti-CD47/anti-PD-L1 multiple antigen binding protein or antigen binding fragment thereof), nucleic acid molecules and/or vectors encoding thereof, host cells comprising nucleic acid molecules and/or vectors encoding thereof, that can be administered either alone or in any combination with another therapy, and in at least some aspects, together with a pharmaceutically acceptable carrier or excipient. In some embodiments, prior to administration of the anti-CD47/anti-PD-L1 multiple antigen binding protein or antigen binding fragment thereof, they may be combined with suitable pharmaceutical carriers and excipients that are well known in the art. The compositions prepared according to the disclosure can be used for the treatment or delaying of worsening of cancer.

[215] In some embodiments, there is provided a method of treating cancer comprising administering to the subject an effective amount of a pharmaceutical composition comprising an isolated anti-CD47/anti-PD-L1 multiple antigen binding protein or antigen binding fragment thereof. In some embodiments, the cancer is a solid tumor (such as colon cancer). In some embodiments, the cancer is a hematological cancer (such as a leukemia, a lymphoma, or a myeloma).

[216] In some embodiments, the pharmaceutical composition is administered systemically (such as intravenously). In some embodiments, the pharmaceutical composition is administered locally (such as intratumorally). In some embodiments, the method further comprises administering to the subject an additional cancer therapy (such as surgery, radiation, chemotherapy, immunotherapy, hormone therapy, or a combination thereof). In some embodiments, the subject is a human. In some embodiments, the method of treating cancer has one or more of the following biological activities: (1) killing cancer cells (including bystander killing); (2) inhibiting proliferation of cancer cells; (3) inducing an immune response in a tumor; (4) reducing tumor size; (5) alleviating one or more symptoms in an individual having cancer; (6) inhibiting tumor metastasis; (7) prolonging survival; (8) prolonging time to cancer progression; and (9) preventing, inhibiting, or reducing the likelihood of the recurrence of a cancer. In some embodiments, the method of killing cancer cells mediated by the pharmaceutical composition described herein can achieve a tumor cell death rate of at least about any of 40%, 50%, 60%, 70%, 80%, 90%, 95%, or more. In some embodiments, the method of killing cancer cells mediated by the pharmaceutical composition described herein can achieve a bystander tumor cell (uninfected by the oncolytic VV) death rate of at least about any of 10%, 20%, 30%, 40%, 50%, 60%, 70%, 80%, 90%, 95%, or more. In some embodiments, the method of reducing tumor size mediated by the pharmaceutical composition described herein can reduce at least about 10% (including, for example, at least about any of 20%, 30%, 40%, 60%, 70%, 80%, 90%, or 100%) of the tumor size. In some embodiments, the method of inhibiting tumor metastasis mediated by

the pharmaceutical composition described herein can inhibit at least about 10% (including, for example, at least about any of 20%, 30%, 40%, 60%, 70%, 80%, 90%, or 100%) of the metastasis. In some embodiments, the method of prolonging survival of an individual (such as a human) mediated by the pharmaceutical composition described herein can prolong the survival of the individual by at least any of 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 18, or 24 months. In some embodiments, the method of prolonging time to cancer progression mediated by the pharmaceutical composition described herein can prolong the time to cancer progression by at least any of 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, or 12 weeks.

[217] The methods described herein are suitable for treating a variety of cancers, including both solid cancer and liquid cancer. The methods are applicable to cancers of all stages, including early stage cancer, non-metastatic cancer, primary cancer, advanced cancer, locally advanced cancer, metastatic cancer, or cancer in remission. The methods described herein may be used as a first therapy, second therapy, third therapy, or combination therapy with other types of cancer therapies known in the art, such as chemotherapy, surgery, hormone therapy, radiation, gene therapy, immunotherapy (such as T-cell therapy), bone marrow transplantation, stem cell transplantation, targeted therapy, cryotherapy, ultrasound therapy, photodynamic therapy, radio-frequency ablation or the like, in an adjuvant setting or a neoadjuvant setting (i.e., the method may be carried out before the primary/definitive therapy). In some embodiments, the method is used to treat a subject who has previously been treated. In some embodiments, the cancer has been refractory to prior therapy. In some embodiments, the method is used to treat a subject who has not previously been treated.

[218] In some embodiments, the method is suitable for treating cancers with aberrant CD47 and/or PD-L1 expression, activity and/or signaling include, by way of non-limiting example, melanoma, prostate cancer, lung cancer, colon cancer, gastric cancer, ovarian cancer, breast cancer, glioblastoma, leukemia, lymphoma, and myeloma. The leukemia can, for example, be selected from the group consisting of an acute lymphocytic leukemia (ALL), acute myeloid leukemia (AML), chronic lymphocytic leukemia (CLL), chronic myelogenous leukemia (CML), Myeloproliferative disorder/neoplasm (MPDS), and myelodysplasia syndrome. The lymphoma can, for example, be selected from the group consisting of a Hodgkin's lymphoma, both indolent and aggressive non-Hodgkin's lymphoma, Burkitt's lymphoma, and follicular lymphoma (small cell and large cell). The myeloma can, for example, be selected from the group consisting of multiple myeloma (MM), giant cell myeloma, heavy-chain myeloma, and light chain or Bence-Jones myeloma.

[219] Thus, in some embodiments, there is provided a method of treating an immunotherapy-responsive solid tumor (such as carcinoma or adenocarcinoma, such as cancers with aberrant CD47 and/or PD-L1 expression, activity and/or signaling), comprising administering to the subject an effective amount of a pharmaceutical composition comprising an isolated anti-CD47/anti-PD-L1 multiple antigen

binding protein or antigen binding fragment thereof. In some embodiments, the cancer is a solid tumor (such as colon cancer). In some embodiments, the cancer is a hematological cancer (such as a leukemia, a lymphoma, or a myeloma).

[220] In some embodiments, the method is suitable for treating cancers with aberrant CD47 and/or PD-1 or PD-L1/PD-L2 expression, activity and/or signaling include, by way of non-limiting example, hematological cancer and/or solid tumors. Some cancers whose growth may be inhibited using the multiple antigen binding proteins or antigen binding fragments thereof of the invention include cancers typically responsive to immunotherapy. Non-limiting examples of other cancers for treatment include melanoma (*e.g.*, metastatic malignant melanoma), renal cancer (*e.g.* clear cell carcinoma), prostate cancer (*e.g.* hormone refractory prostate adenocarcinoma), breast cancer, colon cancer and lung cancer (*e.g.* non-small cell lung cancer). Additionally, the invention includes refractory or recurrent malignancies whose growth may be inhibited using the multiple antigen binding proteins or antigen binding fragments thereof of the invention. Examples of other cancers that may be treated using the multiple antigen binding proteins or antigen binding fragments thereof of the invention include bone cancer, pancreatic cancer, skin cancer, cancer of the head or neck, cutaneous or intraocular malignant melanoma, uterine cancer, ovarian cancer, rectal cancer, cancer of the anal region, stomach cancer, testicular cancer, uterine cancer, carcinoma of the fallopian tubes, carcinoma of the endometrium, carcinoma of the cervix, carcinoma of the vagina, carcinoma of the vulva, Hodgkin's Disease, non-Hodgkin's lymphoma, cancer of the esophagus, cancer of the small intestine, cancer of the endocrine system, cancer of the thyroid gland, cancer of the parathyroid gland, cancer of the adrenal gland, sarcoma of soft tissue, cancer of the urethra, cancer of the penis, chronic or acute leukemias including acute myeloid leukemia, chronic myeloid leukemia, acute lymphoblastic leukemia, chronic lymphocytic leukemia, solid tumors of childhood, lymphocytic lymphoma, cancer of the bladder, cancer of the kidney or ureter, carcinoma of the renal pelvis, neoplasm of the central nervous system (CNS), primary CNS lymphoma, tumor angiogenesis, spinal axis tumor, brain stem glioma, pituitary adenoma, Kaposi's sarcoma, epidermoid cancer, squamous cell cancer, T-cell lymphoma, environmentally induced cancers including those induced by asbestos, and combinations of said cancers. The present invention is also useful for treatment of metastatic cancers, especially metastatic cancers that express PD-L1 (Iwai *et al.* (2005) *Int. Immunol.* 17:133-144).

[221] In certain embodiments, disclosed anti-CD47/anti-PD-L1 multiple antigen binding proteins may be used as therapeutic agents. Such agents may be employed to diagnose, prognose, monitor, treat, alleviate, and/or prevent a disease or pathology associated with aberrant CD47 and/or PD-L1 expression, activity and/or signaling in a subject. A therapeutic regimen is carried out by identifying a subject, *e.g.*, a human patient suffering from (or at risk of developing) a disease or disorder associated with aberrant

CD47 and/or PD-L1 expression, activity and/or signaling, e.g., a cancer or other neoplastic disorder, using standard methods. A multiple antigen binding protein or antigen binding fragment thereof preparation, preferably one having high specificity and high affinity for its target antigen(s), is administered to the subject and will generally have an effect due to its binding with the target. Administration of the multiple antigen binding protein or antigen binding fragment thereof may abrogate or inhibit or interfere with the expression, activity and/or signaling function of the target (e.g., CD47 and/or PD-L1). Administration of the multiple antigen binding protein or antigen binding fragment thereof may abrogate or inhibit or interfere with the binding of the target (e.g., CD47 and/or PD-L1) with an endogenous ligand (e.g., SIRP α and/or PD-1) to which it naturally binds. For example, the multiple antigen binding protein or antigen binding fragment thereof binds to the target and modulates, blocks, inhibits, reduces, antagonizes, neutralizes, or otherwise interferes with CD47 and/or PD-L1 expression, activity and/or signaling.

[222] Dosages and desired drug concentrations of pharmaceutical compositions of the present application may vary depending on the particular use envisioned. The determination of the appropriate dosage or route of administration is well within the skill of an ordinary artisan. Animal experiments provide reliable guidance for the determination of effective doses for human therapy. Interspecies scaling of effective doses can be performed following the principles laid down by Mordenti, J. and Chappell, W. "The Use of Interspecies Scaling in Toxicokinetics," In *Toxicokinetics and New Drug Development*, Yacobi *et al.*, Eds, Pergamon Press, New York 1989, pp. 42-46.

[223] The pharmaceutical compositions of the present application, including but not limited to reconstituted and liquid formulations, are administered to a subject in need of treatment with the anti-CD47/anti-PD-L1 multiple antigen binding protein or antigen binding fragment thereof described herein, preferably a human, in accord with known methods, such as intravenous administration as a bolus or by continuous infusion over a period of time, by intramuscular, intraperitoneal, intracerebrospinal, subcutaneous, intravenous (i.v.), intra-articular, intrasynovial, intrathecal, oral, topical, or inhalation routes. A reconstituted formulation can be prepared by dissolving a lyophilized anti-CD47/anti-PD-L1 multiple antigen binding protein or antigen binding fragment thereof described herein in a diluent such that the protein is dispersed throughout. Exemplary pharmaceutically acceptable (safe and non-toxic for administration to a human) diluents suitable for use in the present application include, but are not limited to, sterile water, bacteriostatic water for injection (BWFI), a pH buffered solution (e.g. phosphate-buffered saline), sterile saline solution, Ringer's solution or dextrose solution, or aqueous solutions of salts and/or buffers.

[224] In some embodiments, the pharmaceutical compositions are administered to the subject by subcutaneous (*i.e.* beneath the skin) administration. For such purposes, the pharmaceutical compositions may be injected using a syringe. However, other devices for administration of the pharmaceutical

compositions are available such as injection devices; injector pens; auto-injector devices, needleless devices; and subcutaneous patch delivery systems.

[225] In some embodiments, the pharmaceutical compositions are administered to the subject intravenously. In some embodiments, the pharmaceutical composition is administered to a subject by infusion, such as intravenous infusion. Infusion techniques for immunotherapy are known in the art (see, *e.g.*, Rosenberg *et al.*, New Eng. J. of Med. 319: 1676 (1988)).

[226] Methods for the screening for multiple antigen binding proteins or antigen binding fragments thereof that possess the desired specificity include, but are not limited to, enzyme linked immunosorbent assay (ELISA) and other immunologically mediated techniques known within the art.

[227] In other embodiments, multiple antigen binding proteins or antigen binding fragments thereof directed against CD47 and/or PD-L1 may be used in methods known within the art relating to the localization and/or quantitation of CD47 and/or PD-L1 (*e.g.*, for use in measuring levels of CD47, CD47 and SIRP α within appropriate physiological samples, and/or measuring levels of PD-L1 for use in diagnostic methods, for use in imaging the protein, and the like).

[228] In other embodiments, an anti-CD47/anti-PD-L1 multiple antigen binding protein or antigen binding fragment thereof can be used to isolate a CD47 and/or PD-L1 polypeptide, by standard techniques, such as immunoaffinity, chromatography or immunoprecipitation. Multiple antigen binding proteins or antigen binding fragments thereof directed against the CD47 protein (or a fragment thereof) and/or PD-L1 protein (or fragment thereof) can be used diagnostically to monitor protein levels in tissue as part of a clinical testing procedure, *e.g.*, to, for example, determine the efficacy of a given treatment regimen.

[229] Detection can be facilitated by coupling (*i.e.*, physically linking) the multiple antigen binding protein or antigen binding fragment thereof to a detectable substance. Examples of detectable substances include various enzymes, prosthetic groups, fluorescent materials, luminescent materials, bioluminescent materials, and radioactive materials. Examples of suitable enzymes include horseradish peroxidase, alkaline phosphatase, β -galactosidase, or acetylcholinesterase; examples of suitable prosthetic group complexes include streptavidin/biotin and avidin/biotin; examples of suitable fluorescent materials include umbelliferone, fluorescein, fluorescein isothiocyanate, rhodamine, dichlorotriazinylamine fluorescein, dansyl chloride or phycoerythrin; an example of a luminescent material includes luminol; examples of bioluminescent materials include luciferase, luciferin, and aequorin, and examples of suitable radioactive material include ^{125}I , ^{131}I , ^{35}S or ^3H .

[230] In some embodiments, the multiple antigen binding protein or antigen binding fragment thereof contains a detectable label. The term “labeled”, regarding the probe or multiple antigen binding protein or antigen binding fragment thereof, is intended to encompass direct labeling of the probe or multiple antigen binding protein or antigen binding fragment thereof by coupling (*i.e.*, physically linking) a

detectable substance to the probe or multiple antigen binding protein or antigen binding fragment thereof, and indirect labeling of the probe or multiple antigen binding protein or antigen binding fragment thereof by reactivity with another reagent that is directly labeled. Examples of indirect labeling include detection of a primary multiple antigen binding protein or antigen binding fragment thereof using a fluorescently-labeled secondary antibody and end-labeling of a DNA probe with biotin such that it can be detected with fluorescently-labeled streptavidin.

[231] The term “biological sample” is intended to include tissues, cells and biological fluids isolated from a subject, as well as tissues, cells and fluids present within a subject. Included within the usage of the term “biological sample,” therefore, is blood and a fraction or component of blood including blood serum, blood plasma, or lymph. That is, the detection method can be used to detect an analyte mRNA, protein, or genomic DNA in a biological sample *in vitro* as well as *in vivo*. For example, *in vitro* techniques for detection of an analyte mRNA include Northern hybridizations and *in situ* hybridizations. *In vitro* techniques for detection of an analyte protein include enzyme linked immunosorbent assays (ELISAs), Western blots, immunoprecipitations, and immunofluorescence. *In vitro* techniques for detection of an analyte genomic DNA include Southern hybridizations. Procedures for conducting immunoassays are described, for example in “ELISA: Theory and Practice: Methods in Molecular Biology”, Vol. 42, J. R. Crowther (Ed.) Human Press, Totowa, NJ, 1995; “Immunoassay”, E. Diamandis and T. Christopoulos, Academic Press, Inc., San Diego, CA, 1996; and “Practice and Theory of Enzyme Immunoassays”, P. Tijssen, Elsevier Science Publishers, Amsterdam, 1985. Furthermore, *in vivo techniques* for detection of an analyte protein include introducing into a subject a labeled anti-analyte protein antibody. For example, the antibody can be labeled with a radioactive marker whose presence and location in a subject can be detected by standard imaging techniques.

V. Methods of preparation

[232] The anti-CD47/anti-PD-L1 multiple antigen binding protein or antigen binding fragment thereof described herein may be prepared using any methods known in the art or as described herein. Also see Example 1.

[233] Methods for making multiple antigen binding proteins, e.g., bispecific antibodies, are known in the art.

[234] Traditionally, the recombinant production of bispecific antibodies is based on the co-expression of two immunoglobulin heavy-chain/light-chain pairs, where the two heavy chains have different specificities (Milstein and Cuello, *Nature*, 305:537-539 (1983)). Because of the random assortment of immunoglobulin heavy and light chains, these hybridomas (quadromas) produce a potential mixture of ten different antibody molecules, of which only one has the correct bispecific structure. The purification of the correct molecule is usually accomplished by affinity chromatography steps. Similar procedures are

disclosed in WO 93/08829, published 13 May 1993, and in Traunecker *et al.*, *EMBO J.*, 10:3655- 3659 (1991).

[235] Antibody variable domains with the desired binding specificities (antibody-antigen combining sites) can be fused to immunoglobulin constant domain sequences. The fusion preferably is with an immunoglobulin heavy-chain constant domain, comprising at least part of the hinge, CH2, and CH3 regions. It is preferred to have the first heavy-chain constant region (CH1) containing the site necessary for light-chain binding present in at least one of the fusions. DNAs encoding the immunoglobulin heavy-chain fusions and, if desired, the immunoglobulin light chain, are inserted into separate expression vectors, and are co-transfected into a suitable host organism. For further details of generating bispecific antibodies see, for example, Suresh *et al.*, *Methods in Enzymology*, 121:210 (1986).

[236] According to another approach described in WO 96/27011, the interface between a pair of antibody molecules can be engineered to maximize the percentage of heterodimers which are recovered from recombinant cell culture. The preferred interface comprises at least a part of the CH3 region of an antibody constant domain. In this method, one or more small amino acid side chains from the interface of the first antibody molecule are replaced with larger side chains (e.g. tyrosine or tryptophan). Compensatory “cavities” of identical or similar size to the large side chain(s) are created on the interface of the second antibody molecule by replacing large amino acid side chains with smaller ones (e.g. alanine or threonine). This provides a mechanism for increasing the yield of the heterodimer over other unwanted end-products such as homodimers.

[237] Bispecific antibodies can be prepared as full-length antibodies or antibody fragments (e.g. F(ab')₂ bispecific antibodies). Techniques for generating bispecific antibodies from antibody fragments have been described in the literature. For example, bispecific antibodies can be prepared using chemical linkage. Brennan *et al.*, *Science* 229:81 (1985) describe a procedure in which intact antibodies are proteolytically cleaved to generate F(ab')₂ fragments. These fragments are reduced in the presence of the dithiol complexing agent sodium arsenite to stabilize vicinal dithiols and prevent intermolecular disulfide formation. The Fab' fragments generated are then converted to thionitrobenzoate (TNB) derivatives. One of the Fab'-TNB derivatives is then reconverted to the Fab'-thiol by reduction with mercaptoethylamine and is mixed with an equimolar amount of the other Fab'-TNB derivative to form the bispecific antibody. The bispecific antibodies produced can be used as agents for the selective immobilization of enzymes.

[238] Additionally, Fab' fragments can be directly recovered from *E. coli* and chemically coupled to form bispecific antibodies. Shalaby *et al.*, *J. Exp. Med.* 175:217-225 (1992) describe the production of a fully humanized bispecific antibody F(ab')₂ molecule. Each Fab' fragment was separately secreted from *E. coli* and subjected to directed chemical coupling *in vitro* to form the bispecific antibody. The bispecific antibody thus formed was able to bind to cells overexpressing the ErbB2 receptor and normal human T

cells, as well as trigger the lytic activity of human cytotoxic lymphocytes against human breast tumor targets.

[239] Various techniques for making and isolating bispecific antibody fragments directly from recombinant cell culture have also been described. For example, bispecific antibodies have been produced using leucine zippers. Kostelny *et al.*, *J. Immunol.* 148(5): 1547-1553 (1992). The leucine zipper peptides from the Fos and Jun proteins were linked to the Fab' portions of two different antibodies by gene fusion. The antibody homodimers were reduced at the hinge region to form monomers and then re-oxidized to form the antibody heterodimers. This method can also be utilized to make antibody homodimers. The "diabody" technology described by Hollinger *et al.*, *Proc. Natl. Acad. Sci. USA* 90:6444-6448 (1993) has provided an alternative mechanism for making bispecific antibody fragments. The fragments comprise a heavy-chain variable domain connected to a light-chain variable domain by a linker which is too short to allow pairing between the two domains on the same chain. Accordingly, the V_H and V_L domains of one fragment are forced to pair with the complementary V_L and V_H domains of another fragment, thereby forming two antigen-binding sites. Another strategy for making bispecific antibody fragments with single-chain Fv (sFv) dimers has also been reported. Gruber *et al.*, *J. Immunol.* 152:5368 (1994).

[240] For recombinant production of the bispecific antibodies, the nucleic acids encoding the bispecific antibodies are isolated and inserted into a replicable vector for further cloning (amplification of the DNA) or for expression. DNA encoding the bispecific antibody is readily isolated and sequenced using conventional procedures (*e.g.*, by using oligonucleotide probes that are capable of binding specifically to genes encoding the heavy and light chains of the antibody). Many vectors are available. The choice of vector depends in part on the host cell to be used. Generally, preferred host cells are of either prokaryotic or eukaryotic (generally mammalian) origin.

VI. Kits and articles of manufacture

[241] Further provided are kits, unit dosages, and articles of manufacture comprising any of the anti-CD47/anti-PD-L1 multiple antigen binding proteins described herein. In some embodiments, a kit is provided comprising any one of the pharmaceutical compositions described herein and preferably provides instructions for its use.

[242] The kits of the present application are in suitable packaging. Suitable packaging includes, but is not limited to, vials, bottles, jars, flexible packaging (*e.g.*, sealed Mylar or plastic bags), and the like. Kits may optionally provide additional components such as buffers and interpretative information. The present application thus also provides articles of manufacture, which include vials (such as sealed vials), bottles, jars, flexible packaging, and the like.

[243] The article of manufacture can comprise a container and a label or package insert on or associated with the container. Suitable containers include, for example, bottles, vials, syringes etc. The

containers may be formed from a variety of materials such as glass or plastic. Generally, the container holds a composition which is effective for treating a disease or disorder described herein and may have a sterile access port (for example the container may be an intravenous solution bag or a vial having a stopper pierceable by a hypodermic injection needle). The label or package insert indicates that the composition is used for treating the particular condition in an individual. The label or package insert will further comprise instructions for administering the composition to the individual. The label may indicate directions for reconstitution and/or use. The container holding the pharmaceutical composition may be a multi-use vial, which allows for repeat administrations (e.g., from 2-6 administrations) of the reconstituted formulation. Package insert refers to instructions customarily included in commercial packages of therapeutic products that contain information about the indications, usage, dosage, administration, contraindications and/or warnings concerning the use of such therapeutic products. Additionally, the article of manufacture may further comprise a second container comprising a pharmaceutically-acceptable buffer, such as bacteriostatic water for injection (BWFI), phosphate-buffered saline, Ringer's solution and dextrose solution. It may further include other materials desirable from a commercial and user standpoint, including other buffers, diluents, filters, needles, and syringes.

[244] The kits or article of manufacture may include multiple unit doses of the pharmaceutical composition and instructions for use, packages in quantities sufficient for storage and use in pharmacies, for example, hospital pharmacies and compounding pharmacies.

EMBODIMENTS

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

[246] Embodiment 1 is an isolated anti-CD47/anti-PD-L1 multiple antigen binding protein or antigen binding fragment thereof comprising:

- (a) a first antigen binding portion comprising a heavy chain variable domain (V_H) and a light chain variable domain (V_L), wherein the V_H and V_L together form an antigen-binding site that specifically binds CD47, and wherein the V_H comprises a heavy chain complementarity determining region 1 (HCDR1), HCDR2, and HCDR3 comprising the amino acid sequences of SEQ ID NO:19, SEQ ID NO:20, and SEQ ID NO:21, respectively, and the V_L comprises a light chain complementarity determining region 1 (LCDR1), LCDR2, and LCDR3 comprising the amino acid sequences of SEQ ID NO:22, SEQ ID NO:23, and SEQ ID NO:24, respectively; and
- (b) a second antigen binding portion comprising a single-domain antibody that specifically binds PD-L1, wherein the single-domain antibody comprises a complementarity determining region 1 (CDR1), CDR2, and CDR3 comprising the amino acid sequences of:
 - (i) SEQ ID NO:25, SEQ ID NO:26, and SEQ ID NO:27, respectively,

- (ii) SEQ ID NO:28, SEQ ID NO:29, and SEQ ID NO:30, respectively, or
- (iii) SEQ ID NO:31, SEQ ID NO:32, and SEQ ID NO:33, respectively;

wherein the first antigen binding portion and the second antigen binding portion are fused to each other.

[247] Embodiment 2 is the isolated anti-CD47/anti-PD-L1 multiple antigen binding protein or antigen binding fragment thereof of embodiment 1, wherein the first antigen binding portion is a full-length antibody comprising two heavy chains and two light chains.

[248] Embodiment 3 is the isolated anti-CD47/anti-PD-L1 multiple antigen binding protein or antigen binding fragment thereof of embodiment 1 or 2, wherein the first antigen binding portion is an antibody fragment comprising a heavy chain comprising the V_H and a light chain comprising the V_L .

[249] Embodiment 4 is the isolated anti-CD47/anti-PD-L1 multiple antigen binding protein or antigen binding fragment thereof of any one of embodiments 1-3, wherein the second antigen binding portion comprises a single polypeptide chain.

[250] Embodiment 5 is the isolated anti-CD47/anti-PD-L1 multiple antigen binding protein or antigen binding fragment thereof of embodiment 4, wherein the carboxy (C)-terminus of the second antigen binding portion is fused to the amino (N)-terminus of at least one heavy chain of the first antigen binding portion.

[251] Embodiment 6 is the isolated anti-CD47/anti-PD-L1 multiple antigen binding protein or antigen binding fragment thereof of embodiment 4, wherein the carboxy (C)-terminus of the second antigen binding portion is fused to the amino (N)-terminus of at least one light chain of the first antigen binding portion.

[252] Embodiment 7 is the isolated anti-CD47/anti-PD-L1 multiple antigen binding protein or antigen binding fragment thereof of embodiment 4, wherein the amino (N)-terminus of the second antigen binding portion is fused to the carboxy (C)-terminus of at least one heavy chain of the first antigen binding portion.

[253] Embodiment 8 is the isolated anti-CD47/anti-PD-L1 multiple antigen binding protein or antigen binding fragment thereof of embodiment 4, wherein the amino (N)-terminus of the second antigen binding portion is fused to the carboxy (C)-terminus of at least one light chain of the first antigen binding portion.

[254] Embodiment 9 is the isolated anti-CD47/anti-PD-L1 multiple antigen binding protein or antigen binding fragment thereof of any one of embodiments 1-8, wherein the heavy chain of the first antigen binding portion comprises an amino acid sequence at least 95% identical to SEQ ID NO:4, and the light chain of the first antigen binding portion comprises an amino acid sequence at least 95% identical to SEQ ID NO:6.

[255] Embodiment 10 is the isolated anti-CD47/anti-PD-L1 multiple antigen binding protein or antigen binding fragment thereof of embodiment 9, wherein the heavy chain of the first antigen binding portion comprises the amino acid sequence of SEQ ID NO:4, and the light chain of the first antigen binding portion comprises the amino acid sequence of SEQ ID NO:6.

[256] Embodiment 11 is the isolated anti-CD47/anti-PD-L1 multiple antigen binding protein or antigen binding fragment thereof of any one of embodiments 1-10, wherein the second antigen binding portion comprises an amino acid sequence at least 95% identical to an amino acid sequence selected from SEQ ID NO:14, SEQ ID NO:16, or SEQ ID NO:18.

[257] Embodiment 12 is the isolated anti-CD47/anti-PD-L1 multiple antigen binding protein or antigen binding fragment thereof of embodiment 11, wherein the second antigen binding portion comprises the amino acid sequence selected from SEQ ID NO:14, SEQ ID NO:16, or SEQ ID NO:18.

[258] Embodiment 13 is the isolated anti-CD47/anti-PD-L1 multiple antigen binding protein or antigen binding fragment thereof of any one of embodiments 1-12, wherein the first antigen binding portion comprises a human, humanized, or chimeric antibody or antigen binding fragment thereof.

[259] Embodiment 14 is the isolated anti-CD47/anti-PD-L1 multiple antigen binding protein or antigen binding fragment thereof of any one of embodiments 1-13, wherein the second antigen binding portion comprising a single-domain antibody that specifically binds PD-L1 is camelid, chimeric, human, partially humanized, or fully humanized.

[260] Embodiment 15 is the isolated anti-CD47/anti-PD-L1 multiple antigen binding protein or antigen binding fragment thereof of any one of embodiments 1-14, wherein the first antigen binding portion comprises an Fc region.

[261] Embodiment 16 is the isolated anti-CD47/anti-PD-L1 multiple antigen binding protein or antigen binding fragment thereof of embodiment 15, wherein the second antigen binding portion is fused to the N-terminus of the Fc region.

[262] Embodiment 17 is the isolated anti-CD47/anti-PD-L1 multiple antigen binding protein or antigen binding fragment thereof of embodiment 15 or 16, wherein the Fc region is an IgG1 Fc.

[263] Embodiment 18 is the isolated anti-CD47/anti-PD-L1 multiple antigen binding protein or antigen binding fragment thereof of embodiment 15 or 16, wherein the Fc region is an IgG4 Fc having an S228P mutation and/or an L235E mutation.

[264] Embodiment 19 is the isolated anti-CD47/anti-PD-L1 multiple antigen binding protein or antigen binding fragment thereof of any one of embodiments 1-18, wherein the first antigen binding portion and the second antigen binding portion are fused to each other via a peptide bond or a peptide linker.

- [265] Embodiment 20 is the isolated anti-CD47/anti-PD-L1 multiple antigen binding protein or antigen binding fragment thereof of embodiment 19, wherein the peptide linker comprises an amino acid sequence selected from SEQ ID NO:8, SEQ ID NO:10, or SEQ ID NO:69.
- [266] Embodiment 21 is an isolated nucleic acid encoding the isolated anti-CD47/anti-PD-L1 multiple antigen binding protein or antigen binding fragment thereof of any one of embodiments 1-20.
- [267] Embodiment 22 is an isolated vector comprising the isolated nucleic acid of embodiment 21.
- [268] Embodiment 23 is a host cell comprising the isolated vector of embodiment 22.
- [269] Embodiment 24 is a pharmaceutical composition comprising the isolated anti-CD47/anti-PD-L1 multiple antigen binding protein or antigen binding fragment thereof of any one of embodiments 1-20, and a pharmaceutical acceptable carrier.
- [270] Embodiment 25 is a method of treating a subject having or at risk of having a CD47 and/or PD-L1-related disease, the method comprising administering to the subject an effective amount of the pharmaceutical composition of embodiment 24.
- [271] Embodiment 26 is the method of embodiment 25, wherein the CD47 and/or PD-L1 related disease is cancer.
- [272] Embodiment 27 is the method of embodiment 26, wherein the cancer is a solid tumor.
- [273] Embodiment 28 is the method of embodiment 26 or 27, wherein the cancer is a colon cancer.
- [274] Embodiment 29 is the method of embodiment 26, wherein the cancer is a hematological cancer.
- [275] Embodiment 30 is the method of embodiment 29, wherein the hematological cancer is leukemia, lymphoma, or myeloma.
- [276] Embodiment 31 is the method of embodiment 30, wherein the leukemia is selected from the group consisting of acute lymphocytic leukemia (ALL), acute myeloid leukemia (AML), chronic lymphocytic leukemia (CLL), chronic myelogenous leukemia (CML), myeloproliferative disorder/neoplasm (MPDS), and myelodysplasia syndrome.
- [277] Embodiment 32 is the method of embodiment 30, wherein the lymphoma is selected from the group consisting of a Hodgkin's lymphoma, both indolent and aggressive non-Hodgkin's lymphoma, Burkitt's lymphoma, and follicular lymphoma (small cell and large cell).
- [278] Embodiment 33 is the method of embodiment 30, wherein the myeloma is selected from the group consisting of multiple myeloma (MM), giant cell myeloma, heavy-chain myeloma, and light chain or Bence-Jones myeloma.
- [279] Embodiment 34 is the method of any one of embodiments 25-33, further comprising administering to the individual an additional cancer therapy.
- [280] Embodiment 35 is the method of embodiment 34, wherein the additional cancer therapy is surgery, radiation, chemotherapy, immunotherapy, hormone therapy, or a combination thereof.

[281] Embodiment 36 is the method of embodiment 25, wherein the PD-L1 related disease is a pathogenic infection.

[282] Embodiment 37 is the method of any one of embodiments 25-36, wherein the pharmaceutical composition is administered systemically or locally.

[283] Embodiment 38 is the method of embodiment 37, wherein the pharmaceutical composition is administered intravenously.

[284] Embodiment 39 is the method of embodiment 37, wherein the pharmaceutical composition is administered intratumorally.

[285] Embodiment 40 is the method of any one of embodiments 25-39, wherein the subject is a human.

EXAMPLES

[286] The examples below are intended to be purely exemplary of the invention and should therefore not be considered to limit the invention in any way. The following examples and detailed description are offered by way of illustration and not by way of limitation.

Example 1: Construction and expression of anti-CD47/anti-PD-L1 SMAB

[287] Based on the single domain antibody fused to monoclonal antibody (SMAB) structure described in WO2018014855A1, a series of anti-CD47/anti-PD-L1 multiple antigen binding proteins was designed using anti-CD47 monoclonal antibody (mAb) and anti-PD-L1 single domain antibody (sdAb). For SMAB construction, three anti-PD-L1 sdAbs were fused to anti-CD47 mAb, respectively. According to the SMAB design, anti-PD-L1 sdAb was located at N- or C-terminus of heavy chain or light chain of anti-CD47 mAb with three kinds of linker (E-linker: EPKSSDKTHTSPPSP (SEQ ID NO:8), E4-linker: ESKYGPPSPSP (SEQ ID NO:69), or G-linker: (G₄S)₃ (SEQ ID NO:10)) for fusion. Each SMAB construct is composed of one fusion polypeptide chain and one native polypeptide chain, and the DNA sequence expressing each polypeptide chain was inserted into a pTT5 vector between EcoRI and HindIII restriction sites. Each plasmid also includes a secretion signal sequence (SEQ ID NO:1 (DNA); SEQ ID NO:2 (amino acid)) for proteins secreted into the growth medium. An anti-PD-L1 sdAb fused to the N-terminus of IgG4 Fc portion (SEQ ID NO:11 (DNA); SEQ ID NO:12 (amino acid)), with mutations S228P and L235E, was used as a control for the *in vitro* bioassay.

[288] CHO-3E7 cells, transfected with expression plasmids, were cultured at 37°C and 100 rpm for 6 days. The supernatant fraction was collected by centrifugation and the SMAB protein was purified through Protein A column.

[289] Table 3: Plasmids expressing anti-CD47/anti-PD-L1 SMAB proteins

Protein	Component	plasmids	SEQ ID NO:
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CD47	H0	pTT5-CD47HC	4
	L0	pTT5-CD47LC	6
PDL1a-E-HC	H1	pTT5-CD47HC-E-PDL1a	36
	L0	pTT5-CD47LC	6
PDL1b-E-HC	H2	pTT5-CD47HC-E-PDL1b	38
	L0	pTT5-CD47LC	6
PDL1c-E-HC	H3	pTT5-CD47HC-E-PDL1c	40
	L0	pTT5-CD47LC	6
PDL1a-E-HN	H4	pTT5-PDL1a-E-CD47HC	42
	L0	pTT5-CD47LC	6
PDL1b-E-HN	H5	pTT5-PDL1b-E-CD47HC	44
	L0	pTT5-CD47LC	6
PDL1c-E-HN	H6	pTT5-PDL1c-E-CD47HC	46
	L0	pTT5-CD47LC	6
PDL1a-E-LC	L1	pTT5-CD47LC-E-PDL1a	52
	H0	pTT5-CD47HC	4
PDL1b-E-LC	L2	pTT5-CD47LC-E-PDL1b	54
	H0	pTT5-CD47HC	4
PDL1c-E-LC	L3	pTT5-CD47LC-E-PDL1c	56
	H0	pTT5-CD47HC	4
PDL1a-E-LN	L4	pTT5-PDL1a-E-CD47LC	58
	H0	pTT5-CD47HC	4
PDL1b-E-LN	L5	pTT5-PDL1b-E-CD47LC	60
	H0	pTT5-CD47HC	4
PDL1c-E-LN	L6	pTT5-PDL1c-E-CD47LC	62
	H0	pTT5-CD47HC	4
PDL1a-G15-HC	H7	pTT5-CD47HC-G15-PDL1a	48
	L0	pTT5-CD47LC	6
PDL1a-G15-HN	H8	pTT5-PDL1a-G15-CD47HC	50
	L0	pTT5-CD47LC	6
PDL1a-G15-LC	L7	pTT5-CD47LC-G15-PDL1a	64
	H0	pTT5-CD47HC	4
PDL1a-G15-LN	L8	pTT5-PDL1a-G15-CD47LC	66
	H0	pTT5-CD47HC	4
PDL1a-E4-LC	L9	pTT5-CD47LC-E4-PDL1a	68
	H0	pTT5-CD47HC	4

[290] Table 4: DNA and amino acid (a.a.) sequences of anti-CD47 monoclonal antibody moiety

	Sequence	SEQ ID NO:
Anti-CD47 heavy chain DNA	GAGGTGCAGCTGGTGCAGTCCGGAGCTGAGGTGAAGAAGCCAGGATCCAGCGTGAA GGTGAGCTGCAAGGCTAGCGGCTACTCTTTCACCCACCATTGGATCCACTGGGTGAG GCAGGCTCCTGGACAGGGACTGGAGTGGATGGGCATGATCGACGCTTCCGATAGCG AGACAAGACTGTCTCAGAAGTTTAAGGACCGGTGACCATCACAGCCGATAAGTCTA CCTCCACAGCTTACATGGAGCTGTCTTCCCTGAGATCCGAGGACACCGCCGTGTACTA TTGTGCTAGGCTGGGCCGGTACTATTTCGATTATTGGGGCCAGGGCACCACAGTGACA GTGAGCTCTGCCAGCACAAAGGGCCCTTCCGTGTTCCCACTGGCTCCCTGCTCCAGAA	3

	GCACATCTGAGTCCACCGCCGCTCTGGGCTGTCTGGTGAAGGACTACTTCCCTGAGCC AGTGACCGTGTCTGGAACAGCGGCGCCCTGACATCTGGCGTGCACACCTTTCCAGCT GTGCTGCAGTCCAGCGGCTGTACTCCCTGTCTTCCGTGGTGACAGTGCCAGCTCTTC CCTGGGCACCAAGACATATACCTGCAACGTGGACCATAAGCCTTCCAATACCAAGGTG GATAAGAGGGTGGAGAGCAAGTACGGACCACCTTGCCACCACATGTCCAGCTCCTGAGT TTGAGGGAGGACCATCCGTGTTCTGTTCCTCCAAAGCCTAAGGACACCCTGATGAT CAGCCGGACACCTGAGGTGACCTGCGTGGTGGTGGACGTGTCTCAGGAGGATCCAGAG GTGCAGTTCAACTGGTACGTGGATGGCGTGGAGGTGCACAATGCTAAGACCAAGCCAA GAGAGGAGCAGTTAATTCCACATACCGCGTGGTGGAGCGTGTGACCGTGTCTGCATCA GGATTGGCTGAACGGCAAGGAGTATAAGTGCAAGGTGTCCAATAAGGGCCTGCCAGC TCTATCGAGAAGACAATCAGCAAGGCTAAGGGACAGCCTAGGGAGCCACAGGTGTACA CCCTGCCCCCTTCTCAGGAGGAGATGACAAAGAACCAGGTGTCCCTGACCTGTCTGGTG AAGGGCTTCTATCCAAGCGACATCGCTGTGGAGTGGGAGTCTAATGGCCAGCCCGAGA ACAATTACAAGACCACACCACCGTGTCTGGACTCTGATGGCTCCTTCTTCTGTATTCTA GGCTGACAGTGGATAAGTCCCGGTGGCAGGAGGGCAACGTGTTAGCTGCTCTGTGAT GCACGAGGCCCTGCACAATCATTATACCCAGAAGTCCCTGAGCCTGTCTCTGGGCAAG					
Anti-CD47 heavy chain a.a.	EVQLVQSGAEVKKPGSSVKVSKASGYSTFTHWHWVRQAPGQGLEWMGMIDASDSETRL SQKFKDRVTITADKSTSTAYMELSSLRSEDAVYYCARLGRYYFDYWGQGTITVTVSSASTK GPSVFPLAPCSRSTSESTAALGCLVKDYFPEPTVSWNSGALTSGVHTFPAVLQSSGLYSLSS VVTVPSSSLGKTYTCNVDHKPSNTKVDKRVESKYGPPCPPCPAPEFEGGPSVFLFPPKPKDT LMISRTPEVTCVVDVVSQEDPEVQFNWYVDGVEVHNAKTKPREEQFNSTYRVVSVLTVLHQ DWLNGKEYKCKVSNKGLPSSIEKTIKAKGPQPREPQVYTLPPSQEEMTKNQVSLTCLVKGFY PSDIAVEWESNGQPENNYKTPPVLDSDGSFFLYSRLTVDKSRWQEGNVFSCSVMEALHNH YTQKSLSLSLGK					4
Anti-CD47 light chain DNA	GAGATCGTGCTGACCCAGTCTCCAGCCACACTGTCTCTGTCCCAGGAGAGAGGGCCAC CCTGAGCTGCCGGGCTTCTGAGAACGTGGGCACATACATCTCCTGGTATCAGCAGAAGC CAGGACAGGCTCCTAGGCTGCTGATCTACGGCGCTAGCAATAGATATACCGGCATCCCT GCTCGCTTCAGCGGATCTGGATCCGGCACAGACTTTACCCTGACAATCTCCAGCCTGGA GCCAGAGGATTCGCCGTGTAATTTGTGGCGAGTCTACGGCCACCTGTATACCTTTGG CGGCGGCACAAAGGTGGAGATCAAGCGAACGGTGGCTGCACCATCTGTCTTCATCTTCC CGCCATCTGATGAGCAGTTGAAATCTGGAAGTGCCTCTGTTGTGTGCTGCTGAATAACT TCTATCCCAGAGAGGGCAAAGTACAGTGGAAAGTGGATAACGCCCTCCAATCGGGTAAC TCCCAGGAGAGTGTACAGAGCAGGACAGCAAGGACAGCACCTACAGCCTCAGCAGCA CCCTGACGCTGAGCAAAGCAGACTACGAGAAACACAAAGTCTACGCCTGCGAAGTCACC CATCAGGGCCTGAGCTCGCCCGTCACAAAGAGCTTCAACAGGGGAGAGTGT					5
Anti-CD47 light chain a.a.	EIVLTQSPATLSLSPGERATLSCRASENVGTYSWYQQKPGQAPRLLIYGASNRYTGIPARFSG SGSGTDFLTISSELPEDFAVYYCGESYGHLTYFGGGTKVEIKRTVAAPSVFIFPPSDEQLKSG TASVVCLNNFYPREAKVQWKVDNALQSGNSQESVTEQDSKDSYSTLSSTLTLKADYEKHK VYACEVTHQGLSPVTKSFNRGEC					6
	Sequence	SEQ ID NO:	Sequence	SEQ ID NO:	Sequence	SEQ ID NO:
Anti-CD47 heavy chain CDR protein	GYSFTHHWIH	19	MIDASDSETRL SQKFKD	20	LGRYYFDY	21
Anti-CD47 light chain CDR a.a.	RASENVGTIYS	22	GASNRYT	23	GESYGHLTY	24

[291] Table 5: DNA and amino acid (a.a.) sequences of anti-PD-L1 single-domain antibody moiety

	Sequence	SEQ ID NO:
PDL1a sdAb DNA	GAGGTGCAGCTGGTGGAAATCCGGCGGAGGCCTGGTCCAGCCTGGCGGCTCTCTGCGGCTGTC CTGCGCCGCTTCTGGCAGAACCTTCGTGACCTACGGCATGGGCTGGTTCGGGCAGGCTCCTGG CAAGGGCAGAGAGTTCTGTGTCGCCATCTCTGGTCCGGCTCCATGACCTCTTACGGCGACTC TGTGAAGGGCAGATTACCATCAGCGCGGATAACGCCAAGAACACACTGTACTCTGCAGATGA ACTCCCTGCGGCCTGAGGACACCGCCGTGTACTACTGCGCCGCTGCCCTGGGCGCTGTCGTGT ACACCACCAGAGAACCCTATACCTACTGGGGACAGGGCACCCCTGGTGACCGTGTCTCTCT	13

PDL1a sdAb a.a.	EVQLVESGGGLVQPGGSLRLSCAASGRFTVITYGMGWFRQAPGKGRFVSAISWSGSMTSYGD SVKGRFTISRDNAKNTLYLQMNSLRPEDTAVYYCAAALGAVVYTTREPYTYWGQGLVTVSS					14
PDL1b sdAb DNA	GAGGTGCAGCTGGTGAATCCGGCGGAGGCCTGGTCCAGCCTGGCGGCTCTCTGCGGCTGTCC TGCGCCGCTTCTGGCAGAACCTTCGTGACCTACGGCATGGGCTGGTTCCGGCAGGCTCCTGGCA AGGGCAGAGAGTTCGTGTCCGCCATCTCCTGGTCCGGCTCCAGACCTCTTACGGCGACTCTGT GAAGGGCAGATTACCATCAGCCGGGATAACGCCAAGAACACACTGTACCTGCAGATGAATC CCTGCGGCCTGAGGACACCGCCGTGTACTACTGCGCCGCTGCCCTGGGCGCTGTCGTGTACACC ACCAGAGAACCCTATACCTACTGGGGACAGGGCACCCCTGGTGACCGTGTCTCT					15
PDL1b sdAb a.a.	EVQLVESGGGLVQPGGSLRLSCAASGRFTVITYGMGWFRQAPGKGRFVSAISWSGSSTSYGD SVKGRFTISRDNAKNTLYLQMNSLRPEDTAVYYCAAALGAVVYTTREPYTYWGQGLVTVSS					16
PDL1c sdAb DNA	GAGGTGCAGCTGGTGAATCCGGCGGAGGCCTGGTGCAGCCTGGCGGCTCTCTGAGACTGTCTT GCGCCGCTTCTGGCCGGACCTTCATCACCTACGCCATCGGCTGGTTCAGACAGGCCCTGGCAA GGGCAGAGAGTTCGTGTCCGCCATCTCCTGGTCCGGCTCTATGACCAGCTACGCCGACTCTGTG AAGGGCAGATTACCATCTCCCGGGATAACGCCAAGAACCCTGTACCTGCAGATGAATTCCC TGAGACCTGAGGACACAGCTGTGTATTACTGCGCCGCTCACCGGGGCGCCATCGCTCCCATCGC TCAGAGCGTGTACACCAACTGGGGCCAGGGAACCCTGGTACCGTGTCCAGC					17
PDL1c sdAb a.a.	EVQLVESGGGLVQPGGSLRLSCAASGRFTITYAIGWFRQAPGKGRFVSAISWSGSMTSYAD SVKGRFTISRDNAKNTLYLQMNSLRPEDTAVYYCAAHRAIAPIAQSVYTNWGQGLVTVSS					18
	Sequence	SEQ ID NO:	Sequence	SEQ ID NO:	Sequence	SEQ ID NO:
PDL1a sdAb CDR a.a.	GRTFVITYGMG	25	AISWSGSMTSYGDSVKG	26	ALGAVVYTTREPYTY	27
PDL1b sdAb CDR a.a.	GRTFVITYGMG	28	AISWSGSSTSYGDSVKG	29	ALGAVVYTTREPYTY	30
PDL1c sdAb CDR a.a.	GRTFITYAIG	31	AISWSGSMTSYADSVKG	32	HRGAIPIAQSVYTN	33

[292] Table 6: DNA and amino acid (a.a.) sequences of secretory signal sequence, linker sequences, and IgG4 Fc sequence

	Sequence	SEQ ID NO:
Secretory Signal Peptide DNA	ATGGGCTGGTCCTGCATCATCCTGTTCTCTGGTGGCTACCGCCACCGGCGTGCCTCC	1
Secretory Signal Peptide a.a.	MGWSCILFLVATATGVHS	2
E-Linker DNA	GAACCTAAGTCTAGCGACAAAACATACCAGCCCCCTAGTCCA	7
E-Linker a.a.	EPKSSDKTHTSPSP	8
G-Linker DNA	GGTGGAGGCGGTAGTGGAGGCGGTGGTTCAGGCGGAGGCGGATCT	9
G-Linker a.a.	GGGSGGGGSGGGGS	10
IgG4 Fc DNA	GAGAGCAAGTACGGACCACCTTGCCACCATGTCCAGCTCCTGAGTTTGAGGGAGGACCAT CCGTGTTCTGTTTCTCCAAAGCCTAAGGACACCCTGATGATCAGCCGGACACCTGAGGT	11

	GACCTGCGTGGTGGTGGACGTGTCTCAGGAGGATCCAGAGGTGCAGTTCAACTGGTACGTG GATGGCGTGGAGGTGCACAATGCTAAGACCAAGCCAAGAGAGGAGCAGTTTAATTCACA TACCGCGTGGTGAAGCGTGTGACCGTGTGTCATCAGGATTGGCTGAACGGCAAGGAGTATA AGTGCAAGGTGTCCAATAAGGGCGCTGCCAGCTCTATCGAGAAGACAATCAGCAAGGCTAA GGGACAGCCTAGGGAGCCACAGGTGTACACCCTGCCCCCTTCTCAGGAGGAGATGACAAAG AACCAGGTGTCCCTGACCTGTCTGGTGAAGGGCTTCTATCCAAGCGACATCGCTGTGGAGTG GGAGTCTAATGGCCAGCCGAGAACAAATTACAAGACCACACCACCCGTGCTGGACTCTGAT GGCTCCTTCTTTCTGTATTCTAGGCTGACAGTGGATAAGTCCCGGTGGCAGGAGGGCAACGT GTTTAGCTGCTCTGTGATGCACGAGGCCCTGCACAATCATTATACCCAGAAGTCCCTGAGCC TGCTCTGGGCAAG	
IgG4 Fc a.a.	ESKYGPPCPPCPAPEFEGGPSVFLFPPKPKDTLMISRTPEVTCVVDVVSQEDPEVQFNWYVDGV EVHNAKTKPREEQFNSTYRVVSVLTVHLQDQLNGKEYCKVSNKGLPSSIEKTKAKGQPRE PQVYTLPPSQEEMTKNQVSLTCLVKGFYPSDIAVEWESNGQPENNYKTPPVLDSDGSFFLYSR LTVDKSRWQEGNVFSCSVMEALHNHYTQKSLSLGLK	12

[293] As mentioned above, three different PD-L1 sdAbs, including anti-PDL1a (SEQ ID NO:13 (DNA); SEQ ID NO:14 (amino acid)), anti-PDL1b (SEQ ID NO:15 (DNA); SEQ ID NO:16 (amino acid)), and anti-PDL1c (SEQ ID NO:17 (DNA); SEQ ID NO:18 (amino acid)), were used for SMAB construction. Anti-CD47 mAb consists of heavy chain called H0 (SEQ ID NO:3 (DNA); SEQ ID NO:4 (amino acid)) and light chain called L0 (SEQ ID NO:5 (DNA); SEQ ID NO:6 (amino acid)). A series of SMABs were designed by fusing different anti-PD-L1 sdAbs to anti-CD47 mAb, respectively, with linker (E-linker: EPKSSDKTHTSPSP (SEQ ID NO:8), E4-linker: ESKYGPPSPSP (SEQ ID NO:69), or G-linker: (G₄S)₃ (SEQ ID NO:10)). Anti-PDL1a sdAb was fused to the C-terminus of heavy chain of H0 by E-linker generating new polypeptide called H1. In the same way, new polypeptides called H2 and H3 were generated by using anti-PDL1b and anti-PDL1c sdAb, respectively. Similarly, these sdAbs were individually fused to the N-terminus of heavy chain of H0 by E-linker generating three new polypeptides called H4, H5 and H6. Also, these sdAbs were separately connected to the C- and N-terminus of light chain of L0 by E-linker generating six new polypeptides called L1, L2, L3, L4, L5 and L6, respectively. Meanwhile, anti-PDL1a sdAb was fused to the C- and N-terminus of heavy chain of H0 by G-linker leading to new polypeptides called H7 and H8. Likewise, anti-PDL1a sdAb was fused to the C- and N-terminus of light chain of L0 by G-linker leading to new polypeptides called L7 and L8. In addition, anti-PDL1a sdAb was fused to the C-terminus of light chain of L0 by E4-linker leading to a new polypeptide called L9.

[294] Using these new fusion proteins, a series of SMABs were generated by combining the new heavy chain fusion protein and native light chain of L0, or by combining the new light chain fusion protein and native heavy chain of H0. The combination of new heavy chain fusion protein and native light chain of L0 led to new SMAB proteins called PDL1a-E-HC (comprising H1(SEQ ID NO:36) and L0 (SEQ ID NO:6)), PDL1b-E-HC (comprising H2 (SEQ ID NO:38) and L0 (SEQ ID NO:6)), PDL1c-E-HC (comprising H3 (SEQ ID NO:40) and L0 (SEQ ID NO:6)), PDL1a-E-HN (comprising H4 (SEQ ID NO:42) and L0 (SEQ ID NO:6)), PDL1b-E-HN (comprising H5 (SEQ ID NO:44) and L0 (SEQ ID

NO:6)), PDL1c-E-HN (comprising H6 (SEQ ID NO:46) and L0 (SEQ ID NO:6)), PDL1a-G15-HC (comprising H7(SEQ ID NO:48) and L0 (SEQ ID NO:6))and PDL1a-G15-HN (comprising H8 (SEQ ID NO:50) and L0 (SEQ ID NO:6)). Combining new light chain fusion protein and native heavy chain of H0 generated new SMAB proteins called PDL1a-E-LC (comprising H0 (SEQ ID NO:4) and L1 (SEQ ID NO:52)), PDL1b-E-LC (comprising H0 (SEQ ID NO:4) and L2 (SEQ ID NO:54)), PDL1c-E-LC (comprising H0 (SEQ ID NO:4) and L3 (SEQ ID NO:56)), PDL1a-E-LN (comprising H0 (SEQ ID NO:4) and L4 (SEQ ID NO:58)), PDL1b-E-LN (comprising H0 (SEQ ID NO:4) and L5 (SEQ ID NO:60)), PDL1c-E-LN(comprising H0 (SEQ ID NO:4) and L6 (SEQ ID NO:62)), PDL1a-G15-LC(comprising H0 (SEQ ID NO:4) and L7(SEQ ID NO:64)), PDL1a-G15-LN (comprising H0 (SEQ ID NO:4) and L8 (SEQ ID NO:66)), and PDL1a-E4-LC (comprising H0 (SEQ ID NO:4) and L9 (SEQ ID NO:68)).

[295] The sdAb-Fc fusion proteins were constructed by linking sdAb to the N-terminus of human IgG4 Fc portion, with mutations S228P and L235E, called IgG4PE, generating new Fc fusion proteins called sdAb-PDL1a-IgG4PE, sdAb-PDL1b-IgG4PE and sdAb-PDL1c-IgG4PE.

[296] FACS binding assay

[297] The binding pattern of multiple antigen binding proteins on CD47 or PD-L1 expressed on CHO-K1 cells was plotted with antibody in 3X serial dilutions, starting at a concentration of 300nM. Antibody-antigen binding curves were generated with geometric mean values. Raw data was plotted with GraphPad Prism v6.02 software with four parameters, best-fit values program to analyze the EC₅₀.

[298] For CD47 binding, anti-PD-L1 sdAb fused to the C-terminus of heavy/light chain of anti-CD47 mAb did not affect CD47 binding. As demonstrated in FIG. 1, the SMAB constructs have similar affinity to CD47 antigen compared to anti-CD47 mAb itself. However, when anti-PD-L1 sdAb is fused to the N-terminus of heavy/light chain of anti-CD47 mAb, the final constructs have lower affinity to the CD47 antigen as compared to an anti-CD47 mAb (FIG. 2).

[299] For PD-L1 binding, when anti-PD-L1 sdAb is fused to the C-terminus of heavy/light chain of anti-CD47 mAb, the final constructs have lower affinity to PD-L1 antigen as compared to the controls of sdAb-PDL1a-IgG4PE, sdAb-PDL1b-IgG4PE, and sdAb-PDL1c-IgG4PE (FIG. 3). However, when anti-PD-L1 sdAb is fused to the N-terminus of heavy/light chain of anti-CD47 mAb, the final constructs have higher affinity to PD-L1 antigen as compared to the controls (FIG. 4).

[300] *In vitro* bioassay

[301] A multiple antigen binding protein can bind to two target antigens, evaluating its synergistic effect is more challenging than checking the response by single antigen targeting. For the *in vitro* bioassay of anti-CD47/anti-PD-L1 multiple antigen binding proteins, CD47 and PD-L1 target response was performed by PD-1/PD-L1 blockade bioassay and anti-CD47 antibody mediated phagocytosis separately.

[302] PD-1/PD-L1 blockade bioassay

[303] The PD-1/PD-L1 blockade bioassay system from Promega (Madison, WI) can be used to measure the potency and stability of antibodies and other biologics designed to block the PD-1/PD-L1 interaction. The assay consists of two genetically engineered cell lines: PD-1 Effector Cells, which are Jurkat T cells expressing human PD-1 and a luciferase reporter driven by an NFAT response element (NFAT-RE), and PD-L1 aAPC/CHO-K1 Cells, which are CHO-K1 cells expressing human PD-L1 and an engineered cell surface protein designed to activate cognate TCRs in an antigen-independent manner. When the two cell types are co-cultured, the PD-1/PD-L1 interaction inhibits TCR signaling and NFAT-RE-mediated luminescence. Addition of either an anti-PD-1 or anti-PD-L1 antibody that blocks the PD-1/PD-L1 interaction releases the inhibitory signal and results in TCR activation and NFAT-RE-mediated luminescence.

[304] For the PD-1/PD-L1 blockade bioassay, a Tecentriq biosimilar was utilized as a reference antibody. Its activity was set as 100%, and the relative activity of samples was calculated by comparison with this reference antibody. Among these SMAB constructs, the SMAB of PDL1a-E-LC exhibited the highest activity, which was even higher than its parent antibody of sdAb-PDL1a-IgG4PE (FIGS. 5-10). The SMAB, PDL1a-E-LC, also showed higher activity when compared with the reference antibody of the Tecentriq biosimilar (FIG. 10). In an additional bioassay with PDL1a-E4-LC, PDL1a-E-LC also showed the best activity among SMAB constructs (FIG. 18).

[305] Bioassay of anti-CD47 antibody mediated phagocytosis

[306] Peripheral blood mononuclear cells (PBMCs) were isolated from human peripheral blood using density gradient method (Ficoll). Monocytes were purified from PBMCs using Pan Monocyte Isolation Kit (Miltenyi Biotec; Bergisch Gladbach, Germany). Monocytes were stimulated to become monocyte-derived macrophages (MDMs) using GM-CSF (Granulocyte-Macrophage Colony Stimulating Factor), and the stimulation was carried out for 14 days. On the 14th day, the target cell line, HL60, was cultured to 90% confluency and labeled with PKH26. PKH26-labeled HL60 cells were seeded into the wells in a 96 well plate. MDMs were digested off the culturing dishes using accutase (Sigma; St. Louis, MO) and added to the HL60-containing wells, with the addition of anti-CD47 antibody samples at serially diluted concentrations, and incubated for one hour at 37°C, allowing cellular phagocytosis to take place. After incubation, MDMs were digested off the culturing dish and stained with fluorochrome-labeled anti-CD11b antibodies. The cells in the wells were analyzed using a BD FACSCalibur flow cytometer. Percentage phagocytosis was calculated by dividing PKH26 and CD11b double positive population by PKH26+ population. The dose response curve was plotted with the percentage phagocytosis against the concentration of anti-CD47 antibody samples, and the curve was analyzed using GraphPad Prism software to obtain EC₅₀ values and other specifications of the data.

[307] For the anti-CD47 antibody mediated phagocytosis assay, the Hu5F9 biosimilar was utilized as a reference antibody. The Hu5F9 biosimilar activity was set as 100%, and the relative activity of samples was calculated by comparison with this reference antibody. Among the SMAB constructs, the SMAB of PDL1a-E-LC exhibited the highest activity (FIGS. 13-16), although it showed lower activity when compared with reference antibody Hu5F9 (FIGS. 11-15). In an additional phagocytosis assay with PDL1a-E4-LC, PDL1a-E-LC also showed the best activity among SMAB constructs (FIG. 17).

PD-L1 protein (SEQ ID NO:34):

FTVTVPKDLVYVEYGSNMIECKFPVEKQLDLAALIVYWEMEDKNIIQFVHGEECLKVQHSSYRQRARLL
KDQLSLGNAALQITDVKLQDAGVYRCMISYGGADYKRITVKVNAPYNKINQRILVVDPTSEHELTCQAE
GYPKAEVIWTSSDHQVLSGKTTTNSKREEKLFNVTSTLRINTTTNEIFYCTFRRLDPEENHTAELVIPPL
AHPPNERTHLVILGAILLCLGVALTFIFRLRKGRMMDVKKCGIQDTNSKKQSDTHLEET

The sequence of anti-CD47/anti-PD-L1 multiple antigen binding proteins

DNA sequence of H1 (SEQ ID NO: 35):

GAGGTGCAGCTGGTGCAGTCCGGAGCTGAGGTGAAGAAGCCAGGATCCAGCGTGAAGGTGAGCTGCA
AGGCTAGCGGCTACTCTTTCACCCACCATTGGATCCACTGGGTGAGGCAGGCTCCTGGACAGGGACTG
GAGTGGATGGGCATGATCGACGCTTCCGATAGCGAGACAAGACTGTCTCAGAAAGTTTAAGGACCGCGT
GACCATCACAGCCGATAAGTCTACCTCCACAGCTTACATGGAGCTGTCTTCCCTGAGATCCGAGGACA
CCGCCGTGTACTATTGTGCTAGGCTGGGCCGGTACTATTTTCGATTATTGGGGCCAGGGCACCACAGTG
ACAGTGAGCTCTGCCAGCACAAAGGGCCCTTCCGTGTTCCCACTGGCTCCCTGCTCCAGAAGCACATC
TGAGTCCACCGCCGCTCTGGGCTGTCTGGTGAAGGACTACTTCCCTGAGCCAGTGACCGTGTCTGGA
ACAGCGGCGCCCTGACATCTGGCGTGCACACCTTTCAGCTGTGCTGCAGTCCAGCGGCCCTGTACTCCC
TGTCTTCCGTGGTGACAGTGCCCAAGCTCTTCCCTGGGCACCAAGACATATACTGCAACGTGGACCAT
AAGCCTTCCAATACCAAGGTGGATAAGAGGGTGGAGAGCAAGTACGGACCACCTTGCCACCATGTC
CAGCTCCTGAGTTTGAGGGAGGACCATCCGTGTTCTGTTTCTCCAAAGCCTAAGGACACCCTGATG
ATCAGCCGGACACCTGAGGTGACCTGCGTGGTGGTGGACGTGTCTCAGGAGGATCCAGAGGTGCAGTT
CAACTGGTACGTGGATGGCGTGGAGGTGCACAATGCTAAGACCAAGCCAAGAGAGGAGCAGTTTAAT
TCCACATACCGCGTGGTGAGCGTGCTGACCGTGCTGCATCAGGATTGGCTGAACGGCAAGGAGTATAA
GTGCAAGGTGTCCAATAAGGGCCTGCCAGCTCTATCGAGAAGACAATCAGCAAGGCTAAGGGACAG
CCTAGGGAGCCACAGGTGTACACCCTGCCCCCTTCTCAGGAGGAGATGACAAAGAACCAGGTGTCCCT
GACCTGTCTGGTGAAGGGCTTCTATCCAAGCGACATCGCTGTGGAGTGGGAGTCTAATGGCCAGCCCG
AGAACAATTACAAGACCACACCACCCGTGCTGGACTCTGATGGCTCCTTCTTTCTGTATTCTAGGCTGA
CAGTGGATAAGTCCCGGTGGCAGGAGGGCAACGTGTTTAGCTGCTCTGTGATGCACGAGGCCCTGCAC
AATCATTATACCCAGAAGTCCCTGAGCCTGTCTCTGGGCAAGGAACCTAAGTCTAGCGACAAAACCTCA
TACCAGCCCCCTAGTCCAGAGGTGCAGCTGGTGAATCCGGCGGAGGCCTGGTCCAGCCTGGCGGCT
CTCTGCGGCTGTCTGCGCCGCTTCTGGCAGAACCTTCGTGACCTACGGCATGGGCTGGTTCCGGCAGG
CTCCTGGCAAGGGCAGAGAGTTCTGTGTCGCCATCTCCTGGTCCGGCTCCATGACCTCTTACGGCGACT
CTGTGAAGGGCAGATTACCATCAGCCGGGATAACGCCAAGAACACACTGTACCTGCAGATGAACTCC
CTGCGGCCTGAGGACACCGCCGTGTACTACTGCGCCGCTGCCCTGGGCGCTGTCTGTACACCACCAG
AGAACCCTATACCTACTGGGGACAGGGCACCTGGTGACCGTGTCTCT

Amino acid sequence of H1 (SEQ ID NO: 36):

EVQLVQSGAEVKKPGSSVKVSKASGYSFTHHWIHWVRQAPGQGLEWMGMIDASDSETRLISQKFKDRVT
ITADKSTSTAYMELSSLRSEDTAVYYCARLGRYYFDYWGQGTITVTVSSASTKGPSVFPLAPCSRSTSESTAA
LGCLVKDYFPEPVTVSWNSGALTSGVHTFPAVLQSSGLYSLSSVTVPSSSLGKTYTCNVDHKPSNTKVD
KRVESKYGPCCPPAPEFEGGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSQEDPEVQFNWYVDGVEVHN
AKTKPREEQFNSTYRVVSVLTVLHQDWLNGKEYKCKVSNKGLPSSIEKTISKAKGQPREPQVYTLPPSQEE
MTKNQVSLTCLVKGFYPSDIAVEWESNGQPENNYKTPPVLDSDGSFFLYSRLTVDKSRWQEGNVFSCSV
MHEALHNHYTQKSLSLSLGKEPKSSDKTHTSPSPSEVQLVESGGGLVQPGGSLRLSCAASGRFTVYGMG

WFRQAPGKGREFVSAISWSGSMTSYGDSVKGRFTISRDNKNTLYLQMNSLRPEDTAVYYCAAALGAVV
YTTREPYTYWGQGLTVTVSS

DNA sequence of H2 (SEQ ID NO: 37):

GAGGTGCAGCTGGTGCAGTCCGGAGCTGAGGTGAAGAAGCCAGGATCCAGCGTGAAGGTGAGCTGCA
AGGCTAGCGGCTACTCTTTACCCACCATTGGATCCACTGGGTGAGGCAGGCTCCTGGACAGGGACTG
GAGTGGATGGGCATGATCGACGCTTCCGATAGCGAGACAAGACTGTCTCAGAAGTTTAAGGACCGCGT
GACCATCACAGCCGATAAGTCTACCTCCACAGCTTACATGGAGCTGTCTTCCCTGAGATCCGAGGACA
CCGCCGTGTACTATTGTGCTAGGCTGGGCCGGTACTATTTTCGATTATTGGGGCCAGGGCACCACAGTG
ACAGTGAGCTCTGCCAGCACAAAGGGCCCTTCCGTGTTCCCACTGGCTCCCTGCTCCAGAAGCACATC
TGAGTCCACCGCCGCTCTGGGCTGTCTGGTGAAGGACTACTTCCCTGAGCCAGTGACCGTGTCTGGA
ACAGCGGCGCCCTGACATCTGGCGTGCACACCTTTCCAGCTGTGCTGCAGTCCAGCGGCCTGTACTCCC
TGTCTTCCGTGGTGACAGTGCCAGCTCTTCCCTGGGCACCAAGACATATACCTGCAACGTGGACCAT
AAGCCTTCCAATACCAAGGTGGATAAGAGGGTGGAGAGCAAGTACGGACCACCTTGCCACCATGTC
CAGCTCCTGAGTTTGAGGGAGGACCATCCGTGTTCTGTTTCTCCAAAGCCTAAGGACACCCTGATG
ATCAGCCGGACACCTGAGGTGACCTGCGTGGTGGTGGACGTGTCTCAGGAGGATCCAGAGGTGCAGTT
CAACTGGTACGTGGATGGCGTGGAGGTGCACAATGCTAAGACCAAGCCAAGAGAGGAGCAGTTTAAT
TCCACATACCGCGTGGTGAGCGTGCTGACCGTGCTGCATCAGGATTGGCTGAACGGCAAGGAGTATAA
GTGCAAGGTGTCCAATAAGGGCCTGCCAGCTCTATCGAGAAGACAATCAGCAAGGCTAAGGGACAG
CCTAGGGAGCCACAGGTGTACACCCTGCCCTTCTCAGGAGGAGATGACAAAGAACCAGGTGTCCCT
GACCTGTCTGGTGAAGGGCTTCTATCCAAGCGACATCGCTGTGGAGTGGGAGTCTAATGGCCAGCCCG
AGAACAATTACAAGACACACCACCCGTGCTGGACTCTGATGGCTCCTTCTTCTGTATTCTAGGCTGA
CAGTGGATAAGTCCCGGTGGCAGGAGGGCAACGTGTTTAGCTGCTCTGTGATGCACGAGGCCCTGCAC
AATCATTATACCCAGAAGTCCCTGAGCCTGTCTCTGGGCAAGGAACCTAAGTCTAGCGACAAAACCTCA
TACCAGCCCCCTAGTCCAGAGGTGCAGCTGGTGAATCCGGCGGAGGCCTGGTCCAGCCTGGCGGCT
CTCTGCGGCTGTCTGCGCCGCTTCTGGCAGAACCTTCGTGACCTACGGCATGGGCTGGTTCCGGCAGG
CTCCTGGCAAGGGCAGAGAGTTCGTGTCCGCCATCTCCTGGTCCGGCTCCAGCACCTCTTACGGCGACT
CTGTGAAGGGCAGATTACCATCAGCCGGGATAACGCCAAGAACACACTGTACCTGCAGATGAACTCC
CTGCGGCCTGAGGACACCGCCGTGTACTACTGCGCCGCTGCCCTGGGCGCTGTCTGTACACCACCAG
AGAACCCTATACCTACTGGGGACAGGGCACCCCTGGTGACCGTGTCTCT

Amino acid sequence of H2 (SEQ ID NO: 38):

EVQLVQSGAEVKKPGSSVKVSCKASGYSFTHHWIHWVRQAPGQGLEWMGMIDASDSETRLISQKFKDRVT
ITADKSTSTAYMELSSLRSEDTAVYYCARLGRYFYFDYWQGTTTVTVSSASTKGPSVFPLAPCSRSTSESTA
LGCLVKDYFPEPVTVSWNSGALTSGVHTFPAVLQSSGLYSLSSVTVVPSSSLGKTYTCNVDHKPSNTKVD
KRVESKYGPPCPPCPAPEFEGGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSDQEDPEVQFNWYVDGVEVHN
AKTKPREEQFNSTYRVVSVLTVLHQDWLNGKEYKCKVSNKGLPSSIEKTISKAKGQPREPQVYTLPPSQEE
MTKNQVSLTCLVKGFYPSDIAVEWESNGQPENNYKTPPVLDSDGSFFLYSRLTVDKSRWQEGNVFSCSV
MHEALHNHYTQKSLSLSLGKEPKSSDKTHTSPSPVEQLVESGGGLVQPGGSLRLSCAASGRFTVITYGMG
WFRQAPGKGREFVSAISWSGSSTSYGDSVKGRFTISRDNKNTLYLQMNSLRPEDTAVYYCAAALGAVV
YTTREPYTYWGQGLTVTVSS

DNA sequence of H3 (SEQ ID NO: 39):

GAGGTGCAGCTGGTGCAGTCCGGAGCTGAGGTGAAGAAGCCAGGATCCAGCGTGAAGGTGAGCTGCA
AGGCTAGCGGCTACTCTTTACCCACCATTGGATCCACTGGGTGAGGCAGGCTCCTGGACAGGGACTG
GAGTGGATGGGCATGATCGACGCTTCCGATAGCGAGACAAGACTGTCTCAGAAGTTTAAGGACCGCGT
GACCATCACAGCCGATAAGTCTACCTCCACAGCTTACATGGAGCTGTCTTCCCTGAGATCCGAGGACA
CCGCCGTGTACTATTGTGCTAGGCTGGGCCGGTACTATTTTCGATTATTGGGGCCAGGGCACCACAGTG
ACAGTGAGCTCTGCCAGCACAAAGGGCCCTTCCGTGTTCCCACTGGCTCCCTGCTCCAGAAGCACATC
TGAGTCCACCGCCGCTCTGGGCTGTCTGGTGAAGGACTACTTCCCTGAGCCAGTGACCGTGTCTGGA
ACAGCGGCGCCCTGACATCTGGCGTGCACACCTTTCCAGCTGTGCTGCAGTCCAGCGGCCTGTACTCCC
TGTCTTCCGTGGTGACAGTGCCAGCTCTTCCCTGGGCACCAAGACATATACCTGCAACGTGGACCAT
AAGCCTTCCAATACCAAGGTGGATAAGAGGGTGGAGAGCAAGTACGGACCACCTTGCCACCATGTC
CAGCTCCTGAGTTTGAGGGAGGACCATCCGTGTTCTGTTTCTCCAAAGCCTAAGGACACCCTGATG
ATCAGCCGGACACCTGAGGTGACCTGCGTGGTGGTGGACGTGTCTCAGGAGGATCCAGAGGTGCAGTT
CAACTGGTACGTGGATGGCGTGGAGGTGCACAATGCTAAGACCAAGCCAAGAGAGGAGCAGTTTAAT

TCCACATACCGCGTGGTGAGCGTGCTGACCGTGCTGCATCAGGATTGGCTGAACGGCAAGGAGTATAA
 GTGCAAGGTGTCCAATAAGGGCCTGCCCAGCTCTATCGAGAAGACAATCAGCAAGGCTAAGGGACAG
 CCTAGGGAGCCACAGGTGTACACCCTGCCCCCTTCTCAGGAGGAGATGACAAAGAACCAGGTGTCCCT
 GACCTGTCTGGTGAAGGGCTTCTATCCAAGCGACATCGCTGTGGAGTGGGAGTCTAATGGCCAGCCCCG
 AGAACAATTACAAGACCACACCACCCGTGCTGGACTCTGATGGCTCCTTCTTTCTGTATTCTAGGCTGA
 CAGTGGATAAGTCCCCGGTGGCAGGAGGGCAACGTGTTTAGCTGCTCTGTGATGCACGAGGCCCTGCAC
 AATCATTATACCCAGAAGTCCCTGAGCCTGTCTCTGGGCAAGGAACCTAAGTCTAGCGACAAAACCTCA
 TACCAGCCCCCTAGTCCAGAGGTGCAGCTGGTGAATCCGGCGGAGGCCTGGTGCAGCCTGGCGGCT
 CTCTGAGACTGTCCTGCGCCGCTTCTGGCCGACCTTCATCACCTACGCCATCGGCTGGTTCAGACAGG
 CCCCTGGCAAGGGCAGAGAGTTCGTGTCCGCCATCTCCTGGTCCGGCTCTATGACCAGCTACGCCGAC
 TCTGTGAAGGGCAGATTACCATCTCCCGGGATAACGCCAAGAACACCCTGTACCTGCAGATGAATTC
 CCTGAGACCTGAGGACACAGCTGTGTATTACTGCGCCGCTCACCGGGCGCCATCGCTCCCATCGCTC
 AGAGCGTGTACACCAACTGGGGCCAGGGAACCTGGTCACCGTGTCCAGC

Amino acid sequence of H3 (SEQ ID NO: 40):

EVQLVQSGAEVKKPGSSVKVSKASGYSFTHHWIHWVRQAPGQGLEWMGMIDASDSETRLSSQKFKDRV
 ITADKSTSTAYMELSSLRSEDTAVYYCARLGRYFYFDYWGQGTITVTVSSASTKGPSVFPLAPCSRSTSE
 LGCLVKDYFPEPVTVSWNSGALTSGVHTFPAVLQSSGLYSLSSVTVPSSSLGKTYTCNVDHKPSNTKVD
 KRVESKYGPPCPPPAPEFEGGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSQEDPEVQFNWYVDGVEVHN
 AKTKPREEQFNSTYRVVSVLTVLHQDWLNGKEYKCKVSNKGLPSSIEKTISKAKGQPREPQVYTLPPSQEE
 MTKNQVSLTCLVKGFYPSDIAVEWESNGQPENNYKTTTPVLDSDGSFFLYSRLTVDKSRWQEGNVFSCV
 MHEALHNHYTQKLSLSLKGEPKSSDKTHTSPSPPEVQLVESGGGLVQPGGSLRLSCAASGRTFTITYAIGWF
 RQAPGKGREFVSAISWSGSMSTYADSVKGRFTISRDNKNTLYLQMNSLRPEDTAVYYCAAHRAIAPIAQ
 SVYTNWGQGLTVTVSS

DNA sequence of H4 (SEQ ID NO: 41):

GAGGTGCAGCTGGTGAATCCGGCGGAGGCCTGGTCCAGCCTGGCGGCTCTCTGCGGCTGTCTGCGC
 CGCTTCTGGCAGAACCTTCGTGACCTACGGCATGGGCTGGTTCGGCAGGCTCCTGGCAAGGGCAGAG
 AGTTCGTGTCCGCCATCTCCTGGTCCGGCTCCATGACCTCTTACGGCGACTCTGTGAAGGGCAGATTCA
 CCATCAGCCGGGATAACGCCAAGAACACACTGTACCTGCAGATGAACCTCCCTGCGGCCTGAGGACACC
 GCCGTGTACTACTGCGCCGCTGCCCTGGGCGCTGTCGTGTACACCACCAGAGAACCCTATACCTACTG
 GGGACAGGGCACCCTGGTGACCGTGTCTCTGAACCTAAGTCTAGCGACAAAACCTCATACCAGCCCCC
 CTAGTCCAGAGGTGCAGCTGGTGCAGTCCGGAGCTGAGGTGAAGAAGCCAGGATCCAGCGTGAAGGT
 GAGCTGCAAGGCTAGCGGCTACTCTTTCACCCACCATTGGATCCACTGGGTGAGGCAGGCTCCTGGAC
 AGGACTGGAGTGGATGGGCATGATCGACGCTTCCGATAGCGAGACAAGACTGTCTCAGAAGTTTAA
 GGACCGCTGACCATCACAGCCGATAAGTCTACCTCCACAGCTTACATGGAGCTGTCTTCCCTGAGAT
 CCGAGGACACCCCGCTGTACTATTGTGCTAGGCTGGGCCGCTACTATTTTCGATTATTGGGGCCAGGGC
 ACCACAGTGACAGTGAGCTCTGCCAGCACAAAGGGCCCTTCCGTGTTCCCACTGGCTCCCTGCTCCAG
 AAGCACATCTGAGTCCACCGCCGCTCTGGGCTGTCTGGTGAAGGACTACTTCCCTGAGCCAGTGACCG
 TGTCTTGAACAGCGGCGCCCTGACATCTGGCGTGCACACCTTCCAGCTGTGCTGCAGTCCAGCGGC
 CTGTACTCCCTGTCTTCCGTGGTGACAGTGCCAGCTCTTCCCTGGGCACCAAGACATATACCTGCAAC
 GTGGACCATAAGCCTTCCAATACCAAGGTGGATAAGAGGGTGGAGAGCAAGTACGGACCACCTTGCC
 CACCATGTCCAGCTCCTGAGTTTGAGGGAGGACCATCCGTGTTCTCTGTTTCCCTCAAAGCCTAAGGACA
 CCCTGATGATCAGCCGACACCTGAGGTGACCTGCGTGGTGGTGGACGTGTCTCAGGAGGATCCAGAG
 GTGCAGTTCAACTGGTACGTGGATGGCGTGGAGGTGCACAATGCTAAGACCAAGCCAAGAGAGGAGC
 AGTTTAATTCCACATACCGCGTGGTGAGCGTGCTGACCGTGCTGCATCAGGATTGGCTGAACGGCAAG
 GAGTATAAGTGCAAGGTGTCCAATAAGGGCCTGCCCAGCTCTATCGAGAAGACAATCAGCAAGGCTA
 AGGGACAGCCTAGGGAGCCACAGGTGTACACCCTGCCCCCTTCTCAGGAGGAGATGACAAAGAACCA
 GGTGTCCCTGACCTGTCTGGTGAAGGGCTTCTATCCAAGCGACATCGCTGTGGAGTGGGAGTCTAATG
 GCCAGCCGAGACATTACAAGACCACACCCGCTGCTGGACTCTGATGCTCCTTCTTCTGTATT
 CTAGGCTGACAGTGGATAAGTCCCGGTGGCAGGAGGGCAACGTGTTTAGCTGCTCTGTGATGCACGAG
 GCCCTGCACAATCATTATACCCAGAAGTCCCTGAGCCTGTCTCTGGGCAAG

Amino acid sequence of H4 (SEQ ID NO: 42):

EVQLVESGGGLVQPGGSLRLSCAASGRTFTVITYGMGWFRQAPGKGREFVSAISWSGSMSTSYGDSVKGRFTI
 SRDNKNTLYLQMNSLRPEDTAVYYCAAALGAVVYTTREPYTYWGQGLTVTVSSSEPKSSDKTHTSPSPSE

VQLVQSGAEVKKPGSSVKVSCKASGYSFTHHWHWVRQAPGQGLEWMGMIDASDSETRLSQKFKDRVTI
TADKSTSTAYMELSSLRSEDTAVYYCARLGRYYFDYWQGQTTVTVSSASTKGPSVFPLAPCSRSTSESTA
LGCLVKDYFPEPVTVSWNSGALTSGVHTFPAVLQSSGLYSLSSVTVPSSSLGKTYTCNVDHKPSNTKVD
KRVESKYGPPCPPPAPEFEGGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSQEDPEVQFNWYVDGVEVHN
AKTKPREEQFNSTYRVVSVLTVLHQDWLNGKEYKCKVSNKGLPSSIEKTISKAKGQPREPQVYTLPPSQEE
MTKNQVSLTCLVKGFYPSDIAVEWESNGQPENNYKTTTPVLDSGDSFFLYSRLTVDKSRWQEGNVFSCSV
MHEALHNHYTQKLSLSLGLK

DNA sequence of H5 (SEQ ID NO: 43):

GAGGTGCAGCTGGTGAATCCGGCGGAGGCCTGGTCCAGCCTGGCGGCTCTCTGCGGCTGTCTGCGC
CGCTTCTGGCAGAACCTTCGTGACCTACGGCATGGGCTGGTTCGGCAGGCTCCTGGCAAGGGCAGAG
AGTTCGTGTCCGCCATCTCCTGGTCCGGCTCCAGCACCTCTTACGGCGACTCTGTGAAGGGCAGATTCA
CCATCAGCCGGGATAACGCCAAGAACACACTGTACCTGCAGATGAACTCCCTGCGGCCTGAGGACACC
GCCGTGTACTACTGCGCCGCTGCCCTGGGCGCTGTCGTGTACACCACCAGAGAACCCTATACCTACTG
GGGACAGGGCACCTGGTGACCGTGTCTCTGAACCTAAGTCTAGCGACAAAACCTCATACCAGCCCCC
CTAGTCCAGAGGTGCAGCTGGTGCAGTCCGGAGCTGAGGTGAAGAAGCCAGGATCCAGCGTGAAGGT
GAGCTGCAAGGCTAGCGGCTACTCTTTCACCCACCATTGGATCCACTGGGTGAGGCAGGCTCCTGGAC
AGGGACTGGAGTGGATGGGCATGATCGACGCTTCCGATAGCGAGACAAGACTGTCTCAGAAGTTTAA
GGACCGCGTGACCATCACAGCCGATAAGTCTACCTCCACAGCTTACATGGAGCTGTCTTCCCTGAGAT
CCGAGGACACCGCGGTGTACTATTGTGCTAGGCTGGGCCGCTACTATTTTCGATTATTGGGGCCAGGGC
AACACAGTGACAGTGTGCTGCCAGCACAAAGGGCCCTTCCGTGTTCCACTGGCTCCCTGCTCCAG
AAGCATCTGAGTCCACCGCCGCTCTGGGCTGTCTGGTGAAGGACTACTTCCCTGAGCCAGTGACCG
TGTCCTGGAACAGCGGCGCCCTGACATCTGGCGTGCACACCTTTCAGCTGTGCTGCAGTCCAGCGGC
CTGTACTCCCTGTCTTCCGTGGTGACAGTGCCAGCTCTTCCCTGGGCACCAAGACATATACCTGCAAC
GTGGACCATAAGCCTTCCAATACCAAGGTGGATAAGAGGGTGGAGAGCAAGTACGGACCACCTTGCC
CACCATGTCCAGCTCCTGAGTTTGAGGGAGGACCATCCGTGTTCTCTTCCCTCAAAGCCTAAGGACA
CCCTGATGATCAGCCGGACACCTGAGGTGACCTGCGTGGTGGTGGACGTGTCTCAGGAGGATCCAGAG
GTGCAGTTCAACTGGTACGTGGATGGCGTGGAGGTGCACAATGCTAAGACCAAGCCAAGAGAGGAGC
AGTTTAATCCACATACCGCGTGGTGAGCGTGTGACCGTGTGTCATCAGGATTGGCTGAACGGCAAG
GAGTATAAGTGCAAGGTGTCCAATAAGGGCCTGCCCAGCTCTATCGAGAAGACAATCAGCAAGGCTA
AGGGACAGCCTAGGGAGCCACAGGTGTACACCCTGCCCCCTTCTCAGGAGGAGATGACAAAGAACCA
GGTGTCCCTGACCTGTCTGGTGAAGGGCTTCTATCCAAGCGACATCGCTGTGGAGTGGGAGTCTAATG
GCCAGCCCGAGAACAAATTACAAGACCACACCACCCGTGCTGGACTCTGATGGCTCCTTCTTTCTGTATT
CTAGGCTGACAGTGGATAAGTCCCGGTGGCAGGAGGGCAACGTGTTTAGCTGCTCTGTGATGCACGAG
GCCCTGCACAATCATTATACCCAGAAGTCCCTGAGCCTGTCTCTGGGCAAG

Amino acid sequence of H5 (SEQ ID NO: 44):

EVQLVESGGGLVQPGGSLRLSCAASGRFTVITYGMGWFRQAPGKGRFVSAISWSGSSTSYGDSVKGRFTIS
RDNAKNTLYLQMNSLRPEDTAVYYCAAALGAVVYTTREPYTYWGQGLTVTVSSEPKSSDKTHTSPSPPEV
QLVQSGAEVKKPGSSVKVSCKASGYSFTHHWHWVRQAPGQGLEWMGMIDASDSETRLSQKFKDRVTIT
ADKSTSTAYMELSSLRSEDTAVYYCARLGRYYFDYWQGQTTVTVSSASTKGPSVFPLAPCSRSTSESTAAL
GCLVKDYFPEPVTVSWNSGALTSGVHTFPAVLQSSGLYSLSSVTVPSSSLGKTYTCNVDHKPSNTKVDK
RVESKYGPPCPPPAPEFEGGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSQEDPEVQFNWYVDGVEVHNA
KTKPREEQFNSTYRVVSVLTVLHQDWLNGKEYKCKVSNKGLPSSIEKTISKAKGQPREPQVYTLPPSQEEM
TKNQVSLTCLVKGFYPSDIAVEWESNGQPENNYKTTTPVLDSGDSFFLYSRLTVDKSRWQEGNVFSCSV
MHEALHNHYTQKLSLSLGLK

DNA sequence of H6 (SEQ ID NO: 45):

GAGGTGCAGCTGGTGAATCCGGCGGAGGCCTGGTGCAGCCTGGCGGCTCTCTGAGACTGTCTGCGC
CGCTTCTGGCCGACCTTCATCACCTACGCCATCGGCTGGTTCAGACAGGCCCTGGCAAGGGCAGAG
AGTTCGTGTCCGCCATCTCCTGGTCCGGCTCTATGACCAGCTACGCCACTCTGTGAAGGGCAGATTCA
CCATCTCCCGGGATAACGCCAAGAACACCCTGTACCTGCAGATGAATTCCCTGAGACCTGAGGACACA
GCTGTGTATTACTGCGCCGCTACCGGGGCGCCATCGCTCCCATCGCTCAGAGCGTGTACACCAACTG
GGGCCAGGGAACCCTGGTACCGTGTCCAGCGAACCTAAGTCTAGCGACAAAACCTCATACCAGCCCCC
CTAGTCCAGAGGTGCAGCTGGTGCAGTCCGGAGCTGAGGTGAAGAAGCCAGGATCCAGCGTGAAGGT
GAGCTGCAAGGCTAGCGGCTACTCTTTCACCCACCATTGGATCCACTGGGTGAGGCAGGCTCCTGGAC

AGGGACTGGAGTGGATGGGCATGATCGACGCTTCCGATAGCGAGACAAGACTGTCTCAGAAGTTTAA
 GGACCGCGTGACCATCACAGCCGATAAGTCTACCTCCACAGCTTACATGGAGCTGTCTTCCCTGAGAT
 CCGAGGACACCGCCGTGTACTATTGTGCTAGGCTGGGCCGGTACTATTTTCGATTATTGGGGCCAGGGC
 ACCACAGTGACAGTGAGCTCTGCCAGCACAAAGGGCCCTTCCGTGTTCCCACTGGCTCCCTGCTCCAG
 AAGCACATCTGAGTCCACCGCCGCTCTGGGCTGTCTGGTGAAGGACTACTTCCCTGAGCCAGTGACCG
 TGTCTCTGGAACAGCGGCCCTGACATCTGGCGTGACACCTTCCAGCTGTGCTGCAGTCCAGCGGC
 CTGTACTCCCTGTCTTCCGTGGTGACAGTGCCAGCTTCCCTGGGCACCAAGACATATACCTGCAAC
 GTGGACCATAAGCCTTCCAATACCAAGGTGGATAAGAGGGTGGAGAGCAAGTACGGACCACCTTGCC
 CACCATGTCCAGCTCCTGAGTTTGAGGGAGGACCATCCGTGTTCTGTTTCCCTCCAAAGCCTAAGGACA
 CCCTGATGATCAGCCGACACCTGAGGTGACCTGCGTGGTGGTGGACGTGTCTCAGGAGGATCCAGAG
 GTGCAGTTCAACTGGTACGTGGATGGCGTGGAGGTGCACAATGCTAAGACCAAGCCAAGAGAGGAGC
 AGTTTAATTCACATAACCGCGTGGTGAAGCGTGTGACCGTGTGCTGCATCAGGATTGGCTGAACGGCAAG
 GAGTATAAGTGCAAGGTGTCCAATAAGGGCCTGCCAGCTCTATCGAGAAGACAATCAGCAAGGCTA
 AGGGACAGCCTAGGGAGCCACAGGTGTACACCTGCCCCCTTCTCAGGAGGAGATGACAAAGAACCA
 GGTGTCCCTGACCTGTCTGGTGAAGGGCTTCTATCCAAGCGACATCGCTGTGGAGTGGGAGTCTAATG
 GCCAGCCCGAGAACAATTACAAGACCACACCACCCGTGCTGGACTCTGATGGCTCCTTCTTTCTGTATT
 CTAGGCTGACAGTGGATAAGTCCCGGTGGCAGGAGGGCAACGTGTTTAGCTGCTCTGTGATGCACGAG
 GCCCTGCACAATCATTATACCCAGAAGTCCCTGAGCCTGTCTCTGGGCAAG

Amino acid sequence of H6 (SEQ ID NO: 46):

EVQLVESGGGLVQPGGSLRLSCAASGRFTITYAIGWFRQAPGKGRFVSAISWSGSMSTSYADSVKGRFTISR
 DNAKNTLYLQMNSLRPEDTAVYYCAHRGAIPIAQSVYTNWGQGLTVTSSEPKSSDKTHTSPSPSEVQL
 VQSGAEVKKPGSSVKVSKASGYSFTHHWIHWVRQAPGQGLEWMGMIDASDSETRLISQKFKDRVITAD
 KSTSTAYMELSSLRSEDTAVYYCARLGRYYFDYWGQGTITVTVSSASTKGPSVFPLAPCSRSTSESTAALGC
 LVKDYFPEPVTVSWNSGALTSGVHTFPAVLQSSGLYSLSSVTVPSSSLGKTYTCNVDPKPSNTKVDKRV
 ESKYGPPCPPCPAPEFEGGPSVFLFPPKPKDTLMISRTPEVTCVVDVVSQEDPEVQFNWYVDGVEVHNAKT
 KPREEQFNSTYRVSVLTVLHQDWLNGKEYKCKVSNKGLPSSIEKTISKAKGQPREPQVYTLPPSQEEMTK
 NQVSLTCLVKGFYPSDIAVEWESNGQPENNYKTPPVLDSDGSFFLYSRLTVDKSRWQEGNVFSCSVMHE
 ALHNHYTQKSLSLGLGK

DNA sequence of H7 (SEQ ID NO: 47):

GAGGTGCAGCTGGTGCAGTCCGGAGCTGAGGTGAAGAAGCCAGGATCCAGCGTGAAGGTGAGCTGCA
 AGGCTAGCGGCTACTCTTTACCCACCATTTGGATCCACTGGGTGAGGCAGGCTCCTGGACAGGGACTG
 GAGTGGATGGGCATGATCGACGCTTCCGATAGCGAGACAAGACTGTCTCAGAAGTTTAAGGACCGCGT
 GACCATCACAGCCGATAAGTCTACCTCCACAGCTTACATGGAGCTGTCTTCCCTGAGATCCGAGGACA
 CCGCCGTGTACTATTGTGCTAGGCTGGGCCGGTACTATTTTCGATTATTGGGGCCAGGGCACACAGTG
 ACAGTGAGCTCTGCCAGCACAAAGGGCCCTTCCGTGTTCCCACTGGCTCCCTGCTCCAGAAGCACATC
 TGAGTCCACCGCCGCTCTGGGCTGTCTGGTGAAGGACTACTTCCCTGAGCCAGTGACCGTGTCTTGGGA
 ACAGCGGCGCCCTGACATCTGGCGTGACACCTTTCAGCTGTGCTGCAGTCCAGCGGCCTGTACTCCC
 TGTCTTCCGTGGTGCAGTGCCAGCTCTTCCCTGGGCACCAAGACATATACCTGCAACGTGGACCAT
 AAGCCTTCCAATACCAAGGTGGATAAGAGGGTGGAGAGCAAGTACGGACCACCTTGCCACCATGTC
 CAGCTCCTGAGTTTGAGGGAGGACCATCCGTGTTCTGTTTCCCTCCAAAGCCTAAGGACACCCTGATG
 ATCAGCCGGACACCTGAGGTGACCTGCGTGGTGGTGGACGTGTCTCAGGAGGATCCAGAGGTGCAGTT
 CAACTGGTACGTGGATGGCGTGGAGGTGCACAATGCTAAGACCAAGCCAAGAGAGGAGCAGTTTAAT
 TCCACATACCGCGTGGTGAAGCGTGTGACCGTGTGCTGCATCAGGATTGGCTGAACGGCAAGGAGTATAA
 GTGCAAGGTGTCCAATAAGGGCCTGCCAGCTCTATCGAGAAGACAATCAGCAAGGCTAAGGGACAG
 CCTAGGGAGCCACAGGTGTACACCCTGCCCCCTTCTCAGGAGGAGATGACAAAGAACCAGGTGTCCCT
 GACCTGTCTGGTGAAGGGCTTCTATCCAAGCGACATCGCTGTGGAGTGGGAGTCTAATGGCCAGCCCCG
 AGAACAATTACAAGACACACACCCGTGCTGGACTCTGATGGCTCCTTCTTTCTGTATTCTAGGCTGA
 CAGTGGATAAGTCCCGGTGGCAGGAGGGCAACGTGTTTAGCTGCTGTGATGCACGAGGCCCTGCAC
 AATCATTATACCCAGAAGTCCCTGAGCCTGTCTCTGGGCAAGGGTGGAGGCGGTAGTGGAGGCGGTGG
 TTCAGGCGGAGGCGGATCTGAGGTGCAGCTGGTGGAAATCCGGCGGAGGCCTGGTCCAGCCTGGCGGC
 TCTCTGCGGCTGTCTGCGCCGCTTCTGGCAGAACCTTCGTGACCTACGGCATGGGCTGGTTCCGGCAG
 GCTCCTGGCAAGGGCAGAGAGTTCGTGTCCGCCATCTCCTGGTCCGGCTCCATGACCTCTTACGGCGA
 CTCTGTGAAGGGCAGATTACCATCAGCCGGGATAACGCCAAGAACACACTGTACCTGCAGATGAACT

CCCTGCGGCCTGAGGACACCGCCGTGTACTACTGCGCCGCTGCCCTGGGCGCTGTCGTGTACACCACC
AGAGAACCTATACCTACTGGGGACAGGGCACCTGGTGACCGTGTCTCT

Amino acid sequence of H7 (SEQ ID NO: 48):

EVQLVQSGAEVKKPGSSVKVSCKASGYSFTHHWHWVRQAPGQGLEWMGMIDASDSETRLSSQKFKDRVT
ITADKSTSTAYMELSSLRSEDTAVYYCARLGRYYFDYWGQGTITVTVSSASTKGPSVFPLAPCSRSTSESTA
LGCLVKDYFPEPVTWNSGALTSGVHTFPAVLQSSGLYSLSSVTVPSSSLGKTYTCNVDPKPSNTKVD
KRVESKYGPPCPPCPAPEFEGGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSQEDPEVQFNWYVDGVEVHN
AKTKPREEQFNSTYRVVSVLTVLHQDWLNGKEYKCKVSNKGLPSSIEKTISKAKGQPREPQVYTLPPSQEE
MTKNQVSLTCLVKGFYPSDIAVEWESNGQPENNYKTPPVLDSDGSFFLYSRLTVDKSRWQEGNVFSCSV
MHEALHNHYTQKSLSLGKGGGSGGGGSGGGGSEVQLVESGGGLVQPGGSLRLSCAASGRFTVITYGM
GWFRQAPGKGRFVSASISWGSMTSYGDSVKGRFTISRDNKNTLYLQMNSLRPEDTAVYYCAAALGAV
VYTTREPYTYWGQGLTVTVSS

DNA sequence of H8 (SEQ ID NO: 49):

GAGGTGCAGCTGGTGAATCCGGCGGAGGCCTGGTCCAGCCTGGCGGCTCTCTGCGGCTGTCTGCGC
CGCTTCTGGCAGAACCTTCGTGACCTACGGCATGGGCTGGTTCGGCAGGCTCCTGGCAAGGGCAGAG
AGTTCGTGTCCGCCATCTCCTGGTCCGGCTCCATGACCTCTTACGGCGACTCTGTGAAGGGCAGATTCA
CCATCAGCCGGGATAACGCCAAGAACACACTGTACCTGCAGATGAACCTCCCTGCGGCCTGAGGACACC
GCCGTGTACTACTGCGCCGCTGCCCTGGGCGCTGTCGTGTACACCACCAGAGAACCCTATACCTACTG
GGCAGAGGCACCCCTGGTGACCGTGTCTCTGGTGGAGGCGGTAGTGGAGGCGGTGTTTCAGGCGGA
GGCGAGTCTGAGGTGCAGCTGGTGCAGTCCGGAGCTGAGGTGAAGAAGCCAGGATCCAGCGTGAAGG
TGAGCTGCAAGGCTAGCGGCTACTCTTTCACCCACCATTGGATCCACTGGGTGAGGCAGGCTCCTGGA
CAGGGACTGGAGTGGATGGGCATGATCGACGCTTCCGATAGCGAGACAAGACTGTCTCAGAAGTTTA
AGGACCGCGTGACCATCACAGCCGATAAGTCTACCTCCACAGCTTACATGGAGCTGTCTTCCCTGAGA
TCCGAGGACACCGCCGTGTACTATTGTGCTAGGCTGGGCCGCTACTATTTTCGATTATTGGGGCCAGGG
CACCACAGTGACAGTGAGCTCTGCCAGCACAAAGGGCCCTTCCGTGTTCCCACTGGCTCCCTGCTCCA
GAAGCACATCTGAGTCCACCGCCGCTCTGGGCTGTCTGGTGAAGGACTACTTCCCTGAGCCAGTGACC
GTGTCTGGAACAGCGGCGCCCTGACATCTGGCGTGCACACCTTTCAGCTGTGCTGCAGTCCAGCGG
CCTGTACTCCCTGTCTTCCGTGGTGACAGTGCCAGCTCTTCCCTGGGCACCAAGACATATACCTGCAA
CGTGGACCATAAGCCTTCCAATACCAAGGTGGATAAGAGGGGTGGAGAGCAAGTACGGACCACCTTGC
CCACCATGTCCAGCTCCTGAGTTTGAGGGAGGACCATCCGTGTTCTGTTTCTCCAAAGCCTAAGGAC
ACCCTGATGATCAGCCGGACACCTGAGGTGACCTGCGTGGTGGTGGACGTGTCTCAGGAGGATCCAGA
GGTGCAGTTCAACTGGTACGTGGATGGCGTGGAGGTGCACAATGCTAAGACCAAGCCAAGAGAGGAG
CAGTTTAATTCCACATAACCGCTGGTGGTGGAGCTGCTGACCGTGTGCTGCATCAGGATTGGCTGAACGGCA
GGAGTATAAGTGCAAGGTGTCCAATAAGGGCCTGCCAGCTCTATCGAGAAGACAATCAGCAAGGCT
AAGGGACAGCCTAGGGAGCCACAGGTGTACACCTGCCCCCTTCTCAGGAGGAGATGACAAAGAACC
AGGTGTCCCTGACCTGTCTGGTGAAGGGCTTCTATCCAAGCGACATCGCTGTGGAGTGGGAGTCTAAT
GGCCAGCCCAGAGAACAATTACAAGACCACACCACCGTGTGACTCTGATGGCTCCTTCTTTCTGTAT
TCTAGGCTGACAGTGATAAGTCCCGGTGGCAGGAGGGCAACGTGTTTAGCTGCTGTGTATGCACGA
GGCCCTGCACAATCATTATACCCAGAAGTCCCTGAGCCTGTCTCTGGGCAAG

Amino acid sequence of H8 (SEQ ID NO: 50):

EVQLVESGGGLVQPGGSLRLSCAASGRFTVITYGMGWFRQAPGKGRFVSASISWGSMTSYGDSVKGRFTI
SRDNKNTLYLQMNSLRPEDTAVYYCAAALGAVVYTTREPYTYWGQGLTVTVSSGGGSGGGGSGGGG
SEVQLVQSGAEVKKPGSSVKVSCKASGYSFTHHWHWVRQAPGQGLEWMGMIDASDSETRLSSQKFKDRV
TITADKSTSTAYMELSSLRSEDTAVYYCARLGRYYFDYWGQGTITVTVSSASTKGPSVFPLAPCSRSTSESTA
ALGCLVKDYFPEPVTWNSGALTSGVHTFPAVLQSSGLYSLSSVTVPSSSLGKTYTCNVDPKPSNTKVD
DKRVESKYGPPCPPCPAPEFEGGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSQEDPEVQFNWYVDGVEVH
NAKTKPREEQFNSTYRVVSVLTVLHQDWLNGKEYKCKVSNKGLPSSIEKTISKAKGQPREPQVYTLPPSQE
EMTKNQVSLTCLVKGFYPSDIAVEWESNGQPENNYKTPPVLDSDGSFFLYSRLTVDKSRWQEGNVFSCSV
MHEALHNHYTQKSLSLGK

DNA sequence of L1 (SEQ ID NO: 51):

GAGATCGTGCTGACCCAGTCTCCAGCCACACTGTCTCTGTCCCCAGGAGAGAGGGCCACCCTGAGCTG
CCGGGCTTCTGAGAACGTGGGCACATACATCTCCTGGTATCAGCAGAAGCCAGGACAGGCTCCTAGGC

TGCTGATCTACGGCGCTAGCAATAGATATACCGGCATCCCTGCTCGCTTCAGCGGATCTGGATCCGGC
ACAGACTTTACCCTGACAATCTCCAGCCTGGAGCCAGAGGATTTGCGCGTGTACTATTGTGGCGAGTC
CTACGGCCACCTGTATACCTTTGGCGGCGGCACAAAGGTGGAGATCAAGCGAACGGTGGCTGCACCAT
CTGTCTTCATCTTCCCGCCATCTGATGAGCAGTTGAAATCTGGAAGTGCCTCTGTTGTGTGCCTGCTGA
ATAACTTCTATCCCAGAGAGGCCAAAGTACAGTGGAAGGTGGATAACGCCCTCCAATCGGGTAACTCC
CAGGAGAGTGTACAGAGCAGGACAGCAAGGACAGCACCTACAGCCTCAGCAGCACCTGACGCTGA
GCAAAGCAGACTACGAGAAACACAAAGTCTACGCCTGCGAAGTCACCCATCAGGGCCTGAGCTCGCC
CGTCACAAAGAGCTTCAACAGGGGAGAGTGTGAACCTAAGTCTAGCGACAAAACATACACCAGCCCC
CCTAGTCCAGAGGTGCAGCTGGTGGAATCCGGCGGAGGCCTGGTCCAGCCTGGCGGCTCTCTGCGGCT
GTCCTGCGCCGCTTCTGGCAGAACCTTCGTGACCTACGGCATGGGCTGGTTCCGGCAGGCTCCTGGCA
AGGGCAGAGAGTTTCGTGTCCGCCATCTCCTGGTCCGGCTCCATGACCTCTTACGGCGACTCTGTGAAG
GGCAGATTACCATCAGCCGGGATAACGCCAAGAACACACTGTACCTGCAGATGAACTCCCTGCGGCC
TGAGGACACCGCCGTGTACTACTGCGCCGCTGCCCTGGGCGCTGTCGTGTACACCACCAGAGAACCCT
ATACCTACTGGGGACAGGGCACCTGGTGACCGTGTCTCT

Amino acid sequence of L1 (SEQ ID NO: 52):

EIVLTQSPATLSLSPGERATLSCRASENVGTIISWYQQKPGQAPRLLIYGASNRYTGIPARFSGSGSGTDFTL
TISSLEPEDFAVYYCGESYGHL YTFGGGTKVEIKRTVAAPSVFIFPPSDEQLKSGTASVVCLLNNFYPREAKV
QWKVDNALQSGNSQESVTEQDSKSTYLSSTLTLSKADYEKHKVYACEVTHQGLSSPVTKSFNRGECEP
KSSDKTHTSPSPPEVQLVESGGGLVQPGGSLRLSCAASGRTFTVYGMGWFRQAPGKGRFVS AISWSGSMT
SYGDSVKGRFTISRDNKNTLYLQMNSLRPEDTAVYYCAAALGAVVYTTREPYTYWGQGLTVTVSS

DNA sequence of L2 (SEQ ID NO: 53):

GAGATCGTGCTGACCCAGTCTCCAGCCACACTGTCTCTGTCCCCAGGAGAGAGGGCCACCCTGAGCTG
CCGGGCTTCTGAGAACGTGGGCACATACATCTCCTGGTATCAGCAGAAGCCAGGACAGGCTCCTAGGC
TGCTGATCTACGGCGCTAGCAATAGATATACCGGCATCCCTGCTCGCTTCAGCGGATCTGGATCCGGC
ACAGACTTTACCCTGACAATCTCCAGCCTGGAGCCAGAGGATTTGCGCGTGTACTATTGTGGCGAGTC
CTACGGCCACCTGTATACCTTTGGCGGCGGCACAAAGGTGGAGATCAAGCGAACGGTGGCTGCACCAT
CTGTCTTCATCTTCCCGCCATCTGATGAGCAGTTGAAATCTGGAAGTGCCTCTGTTGTGTGCCTGCTGA
ATAACTTCTATCCCAGAGAGGCCAAAGTACAGTGGAAGGTGGATAACGCCCTCCAATCGGGTAACTCC
CAGGAGAGTGTACAGAGCAGGACAGCAAGGACAGCACCTACAGCCTCAGCAGCACCTGACGCTGA
GCAAAGCAGACTACGAGAAACACAAAGTCTACGCCTGCGAAGTCACCCATCAGGGCCTGAGCTCGCC
CGTCACAAAGAGCTTCAACAGGGGAGAGTGTGAACCTAAGTCTAGCGACAAAACATACACCAGCCCC
CCTAGTCCAGAGGTGCAGCTGGTGGAATCCGGCGGAGGCCTGGTCCAGCCTGGCGGCTCTCTGCGGCT
GTCCTGCGCCGCTTCTGGCAGAACCTTCGTGACCTACGGCATGGGCTGGTTCCGGCAGGCTCCTGGCA
AGGGCAGAGAGTTTCGTGTCCGCCATCTCCTGGTCCGGCTCCAGCACCTCTTACGGCGACTCTGTGAAG
GGCAGATTACCATCAGCCGGGATAACGCCAAGAACACACTGTACCTGCAGATGAACTCCCTGCGGCC
TGAGGACACCGCCGTGTACTACTGCGCCGCTGCCCTGGGCGCTGTCGTGTACACCACCAGAGAACCCT
ATACCTACTGGGGACAGGGCACCTGGTGACCGTGTCTCT

Amino acid sequence of L2 (SEQ ID NO: 54):

EIVLTQSPATLSLSPGERATLSCRASENVGTIISWYQQKPGQAPRLLIYGASNRYTGIPARFSGSGSGTDFTL
TISSLEPEDFAVYYCGESYGHL YTFGGGTKVEIKRTVAAPSVFIFPPSDEQLKSGTASVVCLLNNFYPREAKV
QWKVDNALQSGNSQESVTEQDSKSTYLSSTLTLSKADYEKHKVYACEVTHQGLSSPVTKSFNRGECEP
KSSDKTHTSPSPPEVQLVESGGGLVQPGGSLRLSCAASGRTFTVYGMGWFRQAPGKGRFVS AISWSGSST
SYGDSVKGRFTISRDNKNTLYLQMNSLRPEDTAVYYCAAALGAVVYTTREPYTYWGQGLTVTVSS

DNA sequence of L3 (SEQ ID NO: 55):

GAGATCGTGCTGACCCAGTCTCCAGCCACACTGTCTCTGTCCCCAGGAGAGAGGGCCACCCTGAGCTG
CCGGGCTTCTGAGAACGTGGGCACATACATCTCCTGGTATCAGCAGAAGCCAGGACAGGCTCCTAGGC
TGCTGATCTACGGCGCTAGCAATAGATATACCGGCATCCCTGCTCGCTTCAGCGGATCTGGATCCGGC
ACAGACTTTACCCTGACAATCTCCAGCCTGGAGCCAGAGGATTTGCGCGTGTACTATTGTGGCGAGTC
CTACGGCCACCTGTATACCTTTGGCGGCGGCACAAAGGTGGAGATCAAGCGAACGGTGGCTGCACCAT
CTGTCTTCATCTTCCCGCCATCTGATGAGCAGTTGAAATCTGGAAGTGCCTCTGTTGTGTGCCTGCTGA
ATAACTTCTATCCCAGAGAGGCCAAAGTACAGTGGAAGGTGGATAACGCCCTCCAATCGGGTAACTCC
CAGGAGAGTGTACAGAGCAGGACAGCAAGGACAGCACCTACAGCCTCAGCAGCACCTGACGCTGA

GCAAAGCAGACTACGAGAAACACAAAGTCTACGCCTGCGAAGTCACCCATCAGGGCCTGAGCTCGCC
CGTCACAAAGAGCTTCAACAGGGGAGAGTGTGAACCTAAGTCTAGCGACAAAACCTCATACCAGCCCC
CCTAGTCCAGAGGTGCAGCTGGTGGAATCCGGCGGAGGCCTGGTGCAGCCTGGCGGCTCTCTGAGACT
GTCCTGCGCCGCTTCTGGCCGGACCTTCATCACCTACGCCATCGGCTGGTTCAGACAGGCCCCCTGGCAA
GGGACAGAGAGTTCGTGTCCGCCATCTCCTGGTCCGGCTCTATGACCAGCTACGCCGACTCTGTGAAGG
GCAGATTACCATCTCCCGGGATAACGCCAAGAACACCCTGTACCTGCAGATGAATCCCTGAGACCT
GAGGACACAGCTGTGTATTACTGCGCCGCTACCGGGGCGCCATCGCTCCCATCGCTCAGAGCGTGTA
CACCAACTGGGGCCAGGGAACCCTGGTCACCGTGTCCAGC

Amino acid sequence of L3 (SEQ ID NO: 56):

EIVLTQSPATLSLSPGERATLSCRAENVGTIYISWYQQKPGQAPRLLIYGASNRYTGIPARFSGSGSGTDFTL
TISSLEPEDFAVYYCGESYGHLYTFGGGTKVEIKRTVAAPSVFIFPPSDEQLKSGTASVVCLLNNFYPREAKV
QWKVDNALQSGNSQESVTEQDSKDYSLSTLTLISKADYEKHKVYACEVTHQGLSSPVTKSFNRGECEP
KSSDKTHTSPSPVEQLVESGGGLVQPGGSLRLSCAASGRTFITYAIGWFRQAPGKGRFVSAISWSGSMST
YADSVKGRFTISRDNAKNTLYLQMNSLRPEDTAVYYCAAHRGAIPIAQSVYTNWGQGTLLVTVSS

DNA sequence of L4 (SEQ ID NO: 57):

GAGGTGCAGCTGGTGGAATCCGGCGGAGGCCTGGTCCAGCCTGGCGGCTCTCTGCGGCTGTCTTGCGC
CGCTTCTGGCAGAACCTTCGTGACCTACGGCATGGGCTGGTTCGGCAGGCTCCTGGCAAGGGCAGAG
AGTTCGTGTCCGCCATCTCCTGGTCCGGCTCCATGACCTCTTACGGCGACTCTGTGAAGGGCAGATTCA
CCATCAGCCGGGATAACGCCAAGAACAACACTGTACCTGCAGATGAACTCCCTGCGGCCTGAGGACACC
GCCGTGTACTACTGCGCCGCTGCCCTGGGCGCTGTCTGTGTACACCACCAGAGAACCTATACCTACTG
GGGACAGGGCACCCCTGGTGACCGTGTCTCTGAACCTAAGTCTAGCGACAAAACCTCATACCAGCCCCC
CTAGTCCAGAGATCGTGCTGACCCAGTCTCCAGCCACACTGTCTCTGTCCCCAGGAGAGAGGGCCACC
CTGAGCTGCCGGGCTTCTGAGAACGTGGGCACATACATCTCCTGGTATCAGCAGAAGCCAGGACAGGC
TCCTAGGCTGCTGATCTACGGCGCTAGCAATAGATATACCGGCATCCCTGCTCGCTTCAGCGGATCTGG
ATCCGGCACAGACTTTACCCTGACAATCTCCAGCCTGGAGCCAGAGGATTTGCGCGTGTACTATTGTG
GCGAGTCCTACGGCCACCTGTATACCTTTGGCGGCGGCACAAAGGTGGAGATCAAGCGAACGGTGGCT
GCACCATCTGTCTTCATCTTCCCGCCATCTGATGAGCAGTTGAAATCTGGAAGTGCCTCTGTTGTGTGC
CTGCTGAATAAATTCTATCCCAGAGAGGCCAAAAGTACAGTGGAAGGTGGATAACGCCCTCCAATCGGG
TAACTCCCAGGAGAGTGTACAGAGCAGGACAGCAAGGACAGCACCTACAGCCTCAGCAGCACCCCTG
ACGCTGAGCAAAGCAGACTACGAGAAACACAAAGTCTACGCCTGCGAAGTCACCCATCAGGGCCTGA
GCTCGCCCGTCACAAAGAGCTTCAACAGGGGAGAGTGT

Amino acid sequence of L4 (SEQ ID NO: 58):

EVQLVESGGGLVQPGGSLRLSCAASGRTFVTYGMGWFRQAPGKGRFVSAISWSGSMSTSYGDSVKGRFTI
SRDNAKNTLYLQMNSLRPEDTAVYYCAAALGAVVYTTREPYTYWGQGTLLVTVSSPEKSSDKTHTSPSP
VLTQSPATLSLSPGERATLSCRAENVGTIYISWYQQKPGQAPRLLIYGASNRYTGIPARFSGSGSGTDFTLTI
SSLEPEDFAVYYCGESYGHLYTFGGGTKVEIKRTVAAPSVFIFPPSDEQLKSGTASVVCLLNNFYPREAKVQ
WKVDNALQSGNSQESVTEQDSKDYSLSTLTLISKADYEKHKVYACEVTHQGLSSPVTKSFNRGEC

DNA sequence of L5 (SEQ ID NO: 59):

GAGGTGCAGCTGGTGGAATCCGGCGGAGGCCTGGTCCAGCCTGGCGGCTCTCTGCGGCTGTCTTGCGC
CGCTTCTGGCAGAACCTTCGTGACCTACGGCATGGGCTGGTTCGGCAGGCTCCTGGCAAGGGCAGAG
AGTTCGTGTCCGCCATCTCCTGGTCCGGCTCCAGCACCTCTTACGGCGACTCTGTGAAGGGCAGATTCA
CCATCAGCCGGGATAACGCCAAGAACAACACTGTACCTGCAGATGAACTCCCTGCGGCCTGAGGACACC
GCCGTGTACTACTGCGCCGCTGCCCTGGGCGCTGTCTGTGTACACCACCAGAGAACCTATACCTACTG
GGGACAGGGCACCCCTGGTGACCGTGTCTCTGAACCTAAGTCTAGCGACAAAACCTCATACCAGCCCCC
CTAGTCCAGAGATCGTGCTGACCCAGTCTCCAGCCACACTGTCTCTGTCCCCAGGAGAGAGGGCCACC
CTGAGCTGCCGGGCTTCTGAGAACGTGGGCACATACATCTCCTGGTATCAGCAGAAGCCAGGACAGGC
TCCTAGGCTGCTGATCTACGGCGCTAGCAATAGATATACCGGCATCCCTGCTCGCTTCAGCGGATCTGG
ATCCGGCACAGACTTTACCCTGACAATCTCCAGCCTGGAGCCAGAGGATTTGCGCGTGTACTATTGTG
GCGAGTCCTACGGCCACCTGTATACCTTTGGCGGCGGCACAAAGGTGGAGATCAAGCGAACGGTGGCT
GCACCATCTGTCTTCATCTTCCCGCCATCTGATGAGCAGTTGAAATCTGGAAGTGCCTCTGTTGTGTGC
CTGCTGAATAAATTCTATCCCAGAGAGGCCAAAAGTACAGTGGAAGGTGGATAACGCCCTCCAATCGGG
TAACTCCCAGGAGAGTGTACAGAGCAGGACAGCAAGGACAGCACCTACAGCCTCAGCAGCACCCCTG

ACGCTGAGCAAAGCAGACTACGAGAAACACAAAGTCTACGCCTGCGAAGTCACCCATCAGGGCCTGA
GCTCGCCCGTCACAAAGAGCTTCAACAGGGGAGAGTGT

Amino acid sequence of L5 (SEQ ID NO: 60):

EVQLVESGGGLVQPGGSLRLSCAASGRFTVITYGMGWFRQAPGKGRFVS AISWSGSSTSYGDSVKGRFTIS
RDNAKNTLYLQMNSLRPEDTAVYYCAAALGAVVYTTREPYTYWGQGLTVTSSEPKSSDKTHTSPSPSEI
VLTSQSPATLSLSPGERATLSCRASENVGTIISWYQQKPGQAPRLLIYGASNRYTGIPARFSGSGSGTDFTLTI
SSLEPEDFAVYYCGESYGHLTYTFGGGTKVEIKRTVAAPSVFIFPPSDEQLKSGTASVVCLLNNFYPREAKVQ
WKVDNALQSGNSQESVTEQDSKDYSLSTLTLSKADYEKHKVYACEVTHQGLSSPVTKSFNRGEC

DNA sequence of L6 (SEQ ID NO: 61):

GAGGTGCAGCTGGTGAATCCGGCGGAGGCCTGGTGCAGCCTGGCGGCTCTCTGAGACTGTCCTGCGC
CGCTTCTGGCCGGACCTTCATCACCTACGCCATCGGCTGGTTCAGACAGGCCCTGGCAAGGGCAGAG
AGTTCGTGTCCGCCATCTCCTGGTCCGGCTCTATGACCAGCTACGCCGACTCTGTGAAGGGCAGATTCA
CCATCTCCCGGGATAACGCCAAGAACACCTGTACCTGCAGATGAATTCCCTGAGACCTGAGGACACA
GCTGTGTATTACTGCGCCGCTCACCGGGGCGCCATCGCTCCCATCGCTCAGAGCGTGTACACCAACTG
GGGCCAGGGAACCCTGGTCACCGTGTCCAGCGAACCTAAGTCTAGCGACAAAATCATACCAGCCCCC
CTAGTCCAGAGATCGTGCTGACCCAGTCTCCAGCCACACTGTCTCTGTCCCCAGGAGAGAGGGCCACC
CTGAGCTGCCGGGCTTCTGAGAACGTGGGCACATACATCTCCTGGTATCAGCAGAAGCCAGGACAGGC
TCCTAGGCTGTGATCTACGGCGCTAGCAATAGATATACCGGCATCCCTGCTCGCTTCAGCGGATCTGG
ATCCGGCAGACAGATTTACCTTGACAATCTCCAGCCTGGAGCCAGAGGATTTCGCCGTGTACTATTGTG
GCGAGTCTACGGCCACCTGTATACCTTTGGCGGCGGCACAAAGGTGGAGATCAAGCGAACCGGTGGCT
GCACCATCTGTCTTCATCTTCCCGCCATCTGATGAGCAGTTGAAATCTGGAAGTGCCTCTGTTGTGTGC
CTGCTGAATAAATTCTATCCCAGAGAGGGCCAAAGTACAGTGGAAGGTGGATAACGCCCTCCAATCGGG
TAACTCCCAGGAGAGTGTACAGAGCAGGACAGCAAGGACAGCACCTACAGCCTCAGCAGCACCTG
ACGCTGAGCAAAGCAGACTACGAGAAACACAAAGTCTACGCCTGCGAAGTCACCCATCAGGGCCTGA
GCTCGCCCGTCACAAAGAGCTTCAACAGGGGAGAGTGT

Amino acid sequence of L6 (SEQ ID NO: 62):

EVQLVESGGGLVQPGGSLRLSCAASGRFTIFYAIGWFRQAPGKGRFVS AISWSGSMTSYADSVKGRFTISR
DNAKNTLYLQMNSLRPEDTAVYYCAAHRGAIAPIAQS VYTNWGQGLTVTSSEPKSSDKTHTSPSPSEIVL
TQSPATLSLSPGERATLSCRASENVGTIISWYQQKPGQAPRLLIYGASNRYTGIPARFSGSGSGTDFTLTISSL
EPEDFAVYYCGESYGHLTYTFGGGTKVEIKRTVAAPSVFIFPPSDEQLKSGTASVVCLLNNFYPREAKVQWK
VDNALQSGNSQESVTEQDSKDYSLSTLTLSKADYEKHKVYACEVTHQGLSSPVTKSFNRGEC

DNA sequence of L7 (SEQ ID NO: 63):

GAGATCGTGCTGACCCAGTCTCCAGCCACACTGTCTCTGTCCCCAGGAGAGAGGGCCACCCTGAGCTG
CCGGGCTTCTGAGAACGTGGGCACATACATCTCCTGGTATCAGCAGAAGCCAGGACAGGCTCCTAGGC
TGCTGATCTACGGCGCTAGCAATAGATATACCGGCATCCCTGCTCGCTTCAGCGGATCTGGATCCGGC
ACAGACTTTACCCTGACAATCTCCAGCCTGGAGCCAGAGGATTTCCCGGTGTACTATTGTGGCGAGTC
CTACGGCCACCTGTATACCTTTGGCGGCGGCACAAAGGTGGAGATCAAGCGAACCGGTGGCTGCACCAT
CTGTCTTCATCTTCCCGCCATCTGATGAGCAGTTGAAATCTGGAAGTGCCTCTGTTGTGTGCCTGCTGA
ATAACTTCTATCCCAGAGAGGCCAAAGTACAGTGGAAGGTGGATAACGCCCTCCAATCGGGTAACTCC
CAGGAGAGTGTACAGAGCAGGACAGCAAGGACAGCACCTACAGCCTCAGCAGCACCTGACGCTGA
GCAAAGCAGACTACGAGAAACACAAAGTCTACGCCTGCGAAGTCACCCATCAGGGCCTGAGCTCGCC
CGTCACAAAGAGCTTCAACAGGGGAGAGTGTGGTGGAGGCGGTAGTGGAGGCGGTGGTTACAGGCGGA
GGCGGATCTGAGGTGCAGCTGGTGAATCCGGCGGAGGCCTGGTCCAGCCTGGCGGCTCTCTGCGGCT
GTCCTGCGCCGCTTCTGGCAGAACCTTCGTGACCTACGGCATGGGCTGGTTCGGCAGGCTCCTGGCA
AGGGCAGAGAGTTCTGTCCGCCATCTCCTGGTCCGGCTCCATGACCTCTTACGGCGACTCTGTGAAG
GGCAGATTACCATCAGCCGGGATAACGCCAAGAACACACTGTACCTGCAGATGAACCTCCTGCGGCC
TGAGGACACCGCGTGTACTACTGCGCCGCTGCCCTGGGCGCTGTCGTGTACACCACAGAGAACCT
ATACCTACTGGGGACAGGGCACCTGGTGACCGTGTCTCT

Amino acid sequence of L7 (SEQ ID NO: 64):

EIVLTQSPATLSLSPGERATLSCRASENVGTIISWYQQKPGQAPRLLIYGASNRYTGIPARFSGSGSGTDFTL
TISSLEPEDFAVYYCGESYGHLTYTFGGGTKVEIKRTVAAPSVFIFPPSDEQLKSGTASVVCLLNNFYPREAKV

QWKVDNALQSGNSQESVTEQDSKDYSLSSLTSLSKADYKHKVYACEVTHQGLSSPVTKSFNRGECGG
GGSGGGGSGGGGSEVQLVESGGGLVQPGGSLRLSAAASGRFTVITYGMGWFRQAPGKGRFVSASISWSGS
MTSYGDSVKGRFTISRDNANKNTLYLQMNSLRPEDTAVYYCAAALGAVVYTTREPYTYWGQGLTVTVSS

DNA sequence of L8 (SEQ ID NO: 65):

GAGGTGCAGCTGGTGGGAATCCGGCGGAGGCCTGGTCCAGCCTGGCGGCTCTCTGCGGCTGTCTGCGC
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AGTTCGTGTCCGCCATCTCCTGGTCCGGCTCCATGACCTCTTACGGCGACTCTGTGAAGGGCAGATTCA
CCATCAGCCGGGATAACGCCAAGAACACACTGTACCTGCAGATGAACTCCCTGCGGCCTGAGGACACC
GCCGTGTACTACTGCGCCGCTGCCCTGGGCGCTGTCGTGTACACCACCAGAGAACCCTATACCTACTG
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CCTGAGCTGCCGGGCTTCTGAGAACGTGGGCACATACATCTCCTGGTATCAGCAGAAGCCAGGACAGG
CTCCTAGGCTGCTGATCTACGGCGCTAGCAATAGATATACCGGCATCCCTGCTCGCTTCAGCGGATCTG
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GGCGAGTCCTACGGCCACCTGTATACCTTTGGCGGCGGCACAAAGGTGGAGATCAAGCGAACGGTGG
CTGCACCATCTGTCTTCATCTTCCCGCCATCTGATGAGCAGTTGAAATCTGGAAGTGCCTCTGTTGTGT
GCCTGCTGAATAACTTCTATCCCAGAGAGGCCAAAGTACAGTGGAAAGGTGGATAACGCCCTCCAATCG
GGTAACTCCCAGGAGAGTGTACAGAGCAGGACAGCAAGGACAGCACCTACAGCCTCAGCAGCACCC
TGACGCTGAGCAAAGCAGACTACGAGAAACACAAAGTCTACGCCTGCGAAGTCACCCATCAGGGCCT
GAGCTCGCCCGTCACAAAGAGCTTCAACAGGGGAGAGTGT

Amino acid sequence of L8 (SEQ ID NO: 66):

EVQLVESGGGLVQPGGSLRLSAAASGRFTVITYGMGWFRQAPGKGRFVSASISWSGSMTSYGDSVKGRFTI
SRDNANKNTLYLQMNSLRPEDTAVYYCAAALGAVVYTTREPYTYWGQGLTVTVSSGGGGGSGGGGSGGGG
SEIVLTQSPATLSLSPGERATLSCRASENVGTIYISWYQKPGQAPRLLIYGASNRYTGIPARFSGSGSDFT
LTISSLEPEDFAVYYCGESYGHLYTFGGGTKVEIKRTVAAPSVFIFPPSDEQLKSGTASVVCLLNNFYPREAK
VQWKVDNALQSGNSQESVTEQDSKDYSLSSLTSLSKADYKHKVYACEVTHQGLSSPVTKSFNRGEC

DNA sequence of L9 (SEQ ID NO: 67):

Gagatcgtgctgaccagctccagccacactgtctctgtccccaggagagaggccacccctgagctgccgggctctgagaacgtgggcacatacatctcctggtatc
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gctgtcgtgtacaccaccagagaacctatactactggggacaggccacctggtgacctgtcctct

Amino acid sequence of L9 (SEQ ID NO: 68):

EIVLTQSPATLSLSPGERATLSCRASENVGTIYISWYQKPGQAPRLLIYGASNRYTGIPARFSGSGS
GTDFTLTISSLEPEDFAVYYCGESYGHLYTFGGGTKVEIKRTVAAPSVFIFPPSDEQLKSGTASVV
CLLNNFYPREAKVQWKVDNALQSGNSQESVTEQDSKDYSLSSLTSLSKADYKHKVYACEV
THQGLSSPVTKSFNRGECESKYGPPSPSPSEVQLVESGGGLVQPGGSLRLSAAASGRFTVITYGMG
WFRQAPGKGRFVSASISWSGSMTSYGDSVKGRFTISRDNANKNTLYLQMNSLRPEDTAVYYCAA
ALGAVVYTTREPYTYWGQGLTVTVSS

Amino acid sequence of E4-linker (SEQ ID NO: 69):

ESKYGPPSPSP

CLAIMS

What is claimed is:

1. An isolated anti-CD47/anti-PD-L1 multiple antigen binding protein or antigen binding fragment thereof comprising:
 - (a) a first antigen binding portion comprising a heavy chain variable domain (V_H) and a light chain variable domain (V_L), wherein the V_H and V_L together form an antigen-binding site that specifically binds CD47, and wherein the V_H comprises a heavy chain complementarity determining region 1 (HCDR1), HCDR2, and HCDR3 comprising the amino acid sequences of SEQ ID NO:19, SEQ ID NO:20, and SEQ ID NO:21, respectively, and the V_L comprises a light chain complementarity determining region 1 (LCDR1), LCDR2, and LCDR3 comprising the amino acid sequences of SEQ ID NO:22, SEQ ID NO:23, and SEQ ID NO:24, respectively; and
 - (b) a second antigen binding portion comprising a single-domain antibody that specifically binds PD-L1;
wherein the first antigen binding portion and the second antigen binding portion are fused to each other.
2. The isolated anti-CD47/anti-PD-L1 multiple antigen binding protein or antigen binding fragment thereof of claim 1, wherein the single-domain antibody comprises a complementarity determining region 1 (CDR1), CDR2, and CDR3 comprising the amino acid sequences of:
 - (i) SEQ ID NO:25, SEQ ID NO:26, and SEQ ID NO:27, respectively;
 - (ii) SEQ ID NO:28, SEQ ID NO:29, and SEQ ID NO:30, respectively; or
 - (iii) SEQ ID NO:31, SEQ ID NO:32, and SEQ ID NO:33, respectively.
3. The isolated anti-CD47/anti-PD-L1 multiple antigen binding protein or antigen binding fragment thereof of claim 1 or 2, wherein the first antigen binding portion is a full-length antibody comprising two heavy chains and two light chains.
4. The isolated anti-CD47/anti-PD-L1 multiple antigen binding protein or antigen binding fragment thereof of any one of claims 1 to 3, wherein the first antigen binding portion is an antibody fragment comprising a heavy chain comprising the V_H and a light chain comprising the V_L .
5. The isolated anti-CD47/anti-PD-L1 multiple antigen binding protein or antigen binding fragment thereof of any one of claims 1 to 4, wherein the second antigen binding portion comprises a single polypeptide chain.
6. The isolated anti-CD47/anti-PD-L1 multiple antigen binding protein or antigen binding fragment thereof of claim 5, wherein the carboxy (C)-terminus of the second antigen binding portion is fused to the amino (N)-terminus of at least one heavy chain of the first antigen binding portion.

7. The isolated anti-CD47/anti-PD-L1 multiple antigen binding protein or antigen binding fragment thereof of claim 5, wherein the carboxy (C)-terminus of the second antigen binding portion is fused to the amino (N)-terminus of at least one light chain of the first antigen binding portion.
8. The isolated anti-CD47/anti-PD-L1 multiple antigen binding protein or antigen binding fragment thereof of claim 5, wherein the amino (N)-terminus of the second antigen binding portion is fused to the carboxy (C)-terminus of at least one heavy chain of the first antigen binding portion.
9. The isolated anti-CD47/anti-PD-L1 multiple antigen binding protein or antigen binding fragment thereof of claim 5, wherein the amino (N)-terminus of the second antigen binding portion is fused to the carboxy (C)-terminus of at least one light chain of the first antigen binding portion.
10. The isolated anti-CD47/anti-PD-L1 multiple antigen binding protein or antigen binding fragment thereof of any one of claims 1 to 9, wherein the heavy chain of the first antigen binding portion comprises an amino acid sequence at least 95% identical to SEQ ID NO:4, and the light chain of the first antigen binding portion comprises an amino acid sequence at least 95% identical to SEQ ID NO:6.
11. The isolated anti-CD47/anti-PD-L1 multiple antigen binding protein or antigen binding fragment thereof of claim 10, wherein the heavy chain of the first antigen binding portion comprises the amino acid sequence of SEQ ID NO:4, and the light chain of the first antigen portion comprises the amino acid sequence of SEQ ID NO:6.
12. The isolated anti-CD47/anti-PD-L1 multiple antigen binding protein or antigen binding fragment thereof of any one of claims 1 to 11, wherein the second antigen binding portion comprises an amino acid sequence at least 95% identical to an amino acid sequence selected from SEQ ID NO:14, SEQ ID NO:16, or SEQ ID NO:18.
13. The isolated anti-CD47/anti-PD-L1 multiple antigen binding protein or antigen binding fragment thereof of claim 12, wherein the second antigen binding portion comprises the amino acid sequence selected from SEQ ID NO:14, SEQ ID NO:16, or SEQ ID NO:18.
14. The isolated anti-CD47/anti-PD-L1 multiple antigen binding protein or antigen binding fragment thereof of any one of claims 1 to 13, wherein the first antigen binding portion comprises a human, humanized, or chimeric antibody or antigen binding fragment thereof.
15. The isolated anti-CD47/anti-PD-L1 multiple antigen binding protein or antigen binding fragment thereof of any one of claims 1 to 14, wherein the second antigen binding portion comprising a single-domain antibody that specifically binds PD-L1 is camelid, chimeric, human, partially humanized, or fully humanized.

16. The isolated anti-CD47/anti-PD-L1 multiple antigen binding protein or antigen binding fragment thereof of any one of claims 1 to 15, wherein the first antigen binding portion and the second antigen binding portion are fused to each other via a peptide bond or a peptide linker.
17. The isolated anti-CD47/anti-PD-L1 multiple antigen binding protein or antigen binding fragment thereof of claim 16, wherein the linker is GS linker or a mutated human IgG1 hinge.
18. The isolated anti-CD47/anti-PD-L1 multiple antigen binding protein or antigen binding fragment thereof of claim 17, wherein the peptide linker comprises an amino acid sequence selected from SEQ ID NO:8, SEQ ID NO:10, or SEQ ID NO:69.
19. The isolated anti-CD47/anti-PD-L1 multiple antigen binding protein or antigen binding fragment thereof of any one of claims 1 to 18, comprising an anti-CD47 full-length antibody and an anti-PD-L1 single-domain antibody, wherein:
 - (a) the N-terminus of the anti-PD-L1 sdAb is fused to the C-terminus of both heavy chains of the anti-CD47 full-length antibody, and wherein the heavy chain fusion polypeptide comprises the amino acid sequence of SEQ ID NOs: 36, 38, 40, or 48 and the light chain polypeptide comprises the amino acid sequence of SEQ ID NO:6;
 - (b) the C-terminus of the anti-PD-L1 sdAb is fused to the N-terminus of both heavy chains of the anti-CD47 full-length antibody, and wherein the heavy chain fusion polypeptide comprises the amino acid sequence of SEQ ID NOs: 42, 44, 46, or 50 and the light chain polypeptide comprises the amino acid sequence of SEQ ID NO:6;
 - (c) the N-terminus of the anti-PD-L1 sdAb is fused to the C-terminus of both light chains of the anti-CD47 full-length antibody, and wherein the light chain fusion polypeptide comprises the amino acid sequence of SEQ ID NOs: 52, 54, 56, 64, or 68 and the heavy chain polypeptide comprises the amino acid sequence of SEQ ID NO:4; or
 - (d) the C-terminus of the anti-PD-L1 sdAb is fused to the N-terminus of both light chains of the anti-CD47 full-length antibody, and wherein the light chain fusion polypeptide comprises the amino acid sequence of SEQ ID NOs: 58, 60, 62, or 66 and the heavy chain polypeptide comprises the amino acid sequence of SEQ ID NO:4.
20. An isolated nucleic acid encoding the isolated anti-CD47/anti-PD-L1 multiple antigen binding protein or antigen binding fragment thereof of any one of claims 1 to 19.
21. An isolated vector comprising the isolated nucleic acid of claim 20.
22. A host cell comprising the isolated vector of claim 21.
23. A pharmaceutical composition comprising the isolated anti-CD47/anti-PD-L1 multiple antigen binding protein or antigen binding fragment thereof of any one of claims 1 to 19, and a pharmaceutical acceptable carrier.

24. A method of treating a subject having or at risk of having a CD47 and/or PD-L1-related disease, the method comprising administering to the subject an effective amount of the pharmaceutical composition of claim 23.
25. The method of claim 24, wherein the CD47 and/or PD-L1 related disease is cancer.
26. The method of claim 25, wherein the cancer is a solid tumor.
27. The method of claim 25 or 26, wherein the cancer is a colon cancer.
28. The method of claim 25, wherein the cancer is a hematological cancer.
29. The method of claim 25 or 28, wherein the hematological cancer is leukemia, lymphoma, or myeloma.
30. The method of claim 29, wherein the leukemia is selected from the group consisting of acute lymphocytic leukemia (ALL), acute myeloid leukemia (AML), chronic lymphocytic leukemia (CLL), chronic myelogenous leukemia (CML), myeloproliferative disorder/neoplasm (MPDS), and myelodysplasia syndrome.
31. The method of claim 29, wherein the lymphoma is selected from the group consisting of a Hodgkin's lymphoma, both indolent and aggressive non-Hodgkin's lymphoma, Burkitt's lymphoma, and follicular lymphoma (small cell and large cell).
32. The method of claim 29, wherein the myeloma is selected from the group consisting of multiple myeloma (MM), giant cell myeloma, heavy-chain myeloma, and light chain or Bence-Jones myeloma.
33. The method of any one of claims 24-32, further comprising administering to the individual an additional cancer therapy.
34. The method of claim 33, wherein the additional cancer therapy is surgery, radiation, chemotherapy, immunotherapy, hormone therapy, or a combination thereof.
35. The method of claim 24, wherein the PD-L1 related disease is a pathogenic infection.
36. The method of any one of claims 24-35, wherein the pharmaceutical composition is administered systemically or locally.
37. The method of claim 36, wherein the pharmaceutical composition is administered intravenously.
38. The method of claim 36, wherein the pharmaceutical composition is administered intratumorally.
39. The method of any one of claims 24-38, wherein the subject is a human.

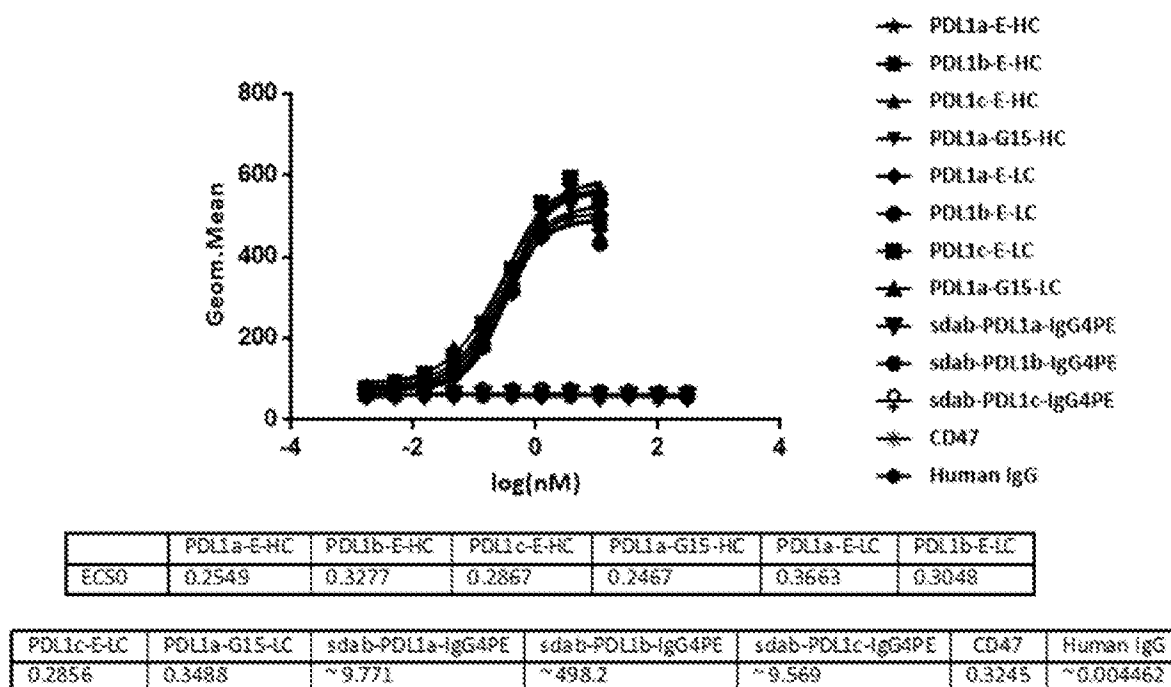


FIG. 1

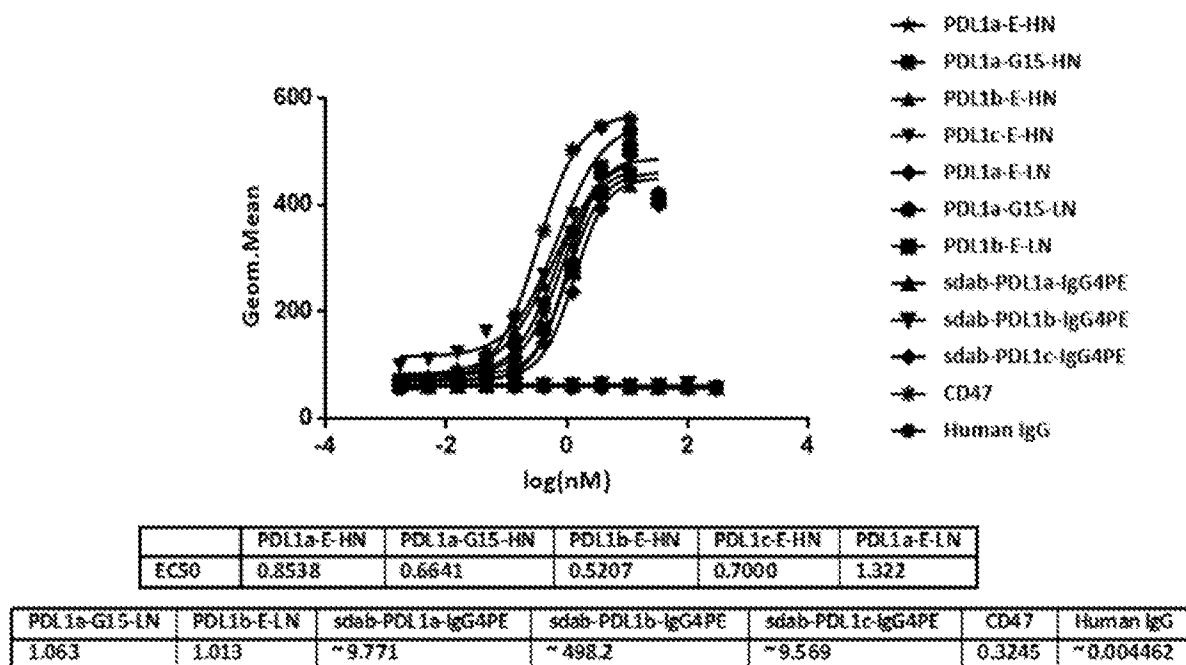


FIG. 2

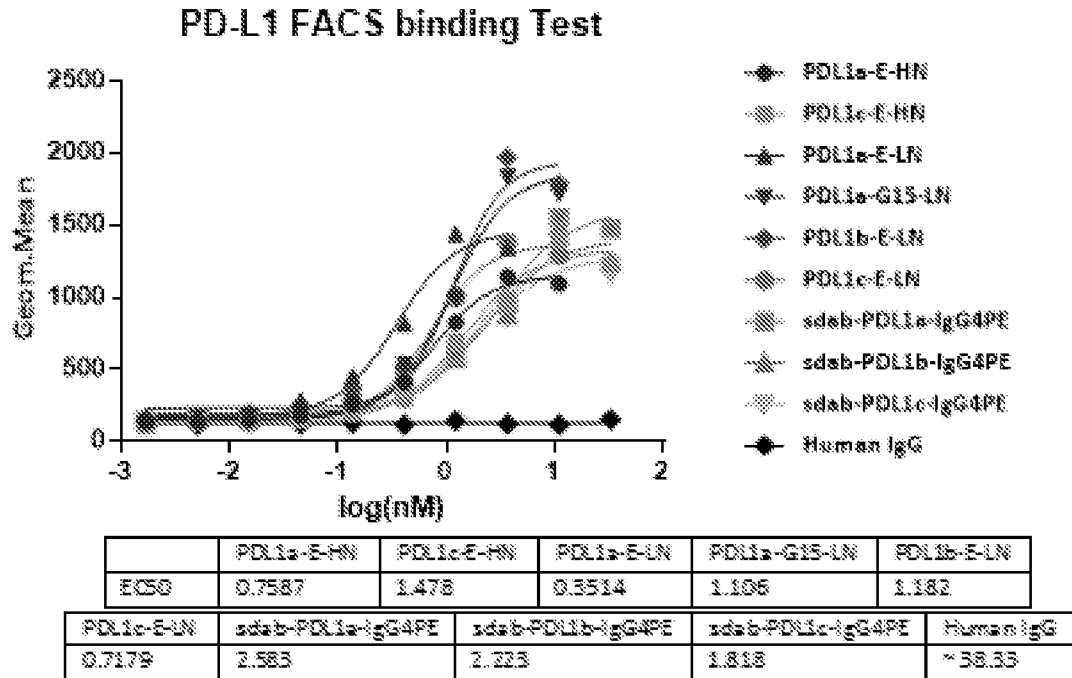


FIG. 3

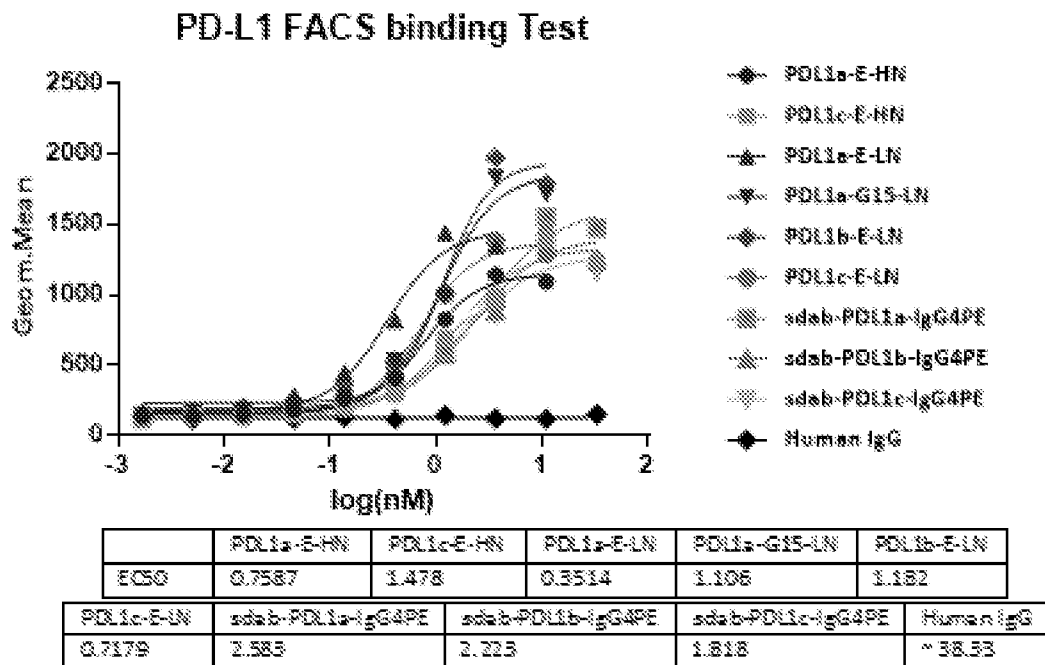


FIG. 4

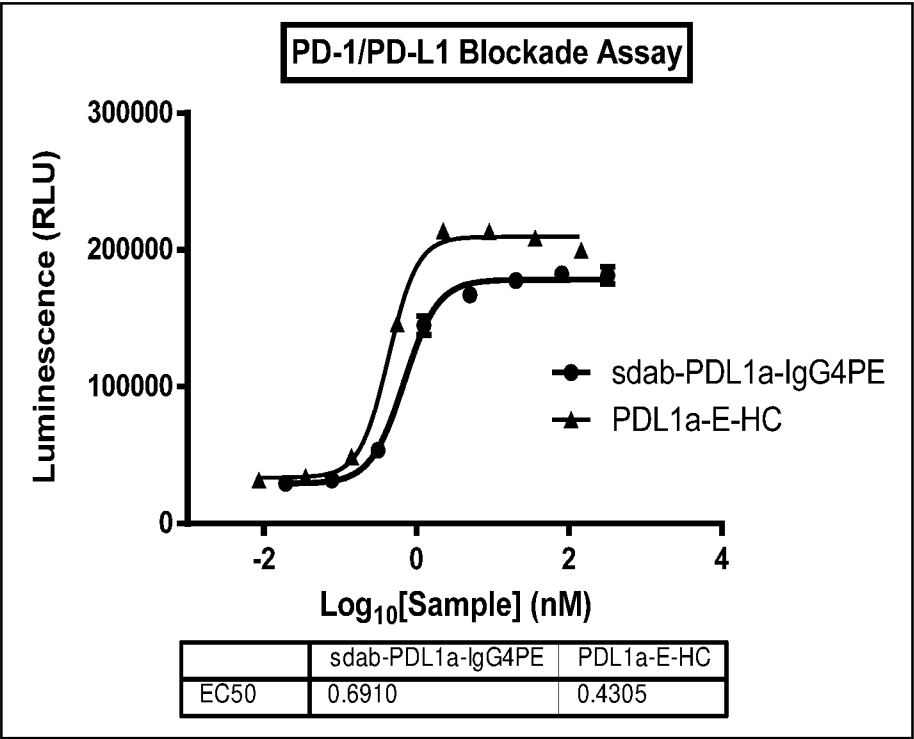


FIG. 5

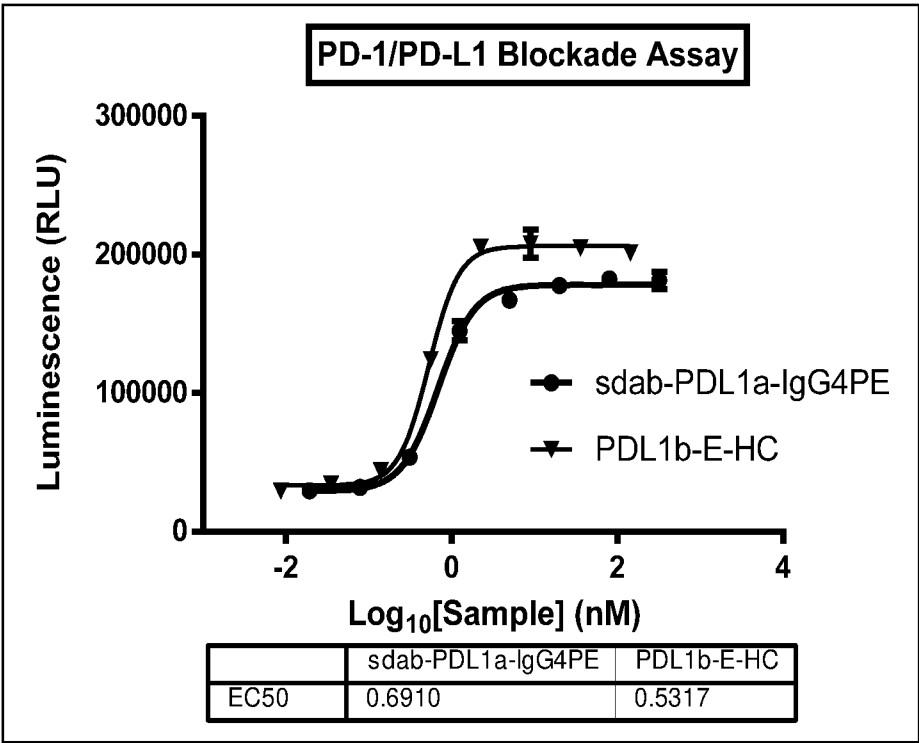


FIG. 6

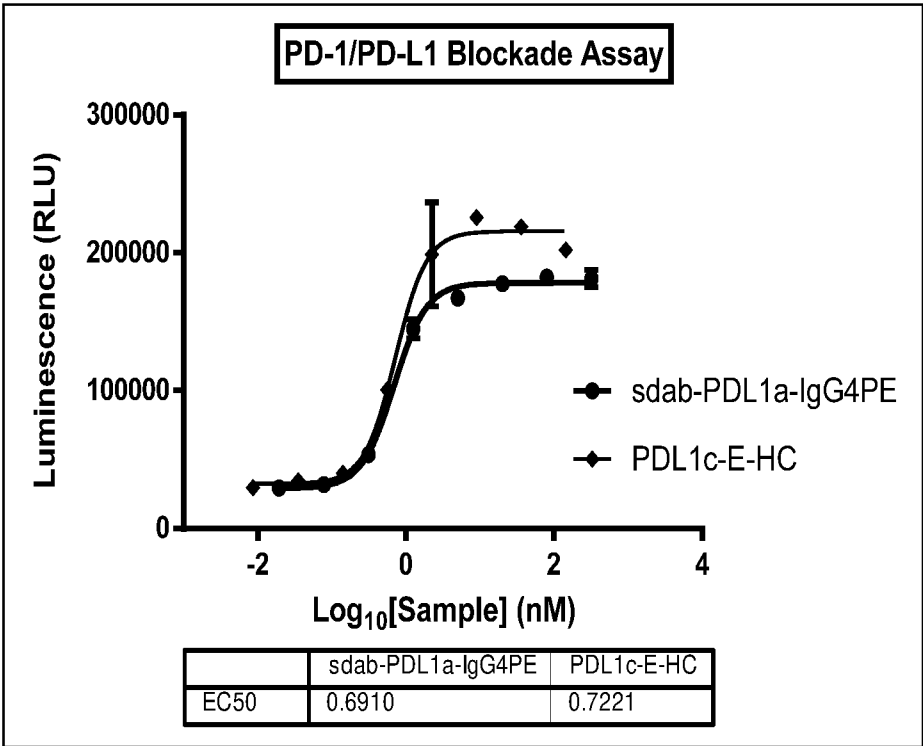


FIG. 7

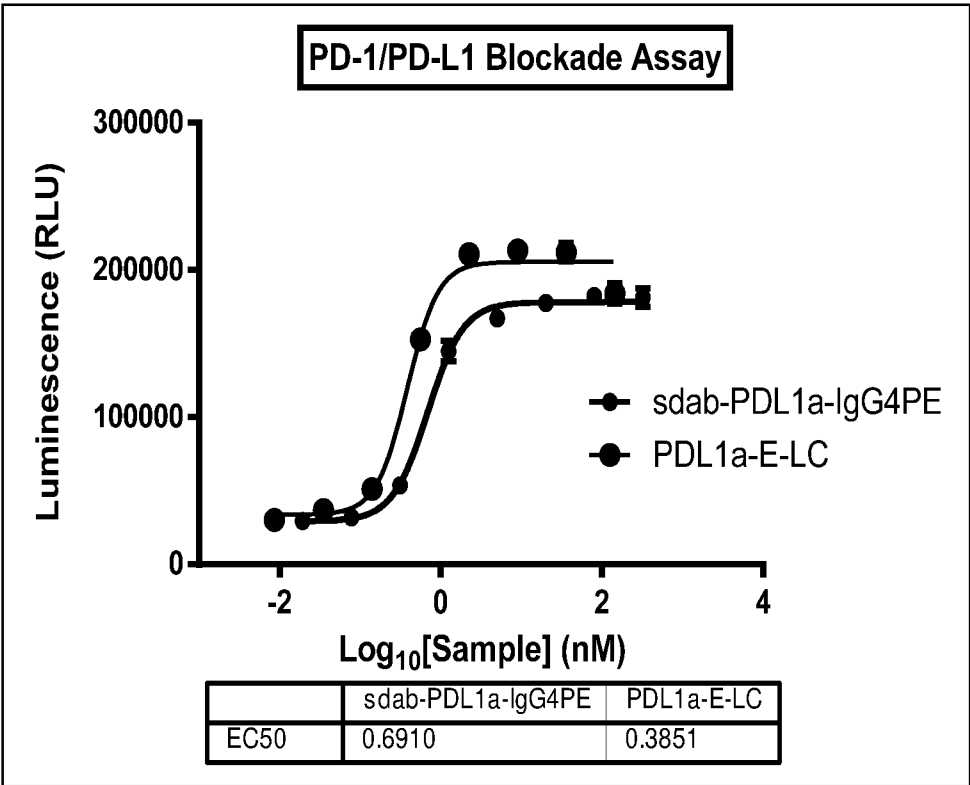


FIG. 8

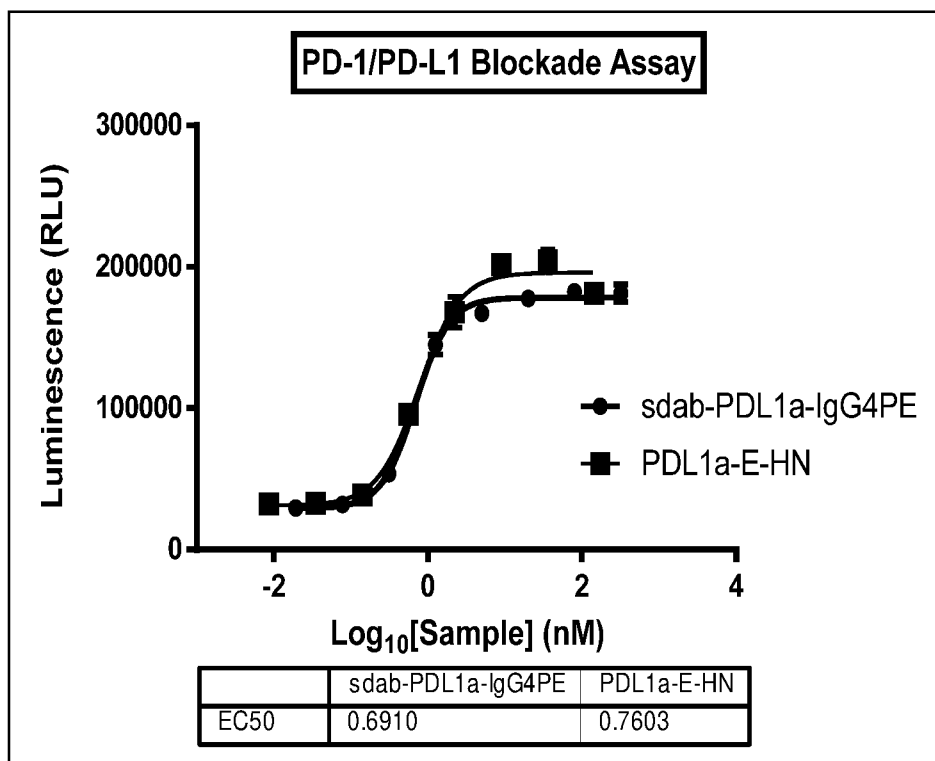


FIG. 9

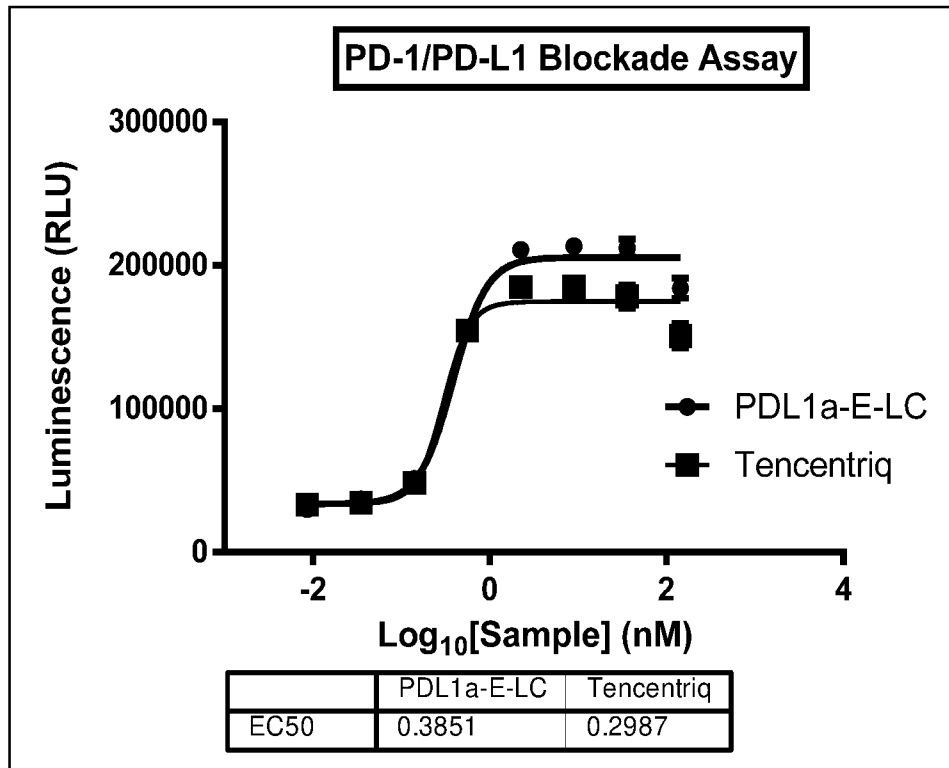


FIG. 10

MDM _ Phagocytosis mediated by CD47 antagonist on HL-60 cells

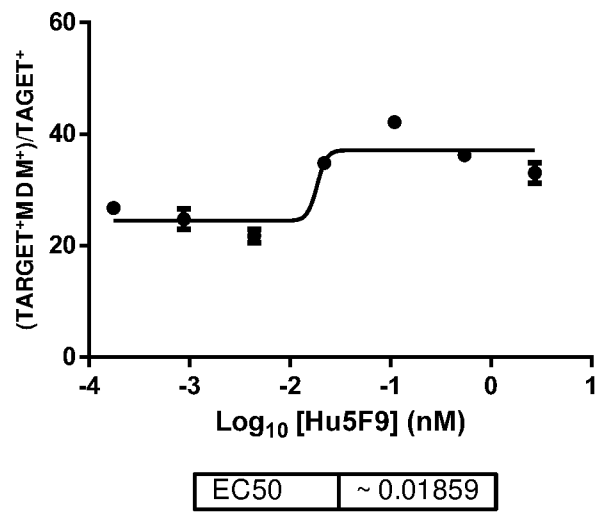


FIG. 11

MDM _ Phagocytosis mediated by CD47 antagonist on HL-60 cells

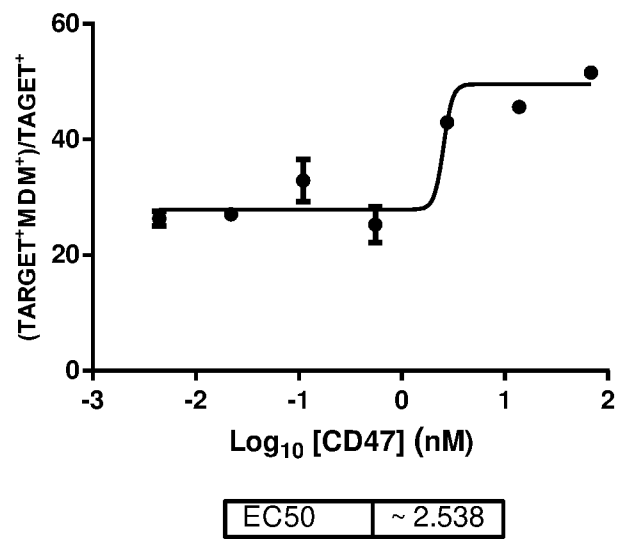


FIG. 12

MDM _ Phagocytosis mediated by CD47 antagonist on HL-60 cells

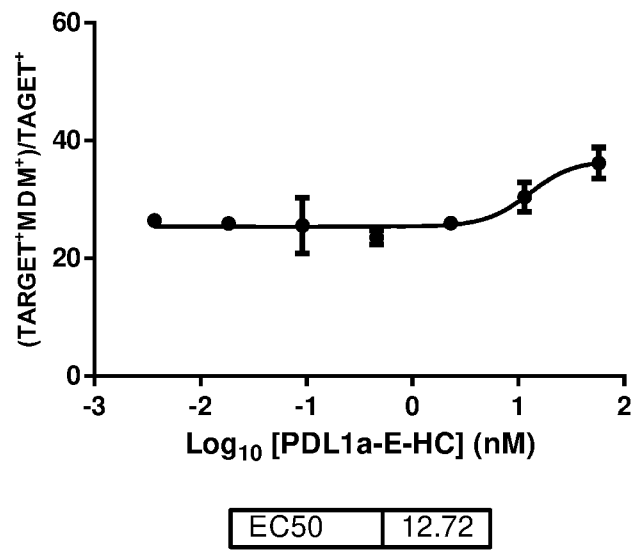


FIG. 13

MDM _ Phagocytosis mediated by CD47 antagonist on HL-60 cells

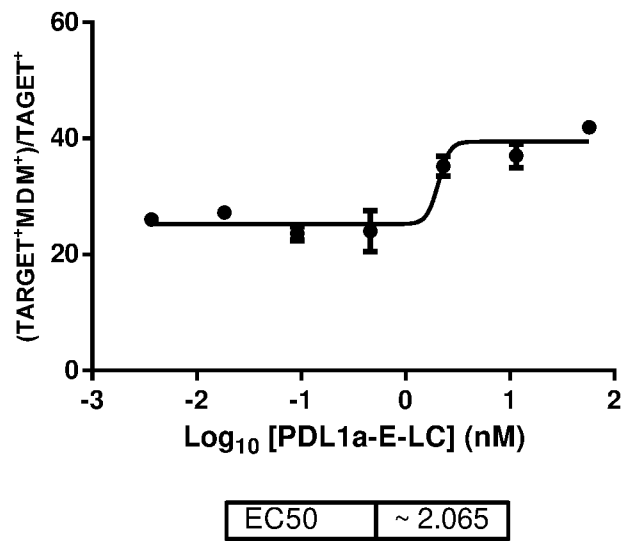


FIG. 14

MDM _ Phagocytosis mediated by CD47 antagonist on HL-60 cells

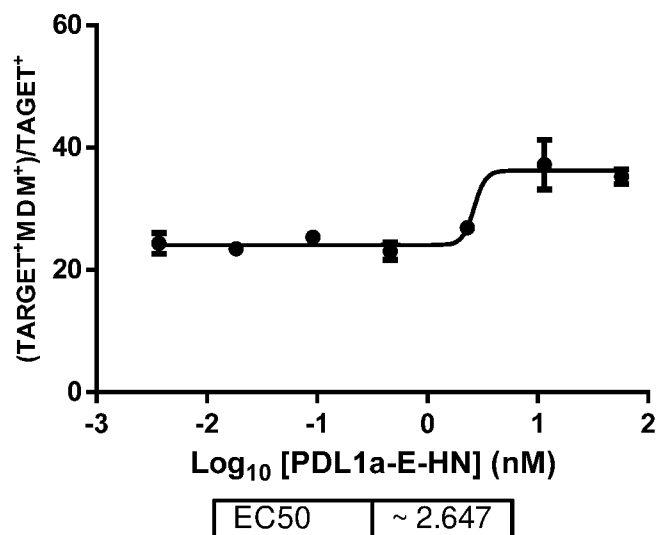
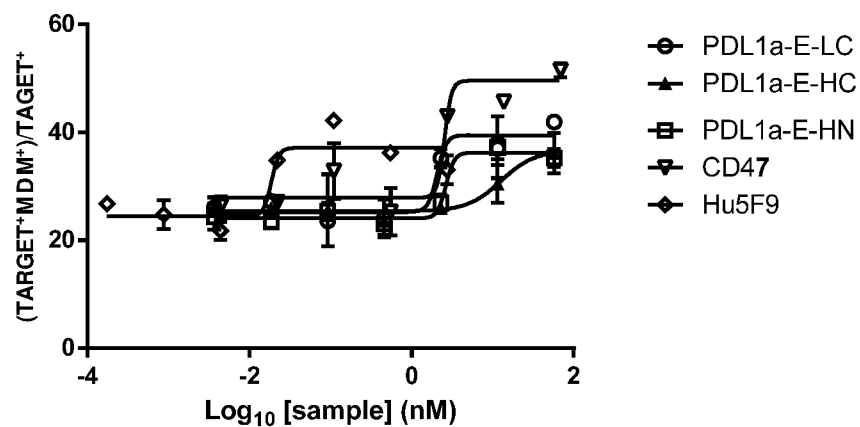


FIG. 15

MDM _ Phagocytosis mediated by CD47 antagonist on HL-60 cells



	PDL1a-E-LC	PDL1a-E-HC	PDL1a-E-HN	CD47	Hu5F9
EC50	~ 2.065	12.72	~ 2.647	~ 2.538	~ 0.01859

FIG. 16

MDM _ Phagocytosis mediated by CD47 antagonist on HL-60 cells

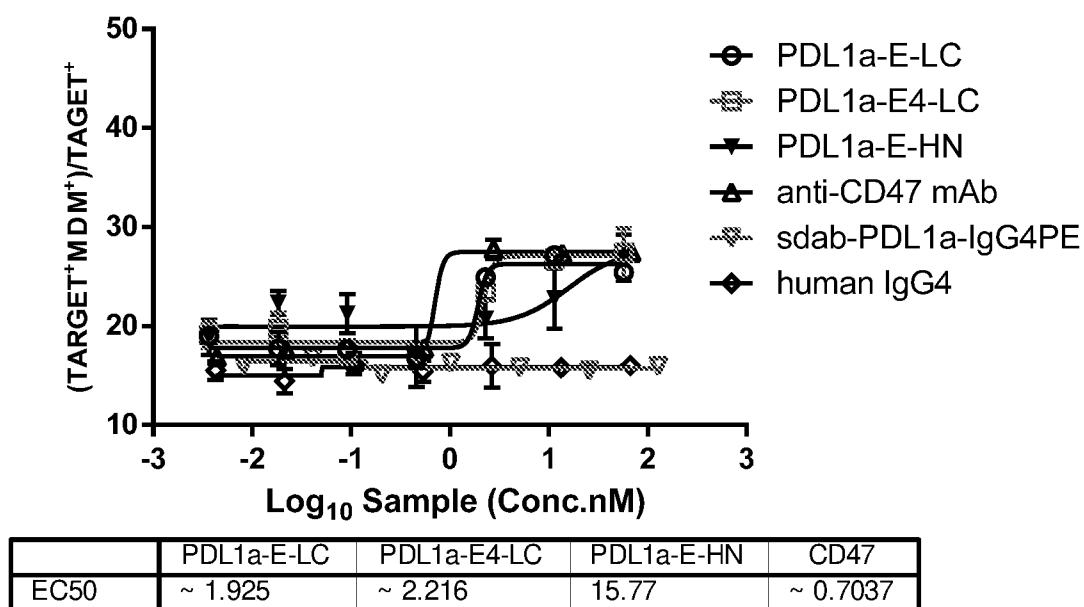


FIG. 17

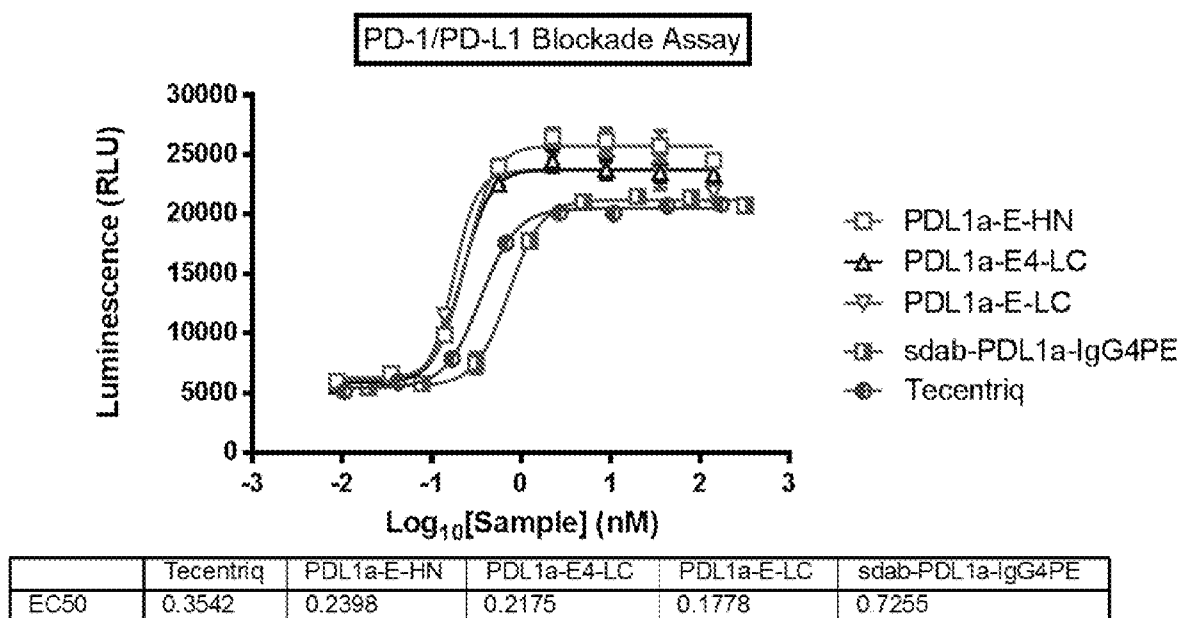


FIG. 18

INTERNATIONAL SEARCH REPORT

International application No.

PCT/CN2020/090753

A. CLASSIFICATION OF SUBJECT MATTER

C07K 19/00(2006.01)i; A61K 39/395(2006.01)i; C12N 15/63(2006.01)i; A61P 35/00(2006.01)i

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

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)

C07K; A61K; C12N; 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;CNKI;ISI Web of Science;Pubmed and keywords: cd47, signal regulatory protein, sirp?, antigen, antibod???, iap, integrin associated protein, pd-11, pd11, b7-h1, cd274, fusion,etc. Genbank;EMBL;Retrieving System for Biological Sequence of Chinese Patent and searched sequences: SEQ ID NOs:4,6,19-24,etc.

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
A	CN 107459578 A (TAIZHOU MABTECH PHARM CO LTD) 12 December 2017 (2017-12-12) the whole document	1-39
A	US 2019125834 A1 (IMMUNEONCO BIOPHARMACEUTICALS SHANGHAI CO LTD) 02 May 2019 (2019-05-02) the whole document	1-39
A	WO 2019073080 A1 (OSE IMMUNOTHERAPEUTICS) 18 April 2019 (2019-04-18) the whole document	1-39
A	WO 2019079549 A1 (FORTY SEVEN INC) 25 April 2019 (2019-04-25) the whole document	1-39
A	WO 2019068302 A1 (BIOCAD JOINT STOCK CO) 11 April 2019 (2019-04-11) the whole document	1-39



Further documents are listed in the continuation of Box C.



See patent family annex.

* Special categories of cited documents:

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

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

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

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

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

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

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

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

“&” document member of the same patent family

Date of the actual completion of the international search

23 July 2020

Date of mailing of the international search report

21 August 2020

Name and mailing address of the ISA/CN

National Intellectual Property Administration, PRC
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Facsimile No. (86-10)62019451

Authorized officer

JIA,Tao

Telephone No. 86-(010)-62411993

INTERNATIONAL SEARCH REPORT

International application No.

PCT/CN2020/090753

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.: **24-39**
because they relate to subject matter not required to be searched by this Authority, namely:
 - [1] Rule 39.1(iv) PCT- Method for treatment of the human or animal body by surgery or therapy.
 - [2] The search of claims 24-39 is based on the subject-matter that could reasonably be expected to be amended, namely "the use of the pharmaceutical composition of claim 23 for manufacturing pharmaceutical preparations for treating a subject having or at risk of having a CD47 and/or PD-L1-related disease".
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/CN2020/090753

Patent document cited in search report			Publication date (day/month/year)	Patent family member(s)			Publication date (day/month/year)
CN	107459578	A	12 December 2017	None			
US	2019125834	A1	02 May 2019	WO	2019080883	A1	02 May 2019
WO	2019073080	A1	18 April 2019	None			
WO	2019079549	A1	25 April 2019	AU	2018351007	A1	21 May 2020
				CA	3078430	A1	25 April 2019
WO	2019068302	A1	11 April 2019	AU	2018345458	A1	14 May 2020
				AR	113342	A1	22 April 2020
				TW	201922781	A	16 June 2019
				CA	3078413	A1	11 April 2019
				CO	2020004199	A2	24 April 2020
				EA	201791961	A1	30 April 2019
				AU	2018345458	A2	21 May 2020