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(54) Title: BISPECIFIC ANTIBODIES TARGETING CD47 AND PD-L1 AND METHODS OF USE THEREOF

(57) Abstract: This disclosure provides novel bispecific antibodies that specifically bind to CD47 and Programmed Death-Ligand 1 (PD-L1). The disclosure further relates to methods of making the bispecific antibodies and nucleic acids encoding the antibodies. The disclosure further relates to therapeutic methods for use of the bispecific antibodies in the treatment of a condition associated with malignant cells expressing CD47 and/or PD-L1 (e.g. cancer).

BISPECIFIC ANTIBODIES TARGETING CD47 AND PD-L1 AND METHODS OF USE THEREOF

RELATED APPLICATIONS

[0001] This application claims the benefit of and priority to U.S. Provisional Application No. 63/317,892, filed on March 8, 2022, and U.S. Provisional Application No. 63/164,237 filed on March 22, 2021, each of which is incorporated herein by reference in its entirety.

FIELD

[0002] The disclosure relates to antibodies that bind to PD-L1 and nucleic acids encoding the antibodies. This disclosure further relates to bispecific antibodies that specifically bind to CD47 and Programmed Death-Ligand 1 (PD-L1). The disclosure further relates to, methods of producing antibodies and therapeutic methods for use of the antibodies in the treatment of a condition associated with malignant cells expressing CD47 and/or PD-L1 (e.g. cancer).

REFERENCE TO SEQUENCE LISTING

[0003] This application is being filed electronically via EFS-Web and includes an electronically submitted sequence listing in .txt format. The .txt file contains a sequence listing entitled “NOVI-047_001WO_SeqList_ST25.txt” created on March 21, 2022, and having a size of ~32 kilobytes. The sequence listing contained in this .txt file is part of the specification and is incorporated herein by reference in its entirety.

BACKGROUND OF THE INVENTION

[0004] CD47 or Integrin-Associated-Protein (IAP) is a ubiquitous 50 kDa transmembrane glycoprotein with multiple functions in cell-cell communication. It interacts with multiple ligands, such as integrins, SIRP α (Signal Regulatory Protein alpha), SIRP γ and thrombospondins

[0005] The widespread expression of CD47 in healthy tissues brings the question of treatment safety and efficacy: First, targeting CD47 with a neutralizing monoclonal antibody (Mab) could affect healthy cells, resulting in severe toxicities as shown in preclinical studies with mice and cynomolgus monkeys. Second, even if severe toxicities could be avoided or mitigated by using alternative formats, broad expression of CD47 could still cause a rapid

elimination of CD47-binding molecules through target-mediated drug disposition resulting in poor pharmacokinetics and decreased efficacy.

[0006] Programmed cell death ligand-1 (PD-L1), also referred to as B7-H1 and CD274, is a transmembrane protein constitutively expressed on both hematopoietic cells, in particular myeloid cells, and non-hematopoietic healthy tissues. It can also be expressed on tumor cells and tumor stroma. In cancer, the expression of the inhibitory receptor PD-1 is considered as a hallmark of exhausted T cells, which exhibit a dysfunctional phenotype due to persistent antigenic and inflammatory stimulation. Furthermore, it has been shown that upregulation of PD-L1 in the tumor microenvironment allows tumors to evade the host immune system, by interacting with PD-1 on T cells. Multiple studies have reported that PD-L1 is expressed in a variety of tumor tissues, either on tumor cells or immune-infiltrating cells or on both. In patients, blocking the interaction of PD-1 with PD-L1 using monoclonal antibodies has proved to be a successful therapy in a range of cancer indications and is widely thought to enhance antitumor T-cell responses by reversing or preventing the onset of T-cell exhaustion, but also by promoting the expansion of T-cells during T-cell priming in the tumor draining lymph nodes. However, despite the considerable improvement in patient outcome that has been achieved with PD-1/PD-L1 checkpoint inhibitors, durable responses to these therapies are observed in only a minority of patients, and intrinsic or acquired resistances are common.

[0007] Accordingly, there exists a need for novel antibodies and therapeutics that enable dual targeting of CD47 and PD-L1 to overcome these obstacles.

SUMMARY OF THE INVENTION

[0008] The disclosure provides bispecific antibodies that specifically bind to CD47 and PD-L1.

[0009] In some aspects, the disclosure provides a bispecific antibody comprising: i) a heavy chain; ii) a first light chain; and iii) a second light chain. In some embodiments, the bispecific antibody disclosed herein comprises a first antigen binding region comprising a heavy chain and a first light chain that specifically binds to CD47 and a second antigen binding region comprising a heavy chain and a second light chain that specifically binds to Programmed Death-ligand 1 (PD-L1).

[0010] In some embodiments, the heavy chain comprises a heavy chain complementarity determining region 1 (CDRH1) comprising an amino acid sequence of SEQ ID NO: 1; a heavy chain complementarity determining region 2 (CDRH2) comprising an amino acid

sequence of SEQ ID NO: 2; and a heavy chain complementarity determining region 3 (CDRH3) comprising an amino acid sequence of SEQ ID NO: 3.

[0011] In some embodiments, a portion of the first light chain is of the kappa type and at least a portion of the second light chain is of the lambda type. In some embodiments, the first light chain comprises at least a Kappa constant region. In some embodiments, the first light chain further comprises a Kappa variable region. In some embodiments, the first light chain further comprises a Lambda variable region.

[0012] In some embodiments, the second light chain comprises at least a Lambda constant region. In some embodiments, the second light chain further comprises a Lambda variable region. In some embodiments, the second light chain further comprises a Kappa variable region.

[0013] In some embodiments the first light chain comprises a Kappa constant region and a Kappa variable region, and wherein the second light chain comprises a Lambda constant region and a Lambda variable region.

[0014] In some embodiments, the first light chain comprises a light chain complementarity determining region 1 (CDRL1) comprising an amino acid sequence of SEQ ID NO: 89; a light chain complementarity determining region 2 (CDRL2) comprising an amino acid sequence of SEQ ID NO: 92; and a light chain complementarity determining region 3 (CDRL3) comprising an amino acid sequence of SEQ ID NO: 96.

[0015] In some embodiments, the second light chain comprises: a CDRL1 comprising an amino acid sequence of SEQ ID NO: 8; a CDRL2 comprising an amino acid sequence of SEQ ID NO: 15; and a CDRL3 comprising an amino acid sequence of SEQ ID NO: 20.

[0016] In some embodiments, the second light chain comprises: a CDRL1 comprising an amino acid sequence of SEQ ID NO: 8; a CDRL2 comprising an amino acid sequence of SEQ ID NO: 15; and a CDRL3 comprising an amino acid sequence of SEQ ID NO: 21.

[0017] In some embodiments, the second light chain comprises: a CDRL1 comprising an amino acid sequence of SEQ ID NO: 8; a CDRL2 comprising an amino acid sequence of SEQ ID NO: 15; and a CDRL3 comprising an amino acid sequence of SEQ ID NO: 22.

[0018] In some embodiments, the second light chain comprises: a CDRL1 comprising an amino acid sequence of SEQ ID NO: 9; a CDRL2 comprising an amino acid sequence of SEQ ID NO: 16; and a CDRL3 comprising an amino acid sequence of SEQ ID NO: 23.

[0019] In some embodiments, the second light chain comprises: a CDRL1 comprising an amino acid sequence of SEQ ID NO: 9; a CDRL2 comprising an amino acid sequence of SEQ ID NO: 16; and a CDRL3 comprising an amino acid sequence of SEQ ID NO: 24.

[0020] In some embodiments, the second light chain comprises: a CDRL1 comprising an amino acid sequence of SEQ ID NO: 9; a CDRL2 comprising an amino acid sequence of SEQ ID NO: 16; and a CDRL3 comprising an amino acid sequence of SEQ ID NO: 25.

[0021] In some embodiments, the second light chain comprises: a CDRL1 comprising an amino acid sequence of SEQ ID NO: 10; a CDRL2 comprising an amino acid sequence of SEQ ID NO: 17; and a CDRL3 comprising an amino acid sequence of SEQ ID NO: 97.

[0022] In some embodiments, the second light chain comprises: a CDRL1 comprising an amino acid sequence of SEQ ID NO: 10; a CDRL2 comprising an amino acid sequence of SEQ ID NO: 17; and a CDRL3 comprising an amino acid sequence of SEQ ID NO: 98.

[0023] In some embodiments, the second light chain comprises: a CDRL1 comprising an amino acid sequence of SEQ ID NO: 11; a CDRL2 comprising an amino acid sequence of SEQ ID NO: 18; and a CDRL3 comprising an amino acid sequence of SEQ ID NO: 97.

[0024] In some embodiments, the second light chain comprises: a CDRL1 comprising an amino acid sequence of SEQ ID NO: 12; a CDRL2 comprising an amino acid sequence of SEQ ID NO: 19; and a CDRL3 comprising an amino acid sequence of SEQ ID NO: 26.

[0025] In some embodiments, the second light chain comprises: a CDRL1 comprising an amino acid sequence of SEQ ID NO: 13; a CDRL2 comprising an amino acid sequence of SEQ ID NO: 19; and a CDRL3 comprising an amino acid sequence of SEQ ID NO: 26.

[0026] In some embodiments, the second light chain comprises: a CDRL1 comprising an amino acid sequence of SEQ ID NO: 14; a CDRL2 comprising an amino acid sequence of SEQ ID NO: 19; and a CDRL3 comprising an amino acid sequence of SEQ ID NO: 27.

[0027] In some embodiments, the second light chain comprises: a CDRL1 comprising an amino acid sequence of SEQ ID NO: 14; a CDRL2 comprising an amino acid sequence of SEQ ID NO: 19; and a CDRL3 comprising an amino acid sequence of SEQ ID NO: 28.

[0028] In some embodiments, the second light chain comprises: a CDRL1 comprising the amino acid sequence of SEQ ID NO: 101; a CDRL2 comprising the amino acid sequence of SEQ ID NO: 18; and a CDRL3 comprising the amino acid sequence of SEQ ID NO: 97.

[0029] In some embodiments, the second light chain comprises: a CDRL1 comprising the amino acid sequence of SEQ ID NO: 102; a CDRL2 comprising the amino acid sequence of SEQ ID NO: 18; and a CDRL3 comprising the amino acid sequence of SEQ ID NO: 97.

[0030] In some embodiments, the second light chain comprises: a CDRL1 comprising the amino acid sequence of SEQ ID NO: 103; a CDRL2 comprising the amino acid sequence of SEQ ID NO: 18; and a CDRL3 comprising the amino acid sequence of SEQ ID NO: 97.

[0031] In some embodiments, the bispecific antibody is human antibody. In some embodiments, the bispecific antibody is an IgG1 antibody. In some embodiments, the isolated bispecific antibody is isolated.

[0032] The disclosure provides a composition comprising the bispecific antibody and a pharmaceutically acceptable carrier.

[0033] The disclosure provides a method of reducing the proliferation of and/or killing a tumor cell comprising contacting the cell with the composition comprising the bispecific antibody. The disclosure also provides a method of treating a cancer in a subject comprising administering to the subject the composition comprising the bispecific antibody.

[0034] The disclosure provides the use of an isolated bispecific antibody described herein for treating, preventing, or delaying the progression of pathologies associated with aberrant CD47 expression or activity, or associated with aberrant CD47-SIRP α expression or activity. In some embodiments, the pathology is cancer. In some embodiments, the cancer is a solid tumor. In some embodiments, the solid tumor is or is derived from breast cancer, ovarian cancer, head and neck cancer, bladder cancer, melanoma, mesothelioma, colorectal cancer, cholangiocarcinoma, pancreatic cancer, lung cancer, leiomyoma, leiomyosarcoma, kidney cancer, glioma, glioblastoma, endometrial cancer, esophageal cancer, biliary gastric cancer, prostate cancer, or combinations thereof.

[0035] The disclosure provides antibodies that specifically bind to PD-L1. In some aspects, the disclosure provides a antibody comprising: i) a heavy chain; and ii) a light chain.

[0036] In some embodiments, the heavy chain comprises a heavy chain complementarity determining region 1 (CDRH1) comprising an amino acid sequence of SEQ ID NO: 1; a heavy chain complementarity determining region 2 (CDRH2) comprising an amino acid sequence of SEQ ID NO: 2; and a heavy chain complementarity determining region 3 (CDRH3) comprising an amino acid sequence of SEQ ID NO: 3.

[0037] In some embodiments, the light chain comprises: a CDRL1 comprising an amino acid sequence of SEQ ID NO: 8; a CDRL2 comprising an amino acid sequence of SEQ ID NO: 15; and a CDRL3 comprising an amino acid sequence of SEQ ID NO: 20.

[0038] In some embodiments, the light chain comprises: a CDRL1 comprising an amino acid sequence of SEQ ID NO: 8; a CDRL2 comprising an amino acid sequence of SEQ ID NO: 15; and a CDRL3 comprising an amino acid sequence of SEQ ID NO: 21.

[0039] In some embodiments, the light chain comprises: a CDRL1 comprising an amino acid sequence of SEQ ID NO: 8; a CDRL2 comprising an amino acid sequence of SEQ ID NO: 15; and a CDRL3 comprising an amino acid sequence of SEQ ID NO: 22.

[0040] In some embodiments, the light chain comprises: a CDRL1 comprising an amino acid sequence of SEQ ID NO: 9; a CDRL2 comprising an amino acid sequence of SEQ ID NO: 16; and a CDRL3 comprising an amino acid sequence of SEQ ID NO: 23.

[0041] In some embodiments, the light chain comprises: a CDRL1 comprising an amino acid sequence of SEQ ID NO: 9; a CDRL2 comprising an amino acid sequence of SEQ ID NO: 16; and a CDRL3 comprising an amino acid sequence of SEQ ID NO: 24.

[0042] In some embodiments, the light chain comprises: a CDRL1 comprising an amino acid sequence of SEQ ID NO: 9; a CDRL2 comprising an amino acid sequence of SEQ ID NO: 16; and a CDRL3 comprising an amino acid sequence of SEQ ID NO: 25.

[0043] In some embodiments, the light chain comprises: a CDRL1 comprising an amino acid sequence of SEQ ID NO: 10; a CDRL2 comprising an amino acid sequence of SEQ ID NO: 17; and a CDRL3 comprising an amino acid sequence of SEQ ID NO: 97.

[0044] In some embodiments, the light chain comprises: a CDRL1 comprising an amino acid sequence of SEQ ID NO: 10; a CDRL2 comprising an amino acid sequence of SEQ ID NO: 17; and a CDRL3 comprising an amino acid sequence of SEQ ID NO: 98.

[0045] In some embodiments, the light chain comprises: a CDRL1 comprising an amino acid sequence of SEQ ID NO: 11; a CDRL2 comprising an amino acid sequence of SEQ ID NO: 18; and a CDRL3 comprising an amino acid sequence of SEQ ID NO: 97.

[0046] In some embodiments, the light chain comprises: a CDRL1 comprising an amino acid sequence of SEQ ID NO: 12; a CDRL2 comprising an amino acid sequence of SEQ ID NO: 19; and a CDRL3 comprising an amino acid sequence of SEQ ID NO: 26.

[0047] In some embodiments, the light chain comprises: a CDRL1 comprising an amino acid sequence of SEQ ID NO: 13; a CDRL2 comprising an amino acid sequence of SEQ ID NO: 19; and a CDRL3 comprising an amino acid sequence of SEQ ID NO: 26.

[0048] In some embodiments, the light chain comprises: a CDRL1 comprising an amino acid sequence of SEQ ID NO: 14; a CDRL2 comprising an amino acid sequence of SEQ ID NO: 19; and a CDRL3 comprising an amino acid sequence of SEQ ID NO: 27.

[0049] In some embodiments, the light chain comprises: a CDRL1 comprising an amino acid sequence of SEQ ID NO: 14; a CDRL2 comprising an amino acid sequence of SEQ ID NO: 19; and a CDRL3 comprising an amino acid sequence of SEQ ID NO: 28.

[0050] In some embodiments, the light chain comprises: a CDRL1 comprising the amino acid sequence of SEQ ID NO: 101; a CDRL2 comprising the amino acid sequence of SEQ ID NO: 18; and a CDRL3 comprising the amino acid sequence of SEQ ID NO: 97.

[0051] In some embodiments, the light chain comprises: a CDRL1 comprising the amino acid sequence of SEQ ID NO: 102; a CDRL2 comprising the amino acid sequence of SEQ ID NO: 18; and a CDRL3 comprising the amino acid sequence of SEQ ID NO: 97.

[0052] In some embodiments, the light chain comprises: a CDRL1 comprising the amino acid sequence of SEQ ID NO: 103; a CDRL2 comprising the amino acid sequence of SEQ ID NO: 18; and a CDRL3 comprising the amino acid sequence of SEQ ID NO: 97.

[0053] In some embodiments, the antibody is human antibody. In some embodiments, the bispecific antibody is an IgG1 antibody. In some embodiments, the antibody is isolated. In some embodiments, the antibody is a F(ab) fragment, a F(ab')₂ fragment, and Fv fragment or a single chain Fv fragment. In some embodiments, the antibody is monospecific. In some embodiments, the antibody is monovalent.

[0054] The disclosure provides a composition comprising the antibody and a pharmaceutically acceptable carrier.

[0055] The disclosure provides a method of reducing the proliferation of and/or killing a tumor cell comprising contacting the cell with the composition comprising the antibody. The disclosure also provides a method of treating a cancer in a subject comprising administering to the subject the composition comprising the antibody.

[0056] The disclosure provides the use of an isolated antibody described herein for treating, preventing, or delaying the progression of pathologies associated with aberrant PD-L1 expression or activity, or associated with aberrant PD-L1 expression or activity. In some embodiments, the pathology is cancer. In some embodiments, the cancer is a solid tumor. In some embodiments, the solid tumor is or is derived from breast cancer, ovarian cancer, head and neck cancer, bladder cancer, melanoma, mesothelioma, colorectal cancer, cholangiocarcinoma, pancreatic cancer, lung cancer, leiomyoma, leiomyosarcoma, kidney cancer, glioma, glioblastoma, endometrial cancer, esophageal cancer, biliary gastric cancer, prostate cancer, or combinations thereof.

BRIEF DESCRIPTION OF THE DRAWINGS

[0057] **FIG. 1A-1E** shows a series of graphs depicting binding, cross-reactivity, and specificity of exemplary CD47xPD-L1 bispecific antibodies and anti-PD-L1 mAbs of the invention (S79 and S100) to PD-L1 isolated from various species. **FIG. 1A** shows binding to recombinant human PD-L1 determined by ELISA assay. **FIG. 1B** shows binding to recombinant cynomolgus monkey PD-L1 determined by ELISA assay. **FIG. 1C** shows binding to recombinant mouse PD-L1 determined by ELISA assay. **FIG. 1D** shows binding to recombinant human PD-L2 determined by ELISA assay. **FIG. 1E** shows a graph depicting blockade of soluble hPD-1 binding to hPD-L1-transfected CHO (hamster) cells by exemplary CD47xPD-L1 bispecific antibodies compared to hIgG4 isotype control antibody, anti-PD-L1 mAbs atezolizumab and avelumab, and an anti-PD-L2 mAb, determined by a competitive binding cell-based assay.

[0058] **FIG. 2A-2C** shows a series of graphs depicting binding of exemplary CD47xPD-L1 bispecific antibodies of the invention to CD47 isolated from various species. **FIG. 2A** shows binding to recombinant human CD47 determined by ELISA assay. **FIG. 2B** shows binding to cynomolgus monkey CD47 determined by ELISA assay. **FIG. 2C** shows binding to recombinant mouse CD47 determined by ELISA assay.

[0059] **FIGS. 2D-2E** shows a series of graphs depicting binding of exemplary CD47xPD-L1 bispecific antibodies of the invention to human CD47⁺PD-L1⁻ tumor cells. **FIG. 2D** shows binding to Raji tumor cells determined by flow cytometry. **FIG. 2E** shows binding to Nalm-6 tumor cells by flow cytometry.

[0060] **FIG. 2F** shows a graph depicting blockage of soluble SIRPa binding to human CD47⁺PD-L1⁻ Nalm-6 tumor cells by one exemplary CD47xPD-L1 bispecific antibody compared to anti-CD47 5F9 analog in a cell-based competitive binding assay.

[0061] **FIG. 3** shows a graph depicting binding of exemplary CD47xPD-L1 bispecific antibodies to human red blood cells isolated from whole blood of healthy donors assessed by flow cytometry compared to anti-CD47 5F9 analog.

[0062] **FIG. 4A-4B** shows a series of graphs depicting binding of exemplary CD47xPD-L1 bispecific antibodies of the invention to HT-1080 tumor cells evaluated by flow cytometry.

[0063] **FIG. 5A-5B** shows a series of graphs depicting PD-1 and SIRPa blocking activity of exemplary CD47xPD-L1 bispecific antibodies binding using human CD47⁺/PD-L1⁺ HT-1080 tumor cells. **FIG. 5A** shows blockage of blockage of PD-1 on HT-1080 tumor cells. **FIG. 5B** shows blockage of SIRPa blocking on HT-1080 tumor cells.

[0064] **FIG. 6A-6B** shows a series of graphs depicting phagocytosis of tumor cells mediated

by exemplary CD47xPD-L1 bispecific antibodies in a X assay. **FIG. 6A** shows phagocytosis of N87 tumor cells. **FIG. 6B** shows phagocytosis of HT-1080 tumor cells.

[0065] **FIG. 7A-7C** shows a series of graphs depicting killing of tumor cells by exemplary CD47xPD-L1 bispecific antibodies by antibody-dependent cellular phagocytosis assay. **FIG. 7A** shows killing of H226 tumor cells. **FIG. 7B** shows killing of N87 tumor cells. **FIG. 7C** shows killing of A375 tumor cells.

[0066] **FIG. 8.** T-cell activation induced by selected CD47xPD-L1 bispecific antibodies assessed in the *Staphylococcus* enterotoxin A (SEA) PBMC stimulation assay. IL-2 concentration in the supernatant, harvested after 96h of incubation, was quantified by ELISA. An irrelevant IgG1 isotype control antibody, anti-PD-L1 mAbs avelumab and atezolizumab as well as monovalent CD47 control K2 were tested for comparison.

[0067] **FIG. 9.** In vivo anti-tumor efficacy of anti-PD-L1 S79 mAb in the MC38 colon carcinoma model implanted in immunocompetent C57BL/6 mice. Eight days after subcutaneous tumor cell implantation, mice were administered intraperitoneally every 3 days a dose of 10 mg/kg of anti-PD-L1 mAb S79 or an irrelevant IgG1 control (mice received a maximum of 3 doses). Results show individual tumor growth curves of 8 mice per group. Black arrows correspond to treatment injection.

DETAILED DESCRIPTION

[0068] The present disclosure provides bispecific antibodies that bind to CD47 and PD-L1. Specifically, the bispecific antibody includes a first antigen binding region that binds CD47 and block the SIRPa/CD47 interaction and a second antigen binding region that binds PD-L1 and block PD-1/PD-L1 interaction.

[0069] Cancer cells adopt multiple mechanisms to escape immune surveillance. Several studies demonstrated that CD47 and PD-L1 expression on tumor cells was concomitantly regulated to suppress immune response. Thus, activating innate or adaptive immunity alone may be insufficient to eradicate tumors and harnessing both immune responses may present a more effective strategy to induce durable anti-tumor activity. Therefore, combinations between anti-PD-L1 or anti-PD-1 antibodies and anti-CD47 antibodies are currently being explored in the clinic. However, the expression of CD47 on many healthy cells such as hematopoietic cells, red blood cell and platelets provides a strong antigen sink that affects the pharmacokinetics and compromised safety profile of these agents.

[0070] One way to overcome the ubiquity of CD47 expression is provided by dual-targeting

bispecific antibodies (bsAbs), which bind to two different antigens on the surface of the same cell. The bispecific antibodies bind their targets (i.e. CD47 and PD-L1) with different affinity in a monovalent engagement. Specifically, the bispecific antibody binds PD-L1 with high affinity allowing for monovalent binding. In contrast, the bispecific antibody binds CD47 at a low affinity, that is an affinity that is sufficient to inhibit CD47/SIRP α only upon PD-L1 co-engagement. However, when both targets are expressed on the same cell, the bispecific antibodies can simultaneously block their respective receptor interaction (i.e., SIRP α and PD-L1). This design allows the bispecific antibodies of the invention to preferentially inhibit CD47 only on PD-L1 positive cells. As a consequence, this design confers high selectivity towards cells expressing both antigens as compared to cells that express just one single antigen.

[0071] Additionally, the bispecific antibodies described herein require a functional Fc portion to recruit macrophages and/or other immune effector cells. For example, the bispecific antibody is of the IgG1 isotype..

[0072] CD47

[0073] CD47 or Integrin-Associated-Protein (IAP) is a ubiquitous 50 kDa transmembrane glycoprotein with multiple functions in cell-cell communication. It interacts with multiple ligands, such as, for example, integrins, and/or SIRP α . In the context of the innate immune system, CD47 functions as a marker of self, transmitting an inhibitory “don’t kill me” signal through binding to SIRP α expressed by myeloid cells, such as macrophages, neutrophils, and dendritic cells but also NK cells (Deuse T *et al.*, The SIRP α –CD47 immune checkpoint in NK cells, J Exp Med 2021 Vol. 218 No. 3). The role of widespread expression of CD47 in the physiological situation is therefore to protect healthy cells against the elimination by the innate immune system

[0074] Tumor cells hijack this immunosuppressive mechanism by overexpressing CD47, which efficiently helps them to escape immune surveillance and killing by innate immune cells. CD47 expression is upregulated in most human cancers (e.g., NHL, AML, breast, colon, glioblastoma, glioma, ovarian, bladder and prostate cancers) and increased levels of CD47 expression clearly correlate with aggressive disease and poor survival. Thus, targeting CD47 would be useful in treating, delaying the progression of, or otherwise ameliorating a symptom of cancer.

[0075] However, the widespread expression of CD47 in healthy tissues brings the question of treatment safety and efficacy: First, targeting CD47 with a neutralizing monoclonal antibody (mAb) could affect healthy cells, resulting in severe hematological toxicities

(anemia and thrombocytopenia) as shown in preclinical studies with mice and cynomolgus monkeys. Second, even if severe toxicities could be avoided or mitigated by using alternative antibody formats broad expression of CD47 could still cause a rapid elimination of CD47-binding molecules through target-mediated drug disposition resulting in poor pharmacokinetics and decreased efficacy.

PROGRAMMED CELL DEATH LIGAND-1 (PD-L1)

[0076] Programmed cell death ligand-1 (PD-L1), also referred to as B7-H1 and CD274, is a transmembrane protein constitutively expressed on both hematopoietic cells, in particular myeloid cells, and non-hematopoietic healthy tissues. It can also be expressed on tumor cells and tumor stroma. Various inflammatory stimuli, such as IFN γ , TNF α or LPS, induce PD-L1 expression on immune cells, endothelial cells and epithelial lineages, including tumor cells deriving from these lineages. PD-L1 acts both as a ligand of Programmed cell death-1 (PD-1), which is expressed on the surface of activated lymphocytes, and of B7.1 (also known as CD80), expressed by antigen-presenting cells, especially dendritic cells and macrophages.

The engagement of PD-1 by PD-L1 on T cells is considered as an immune checkpoint, by counteracting T cell-activating signals, results in the inhibition of proliferation, cytokine production and release and cytotoxicity of T-cells. In fact, PD-1 has been shown to suppress T-cell activation at least in part through the inhibition of CD28 signaling, a major co-stimulatory pathway required for optimal activation of T cells. Therefore, the PD-1/PD-L1 pathway, by regulating the magnitude and the functional activity of the T-cell response, play a critical role in physiological conditions in limiting tissue damage during inflammatory reactions, and in maintaining self-tolerance. In pathological circumstances, it is involved in the development of tumor immunity and autoimmune diseases.

[0077] In cancer, the expression of the inhibitory receptor PD-1 is considered as a hallmark of exhausted T cells, which exhibit a dysfunctional phenotype due to persistent antigenic and inflammatory stimulation. Furthermore, it has been shown that upregulation of PD-L1 in the tumor microenvironment allows tumors to evade the host immune system, by interacting with PD-1 on T cells. Multiple studies have reported that PD-L1 is expressed in a variety of tumor tissues, either on tumor cells or immune-infiltrating cells or on both. In patients, blocking the interaction of PD-1 with PD-L1 using monoclonal antibodies has proved to be a successful therapy in a range of cancer indications and is widely thought to enhance antitumor T-cell responses by reversing or preventing the onset of T-cell exhaustion, but also by promoting the expansion of T-cells during T-cell priming in the tumor draining lymph nodes. However,

despite the considerable improvement in patient outcome that has been achieved with PD-1/PD-L1 checkpoint inhibitors, durable responses to these therapies are observed in only a minority of patients, and intrinsic or acquired resistances are common.

EXEMPLARY BISPECIFIC ANTIBODIES THAT BIND TO CD47 AND PD-L1

[0078] The bispecific antibodies of the invention have one antigen binding region that is specific for CD47 and a second antigen binding region that is specific for PD-L1. But another way the bispecific antibodies are monovalent for CD47 and PD-L1. The bispecific antibodies share a common heavy chain. The heavy chains are native heavy chains (i.e., does not contain any mutations) The heavy chains are of the IgG1 or IgG3 isotype which effector function (ADCC and/ or C1q binding) are of high potency.. Optionally, the bispecific antibodies have light chains of different types. For example, one light chain is a kappa light and the other light chain is a lambda light chain (i.e., $\kappa\lambda$ -body) Differing light chains allows the bispecific to be purified easily using kappa and lambda select resins.

[0079] Exemplary CD47 antibodies from which the CD7 antigen binding region can be derived from include the K2 antibody, Exemplary, PD-L1 antibodies from which the PD-L1 antigen binding region can be derived from include the S8 antibody, the S9 antibody, the S37 antibody, the S14 antibody, the S15 antibody, the S17 antibody, the S57 antibody, the S58 antibody, the S28 antibody, the S30 antibody, the S94 antibody, the S23 antibody, the S46 antibody, the S71 antibody, the S79 antibody, the S93 antibody, the S96 antibody and the S100 antibody.

[0080] In some embodiments, exemplary bispecific antibodies of the invention that include at least a first antigen binding region that binds CD47 include a combination of heavy chain and complementarity determining regions and light chain complementarity determining regions (CDRs) selected from the CDR sequences shown in Tables 1, 2 and 3. The CDRs shown in Tables 1, 2 and 3 are defined according to the IMGT nomenclature (See IMGT[®], the international ImMunoGeneTics information system[®]. Available online: <http://www.imgt.org/>).

[0081] In some embodiments, exemplary bispecific antibodies of the invention that includes a heavy chain comprising a combination of heavy chain CDR amino acid sequences selected from the CDRH1, CDRH2 and CDRH3 amino acid sequences shown in Table 1, at least a first light chain with a set of first light chain CDR amino acid sequences selected from the CDRL1, CDRL2 and CDRL3 amino acid sequences shown in Tables 2 and at least a second

light chain with a set of second light chain CDR amino acid sequences selected from CDRL1, CDRL2 and CDRL3 sequences Table 3.

[0082] In some embodiments, exemplary bispecific antibodies of the invention that include a first antigen binding region that binds CD47 and a second antigen binding region that binds PD-L1, wherein the first antigen binding region includes the combination of heavy chain complementarity determining regions (CDRs) shown in Table 1 and a combination of the light chain CDRs selected from the CDR sequences shown in Table 2, and wherein the second antigen binding region includes the combination of heavy chain complementarity determining regions (CDRs) shown in Table 1 and a combination of the light chain CDRs selected from the CDR sequences shown in Table 3.

[0083] Table 1. Common Heavy Chain CDRs

CDRH1	CDRH2	CDRH3
GFTFSSYA (SEQ ID NO: 1)	ISGSGGST (SEQ ID NO: 2)	AKSYGAFDY (SEQ ID NO: 3)

[0084] Table 2. Anti-CD47 Kappa Light Chain CDRs

Kappa Chain	CDRL1	CDRL2	CDRL3
KA3 (K2)	QSISSY (SEQ ID NO : 89)	AAS (SEQ ID NO : 92)	QQMHPRAPKT (SEQ ID NO : 96)

[0085] Table 3. Anti-PD-L1 Lambda Light Chain CDRs

Lambda Light Chain	CDRL1	CDRL2	CDRL3
S8	SSNIRDSF (SEQ ID NO: 8)	ATN (SEQ ID NO: 15)	AAWHPPYYTL (SEQ ID NO: 20)
S9	SSNIRDSF (SEQ ID NO: 8)	ATN (SEQ ID NO: 15)	ASWWPYGTV (SEQ ID NO: 21)
S37	SSNIRDSF (SEQ ID NO: 8)	ATN (SEQ ID NO: 15)	ASWWPFGTV (SEQ ID NO: 22)
S14	SSDVVKNNF (SEQ ID NO: 9)	FGS (SEQ ID NO: 16)	SSWDMPALF (SEQ ID NO: 23)
S15	SSDVVKNNF (SEQ ID NO: 9)	FGS (SEQ ID NO: 16)	SSWDEPDRP (SEQ ID NO: 24)
S17	SSDVVKNNF (SEQ ID NO: 9)	FGS (SEQ ID NO: 16)	SSWDLPFML (SEQ ID NO: 25)
S57	SSDVVKNNF (SEQ ID NO: 9)	FGS (SEQ ID NO: 16)	SSWDEPDRP (SEQ ID NO: 24)
S58	SSDVVKNNF (SEQ ID NO: 9)	FGS (SEQ ID NO: 16)	SSWDEPDRP (SEQ ID NO: 24)
S28	SSNIAHKP	HDN	AAWDFATWPATEV

	(SEQ ID NO: 10)	(SEQ ID NO: 17)	(SEQ ID NO: 97)
S30	SSNIAHKP (SEQ ID NO: 10)	HDN (SEQ ID NO: 17)	AAWDFSRWPATEV (SEQ ID NO: 98)
S94	SVDIAHKP (SEQ ID NO: 11)	HDT (SEQ ID NO: 18)	AAWDFATWPATEV (SEQ ID NO: 97)
S23	SSDVAKIPL (SEQ ID NO: 12)	FAS (SEQ ID NO: 19)	SSWDNAGDGHV (SEQ ID NO: 26)
S46	SSDVLRPPL (SEQ ID NO: 13)	FAS (SEQ ID NO: 19)	SSWDNAGDGHV (SEQ ID NO: 26)
S71	SSDVFRPPL (SEQ ID NO: 14)	FAS (SEQ ID NO: 19)	SSWDQSGDGHV (SEQ ID NO: 27)
S79	SSDVFRPPL (SEQ ID NO: 14)	FAS (SEQ ID NO: 19)	SSWDHTGDGHV (SEQ ID NO: 28)
S93	SDHIAHKP (SEQ ID NO: 101)	HDT (SEQ ID NO: 18)	AAWDFATWPATEV (SEQ ID NO: 97)
S96	SADIAHKP (SEQ ID NO: 102)	HDT (SEQ ID NO: 18)	AAWDFATWPATEV (SEQ ID NO: 97)
S100	SSNIENKP (SEQ ID NO: 103)	HDT (SEQ ID NO: 18)	AAWDFATWPATEV (SEQ ID NO: 97)

[0086] In some embodiments, the **K2 x S8** bispecific antibody has a heavy chain comprising a CDRH1 comprising the amino acid sequence of SEQ ID NO: 1, a CDRH2 comprising the amino acid sequence of SEQ ID NO: 2, a CDRH3 comprising the amino acid sequence of SEQ ID NO: 3, a kappa light chain comprising a CDRL1 comprising the amino acid sequence of SEQ ID NO: 89, a CDRL2 comprising the amino acid sequence of SEQ ID NO: 92, and a CDRL3 comprising the amino acid sequence of SEQ ID NO: 96, and a lambda light chain comprising a CDRL1 comprising the amino acid sequence of SEQ ID NO: 8, a CDRL2 comprising the amino acid sequence of SEQ ID NO: 15, and a CDRL3 comprising the amino acid sequence of SEQ ID NO: 20.

[0087] In some embodiments, the **K2 x S8** bispecific antibody has a heavy chain variable region comprising the amino acid sequence of SEQ ID NO: 6 encoded by the nucleic acid sequence of SEQ ID NO: 7, a kappa light chain variable region comprising the amino acid sequence of SEQ ID NO: 105 encoded by the nucleic acid sequence of SEQ ID NO: 106, and a lambda light chain variable region comprising the amino acid sequence of SEQ ID NO: 31 encoded by the nucleic acid sequence of SEQ ID NO: 32.

[0088] In some embodiments, the **K2 x S8** bispecific antibody has a heavy chain variable and constant region comprising the amino acid sequence of SEQ ID NO: 4 encoded by the nucleic acid sequence of SEQ ID NO: 5, a kappa light chain comprising the amino acid sequence of SEQ ID NO: 107 encoded by the nucleic acid sequence of SEQ ID NO: 104, and a lambda light chain comprising the amino acid sequence of SEQ ID NO: 29 encoded by the nucleic acid sequence shown in SEQ ID NO: 30.

[0089] In some embodiments, the **K2 x S9** bispecific antibody has a heavy chain comprising a CDRH1 comprising the amino acid sequence of SEQ ID NO: 1, a CDRH2 comprising the amino acid sequence of SEQ ID NO: 2, a CDRH3 comprising the amino acid sequence of SEQ ID NO: 3, a kappa light chain comprising a CDRL1 comprising the amino acid sequence of SEQ ID NO: 89, a CDRL2 comprising the amino acid sequence of SEQ ID NO: 92, and a CDRL3 comprising the amino acid sequence of SEQ ID NO: 96, and a lambda light chain comprising a CDRL1 comprising the amino acid sequence of SEQ ID NO: 8, a CDRL2 comprising the amino acid sequence of SEQ ID NO: 15, and a CDRL3 comprising the amino acid sequence of SEQ ID NO: 21.

[0090] In some embodiments, the **K2 x S9** bispecific antibody has a heavy chain variable region comprising the amino acid sequence of SEQ ID NO: 6 encoded by the nucleic acid sequence of SEQ ID NO: 7, a kappa light chain variable region comprising the amino acid sequence of SEQ ID NO: 105 encoded by the nucleic acid sequence of SEQ ID NO: 106, and a lambda light chain variable region comprising the amino acid sequence of SEQ ID NO: 35 encoded by the nucleic acid sequence of SEQ ID NO: 36.

[0091] In some embodiments, the **K2 x S9** bispecific antibody has a heavy chain variable and constant region comprising the amino acid sequence of SEQ ID NO: 4 encoded by the nucleic acid sequence of SEQ ID NO: 5, a kappa light chain comprising the amino acid sequence of SEQ ID NO: 107 encoded by the nucleic acid sequence of SEQ ID NO: 104, and a lambda light chain comprising the amino acid sequence of SEQ ID NO: 33 encoded by the nucleic acid sequence shown in SEQ ID NO: 34.

[0092] In some embodiments, the **K2 x S37** bispecific antibody has a heavy chain comprising a CDRH1 comprising the amino acid sequence of SEQ ID NO: 1, a CDRH2 comprising the amino acid sequence of SEQ ID NO: 2, a CDRH3 comprising the amino acid sequence of SEQ ID NO: 3, a kappa light chain comprising a CDRL1 comprising the amino acid sequence of SEQ ID NO: 89, a CDRL2 comprising the amino acid sequence of SEQ ID NO: 92, and a CDRL3 comprising the amino acid sequence of SEQ ID NO: 96, and a lambda light chain comprising a CDRL1 comprising the amino acid sequence of SEQ ID NO: 8, a CDRL2 comprising the amino acid sequence of SEQ ID NO: 15, and a CDRL3 comprising the amino acid sequence of SEQ ID NO: 22.

[0093] In some embodiments, the **K2 x S37** bispecific antibody has a heavy chain variable region comprising the amino acid sequence of SEQ ID NO: 6 encoded by the nucleic acid sequence of SEQ ID NO: 7, a kappa light chain variable region comprising the amino acid

sequence of SEQ ID NO: 105 encoded by the nucleic acid sequence of SEQ ID NO: 106, and a lambda light chain variable region comprising the amino acid sequence of SEQ ID NO: 39 encoded by the nucleic acid sequence of SEQ ID NO: 40.

[0094] In some embodiments, the **K2 x S37** bispecific antibody has a heavy chain variable and constant region comprising the amino acid sequence of SEQ ID NO: 4 encoded by the nucleic acid sequence of SEQ ID NO: 5, a kappa light chain comprising the amino acid sequence of SEQ ID NO: 107 encoded by the nucleic acid sequence of SEQ ID NO: 104, and a lambda light chain comprising the amino acid sequence of SEQ ID NO: 37 encoded by the nucleic acid sequence shown in SEQ ID NO: 38.

[0095] In some embodiments, the **K2 x S14** bispecific antibody has a heavy chain comprising a CDRH1 comprising the amino acid sequence of SEQ ID NO: 1, a CDRH2 comprising the amino acid sequence of SEQ ID NO: 2, a CDRH3 comprising the amino acid sequence of SEQ ID NO: 3, a kappa light chain comprising a CDRL1 comprising the amino acid sequence of SEQ ID NO: 89, a CDRL2 comprising the amino acid sequence of SEQ ID NO: 92, and a CDRL3 comprising the amino acid sequence of SEQ ID NO: 96, and a lambda light chain comprising a CDRL1 comprising the amino acid sequence of SEQ ID NO: 9, a CDRL2 comprising the amino acid sequence of SEQ ID NO: 16, and a CDRL3 comprising the amino acid sequence of SEQ ID NO: 23.

[0096] In some embodiments, the **K2 x S14** bispecific antibody has a heavy chain variable region comprising the amino acid sequence of SEQ ID NO: 6 encoded by the nucleic acid sequence of SEQ ID NO: 7, a kappa light chain variable region comprising the amino acid sequence of SEQ ID NO: 105 encoded by the nucleic acid sequence of SEQ ID NO: 106, and a lambda light chain variable region comprising the amino acid sequence of SEQ ID NO: 43 encoded by the nucleic acid sequence of SEQ ID NO: 44.

[0097] In some embodiments, the **K2 x S14** bispecific antibody has a heavy chain variable and constant region comprising the amino acid sequence of SEQ ID NO: 4 encoded by the nucleic acid sequence of SEQ ID NO: 5, a kappa light chain comprising the amino acid sequence of SEQ ID NO: 107 encoded by the nucleic acid sequence of SEQ ID NO: 104, and a lambda light chain comprising the amino acid sequence of SEQ ID NO: 41 encoded by the nucleic acid sequence shown in SEQ ID NO: 42.

[0098] In some embodiments, the **K2 x S15** bispecific antibody has a heavy chain comprising a CDRH1 comprising the amino acid sequence of SEQ ID NO: 1, a CDRH2 comprising the amino acid sequence of SEQ ID NO: 2, a CDRH3 comprising the amino

acid sequence of SEQ ID NO: 3, a kappa light chain comprising a CDRL1 comprising the amino acid sequence of SEQ ID NO: 89, a CDRL2 comprising the amino acid sequence of SEQ ID NO: 92, and a CDRL3 comprising the amino acid sequence of SEQ ID NO: 96, and a lambda light chain comprising a CDRL1 comprising the amino acid sequence of SEQ ID NO: 9, a CDRL2 comprising the amino acid sequence of SEQ ID NO: 16, and a CDRL3 comprising the amino acid sequence of SEQ ID NO: 24.

[0099] In some embodiments, the **K2 x S15** bispecific antibody has a heavy chain variable region comprising the amino acid sequence of SEQ ID NO: 6 encoded by the nucleic acid sequence of SEQ ID NO: 7, a kappa light chain variable region comprising the amino acid sequence of SEQ ID NO: 105 encoded by the nucleic acid sequence of SEQ ID NO: 106, and a lambda light chain variable region comprising the amino acid sequence of SEQ ID NO: 47 encoded by the nucleic acid sequence of SEQ ID NO: 48.

[00100] In some embodiments, the **K2 x S15** bispecific antibody has a heavy chain variable and constant region comprising the amino acid sequence of SEQ ID NO: 4 encoded by the nucleic acid sequence of SEQ ID NO: 5, a kappa light chain comprising the amino acid sequence of SEQ ID NO: 107 encoded by the nucleic acid sequence of SEQ ID NO: 104, and a lambda light chain comprising the amino acid sequence of SEQ ID NO: 45 encoded by the nucleic acid sequence shown in SEQ ID NO: 46.

[00101] In some embodiments, the **K2 x S17** bispecific antibody has a heavy chain comprising a CDRH1 comprising the amino acid sequence of SEQ ID NO: 1, a CDRH2 comprising the amino acid sequence of SEQ ID NO: 2, a CDRH3 comprising the amino acid sequence of SEQ ID NO: 3, a kappa light chain comprising a CDRL1 comprising the amino acid sequence of SEQ ID NO: 89, a CDRL2 comprising the amino acid sequence of SEQ ID NO: 92, and a CDRL3 comprising the amino acid sequence of SEQ ID NO: 96, and a lambda light chain comprising a CDRL1 comprising the amino acid sequence of SEQ ID NO: 9, a CDRL2 comprising the amino acid sequence of SEQ ID NO: 16, and a CDRL3 comprising the amino acid sequence of SEQ ID NO: 25.

[00102] In some embodiments, the **K2 x S17** bispecific antibody has a heavy chain variable region comprising the amino acid sequence of SEQ ID NO: 6 encoded by the nucleic acid sequence of SEQ ID NO: 7, a kappa light chain variable region comprising the amino acid sequence of SEQ ID NO: 105 encoded by the nucleic acid sequence of SEQ ID NO: 106, and a lambda light chain variable region comprising the amino acid sequence of SEQ ID NO: 51 encoded by the nucleic acid sequence of SEQ ID NO: 52.

[00103] In some embodiments, the **K2 x S17** bispecific antibody has a heavy chain variable and constant region comprising the amino acid sequence of SEQ ID NO: 4 encoded by the nucleic acid sequence of SEQ ID NO: 5, a kappa light chain comprising the amino acid sequence of SEQ ID NO: 107 encoded by the nucleic acid sequence of SEQ ID NO: 104, and a lambda light chain comprising the amino acid sequence of SEQ ID NO: 49 encoded by the nucleic acid sequence shown in SEQ ID NO: 50.

[00104] In some embodiments, the **K2 x S57** bispecific antibody has a heavy chain comprising a CDRH1 comprising the amino acid sequence of SEQ ID NO: 1, a CDRH2 comprising the amino acid sequence of SEQ ID NO: 2, a CDRH3 comprising the amino acid sequence of SEQ ID NO: 3, a kappa light chain comprising a CDRL1 comprising the amino acid sequence of SEQ ID NO: 89, a CDRL2 comprising the amino acid sequence of SEQ ID NO: 92, and a CDRL3 comprising the amino acid sequence of SEQ ID NO: 96, and a lambda light chain comprising a CDRL1 comprising the amino acid sequence of SEQ ID NO: 9, a CDRL2 comprising the amino acid sequence of SEQ ID NO: 16, and a CDRL3 comprising the amino acid sequence of SEQ ID NO: 24.

[00105] In some embodiments, the **K2 x S57** bispecific antibody has a heavy chain variable region comprising the amino acid sequence of SEQ ID NO: 6 encoded by the nucleic acid sequence of SEQ ID NO: 7, a kappa light chain variable region comprising the amino acid sequence of SEQ ID NO: 105 encoded by the nucleic acid sequence of SEQ ID NO: 106, and a lambda light chain variable region comprising the amino acid sequence of SEQ ID NO: 55 encoded by the nucleic acid sequence of SEQ ID NO: 56.

[00106] In some embodiments, the **K2 x S57** bispecific antibody has a heavy chain variable and constant region comprising the amino acid sequence of SEQ ID NO: 4 encoded by the nucleic acid sequence of SEQ ID NO: 5, a kappa light chain comprising the amino acid sequence of SEQ ID NO: 107 encoded by the nucleic acid sequence of SEQ ID NO: 104, and a lambda light chain comprising the amino acid sequence of SEQ ID NO: 53 encoded by the nucleic acid sequence shown in SEQ ID NO: 54.

[00107] In some embodiments, the **K2 x S58** bispecific antibody has a heavy chain comprising a CDRH1 comprising the amino acid sequence of SEQ ID NO: 1, a CDRH2 comprising the amino acid sequence of SEQ ID NO: 2, a CDRH3 comprising the amino acid sequence of SEQ ID NO: 3, a kappa light chain comprising a CDRL1 comprising the amino acid sequence of SEQ ID NO: 89, a CDRL2 comprising the amino acid sequence of SEQ ID NO: 92, and a CDRL3 comprising the amino acid sequence of SEQ ID NO: 96, and

a lambda light chain comprising a CDRL1 comprising the amino acid sequence of SEQ ID NO: 9, a CDRL2 comprising the amino acid sequence of SEQ ID NO: 16, and a CDRL3 comprising the amino acid sequence of SEQ ID NO: 24.

[00108] In some embodiments, the **K2 x S58** bispecific antibody has a heavy chain variable region comprising the amino acid sequence of SEQ ID NO: 6 encoded by the nucleic acid sequence of SEQ ID NO: 7, a kappa light chain variable region comprising the amino acid sequence of SEQ ID NO: 105 encoded by the nucleic acid sequence of SEQ ID NO: 106, and a lambda light chain variable region comprising the amino acid sequence of SEQ ID NO: 59 encoded by the nucleic acid sequence of SEQ ID NO: 60.

[00109] In some embodiments, the **K2 x S58** bispecific antibody has a heavy chain variable and constant region comprising the amino acid sequence of SEQ ID NO: 4 encoded by the nucleic acid sequence of SEQ ID NO: 5, a kappa light chain comprising the amino acid sequence of SEQ ID NO: 107 encoded by the nucleic acid sequence of SEQ ID NO: 104, and a lambda light chain comprising the amino acid sequence of SEQ ID NO: 57 encoded by the nucleic acid sequence shown in SEQ ID NO: 58.

[00110] In some embodiments, the **K2 x S28** bispecific antibody has a heavy chain comprising a CDRH1 comprising the amino acid sequence of SEQ ID NO: 1, a CDRH2 comprising the amino acid sequence of SEQ ID NO: 2, a CDRH3 comprising the amino acid sequence of SEQ ID NO: 3, a kappa light chain comprising a CDRL1 comprising the amino acid sequence of SEQ ID NO: 89, a CDRL2 comprising the amino acid sequence of SEQ ID NO: 92, and a CDRL3 comprising the amino acid sequence of SEQ ID NO: 96, and a lambda light chain comprising a CDRL1 comprising the amino acid sequence of SEQ ID NO: 10, a CDRL2 comprising the amino acid sequence of SEQ ID NO: 17, and a CDRL3 comprising the amino acid sequence of SEQ ID NO: 97.

[00111] In some embodiments, the **K2 x S28** bispecific antibody has a heavy chain variable region comprising the amino acid sequence of SEQ ID NO: 6 encoded by the nucleic acid sequence of SEQ ID NO: 7, a kappa light chain variable region comprising the amino acid sequence of SEQ ID NO: 105 encoded by the nucleic acid sequence of SEQ ID NO: 106, and a lambda light chain variable region comprising the amino acid sequence of SEQ ID NO: 63 encoded by the nucleic acid sequence of SEQ ID NO: 64.

[00112] In some embodiments, the **K2 x S28** bispecific antibody has a heavy chain variable and constant region comprising the amino acid sequence of SEQ ID NO: 4 encoded by the nucleic acid sequence of SEQ ID NO: 5, a kappa light chain comprising the amino acid

sequence of SEQ ID NO: 107 encoded by the nucleic acid sequence of SEQ ID NO: 104, and a lambda light chain comprising the amino acid sequence of SEQ ID NO: 61 encoded by the nucleic acid sequence shown in SEQ ID NO: 62.

[00113] In some embodiments, the **K2 x S30** bispecific antibody has a heavy chain comprising a CDRH1 comprising the amino acid sequence of SEQ ID NO: 1, a CDRH2 comprising the amino acid sequence of SEQ ID NO: 2, a CDRH3 comprising the amino acid sequence of SEQ ID NO: 3, a kappa light chain comprising a CDRL1 comprising the amino acid sequence of SEQ ID NO: 89, a CDRL2 comprising the amino acid sequence of SEQ ID NO: 92, and a CDRL3 comprising the amino acid sequence of SEQ ID NO: 96, and a lambda light chain comprising a CDRL1 comprising the amino acid sequence of SEQ ID NO: 10, a CDRL2 comprising the amino acid sequence of SEQ ID NO: 17, and a CDRL3 comprising the amino acid sequence of SEQ ID NO: 98.

[00114] In some embodiments, the **K2 x S30** bispecific antibody has a heavy chain variable region comprising the amino acid sequence of SEQ ID NO: 6 encoded by the nucleic acid sequence of SEQ ID NO: 7, a kappa light chain variable region comprising the amino acid sequence of SEQ ID NO: 105 encoded by the nucleic acid sequence of SEQ ID NO: 106, and a lambda light chain variable region comprising the amino acid sequence of SEQ ID NO: 67 encoded by the nucleic acid sequence of SEQ ID NO: 68.

[00115] In some embodiments, the **K2 x S30** bispecific antibody has a heavy chain variable and constant region comprising the amino acid sequence of SEQ ID NO: 4 encoded by the nucleic acid sequence of SEQ ID NO: 5, a kappa light chain comprising the amino acid sequence of SEQ ID NO: 107 encoded by the nucleic acid sequence of SEQ ID NO: 104, and a lambda light chain comprising the amino acid sequence of SEQ ID NO: 65 encoded by the nucleic acid sequence shown in SEQ ID NO: 66.

[00116] In some embodiments, the **K2 x S94** bispecific antibody has a heavy chain comprising a CDRH1 comprising the amino acid sequence of SEQ ID NO: 1, a CDRH2 comprising the amino acid sequence of SEQ ID NO: 2, a CDRH3 comprising the amino acid sequence of SEQ ID NO: 3, a kappa light chain comprising a CDRL1 comprising the amino acid sequence of SEQ ID NO: 89, a CDRL2 comprising the amino acid sequence of SEQ ID NO: 92, and a CDRL3 comprising the amino acid sequence of SEQ ID NO: 96, and a lambda light chain comprising a CDRL1 comprising the amino acid sequence of SEQ ID NO: 11, a CDRL2 comprising the amino acid sequence of SEQ ID NO: 18, and a CDRL3 comprising the amino acid sequence of SEQ ID NO: 97.

[00117] In some embodiments, the **K2 x S94** bispecific antibody has a heavy chain variable region comprising the amino acid sequence of SEQ ID NO: 6 encoded by the nucleic acid sequence of SEQ ID NO: 7, a kappa light chain variable region comprising the amino acid sequence of SEQ ID NO: 105 encoded by the nucleic acid sequence of SEQ ID NO: 106, and a lambda light chain variable region comprising the amino acid sequence of SEQ ID NO: 71 encoded by the nucleic acid sequence of SEQ ID NO: 72.

[00118] In some embodiments, the **K2 x S94** bispecific antibody has a heavy chain variable and constant region comprising the amino acid sequence of SEQ ID NO: 4 encoded by the nucleic acid sequence of SEQ ID NO: 5, a kappa light chain comprising the amino acid sequence of SEQ ID NO: 107 encoded by the nucleic acid sequence of SEQ ID NO: 104, and a lambda light chain comprising the amino acid sequence of SEQ ID NO: 69 encoded by the nucleic acid sequence shown in SEQ ID NO: 70.

[00119] In some embodiments, the **K2 x S23** bispecific antibody has a heavy chain comprising a CDRH1 comprising the amino acid sequence of SEQ ID NO: 1, a CDRH2 comprising the amino acid sequence of SEQ ID NO: 2, a CDRH3 comprising the amino acid sequence of SEQ ID NO: 3, a kappa light chain comprising a CDRL1 comprising the amino acid sequence of SEQ ID NO: 89, a CDRL2 comprising the amino acid sequence of SEQ ID NO: 92, and a CDRL3 comprising the amino acid sequence of SEQ ID NO: 96, and a lambda light chain comprising a CDRL1 comprising the amino acid sequence of SEQ ID NO: 12, a CDRL2 comprising the amino acid sequence of SEQ ID NO: 19, and a CDRL3 comprising the amino acid sequence of SEQ ID NO: 26.

[00120] In some embodiments, the **K2 x S23** bispecific antibody has a heavy chain variable region comprising the amino acid sequence of SEQ ID NO: 6 encoded by the nucleic acid sequence of SEQ ID NO: 7, a kappa light chain variable region comprising the amino acid sequence of SEQ ID NO: 105 encoded by the nucleic acid sequence of SEQ ID NO: 106, and a lambda light chain variable region comprising the amino acid sequence of SEQ ID NO: 75 encoded by the nucleic acid sequence of SEQ ID NO: 76.

[00121] In some embodiments, the **K2 x S23** bispecific antibody has a heavy chain variable and constant region comprising the amino acid sequence of SEQ ID NO: 4 encoded by the nucleic acid sequence of SEQ ID NO: 5, a kappa light chain comprising the amino acid sequence of SEQ ID NO: 107 encoded by the nucleic acid sequence of SEQ ID NO: 104, and a lambda light chain comprising the amino acid sequence of SEQ ID NO: 73 encoded by the nucleic acid sequence shown in SEQ ID NO: 74.

[00122] In some embodiments, the **K2 x S46** bispecific antibody has a heavy chain comprising a CDRH1 comprising the amino acid sequence of SEQ ID NO: 1, a CDRH2 comprising the amino acid sequence of SEQ ID NO: 2, a CDRH3 comprising the amino acid sequence of SEQ ID NO: 3, a kappa light chain comprising a CDRL1 comprising the amino acid sequence of SEQ ID NO: 89, a CDRL2 comprising the amino acid sequence of SEQ ID NO: 92, and a CDRL3 comprising the amino acid sequence of SEQ ID NO: 96, and a lambda light chain comprising a CDRL1 comprising the amino acid sequence of SEQ ID NO: 13, a CDRL2 comprising the amino acid sequence of SEQ ID NO: 19, and a CDRL3 comprising the amino acid sequence of SEQ ID NO: 26.

[00123] In some embodiments, the **K2 x S46** bispecific antibody has a heavy chain variable region comprising the amino acid sequence of SEQ ID NO: 6 encoded by the nucleic acid sequence of SEQ ID NO: 7, a kappa light chain variable region comprising the amino acid sequence of SEQ ID NO: 105 encoded by the nucleic acid sequence of SEQ ID NO: 106, and a lambda light chain variable region comprising the amino acid sequence of SEQ ID NO: 79 encoded by the nucleic acid sequence of SEQ ID NO: 80.

[00124] In some embodiments, the **K2 x S46** bispecific antibody has a heavy chain variable and constant region comprising the amino acid sequence of SEQ ID NO: 4 encoded by the nucleic acid sequence of SEQ ID NO: 5, a kappa light chain comprising the amino acid sequence of SEQ ID NO: 107 encoded by the nucleic acid sequence of SEQ ID NO: 104, and a lambda light chain comprising the amino acid sequence of SEQ ID NO: 77 encoded by the nucleic acid sequence shown in SEQ ID NO: 78.

[00125] In some embodiments, the **K2 x S71** bispecific antibody has a heavy chain comprising a CDRH1 comprising the amino acid sequence of SEQ ID NO: 1, a CDRH2 comprising the amino acid sequence of SEQ ID NO: 2, a CDRH3 comprising the amino acid sequence of SEQ ID NO: 3, a kappa light chain comprising a CDRL1 comprising the amino acid sequence of SEQ ID NO: 89, a CDRL2 comprising the amino acid sequence of SEQ ID NO: 92, and a CDRL3 comprising the amino acid sequence of SEQ ID NO: 96, and a lambda light chain comprising a CDRL1 comprising the amino acid sequence of SEQ ID NO: 14, a CDRL2 comprising the amino acid sequence of SEQ ID NO: 19, and a CDRL3 comprising the amino acid sequence of SEQ ID NO: 27.

[00126] In some embodiments, the **K2 x S71** bispecific antibody has a heavy chain variable region comprising the amino acid sequence of SEQ ID NO: 6 encoded by the nucleic acid sequence of SEQ ID NO: 7, a kappa light chain variable region comprising the amino acid

sequence of SEQ ID NO: 105 encoded by the nucleic acid sequence of SEQ ID NO: 106, and a lambda light chain variable region comprising the amino acid sequence of SEQ ID NO: 83 encoded by the nucleic acid sequence of SEQ ID NO: 84.

[00127] In some embodiments, the **K2 x S71** bispecific antibody has a heavy chain variable and constant region comprising the amino acid sequence of SEQ ID NO: 4 encoded by the nucleic acid sequence of SEQ ID NO: 5, a kappa light chain comprising the amino acid sequence of SEQ ID NO: 107 encoded by the nucleic acid sequence of SEQ ID NO: 104, and a lambda light chain comprising the amino acid sequence of SEQ ID NO: 81 encoded by the nucleic acid sequence shown in SEQ ID NO: 82.

[00128] In some embodiments, the **K2 x S79** bispecific antibody has a heavy chain comprising a CDRH1 comprising the amino acid sequence of SEQ ID NO: 1, a CDRH2 comprising the amino acid sequence of SEQ ID NO: 2, a CDRH3 comprising the amino acid sequence of SEQ ID NO: 3, a kappa light chain comprising a CDRL1 comprising the amino acid sequence of SEQ ID NO: 89, a CDRL2 comprising the amino acid sequence of SEQ ID NO: 92, and a CDRL3 comprising the amino acid sequence of SEQ ID NO: 96, and a lambda light chain comprising a CDRL1 comprising the amino acid sequence of SEQ ID NO: 14, a CDRL2 comprising the amino acid sequence of SEQ ID NO: 19, and a CDRL3 comprising the amino acid sequence of SEQ ID NO: 28.

[00129] In some embodiments, the **K2 x S79** bispecific antibody has a heavy chain variable region comprising the amino acid sequence of SEQ ID NO: 6 encoded by the nucleic acid sequence of SEQ ID NO: 7, a kappa light chain variable region comprising the amino acid sequence of SEQ ID NO: 105 encoded by the nucleic acid sequence of SEQ ID NO: 106, and a lambda light chain variable region comprising the amino acid sequence of SEQ ID NO: 87 encoded by the nucleic acid sequence of SEQ ID NO: 88.

[00130] In some embodiments, the **K2 x S79** bispecific antibody has a heavy chain variable and constant region comprising the amino acid sequence of SEQ ID NO: 4 encoded by the nucleic acid sequence of SEQ ID NO: 5, a kappa light chain comprising the amino acid sequence of SEQ ID NO: 107 encoded by the nucleic acid sequence of SEQ ID NO: 104, and a lambda light chain comprising the amino acid sequence of SEQ ID NO: 85 encoded by the nucleic acid sequence shown in SEQ ID NO: 86.

[00131] In some embodiments, the **K2 x S93** bispecific antibody has a heavy chain comprising a CDRH1 comprising the amino acid sequence of SEQ ID NO: 1, a CDRH2 comprising the amino acid sequence of SEQ ID NO: 2, a CDRH3 comprising the amino

acid sequence of SEQ ID NO: 3, a kappa light chain comprising a CDRL1 comprising the amino acid sequence of SEQ ID NO: 89, a CDRL2 comprising the amino acid sequence of SEQ ID NO: 92, and a CDRL3 comprising the amino acid sequence of SEQ ID NO: 96, and a lambda light chain comprising a CDRL1 comprising the amino acid sequence of SEQ ID NO: 101, a CDRL2 comprising the amino acid sequence of SEQ ID NO: 18, and a CDRL3 comprising the amino acid sequence of SEQ ID NO: 97.

[00132] In some embodiments, the **K2 x S93** bispecific antibody has a heavy chain variable region comprising the amino acid sequence of SEQ ID NO: 6 encoded by the nucleic acid sequence of SEQ ID NO: 7, a kappa light chain variable region comprising the amino acid sequence of SEQ ID NO: 105 encoded by the nucleic acid sequence of SEQ ID NO: 106, and a lambda light chain variable region comprising the amino acid sequence of SEQ ID NO: 91 encoded by the nucleic acid sequence of SEQ ID NO: 109.

[00133] In some embodiments, the **K2 x S93** bispecific antibody has a heavy chain variable and constant region comprising the amino acid sequence of SEQ ID NO: 4 encoded by the nucleic acid sequence of SEQ ID NO: 5, a kappa light chain comprising the amino acid sequence of SEQ ID NO: 107 encoded by the nucleic acid sequence of SEQ ID NO: 104, and a lambda light chain comprising the amino acid sequence of SEQ ID NO: 108 encoded by the nucleic acid sequence shown in SEQ ID NO: 90.

[00134] In some embodiments, the **K2 x S96** bispecific antibody has a heavy chain comprising a CDRH1 comprising the amino acid sequence of SEQ ID NO: 1, a CDRH2 comprising the amino acid sequence of SEQ ID NO: 2, a CDRH3 comprising the amino acid sequence of SEQ ID NO: 3, a kappa light chain comprising a CDRL1 comprising the amino acid sequence of SEQ ID NO: 89, a CDRL2 comprising the amino acid sequence of SEQ ID NO: 92, and a CDRL3 comprising the amino acid sequence of SEQ ID NO: 96, and a lambda light chain comprising a CDRL1 comprising the amino acid sequence of SEQ ID NO: 102, a CDRL2 comprising the amino acid sequence of SEQ ID NO: 18, and a CDRL3 comprising the amino acid sequence of SEQ ID NO: 97.

[00135] In some embodiments, the **K2 x S96** bispecific antibody has a heavy chain variable region comprising the amino acid sequence of SEQ ID NO: 6 encoded by the nucleic acid sequence of SEQ ID NO: 7, a kappa light chain variable region comprising the amino acid sequence of SEQ ID NO: 105 encoded by the nucleic acid sequence of SEQ ID NO: 106, and a lambda light chain variable region comprising the amino acid sequence of SEQ ID NO: 95 encoded by the nucleic acid sequence of SEQ ID NO: 110.

[00136] In some embodiments, the **K2 x S96** bispecific antibody has a heavy chain variable and constant region comprising the amino acid sequence of SEQ ID NO: 4 encoded by the nucleic acid sequence of SEQ ID NO: 5, a kappa light chain comprising the amino acid sequence of SEQ ID NO: 107 encoded by the nucleic acid sequence of SEQ ID NO: 104, and a lambda light chain comprising the amino acid sequence of SEQ ID NO: 93 encoded by the nucleic acid sequence shown in SEQ ID NO: 94.

[00137] In some embodiments, the **K2 x S100** bispecific antibody has a heavy chain comprising a CDRH1 comprising the amino acid sequence of SEQ ID NO: 1, a CDRH2 comprising the amino acid sequence of SEQ ID NO: 2, a CDRH3 comprising the amino acid sequence of SEQ ID NO: 3, a kappa light chain comprising a CDRL1 comprising the amino acid sequence of SEQ ID NO: 89, a CDRL2 comprising the amino acid sequence of SEQ ID NO: 92, and a CDRL3 comprising the amino acid sequence of SEQ ID NO: 96, and a lambda light chain comprising a CDRL1 comprising the amino acid sequence of SEQ ID NO: 103, a CDRL2 comprising the amino acid sequence of SEQ ID NO: 18, and a CDRL3 comprising the amino acid sequence of SEQ ID NO: 97.

[00138] In some embodiments, the **K2 x S100** bispecific antibody has a heavy chain variable region comprising the amino acid sequence of SEQ ID NO: 6 encoded by the nucleic acid sequence of SEQ ID NO: 7, a kappa light chain variable region comprising the amino acid sequence of SEQ ID NO: 105 encoded by the nucleic acid sequence of SEQ ID NO: 106, and a lambda light chain variable region comprising the amino acid sequence of SEQ ID NO: 99 encoded by the nucleic acid sequence of SEQ ID NO: 100.

[00139] In some embodiments, the **K2 x S100** bispecific antibody has a heavy chain variable and constant region comprising the amino acid sequence of SEQ ID NO: 4 encoded by the nucleic acid sequence of SEQ ID NO: 5, a kappa light chain comprising the amino acid sequence of SEQ ID NO: 107 encoded by the nucleic acid sequence of SEQ ID NO: 104, and a lambda light chain comprising the amino acid sequence of SEQ ID NO: 113 encoded by the nucleic acid sequence shown in SEQ ID NO: 114.

[00140] Each of the exemplary anti-CD47, anti-PD-L1, monospecific and the anti-CD47 and anti-PD-L1 bispecific antibodies described herein include a common heavy chain (HC), one kappa chain or one lambda chain for anti-CD47 and anti-PD-L1 antibodies, one kappa and one lambda light chains (LC) for monospecific bispecific antibodies, as shown in the amino acid and corresponding nucleic acid sequences listed below. Each of the exemplary anti-CD47, anti-PD-L1, monovalent and bispecific antibodies described below includes a heavy

chain variable domain (VH), one kappa light chain variable domain or one lambda light chain variable domain for anti-CD47 and anti-PD-L1 antibodies, one kappa light chain variable domain and one lambda light chain variable domains (VL) for monovalent and bispecific antibodies, as shown in the amino acid and corresponding nucleic acid sequences listed below.

[00141] While antibody sequences below are provided herein as examples, it is to be understood that these sequences can be used to generate bispecific antibodies using any of a variety of art-recognized techniques. Examples of bispecific formats include but are not limited to bispecific IgG based on Fab arm exchange (Gramer et al., 2013 MAbs. 5(6)); the CrossMab format (Klein C et al., 2012 MAbs 4(6)); multiple formats based on forced heterodimerization approaches such as SEED technology (Davis JH et al., 2010 Protein Eng Des Sel. 23(4):195-202), electrostatic steering (Gunasekaran K et al., J Biol Chem. 2010 285(25):19637-46.) or knob-into-hole (Ridgway JB et al., Protein Eng. 1996 9(7):617-21.) or other sets of mutations preventing homodimer formation (Von Kreudenstein TS et al., 2013 MAbs. 5(5):646-54.); fragment based bispecific formats such as tandem scFv (such asBiTEs) (Wolf E et al., 2005 Drug Discov. Today 10(18):1237-44.); bispecific tetravalent antibodies (Pörtner LM et al., 2012 Cancer Immunol Immunother. 61(10):1869-75.); dual affinity retargeting molecules (Moore PA et al., 2011 Blood.117(17):4542-51), diabodies (Kontermann RE et al., Nat Biotechnol. 1997 15(7):629-31).

[00142] The exemplary anti-CD47, anti-PD-L1, monospecific and bispecific antibodies include a heavy chain variable region and common region comprising an amino acid sequence of SEQ ID NO: 4 which is encoded by the nucleic acid sequence of SEQ ID NO: 5.

>VHCH IGHV3-23 hIgG1-AA (SEQ ID NO: 4)

EVQLLESGGGLVQPGGSLRLSCAAS**GFTFSSY**AMSWVRQAPGKGLEWVSA**ISGSGGST**YYADSVKGR
FTISRDNSKNTLYLQMNSLRAEDTAVYYC**AKSYGAFDY**WGQGTLVTVSSASTKGPSVFPLAPSSKST
SGGTAALGCLVKDYFPEPVTFSWNSGALTSGVHTFPAVLQSSGLYSLSSVVTVPSSSLGTQTYICNV
NHKPSNTKVDKRVEPKSCDKTHTCPPCPAPELLGGPSVFLFPPKPKDTLMISRTPEVTCVVDVDSHE
DPEVKFNWYVDGVEVHNAKTKPREEQYNSTYRVVSVLTVLHQDWLNGKEYKCKVSNKALPAPIEKT
ISKAKGQPREPQVYTLPPSREEMTKNQVSLTCLVKGFYPSDIAVEWESNGQPENNYKTTPPVLDSDGS
FFLYSKLTVDKSRWQQGNVFSFSVMHEALHNHYTQKSLSLSPG

>VHCH IGHV3-23 hIgG1-NT (SEQ ID NO: 5)

GAGGTGCAGCTGTTGGAGTCTGGGGGAGGCTTGGTACAGCCTGGGGGGTCCCTGAGACTCTCCTGTG
CAGCCTCTGGATTACCTTTAGCAGCTATGCCATGAGCTGGGTCCGCCAGGCTCCAGGGAAGGGGCT
GGAGTGGGTCTCAGCTATTAGTGGTAGTGGTGGTAGCACATACTACGCAGACTCCGTGAAGGGCCGG
TTCACCATCTCCAGAGACAATTCCAAGAACACGCTGTATCTGCAAATGAACAGCCTGAGAGCCGAGG
ACACGGCCGTATATTACTGTGCGAAAAGTTATGGTGCTTTTGACTACTGGGGCCAGGGAACCCTGGT

CACAGTCTCGAGCGCCTCCACCAAGGGCCCATCGGTCTTCCCCCTGGCACCCCTCCTCCAAGAGCACC
TCTGGGGGCACAGCGGCCCTGGGCTGCCTGGTCAAGGACTACTTCCCCGAACCGGTGACAGTCTCGT
GGAAGTCAAGGAGCCCTGACCAGCGCGTGCACACCTTCCCGGCTGTCTACAGTCTCAGGACTCTA
CTCCCTCAGCAGCGTGGTGACTGTGCCCTCCAGCAGCTTGGGCACCCAGACCTACATCTGCAACGTG
AATCACAAGCCCAGCAACACCAAGGTGGACAAGAGAGTTGAGCCCAAATCTTGTGACAAAACCTCACA
CATGCCCCACCGTGCCAGCACCTGAACTCCTGGGGGGACCGTCAGTCTTCTCTTCCCCCCCCAAAACC
CAAGGACACCCCTCATGATCTCCCGGACCCCTGAGGTACATGCGTGGTGGTGGACGTGAGCCACGAA
GACCCTGAGGTCAAGTTCAACTGGTACGTGGACGGCGTGGAGGTGCATAATGCCAAGACAAAGCCGC
GGGAGGAGCAGTACAACAGCACGTACCGTGTGGTACGCGTCTCACCCTCCTGCACCAGGACTGGCT
GAATGGCAAGGAGTACAAGTGCAAGGTCTCCAACAAAGCCCTCCAGCCCCCATCGAGAAAACCATC
TCCAAAGCCAAAGGGCAGCCCCGAGAACCACAGGTGTATACCCTGCCCCCATCTCGGGAGGAGATGA
CCAAGAACCAGGTGAGCCTGACTTGCCTGGTCAAAGGCTTCTATCCAGCGACATCGCCGTGGAGTG
GGAGAGCAACGGGCAGCCGGAGAACAACACTACAAGACCACGCCTCCCGTGCTGGACTCCGACGGCTCC
TTCTTCTCTATAGCAAGCTCACCGTGGACAAGTCCAGGTGGCAGCAGGGGAACGTCTTCTCATGCT
CCGTGATGCATGAGGCTCTGCACAACCACTACACGCAGAAGAGCCTCTCCCTGTCTCCGGGTAA

[00143] The exemplary anti-CD47, anti-PD-L1, monospecific and bispecific antibodies include a heavy chain variable region comprising an amino acid sequence of SEQ ID NO: 6 which is encoded by the nucleic acid sequence of SEQ ID NO: 7.

>VH IGHV3-23 hIgG1-AA (SEQ ID NO: 6)

EVQLLESQGGGLVQPGGSLRLSCAAS**GFTFSSY**AMSWVRQAPGKGLEWVSA**ISGSGGST**YYADSVKGR
FTISRDN SKNTLYLQMN SLRAEDTAVYYC**AKSYGAFDY**WGQGLVTVSS

>VHCH IGHV3-23 hIgG1-NT (SEQ ID NO: 7)

GAGGTGCAGCTGTTGGAGTCTGGGGGAGGCTTGGTACAGCCTGGGGGGTCCCTGAGACTCTCCTGTG
CAGCCTCTGGATTACCTTTAGCAGCTATGCCATGAGCTGGGTCCGCCAGGCTCCAGGGAAGGGCT
GGAGTGGGTCTCAGCTATTAGTGGTAGTGGTGGTAGCACATACTACGCAGACTCCGTGAAGGGCCGG
TTCACCATCTCCAGAGACAATTCCAAGAACACGCTGTATCTGCAAATGAACAGCCTGAGAGCCGAGG
ACACGGCCGTATATTACTGTGCGAAAAGTTATGGTGCTTTTGACTACTGGGGCCAGGGAACCCTGGT
CACAGTCTCGAGC

[00144] ANTI-CD47 ANTIBODIES

[00145] Exemplary anti-CD47 antibody sequences are shown below. Light chain variable regions are shown in *italicized underlined* text. CDR sequences are shown in **underlined** text.

[00146] The “K2” or “Ka3” or “K2_KA3 VKCK aCD47 IGKV1-39” antibody has a heavy chain variable region and heavy chain common region comprising an amino acid sequence of SEQ ID NO: 4, which is encoded by a nucleic acid sequence of SEQ ID NO: 5, and a kappa light chain comprising an amino acid sequence of SEQ ID NO: 107, which is encoded by a nucleic acid sequence of SEQ ID NO: 104.

>K2_KA3 VKCK aCD47 IGKV1-39-AA (SEQ ID NO: 107)

DIQMTQSPSSLSASVGDRVTITCRASQSISSYLNWYQQKPGKAPKLLIYAASSLQSGVPSRFSGSGS
GTDFTLTISSLQPEDFATYYCQQMHPRAPKTFGQGTKVEIKRTVAAPSVFIFPPSDEQLKSGTASV
 CLLNNFYPREAKVQWKVDNALQSGNSQESVTEQDSKDSYLSSTLTLSKADYEKHKVYACEVTHQG
 LSSPVTKSFNRGEC

>K2_KA3 VKCK aCD47 IGKV1-39-NT (SEQ ID NO: 104)

ATGAGTGTGCCCCTCAGGTCCTGGGGTTGCTGCTGCTGTGGCTTACAGATGCCAGATGTGACATCC
 AGATGACCCAGTCTCCATCCTCCCTGTCTGCATCTGTAGGAGACAGAGTCACCATCACTTGCCGGGC
 AAGTCAGAGCATTAGCAGCTATTTAAATTGGTATCAGCAGAAACCAGGGAAAGCCCCTAAGCTCCTG
 ATCTATGCTGCATCCAGTTTGCAAAGTGGGGTCCCATCAAGGTTCAAGTGGCAGTGGATCTGGGACAG
 ATTTCACTCTCACCATCAGCAGTCTGCAACCTGAAGATTTTGCAACTTACTACTGTCAGCAGATGCA
 CCCGCGCGCCCCGAAGACCTTCGGCCAAGGGACCAAGGTGGAAATCAAACGTACGGTGGCTGCACCA
 TCTGTCTTCATCTTCCCGCCATCTGATGAGCAGTTGAAATCTGGAAGTGCCTCTGTTGTGTGCCTGC
 TGAATAACTTCTATCCCAGAGAGGCCAAAGTACAGTGGAAGGTGGATAACGCCCTCCAATCGGGTAA
 CTCCCAGGAGAGTGTACAGAGCAGGACAGCAAGGACAGCACCTACAGCCTCAGCAGCACCTGACG
 CTGAGCAAAGCAGACTACGAGAAACACAAAGTCTACGCCTGCGAAGTCACCCATCAGGGCCTGAGCT
 CGCCCGTCACAAAGAGCTTCAACAGGGGAGAGTGTTAA

[00147] The “K2” or “Ka3” or “K2_KA3 VKCK aCD47 IGKV1-39” antibody has a heavy chain variable region comprising an amino acid sequence of SEQ ID NO: 6 which is encoded by a nucleic acid sequence of SEQ ID NO: 7 and a kappa light chain variable region comprising an amino acid sequence of SEQ ID NO: 105 which is encoded by a nucleic acid sequence of SEQ ID NO: 106.

>K2_KA3 VKCK aCD47 IGKV1-39-AA (SEQ ID NO: 105)

DIQMTQSPSSLSASVGDRVTITCRASQSISSYLNWYQQKPGKAPKLLIYAAASSLQSGVPSRFSGSGS
 GTDFTLTISSLQPEDFATYYCQQMHPRAPKTFGQGTKVEIK

>K2_KA3 VKCK aCD47 IGKV1-39-NT (SEQ ID NO: 106)

GACATCCAGATGACCCAGTCTCCATCCTCCCTGTCTGCATCTGTAGGAGACAGAGTCACCATCACTT
 GCCGGGCAAGTCAGAGCATTAGCAGCTATTTAAATTGGTATCAGCAGAAACCAGGGAAAGCCCCTAA
 GCTCCTGATCTATGCTGCATCCAGTTTGCAAAGTGGGGTCCCATCAAGGTTCAAGTGGCAGTGGATCT
 GGGACAGATTTCACTCTCACCATCAGCAGTCTGCAACCTGAAGATTTTGCAACTTACTACTGTCAGC
 AGATGCACCCGCGCGCCCCGAAGACCTTCGGCCAAGGGACCAAGGTGGAAATCAAA

ANTI-PD-L1 ANTIBODIES

[00148] Exemplary anti-PD-L1 antibody sequences are shown below. Light chain variable regions are shown in *italicized underlined* text. CDR sequences are shown in **bolded underlined** text.

[00149] The “S8” or “S8_Sa10_1A9_VLCL2 aPDL1 IGLV1-44” antibody has a heavy chain variable region and heavy chain common region comprising an amino acid sequence of SEQ ID NO: 4, which is encoded by a nucleic acid sequence of SEQ ID NO: 5, and a lambda light chain comprising an amino acid sequence of SEQ ID NO: 29, which is encoded by a nucleic acid sequence of SEQ ID NO: 30.

>S8_Sa10_1A9_VLCL2 aPDL1 IGLV1-44-AA (SEQ ID NO: 29)

QSVLTQPPSASGTPGQRTISCSGSSSNIRDSFVNWYQQLPGTAPKLLIYATNIRPSGVDP
RFSGSKSGTSASLAISGLQSEDEADYYCAAWHPYYTLFGGGTKLTVLGQPKAAPSVTLFPP
 SSEELQANKATLVCLISDFYPGAVTVAWKADSSPVKAGVETTTPSKQSNKYAASSYLSLT
 PEQWKSHRSYSCQVTHEGSTVEKTVAPTECS

>S8_Sa10_1A9_VLCL2 aPDL1 IGLV1-44-NT (SEQ ID NO: 30)

CAGTCTGTGCTGACTCAGCCACCCTCAGCGTCTGGGACCCCCGGGCAGAGGGTCACCATCT
 CTTGTTCTGGAAGCAGCTCCAACATCAGGGATAGTTTTGTAAACTGGTACCAGCAGCTCCC
 AGGAACGGCCCCCAAACCTCCTCATCTATGCTACGAATATTCGGCCCTCAGGGGTCCCTGAC
 CGATTCTCTGGCTCCAAGTCTGGCACCTCAGCCTCCCTGGCCATCAGTGGGCTCCAGTCTG
 AGGATGAGGCTGATTATTACTGTGCAGCATGGCACCCGTATTACACGTTGTTTCGGCGGAGG
 GACCAAGCTGACCGTCCTAGGTCAGCCCAAGGCTGCCCCCTCGGTCACTCTGTTCCCGCCC
 TCCTCTGAGGAGCTTCAAGCCAACAAGGCCACACTGGTGTGTCTCATAAGTGACTTCTACC
 CGGGAGCCGTGACAGTGGCTTGGAAAGCAGATAGCAGCCCCGTCAAGGCGGGAGTGGAGAC
 CACCACACCCTCCAAACAAAGCAACAACAAGTACGCGGCCAGCAGCTATCTGAGCCTGACG
 CCTGAGCAGTGGAAGTCCACAGAAGCTACAGCTGCCAGGTCACGCATGAAGGGAGCACCG
 TGGAGAAGACAGTGGCCCCCTACAGAATGTTCA

[00150] The “S8” or “S8_Sa10_1A9_VLCL2 aPDL1 IGLV1-44” antibody has a heavy chain variable region comprising an amino acid sequence of SEQ ID NO: 6 which is encoded by a nucleic acid sequence of SEQ ID NO: 7 and a lambda light chain variable region comprising an amino acid sequence of SEQ ID NO: 31 which is encoded by a nucleic acid sequence of SEQ ID NO: 32.

>S8_Sa10_1A9_VLCL2 aPDL1 IGLV1-44-AA (SEQ ID NO: 31)

QSVLTQPPSASGTPGQRTVITSCSGSSSNIRDSFVNWWYQQLPGTAPKLLIYATNIRPSGVDP
RFSGSKSGTSASLAISGLQSEDEADYYCAAWHPPYYTLFGGGTKLTVLGQPKAAPSVTL

>S8_Sa10_1A9_VLCL2 aPDL1 IGLV1-44-NT (SEQ ID NO: 32)

CAGTCTGTGCTGACTCAGCCACCCTCAGCGTCTGGGACCCCCGGGCAGAGGGTCACCATCT
CTTGTTCTGGAAGCAGCTCCAACATCAGGGATAGTTTTGTAACTGGTACCAGCAGCTCCC
AGGAACGGCCCCCAAACCTCCTCATCTATGCTACGAATATTCGGCCCTCAGGGGTCCCTGAC
CGATTCTCTGGCTCCAAGTCTGGCACCTCAGCCTCCCTGGCCATCAGTGGGCTCCAGTCTG
AGGATGAGGCTGATTATTACTGTGCAGCATGGCACCCGTATTACACGTTGTTTCGGCGGAGG
GACCAAGCTGACCGTCCTAGGTCAGCCCAAGGCTGCCCCCTCGGTCACTCTG

[00151] The “S9” or “S9_Sa10_1D9_VLCL2 aPDL1 IGLV1-44-AA” antibody has a heavy chain variable region and heavy chain common region comprising an amino acid sequence of SEQ ID NO: 4, which is encoded by a nucleic acid sequence of SEQ ID NO: 5, and a lambda light chain comprising an amino acid sequence of SEQ ID NO: 33, which is encoded by a nucleic acid sequence of SEQ ID NO: 34.

>S9_Sa10_1D9_VLCL2 aPDL1 IGLV1-44-AA (SEQ ID NO: 33)

QSVLTQPPSASGTPGQRTVITSCSGSSSNIRDSFVNWWYQQLPGTAPKLLIYATNIRPSGVDP
RFSGSKSGTSASLAISGLQSEDEADYYCASWWPYGTVFGGGTKLTVLGQPKAAPSVTLFPP
SSEELQANKATLVCLISDFYPGAVTVAWKADSSPVKAGVETTTPSKQSNKYAASSYLSLT
PEQWKSHRSYSCQVTHEGSTVEKTVAPTECS

>S9_Sa10_1D9_VLCL2 aPDL1 IGLV1-44-NT (SEQ ID NO: 34)

CAGTCTGTGCTGACTCAGCCACCCTCAGCGTCTGGGACCCCCGGGCAGAGGGTCACCATCT
CTTGTTCTGGAAGCAGCTCCAACATCAGGGATAGTTTTGTAACTGGTACCAGCAGCTCCC
AGGAACGGCCCCCAAACCTCCTCATCTATGCTACGAATATTCGGCCCTCAGGGGTCCCTGAC
CGATTCTCTGGCTCCAAGTCTGGCACCTCAGCCTCCCTGGCCATCAGTGGGCTCCAGTCTG
AGGATGAGGCTGATTATTACTGTGCATCGTGGTGGCCGTACGGTACTGTGTTTCGGCGGAGG
GACCAAGCTGACCGTCCTAGGTCAGCCCAAGGCTGCCCCCTCGGTCACTCTGTTCCCGCCC
TCCTCTGAGGAGCTTCAAGCCAACAAGGCCACACTGGTGTGTCTCATAAGTGACTTCTACC
CGGGAGCCGTGACAGTGGCTTGAAAGCAGATAGCAGCCCCGTCAAGGCGGGAGTGGAGAC

CACCACACCCTCCAAACAAAGCAACAACAAGTACGCGGCCAGCAGCTATCTGAGCCTGACG
CCTGAGCAGTGGAAGTCCCACAGAAGCTACAGCTGCCAGGTCACGCATGAAGGGAGCACCG
TGGAGAAGACAGTGGCCCCCTACAGAATGTTCA

[00152] The “S9” or “S9_Sa10_1D9_VLCL2 aPDL1 IGLV1-44-AA” antibody has a heavy chain variable region comprising an amino acid sequence of SEQ ID NO: 6 which is encoded by a nucleic acid sequence of SEQ ID NO: 7 and a lambda light chain variable region comprising an amino acid sequence of SEQ ID NO: 35 which is encoded by a nucleic acid sequence of SEQ ID NO: 36.

>**S9_Sa10_1D9_VLCL2 aPDL1 IGLV1-44-AA** (SEQ ID NO: 35)
QSVLTQPPSASGTPGQRVITISCSGSSSNIRDSFVNWFYQQLPGTAPKLLIYATNIRPSGVDP
RFSGSKSGTSASLAISGLQSEDEADYYCASWWPYGTVFGGGTKLTVLGQPKAAPSVTL

>**S9_Sa10_1D9_VLCL2 aPDL1 IGLV1-44-NT** (SEQ ID NO: 36)
CAGTCTGTGCTGACTCAGCCACCCTCAGCGTCTGGGACCCCCGGGCAGAGGGTCACCATCT
CTTGTTCTGGAAGCAGCTCCAACATCAGGGATAGTTTTGTAACTGGTACCAGCAGCTCCC
AGGAACGGCCCCCAAACCTCCTCATCTATGCTACGAATATTCGGCCCTCAGGGGTCCCTGAC
CGATTCTCTGGCTCCAAGTCTGGCACCTCAGCCTCCCTGGCCATCAGTGGGCTCCAGTCTG
AGGATGAGGCTGATTATTACTGTGCATCGTGGTGGCCGTACGGTACTGTGTTTCGGCGGAGG
GACCAAGCTGACCGTCCTAGGTCAGCCCAAGGCTGCCCCCTCGGTCACTCTG

[00153] The “S37” or “S37_Sa10_1D7_VLCL2 aPDL1 IGLV1-44” antibody has a heavy chain variable region and heavy chain common region comprising an amino acid sequence of SEQ ID NO: 4, which is encoded by a nucleic acid sequence of SEQ ID NO: 5, and a lambda light chain comprising an amino acid sequence of SEQ ID NO: 37, which is encoded by a nucleic acid sequence of SEQ ID NO: 38.

>**S37_Sa10_1D7_VLCL2 aPDL1 IGLV1-44-AA** (SEQ ID NO: 37)
QSVLTQPPSASGTPGQRVITISCSGSSSNIRDSFVNWFYQQLPGTAPKLLIYATNIRPSGVDP
RFSGSKSGTSASLAISGLQSEDEADYYCASWWPFGTVFGGGTKLTVLGQPKAAPSVTLFPP
SSEELQANKATLVCLISDFYPGAVTVAWKADSSPVKAGVETTTPSKQSNNKYAASSYLST
PEQWKSHRSYSCQVTHEGSTVEKTVAPTECS

>S37_ Sa10_1D7_VLCL2 aPDL1 IGLV1-44-NT (SEQ ID NO: 38)

CAGTCTGTGCTGACTCAGCCACCCTCAGCGTCTGGGACCCCCGGGCAGAGGGTCACCATCT
CTTGTTCTGGAAGCAGCTCCAACATCAGGGATAGTTTTGTAACTGGTACCAGCAGCTCCC
AGGAACGGCCCCCAAACCTCCTCATCTATGCTACGAATATTCGGCCCTCAGGGGTCCCTGAC
CGATTCTCTGGCTCCAAGTCTGGCACCTCAGCCTCCCTGGCCATCAGTGGGCTCCAGTCTG
AGGATGAGGCTGATTATTACTGTGCATCCTGGTGGCCGTTTCGGTACTGTGTTTCGGCGGAGG
GACCAAGCTGACCGTCCTAGGTCAGCCCAAGGCTGCCCCCTCGGTCACTCTGTTCCCGCCC
TCCTCTGAGGAGCTTCAAGCCAACAAGGCCACACTGGTGTGTCTCATAAGTGACTTCTACC
CGGGAGCCGTGACAGTGGCTTGGAAGCAGATAGCAGCCCCGTCAAGGCGGGAGTGGAGAC
CACCACACCCTCCAAACAAGCAACAACAAGTACGCGGCCAGCAGCTATCTGAGCCTGACG
CCTGAGCAGTGGAAGTCCCACAGAAGCTACAGCTGCCAGGTCACGCATGAAGGGAGCACCG
TGGAGAAGACAGTGGCCCCCTACAGAATGTTCA

[00154] The “S37” or “S37_ Sa10_1D7_VLCL2 aPDL1 IGLV1-44” antibody has a heavy chain variable region comprising an amino acid sequence of SEQ ID NO: 6 which is encoded by a nucleic acid sequence of SEQ ID NO: 7 and a lambda light chain variable region comprising an amino acid sequence of SEQ ID NO: 39 which is encoded by a nucleic acid sequence of SEQ ID NO: 40.

>S37_ Sa10_1D7_VLCL2 aPDL1 IGLV1-44-AA (SEQ ID NO: 39)

QSVLTQPPSASGTPGQRTVITSCSGSSSNIRDSFVNWYQQLPGTAPKLLIYATNIRPSGVPD
RFSGSKSGTSASLAISGLQSEDEADYYCASWWPFGTVFGGGTKLTVLGQPKAAPSVTL

>S37_ Sa10_1D7_VLCL2 aPDL1 IGLV1-44-NT (SEQ ID NO: 40)

CAGTCTGTGCTGACTCAGCCACCCTCAGCGTCTGGGACCCCCGGGCAGAGGGTCACCATCT
CTTGTTCTGGAAGCAGCTCCAACATCAGGGATAGTTTTGTAACTGGTACCAGCAGCTCCC
AGGAACGGCCCCCAAACCTCCTCATCTATGCTACGAATATTCGGCCCTCAGGGGTCCCTGAC
CGATTCTCTGGCTCCAAGTCTGGCACCTCAGCCTCCCTGGCCATCAGTGGGCTCCAGTCTG
AGGATGAGGCTGATTATTACTGTGCATCCTGGTGGCCGTTTCGGTACTGTGTTTCGGCGGAGG
GACCAAGCTGACCGTCCTAGGTCAGCCCAAGGCTGCCCCCTCGGTCACTCTG

[00155] The “S14” or “S14_ Sh3_1C6_VLCL2 aPDL1 IGLV2-23” antibody has a heavy chain variable region and heavy chain common region comprising an amino acid sequence of SEQ ID NO: 4, which is encoded by a nucleic acid sequence of SEQ ID NO: 5, and a lambda

light chain comprising an amino acid sequence of SEQ ID NO: 41, which is encoded by a nucleic acid sequence of SEQ ID NO: 42.

>**S14_ Sh3_1C6_VLCL2 aPDL1 IGLV2-23-AA** (SEQ ID NO: 41)

QSALTQPASVSGSPGQSITISCTGTSSDVVKNNFVSWYQQHPGKAPKLMIIYFGSVRPSGVS
NRFSKGSGNTASLTISGLQAEDEADYYCSSWDMPALFFGGGTKLTVLGQPKAAPSVTLFPP
 SSEELQANKATLVCLISDFYPGAVTVAWKADSSPVKAGVETTTPSKQSNKYAASSYLSLT
 PEQWKSHRSYSCQVTHEGSTVEKTVAPTECS

>**S14_ Sh3_1C6_VLCL2 aPDL1 IGLV2-23-NT** (SEQ ID NO: 42)

CAGTCTGCCCTGACTCAGCCTGCCTCCGTGTCTGGGTCTCCTGGACAGTCGATCACCATCT
 CCTGCACTGGAACCAGCAGTGACGTTGTTAAGAATAATTTTGTCTCCTGGTACCAACAGCA
 CCCAGGCAAAGCCCCCAAACATCATGATTTATTTTGGGAGTGTTTCGGCCCTCAGGGGTTTCT
 AATCGCTTCTCTGGCTCCAAGTCTGGCAACACGGCCTCCCTGACCATCTCTGGGCTCCAGG
 CTGAGGACGAGGCTGATTATTACTGCAGCTCATGGGATATGCCTGCGCTTTTCTTCGGCGG
 AGGGACCAAGCTGACCGTCCTAGGTCAGCCCAAGGCTGCCCCCTCGGTCACTCTGTTCCCG
 CCCTCCTCTGAGGAGCTTCAAGCCAACAAGGCCACACTGGTGTGTCTCATAAGTGAATTCT
 ACCCGGGAGCCGTGACAGTGGCTTGGAAGCAGATAGCAGCCCCGTCAAGGCGGGAGTGGA
 GACCACCACACCCTCCAAACAAGCAACAACAAGTACGCGGCCAGCAGCTATCTGAGCCTG
 ACGCCTGAGCAGTGGAAGTCCCACAGAAGCTACAGCTGCCAGGTCACGCATGAAGGGAGCA
 CCGTGGAGAAGACAGTGGCCCCCTACAGAATGTTCA

[00156] The “S14” or “S14_ Sh3_1C6_VLCL2 aPDL1 IGLV2-23” antibody has a heavy chain variable region comprising an amino acid sequence of SEQ ID NO: 6 which is encoded by a nucleic acid sequence of SEQ ID NO: 7 and a lambda light chain variable region comprising an amino acid sequence of SEQ ID NO: 43 which is encoded by a nucleic acid sequence of SEQ ID NO: 44.

>**S14_ Sh3_1C6_VLCL2 aPDL1 IGLV2-23-AA** (SEQ ID NO: 43)

QSALTQPASVSGSPGQSITISCTGTSSDVVKNNFVSWYQQHPGKAPKLMIIYFGSVRPSGVS
 NRFSKGSGNTASLTISGLQAEDEADYYCSSWDMPALFFGGGTKLTVLGQPKAAPSVTL

>**S14_ Sh3_1C6_VLCL2 aPDL1 IGLV2-23-NT** (SEQ ID NO: 44)

CAGTCTGCCCTGACTCAGCCTGCCTCCGTGTCTGGGTCTCCTGGACAGTCGATCACCATCT
 CCTGCACTGGAACCAGCAGTGACGTTGTTAAGAATAATTTTGTCTCCTGGTACCAACAGCA
 CCCAGGCAAAGCCCCCAAACATCATGATTTATTTTGGGAGTGTTTCGGCCCTCAGGGGTTTCT
 AATCGCTTCTCTGGCTCCAAGTCTGGCAACACGGCCTCCCTGACCATCTCTGGGCTCCAGG
 CTGAGGACGAGGCTGATTATTACTGCAGCTCATGGGATATGCCTGCGCTTTTCTTCGGCGG
 AGGGACCAAGCTGACCGTCCTAGGTCAGCCCAAGGCTGCCCCCTCGGTCACTCTG

[00157] The “S15” or “S15_ Sh3_1E2_VLCL2 aPDL1 IGLV2-23” antibody has a heavy chain variable region and heavy chain common region comprising an amino acid sequence of SEQ ID NO: 4, which is encoded by a nucleic acid sequence of SEQ ID NO: 5, and a lambda light chain comprising an amino acid sequence of SEQ ID NO: 45, which is encoded by a nucleic acid sequence of SEQ ID NO: 46.

>S15_ Sh3_1E2_VLCL2 aPDL1 IGLV2-23-AA (SEQ ID NO: 45)
QSALTQPASVSGSPGQSITISCTGTSSDVVKNNFVSWYQQHPGKAPKLMIFYGSRPSGVS
NRFSGSKSGNTASLTISGLQAEDEADYYCSSWDEPDRPFGGGTKLTVLGQPKAAPSVTLFP
 PSSEELQANKATLVCLISDFYPGAVTVAWKADSSPVKAGVETTTPSKQSNNKYAASSYLSL
 TPEQWKSHRSYSCQVTHEGSTVEKTVAPTECS

>S15_ Sh3_1E2_VLCL2 aPDL1 IGLV2-23-NT (SEQ ID NO: 46)
 CAGTCTGCCCTGACTCAGCCTGCCTCCGTGTCTGGGTCTCCTGGACAGTCGATCACCATCT
 CCTGCACTGGAACCAGCAGTGACGTTGTTAAGAATAATTTTGTCTCCTGGTACCAACAGCA
 CCCAGGCAAAGCCCCCAAACATCATGATTTATTTTGGGAGTGTTTCGGCCCTCAGGGGTTTCT
 AATCGCTTCTCTGGCTCCAAGTCTGGCAACACGGCCTCCCTGACCATCTCTGGGCTCCAGG
 CTGAGGACGAGGCTGATTATTACTGCAGCTCATGGGATGAGCCGGACAGGCCCTTCGGCGG
 AGGGACCAAGCTGACCGTCCTAGGTCAGCCCAAGGCTGCCCCCTCGGTCACTCTGTTCCCG
 CCCTCCTCTGAGGAGCTTCAAGCCAACAAGGCCACACTGGTGTGTCTCATAAGTGAATTCT
 ACCCGGGAGCCGTGACAGTGGCTTGGAAGCAGATAGCAGCCCCGTCAAGGCGGGAGTGGA
 GACCACCACACCCTCCAAACAAAGCAACAACAAGTACGCGGCCAGCAGCTATCTGAGCCTG
 ACGCCTGAGCAGTGGAAGTCCCACAGAAGCTACAGCTGCCAGGTCACGCATGAAGGGAGCA
 CCGTGGAGAAGACAGTGGCCCCTACAGAATGTTCA

[00158] The “S15” or “S15_ Sh3_1E2_VLCL2 aPDL1 IGLV2-23” antibody has a heavy chain variable region comprising an amino acid sequence of SEQ ID NO: 6 which is encoded

by a nucleic acid sequence of SEQ ID NO: 7 and a lambda light chain variable region comprising an amino acid sequence of SEQ ID NO: 47 which is encoded by a nucleic acid sequence of SEQ ID NO: 48.

>S15_ Sh3_1E2_VLCL2 aPDL1 IGLV2-23-AA (SEQ ID NO: 47)

QSALTQPASVSGSPGQSITISCTGTSSDVVKNNFVSWYQQHPGKAPKLMYFGSVRPSGVS
NRFSGSKSGNTASLTISGLQAEDEADYYCSSWDEPDRPFGGGTKLTVLGQPKAAPSVTL

>S15_ Sh3_1E2_VLCL2 aPDL1 IGLV2-23-NT (SEQ ID NO: 48)

CAGTCTGCCCTGACTCAGCCTGCCTCCGTGTCTGGGTCTCCTGGACAGTCGATCACCATCT
CCTGCACTGGAACCAGCAGTGACGTTGTAAAGAATAATTTGTCTCCTGGTACCAACAGCA
CCCAGGCAAAGCCCCCAAACATCATGATTTATTTTGGGAGTGTTTCGGCCCTCAGGGGTTTCT
AATCGCTTCTCTGGCTCCAAGTCTGGCAACACGGCCTCCCTGACCATCTCTGGGCTCCAGG
CTGAGGACGAGGCTGATTATTACTGCAGCTCATGGGATGAGCCGGACAGGCCCTTCGGCGG
AGGGACCAAGCTGACCGTCCTAGGTCAGCCCAAGGCTGCCCCCTCGGTCACCTCTG

[00159] The “S17” or “S17_ Sh3_1D9_VLCL2 aPDL1 IGLV2-23” antibody has a heavy chain variable region and heavy chain common region comprising an amino acid sequence of SEQ ID NO: 4, which is encoded by a nucleic acid sequence of SEQ ID NO: 5, and a lambda light chain comprising an amino acid sequence of SEQ ID NO: 49, which is encoded by a nucleic acid sequence of SEQ ID NO: 50.

>S17_ Sh3_1D9_VLCL2 aPDL1 IGLV2-23-AA (SEQ ID NO: 49)

QSALTQPASVSGSPGQSITISCTGTSSDVVKNNFVSWYQQHPGKAPKPMIYFGSVRPSGVS
NRFSGSKSGNTASLTISGLQAEDEADYYCSSWDLPLFMFGXGTLTVLGQPKAAPSVTLFP
PSSEELQANKATLVCLISDFYPGAVTVAWKADSSPVKAGVETTTPSKQSNNKYAASSYLSL
TPEQWKSHRSYSCQVTHEGSTVEKTVAPTECS

>S17_ Sh3_1D9_VLCL2 aPDL1 IGLV2-23-NT (SEQ ID NO: 50)

CAGTCTGCCCTGACTCAGCCTGCCTCCGTGTCTGGGTCTCCTGGACAGTCGATCACCATCT
CCTGCACTGGAACCAGCAGTGACGTTGTAAAGAATAATTTGTCTCCTGGTACCAACAGCA
CCCAGGCAAAGCCCCCAAACCATGATTTATTTTGGGAGTGTTTCGGCCCTCAGGGGTTTCT
AATCGCTTCTCTGGCTCCAAGTCTGGCAACACGGCCTCCCTGACCATCTCTGGGCTCCAGG
CTGAGGACGAGGCTGATTATTACTGCAGCTCATGGGATCTCCCTTTCTTATGTTTCGGCGG

RGGGACCAAGCTGACCGTCCTAGGTCAGCCCAAGGCTGCCCCCTCGGTCACTCTGTTCCCG
 CCCTCCTCTGAGGAGCTTCAAGCCAACAAGGCCACACTGGTGTGTCTCATAAGTGA CT TCT
 ACCCGGGAGCCGTGACAGTGGCTTGGAAAGCAGATAGCAGCCCCGTCAAGGCGGGAGTGGA
 GACCACCACACCCTCCAAACAAGCAACAACAAGTACGCGGCCAGCAGCTATCTGAGCCTG
 ACGCCTGAGCAGTGGAAAGTCCCACAGAAGCTACAGCTGCCAGGTCACGCATGAAGGGAGCA
 CCGTGGAGAAGACAGTGGCCCCCTACAGAATGTTCA

[00160] The “S17” or “S17_ Sh3_1D9_VLCL2 aPDL1 IGLV2-23” antibody has a heavy chain variable region comprising an amino acid sequence of SEQ ID NO: 6 which is encoded by a nucleic acid sequence of SEQ ID NO: 7 and a lambda light chain variable region comprising an amino acid sequence of SEQ ID NO: 51 which is encoded by a nucleic acid sequence of SEQ ID NO: 52.

>S17_ Sh3_1D9_VLCL2 aPDL1 IGLV2-23-AA (SEQ ID NO: 51)
 QSALTQPASVSGSPGQSITISCTGTSSDVVKNNF VSWYQQHPGKAPKPMIYFGSVRPSGVS
 NRFSGSKSGNTASLTISGLQAEDEADYYCSSWDLPLFMFGXGTKLTVLGQPKAAPSVTL

>S17_ Sh3_1D9_VLCL2 aPDL1 IGLV2-23-NT (SEQ ID NO: 52)
 CAGTCTGCCCTGACTCAGCCTGCCTCCGTGTCTGGGTCTCCTGGACAGTCGATCACCATCT
 CCTGCACTGGAACCAGCAGTGACGTTGTAAAGAATAATTTTGTCTCCTGGTACCAACAGCA
 CCCAGGCAAAGCCCCCAAACCCATGATTTATTTTGGGAGTGTTTCGGCCCTCAGGGGTTTCT
 AATCGCTTCTCTGGCTCCAAGTCTGGCAACACGGCCTCCCTGACCATCTCTGGGCTCCAGG
 CTGAGGACGAGGCTGATTATTACTGCAGCTCATGGGATCTCCCTTTCCTTATGTTTCGGCGG
 RGGGACCAAGCTGACCGTCCTAGGTCAGCCCAAGGCTGCCCCCTCGGTCACTCTG

[00161] The “S57” or “S57_ Sh3_2D9_VLCL2 aPDL1 IGLV2-23” antibody has a heavy chain variable region and heavy chain common region comprising an amino acid sequence of SEQ ID NO: 4, which is encoded by a nucleic acid sequence of SEQ ID NO: 5, and a lambda light chain comprising an amino acid sequence of SEQ ID NO: 53, which is encoded by a nucleic acid sequence of SEQ ID NO: 54.

>S57_ Sh3_2D9_VLCL2 aPDL1 IGLV2-23-AA (SEQ ID NO: 53)
QSALTQPASVSGSPGQSITISCTSISSDVVKNNFVSWYQQHPGKAPKLMIIYFGSVTADGVS
NRFSGSKSGNTASLTISGLQAEDEADYYCSSWDEPDRPFGGGTKLTVLGQPKAAPSVTLFP

PSSEELQANKATLVCLISDFYPGAVTVAWKADSSPVKAGVETTTTPSKQSNNKYAASSYLSL
TPEQWKSHRSYSCQVTHEGSTVEKTVAPTECS

>S57_ Sh3_2D9_VLCL2 aPDL1 IGLV2-23-NT (SEQ ID NO: 54)

CAGTCTGCCCTGACTCAGCCTGCCTCCGTGTCTGGGTCTCCTGGACAGTCGATCACCATCT
CCTGCACTAGTATTAGCAGTGACGTTGTTAAGAATAATTTTGTCTCTTGGTACCAACAGCA
CCCAGGCAAAGCCCCCAAACATCATGATTTATTTTGGGAGTGTTACTGCTGATGGGGTTTCT
AATCGCTTCTCTGGCTCCAAGTCTGGCAACACGGCCTCCCTGACCATCTCTGGGCTCCAGG
CTGAGGACGAGGCTGATTATTACTGCAGCTCATGGGATGAGCCGGACAGGCCCTTCGGCGG
AGGGACCAAGCTGACCGTCCTAGGTCAGCCCAAGGCTGCCCCCTCGGTCACTCTGTTCCCG
CCCTCCTCTGAGGAGCTTCAAGCCAACAAGGCCACACTGGTGTGTCTCATAAGTGACTTCT
ACCCGGGAGCCGTGACAGTGGCTTGGAAAGCAGATAGCAGCCCCGTCAAGGCGGGAGTGGA
GACCACCACACCCTCCAAACAAAGCAACAACAAGTACGCGGCCAGCAGCTATCTGAGCCTG
ACGCCTGAGCAGTGGAAGTCCCACAGAAGCTACAGCTGCCAGGTCACGCATGAAGGGAGCA
CCGTGGAGAAGACAGTGGCCCCCTACAGAATGTTCA

[00162] The “S57” or “S57_ Sh3_2D9_VLCL2 aPDL1 IGLV2-23” antibody has a heavy chain variable region comprising an amino acid sequence of SEQ ID NO: 6 which is encoded by a nucleic acid sequence of SEQ ID NO: 7 and a lambda light chain variable region comprising an amino acid sequence of SEQ ID NO: 55 which is encoded by a nucleic acid sequence of SEQ ID NO: 56.

>S57_ Sh3_2D9_VLCL2 aPDL1 IGLV2-23-AA (SEQ ID NO: 55)

QSALTQPASVSGSPGQSITISCTSISSDVVKNNFVSWYQQHPGKAPKLMIIYFGSVTADGVS
NRFSGSKSGNTASLTISGLQAEDEADYYCSSWDEPDRPFGGGTKLTVLGQPKAAPSVTL

>S57_ Sh3_2D9_VLCL2 aPDL1 IGLV2-23-NT (SEQ ID NO: 56)

CAGTCTGCCCTGACTCAGCCTGCCTCCGTGTCTGGGTCTCCTGGACAGTCGATCACCATCT
CCTGCACTAGTATTAGCAGTGACGTTGTTAAGAATAATTTTGTCTCTTGGTACCAACAGCA
CCCAGGCAAAGCCCCCAAACATCATGATTTATTTTGGGAGTGTTACTGCTGATGGGGTTTCT
AATCGCTTCTCTGGCTCCAAGTCTGGCAACACGGCCTCCCTGACCATCTCTGGGCTCCAGG
CTGAGGACGAGGCTGATTATTACTGCAGCTCATGGGATGAGCCGGACAGGCCCTTCGGCGG
AGGGACCAAGCTGACCGTCCTAGGTCAGCCCAAGGCTGCCCCCTCGGTCACTCTG

[00163] The “S58” or “S58_ Sh3_1G5_VLCL2 aPDL1 IGLV2-23” antibody has a heavy chain variable region and heavy chain common region comprising an amino acid sequence of SEQ ID NO: 4, which is encoded by a nucleic acid sequence of SEQ ID NO: 5, and a lambda light chain comprising an amino acid sequence of SEQ ID NO: 57, which is encoded by a nucleic acid sequence of SEQ ID NO: 58.

>S58_ Sh3_1G5_VLCL2 aPDL1 IGLV2-23-AA (SEQ ID NO: 57)

QSALTQPASVSGSPGQSITISCNSPSSDVVKNNFVSWYQQHPGKAPKLMIFYG
SVTGPGVS
NRFSGSKSGNTASLTISGLQAEDEADYYC
SSWDEPDRPF
 GGGTKLTVLGQPKAAPSVTLF
 PSSEELQANKATLVCLISDFYPGAVTVAWKADSSPVKAGVETTTPSKQSNNKYAASSYLSL
 TPEQWKSHRSYSCQVTHEGSTVEKTVAPTECS

>S58_ Sh3_1G5_VLCL2 aPDL1 IGLV2-23-NT (SEQ ID NO: 58)

CAGTCTGCCCTGACTCAGCCTGCCTCCGTGTCTGGGTCTCCTGGACAGTCGATCACCATCT
 CCTGCAATAGTCCTAGCAGTGACGTTGTAAAGAATAATTTGTCTCTTGGTACCAACAGCA
 CCCAGGCAAAGCCCCAACTCATGATTTATTTTGGGAGTGTTACTGGTCCTGGGGTTTCT
 AATCGCTTCTCTGGCTCCAAGTCTGGCAACACGGCCTCCCTGACCATCTCTGGGCTCCAGG
 CTGAGGACGAGGCTGATTATTACTGCAGCTCATGGGATGAGCCGGACAGGCCCTTCGGCGG
 AGGGACCAAGCTGACCGTCCTAGGTCAGCCCAAGGCTGCCCCCTCGGTCACTCTGTTCCCG
 CCCTCCTCTGAGGAGCTTCAAGCCAACAAGGCCACACTGGTGTGTCTCATAAGTGAATTCT
 ACCCGGGAGCCGTGACAGTGGCTTGGAAAGCAGATAGCAGCCCCGTCAAGGCGGGAGTGGA
 GACCACCACACCCTCCAAACAAGCAACAACAAGTACGCGGCCAGCAGCTATCTGAGCCTG
 ACGCCTGAGCAGTGGAAGTCCCACAGAAGCTACAGCTGCCAGGTCACGCATGAAGGGAGCA
 CCGTGGAGAAGACAGTGGCCCCTACAGAATGTTCA

[00164] The “S58” or “S58_ Sh3_1G5_VLCL2 aPDL1 IGLV2-23” antibody has a heavy chain variable region comprising an amino acid sequence of SEQ ID NO: 6 which is encoded by a nucleic acid sequence of SEQ ID NO: 7 and a lambda light chain variable region comprising an amino acid sequence of SEQ ID NO: 59 which is encoded by a nucleic acid sequence of SEQ ID NO: 60.

>S58_ Sh3_1G5_VLCL2 aPDL1 IGLV2-23-AA (SEQ ID NO: 59)

QSALTQPASVSGSPGQSITISCNSPSSDVVKNNFVSWYQQHPGKAPKLMIFYFGSSVTGPGVS
 NRFSGSKSGNTASLTISGLQAEDEADYYCSSWDEPDRPFGGGTKLTVLGQPKAAPSVTL

>S58_ Sh3_1G5_VLCL2 aPDL1 IGLV2-23-NT (SEQ ID NO: 60)

CAGTCTGCCCTGACTCAGCCTGCCTCCGTGTCTGGGTCTCCTGGACAGTCGATCACCATCT
CCTGCAATAGTCCTAGCAGTGACGTTGTAAAGAATAATTTTGTCTCTTGGTACCAACAGCA
CCCAGGCAAAGCCCCCAAACATCATGATTTATTTTGGGAGTGTTACTGGTCCTGGGGTTTCT
AATCGCTTCTCTGGCTCCAAGTCTGGCAACACGGCCTCCCTGACCATCTCTGGGCTCCAGG
CTGAGGACGAGGCTGATTATTACTGCAGCTCATGGGATGAGCCGGACAGGCCCTTCGGCGG
AGGGACCAAGCTGACCGTCCTAGGTCAGCCCAAGGCTGCCCCCTCGGTCACTCTG

[00165] The “S28” or “S28_Sa2_1G7_VLCL2 aPDL1 IGLV2-44” antibody has a heavy chain variable region and heavy chain common region comprising an amino acid sequence of SEQ ID NO: 4, which is encoded by a nucleic acid sequence of SEQ ID NO: 5, and a lambda light chain comprising an amino acid sequence of SEQ ID NO: 61, which is encoded by a nucleic acid sequence of SEQ ID NO: 62.

>S28_Sa2_1G7_VLCL2 aPDL1 IGLV2-44-AA (SEQ ID NO: 61)

QSVLTQPPSASGTPGQRVITISCSGSSSNIAHKPVNWYQQLPGTAPKLLIYHDNSRPSGVPD
RFSGSKSGTSASLAISGLQSEDEADYYCAAWDFATWPATEVFVGGGTKLTVLGQPKAAPSVT
LFPPSSEELQANKATLVCLISDFYPGAVTVAWKADSSPVKAGVETTTPSKQSNKYAASSY
LSLTPEQWKSHRSYSCQVTHEGSTVEKTVAPTECS

>S28_Sa2_1G7_VLCL2 aPDL1 IGLV2-44-NT (SEQ ID NO: 62)

CAGTCTGTGCTGACTCAGCCACCCTCAGCGTCTGGGACCCCCGGGCAGAGGGTCACCATCT
CTTGTTCTGGAAGCAGCTCCAACATCGCTCATAAGCCTGTAAACTGGTACCAGCAGCTCCC
AGGAACGGCCCCCAAACCTCCTCATCTATCATGATAATTCTCGGCCCTCAGGGGTCCCTGAC
CGATTCTCTGGCTCCAAGTCTGGCACCTCAGCCTCCCTGGCCATCAGTGGGCTCCAGTCTG
AGGATGAGGCTGATTATTACTGTGCAGCATGGGATTTTCGCGACGTGGCCGGCTACTGAGGT
GTTTCGGCGGAGGGACCAAGCTGACCGTCCTAGGTCAGCCCAAGGCTGCCCCCTCGGTCACT
CTGTTCCCGCCCTCCTCTGAGGAGCTTCAAGCCAACAAGGCCACACTGGTGTGTCTCATAA
GTGACTTCTACCCGGGAGCCGTGACAGTGGCTTGAAAGCAGATAGCAGCCCCGTCAAGGC
GGGAGTGGAGACCACACACCCTCCAACAAAGCAACAACAAGTACGCGGCCAGCAGCTAT
CTGAGCCTGACGCCTGAGCAGTGGAAGTCCCACAGAAGCTACAGCTGCCAGGTCACGCATG
AAGGGAGCACCGTGGAGAAGACAGTGGCCCCTACAGAATGTTCA

[00166] The “S28” or “S28_Sa2_1G7_VLCL2 aPDL1 IGLV2-44” antibody has a heavy chain variable region comprising an amino acid sequence of SEQ ID NO: 6 which is encoded by a nucleic acid sequence of SEQ ID NO: 7 and a lambda light chain variable region comprising an amino acid sequence of SEQ ID NO: 63 which is encoded by a nucleic acid sequence of SEQ ID NO: 64.

>S28_Sa2_1G7_VLCL2 aPDL1 IGLV2-44-AA (SEQ ID NO: 63)

QSVLTQPPSASGTPGQRTISCSGSSSNIAHKPVNWYQQLPGTAPKLLIYHDNSRPSGVDP
RFSGSKSGTSASLAISGLQSEDEADYYCAAWDFATWPATEVFGGGTKLTVLGQPKAAPSVT
L

>S28_Sa2_1G7_VLCL2 aPDL1 IGLV2-44-NT (SEQ ID NO: 64)

CAGTCTGTGCTGACTCAGCCACCCTCAGCGTCTGGGACCCCCGGGCAGAGGGTCACCATCT
CTTGTTCTGGAAGCAGCTCCAACATCGCTCATAAGCCTGTAACTGGTACCAGCAGCTCCC
AGGAACGGCCCCCAAACCTCCTCATCTATCATGATAATTCTCGGCCCTCAGGGGTCCCTGAC
CGATTCTCTGGCTCCAAGTCTGGCACCTCAGCCTCCCTGGCCATCAGTGGGCTCCAGTCTG
AGGATGAGGCTGATTATTACTGTGCAGCATGGGATTTGCGACGTGGCCGGCTACTGAGGT
GTTTCGGCGGAGGGACCAAGCTGACCGTCCTAGGTCAGCCCAAGGCTGCCCCCTCGGTCACT
CTG

[00167] The “S30” or “S30_Sa2_C10_VLCL2 aPDL1 IGLV2-44” antibody has a heavy chain variable region and heavy chain common region comprising an amino acid sequence of SEQ ID NO: 4, which is encoded by a nucleic acid sequence of SEQ ID NO: 5, and a lambda light chain comprising an amino acid sequence of SEQ ID NO: 65, which is encoded by a nucleic acid sequence of SEQ ID NO: 66.

>S30_Sa2_C10_VLCL2 aPDL1 IGLV2-44-AA (SEQ ID NO: 65)

QSVLTQPPSASGTPGQRTISCSGSSSNIAHKPVNWYQQLPGTAPKLLIYHDNSRPSGVDP
RFSGSRSGTSASLAISGLQSEDEADYYCAAWDFSRWPATEVFGGGTKLTVLGQPKAAPSVT
LFPSPSEELQANKATLVCLISDFYPGAVTVAWKADSSPVKAGVETTTPSKQSNNKYAASSY
LSLTPEQWKSHRYSYSCQVTHEGSTVEKTVAPTECS

>S30_Sa2_C10_VLCL2 aPDL1 IGLV2-44-NT (SEQ ID NO: 66)

CAGTCTGTGCTGACTCAGCCACCCTCAGCGTCTGGGACCCCCGGGCAGAGGGTCACCATCT
 CTTGTTCTGGAAGCAGCTCCAACATCGCTCATAAGCCTGTAAACTGGTACCAGCAGCTCCC
 AGGAACGGCCCCCAAACCTCCTCATCTATCATGATAATTCTCGGCCCTCAGGGGTCCCTGAC
 CGATTCTCTGGCTCCAGGTCTGGCACCTCAGCCTCCCTGGCCATCAGTGGGCTCCAGTCTG
 AGGATGAGGCTGATTATTACTGTGCAGCATGGGATTTAGCCGCTGGCCGGCTACTGAGGT
 GTTCGGCGGAGGGACCAAGCTGACCGTCCTAGGTCAGCCCAAGGCTGCCCCCTCGGTCACT
 CTGTTCCCGCCCTCCTCTGAGGAGCTTCAAGCCAACAAGGCCACACTGGTGTGTCTCATAA
 GTGACTTCTACCCGGGAGCCGTGACAGTGGCTTGGAAGCAGATAGCAGCCCCGTCAAGGC
 GGGAGTGGAGACCACCACACCCTCCAAACAAAGCAACAACAAGTACGCGGCCAGCAGCTAT
 CTGAGCCTGACGCCTGAGCAGTGGAAAGTCCCACAGAAGCTACAGCTGCCAGGTCACGCATG
 AAGGGAGCACCGTGGAGAAGACAGTGGCCCCTACAGAATGTTCA

[00168] The “S30” or “S30_ Sa2_C10_VLCL2 aPDL1 IGLV2-44” antibody has a heavy chain variable region comprising an amino acid sequence of SEQ ID NO: 6 which is encoded by a nucleic acid sequence of SEQ ID NO: 7 and a lambda light chain variable region comprising an amino acid sequence of SEQ ID NO: 67 which is encoded by a nucleic acid sequence of SEQ ID NO: 68.

>S30_ Sa2_C10_VLCL2 aPDL1 IGLV2-44-AA (SEQ ID NO: 67)

QSVLTQPPSASGTPGQRVTISSCSGSSSNIAHKPVNWFYQQLPGTAPKLLIYHDNSRPSGVPD
 RFSGSRSGTSASLAISGLQSEDEADYYCAAWDFSRWPATEVFGGGTKLTVLGQPKAAPSVT
 L

>S30_ Sa2_C10_VLCL2 aPDL1 IGLV2-44-NT (SEQ ID NO: 68)

CAGTCTGTGCTGACTCAGCCACCCTCAGCGTCTGGGACCCCCGGGCAGAGGGTCACCATCT
 CTTGTTCTGGAAGCAGCTCCAACATCGCTCATAAGCCTGTAAACTGGTACCAGCAGCTCCC
 AGGAACGGCCCCCAAACCTCCTCATCTATCATGATAATTCTCGGCCCTCAGGGGTCCCTGAC
 CGATTCTCTGGCTCCAGGTCTGGCACCTCAGCCTCCCTGGCCATCAGTGGGCTCCAGTCTG
 AGGATGAGGCTGATTATTACTGTGCAGCATGGGATTTAGCCGCTGGCCGGCTACTGAGGT
 GTTCGGCGGAGGGACCAAGCTGACCGTCCTAGGTCAGCCCAAGGCTGCCCCCTCGGTCACT
 CTG

[00169] The “S94” or “S94_Sa2_G11_VLCL2 aPDL1 IGLV1-44” antibody has a heavy chain variable region and heavy chain common region comprising an amino acid sequence of

SEQ ID NO: 4, which is encoded by a nucleic acid sequence of SEQ ID NO: 5, and a lambda light chain comprising an amino acid sequence of SEQ ID NO: 69, which is encoded by a nucleic acid sequence of SEQ ID NO: 70.

>S94_Sa2_G11_VLCL2 aPDL1 IGLV1-44-AA (SEQ ID NO: 69)

QSVLTQPPSASGTPGQRVTISGISGSVDIAHKPVNWWYQQLPGTAPKLLIYHDTSTPDGVPDRFSGSK
SGTSASLAISGLQSEDEADYYCAAWDFATWPATEVFGGGTKLTVLGQPKAAPSVTLFPPSSEELQAN
 KATLVCLISDFYPGAVTVAWKADSSPVKAGVETTPSKQSNKYAASSYLSLTPEQWKSHRSYSCQV
 THEGSTVEKTVAPTECS

>S94_Sa2_G11_VLCL2 aPDL1 IGLV1-44-NT (SEQ ID NO: 70)

CAGTCTGTGCTGACTCAGCCACCCTCAGCGTCTGGGACCCCCGGGCAGAGGGTCACCATCTCTTGTA
 TTAGTGGTAGCGTTGATATCGCTCATAAGCCTGTAACTGGTACCAGCAGCTCCCAGGAACGGCCCC
 CAACTCCTCATCTATCATGATACCTCTACTCCTGATGGGGTCCCTGACCGATTCTCTGGCTCCAAG
 TCTGGCACCTCAGCCTCCCTGGCCATCAGTGGGCTCCAGTCTGAGGATGAGGCTGATTATTACTGTG
 CAGCATGGGATTTTCGCGACGTGGCCGGCTACTGAGGTGTTTCGGCGGAGGGACCAAGCTGACCGTCCCT
 AGGTCAGCCCCAAGGCTGCCCCCTCGGTCACTCTGTTCCCGCCCTCCTCTGAGGAGCTTCAAGCCAAC
 AAGGCCACACTGGTGTGTCTCATAAGTGACTTCTACCCGGGAGCCGTGACAGTGGCTTGGAAAGCAG
 ATAGCAGCCCCGTCAAGGCGGAGTGAGAGACCACACACCCTCCAAACAAAGCAACAACAAGTACGC
 GGCCAGCAGCTATCTGAGCCTGACGCCTGAGCAGTGGAAGTCCCACAGAAGCTACAGCTGCCAGGTC
 ACGCATGAAGGGAGCACCGTGGAGAAGACAGTGGCCCCCTACAGAATGTTTCATAA

[00170] The “S94” or “S94_Sa2_G11_VLCL2 aPDL1 IGLV1-44” antibody has a heavy chain variable region comprising an amino acid sequence of SEQ ID NO: 6 which is encoded by a nucleic acid sequence of SEQ ID NO: 7 and a lambda light chain variable region comprising an amino acid sequence of SEQ ID NO: 71 which is encoded by a nucleic acid sequence of SEQ ID NO: 72.

>S94_Sa2_G11_VLCL2 aPDL1 IGLV1-44-AA (SEQ ID NO: 71)

QSVLTQPPSASGTPGQRVTISGISGSVDIAHKPVNWWYQQLPGTAPKLLIYHDTSTPDGVPDRFSGSK
 SGTSASLAISGLQSEDEADYYCAAWDFATWPATEVFGGGTKLTVLGQPKAAPSVTL

>S94_Sa2_G11_VLCL2 aPDL1 IGLV1-44-NT (SEQ ID NO: 72)

CAGTCTGTGCTGACTCAGCCACCCTCAGCGTCTGGGACCCCCGGGCAGAGGGTCACCATCTCTTGTA
 TTAGTGGTAGCGTTGATATCGCTCATAAGCCTGTAACTGGTACCAGCAGCTCCCAGGAACGGCCCC

CAAACCTCCTCATCTATCATGATACCTCTACTCCTGATGGGGTCCCTGACCGATTCTCTGGCTCCAAG
TCTGGCACCTCAGCCTCCCTGGCCATCAGTGGGCTCCAGTCTGAGGATGAGGCTGATTATTACTGTG
CAGCATGGGATTTTCGCGACGTGGCCGGCTACTGAGGTGTTTCGGCGGAGGGACCAAGCTGACCGTCCCT
AGGTCAGCCCAAGGCTGCCCCCTCGGTCACTCTG

[00171] The “S23” or “S23_ Sc3_1H4_VLCL2 aPDL1 IGLV2-23” antibody has a heavy chain variable region and heavy chain common region comprising an amino acid sequence of SEQ ID NO: 4, which is encoded by a nucleic acid sequence of SEQ ID NO: 5, and a lambda light chain comprising an amino acid sequence of SEQ ID NO: 73, which is encoded by a nucleic acid sequence of SEQ ID NO: 74.

>S23_ Sc3_1H4_VLCL2 aPDL1 IGLV2-23-AA (SEQ ID NO: 73)

QSALTQPASVSGSPGQSITISCTGTSSDVAKIPLVSWYQQHPGKAPKLMIFYFASLRPSGVS
NRFSGSKSGNTASLTISGLQAEDEADYYCSSWDNAGDGHVFGGGTKLTVLGQPKAAPSVTL
FPPSSEELQANKATLVCLISDFYPGAVTVAWKADSSPVKAGVETTTPSKQSNKNYAASSYL
SLTPEQWKSHRSYSCQVTHEGSTVEKTVAPTECS

>S23_ Sc3_1H4_VLCL2 aPDL1 IGLV2-23-NT (SEQ ID NO: 74)

CAGTCTGCCCTGACTCAGCCTGCCTCCGTGTCTGGGTCTCCTGGACAGTCGATCACCATCT
CCTGCACTGGAACCAGCAGTGACGTTGCTAAGATTCCTCTTGTCTCCTGGTACCAACAGCA
CCCAGGCAAAGCCCCCAAACATCATGATTTATTTTGCTAGTCTTCGGCCCTCAGGGGTTTCT
AATCGCTTCTCTGGCTCCAAGTCTGGCAACACGGCCTCCCTGACCATCTCTGGGCTCCAGG
CTGAGGACGAGGCTGATTATTACTGCAGCTCATGGGATAATGCTGGTGATGGGCATGTGTT
CGGCGGAGGGACCAAGCTGACCGTCCTAGGTCAGCCCAAGGCTGCCCCCTCGGTCACTCTG
TTCCCGCCCTCCTCTGAGGAGCTTCAAGCCAACAAGGCCACACTGGTGTGTCTCATAAGTG
ACTTCTACCCGGGAGCCGTGACAGTGGCTTGGAAAGCAGATAGCAGCCCCGTCAAGGCGGG
AGTGGAGACCACCACACCCTCCAAACAAAGCAACAACAAGTACGCGGCCAGCAGCTATCTG
AGCCTGACGCCTGAGCAGTGGAAGTCCCACAGAAGCTACAGCTGCCAGGTCACGCATGAAG
GGAGCACCGTGGAGAAGACAGTGGCCCCCTACAGAATGTTCA

[00172] The “S23” or “S23_ Sc3_1H4_VLCL2 aPDL1 IGLV2-23” antibody has a heavy chain variable region comprising an amino acid sequence of SEQ ID NO: 6 which is encoded by a nucleic acid sequence of SEQ ID NO: 7 and a lambda light chain variable region

comprising an amino acid sequence of SEQ ID NO: 75 which is encoded by a nucleic acid sequence of SEQ ID NO: 76.

>**S23_ Sc3_1H4_VLCL2 aPDL1 IGLV2-23-AA** (SEQ ID NO: 75)

QSALTQPASVSGSPGQSITISCTGTSSDVAKIPL VSWYQQHPGKAPKLMIIYFASLRPSGVS
NRFSGSKSGNTASLTISGLQAEDEADYYCSSWDNAGDGHVFGGGTKLTVLGQPKAAPSVTL

>**S23_ Sc3_1H4_VLCL2 aPDL1 IGLV2-23-NT** (SEQ ID NO: 76)

CAGTCTGCCCTGACTCAGCCTGCCTCCGTGTCTGGGTCTCCTGGACAGTCGATCACCATCT
CCTGCACTGGAACCAGCAGTGACGTTGCTAAGATTCTCTTGTCTCCTGGTACCAACAGCA
CCCAGGCAAAGCCCCCAAACATCATGATTTATTTTGCTAGTCTTCGGCCCTCAGGGGTTTCT
AATCGCTTCTCTGGCTCCAAGTCTGGCAACACGGCCTCCCTGACCATCTCTGGGCTCCAGG
CTGAGGACGAGGCTGATTATTACTGCAGCTCATGGGATAATGCTGGTGATGGGCATGTGTT
CGGCGGAGGGACCAAGCTGACCGTCCTAGGTCAGCCCAAGGCTGCCCCCTCGGTCACTCTG

[00173] The “S46” or “S46_ Sc3_1E4_VLCL2 aPDL1 IGLV2-23” antibody has a heavy chain variable region and heavy chain common region comprising an amino acid sequence of SEQ ID NO: 4, which is encoded by a nucleic acid sequence of SEQ ID NO: 5, and a lambda light chain comprising an amino acid sequence of SEQ ID NO: 77, which is encoded by a nucleic acid sequence of SEQ ID NO: 78.

>**S46_ Sc3_1E4_VLCL2 aPDL1 IGLV2-23-AA** (SEQ ID NO: 77)

QSALTQPASVSGSPGQSITISCTGTSSDVL RPPLVSWYQQHPGKAPKLMIIYFASLRPSGVS
NRFSGSKSGNTASLTISGLQAEDEADYYCSSWDNAGDGHVFGGGTKLTVLGQPKAAPSVTL
FPPSSEELQANKATLVCLISDFYPGAVTVAWKADSSPVKAGVETTTPSKQSNKYAASSYL
SLTPEQWKSHRSYSCQVTHEGSTVEKTVAPTECS

>**S46_ Sc3_1E4_VLCL2 aPDL1 IGLV2-23-NT** (SEQ ID NO: 78)

CAGTCTGCCCTGACTCAGCCTGCCTCCGTGTCTGGGTCTCCTGGACAGTCGATCACCATCT
CCTGCACTGGAACCAGCAGTGACGTTCTTAGGCCTCCTCTTGTCTCCTGGTACCAACAGCA
CCCAGGCAAAGCCCCCAAACATCATGATTTATTTTGCTAGTCTTCGGCCCTCAGGGGTTTCT
AATCGCTTCTCTGGCTCCAAGTCTGGCAACACGGCCTCCCTGACCATCTCTGGGCTCCAGG
CTGAGGACGAGGCTGATTATTACTGCAGCTCATGGGATAATGCTGGTGATGGGCATGTGTT
CGGCGGAGGGACCAAGCTGACCGTCCTAGGTCAGCCCAAGGCTGCCCCCTCGGTCACTCTG

TTCCCGCCCTCCTCTGAGGAGCTTCAAGCCAACAAGGCCACACTGGTGTGTCTCATAAGTG
 ACTTCTACCCGGGAGCCGTGACAGTGGCTTGGAAAGCAGATAGCAGCCCCGTCAAGGCGGG
 AGTGGAGACCACCACACCCTCCAAACAAAGCAACAACAAGTACGCGGCCAGCAGCTATCTG
 AGCCTGACGCCTGAGCAGTGGAAAGTCCACAGAAGCTACAGCTGCCAGGTCACGCATGAAG
 GGAGCACCGTGGAGAAGACAGTGGCCCCCTACAGAATGTTCA

[00174] The “S46” or “S46_ Sc3_1E4_VLCL2 aPDL1 IGLV2-23” antibody has a heavy chain variable region comprising an amino acid sequence of SEQ ID NO: 6 which is encoded by a nucleic acid sequence of SEQ ID NO: 7 and a lambda light chain variable region comprising an amino acid sequence of SEQ ID NO: 79 which is encoded by a nucleic acid sequence of SEQ ID NO: 80.

>S46_ Sc3_1E4_VLCL2 aPDL1 IGLV2-23-AA (SEQ ID NO: 79)
 QSALTQPASVSGSPGQSITISCTGTSSDVLRPPLLSWYQQHPGKAPKLMIYFASLRPSGVS
 NRFSGSKSGNTASLTISGLQAEDEADYYCSSWDNAGDGHVFGGGTKLTVLGQPKAAPSVTL

>S46_ Sc3_1E4_VLCL2 aPDL1 IGLV2-23-NT (SEQ ID NO: 80)
 CAGTCTGCCCTGACTCAGCCTGCCTCCGTGTCTGGGTCTCCTGGACAGTCGATCACCATCT
 CCTGCACTGGAACCAGCAGTGACGTTCTTAGGCCTCCTCTTGTCTCCTGGTACCAACAGCA
 CCCAGGCAAAGCCCCCAAACATCATGATTTATTTTGCTAGTCTTCGGCCCTCAGGGGTTTCT
 AATCGCTTCTCTGGCTCCAAGTCTGGCAACACGGCCTCCCTGACCATCTCTGGGCTCCAGG
 CTGAGGACGAGGCTGATTATTACTGCAGCTCATGGGATAATGCTGGTGATGGGCATGTGTT
 CGGCGGAGGGACCAAGCTGACCGTCCTAGGTCAGCCCAAGGCTGCCCCCTCGGTCACTCTG

[00175] The “S71” or “S71_ Sc3_2C6_VLCL2 aPDL1 IGLV2-23” antibody has a heavy chain variable region and heavy chain common region comprising an amino acid sequence of SEQ ID NO: 4, which is encoded by a nucleic acid sequence of SEQ ID NO: 5, and a lambda light chain comprising an amino acid sequence of SEQ ID NO: 81, which is encoded by a nucleic acid sequence of SEQ ID NO: 82.

>S71_ Sc3_2C6_VLCL2 aPDL1 IGLV2-23-AA (SEQ ID NO: 81)
QSALTQPASVSGSPGQSITISCTGTSSDVFRPPLLSWYQQHPGKAPKLMIYFASLRPSGVS
NRFSGSKSGNTASLTISGLQAEDEADYYCSSWDQSGDGHVFGGGTKLTVLGQPKAAPSVTL

FPPSSEELQANKATLVCLISDFYPGAVTVAWKADSSPVKAGVETTTPSKQSNNKYAASSYL
SLTPEQWKSHRSYSCQVTHEGSTVEKTVAPTECS

>**S71_ Sc3_2C6_VLCL2 aPDL1 IGLV2-23-NT** (SEQ ID NO: 82)

CAGTCTGCCCTGACTCAGCCTGCCTCCGTGTCTGGGTCTCCTGGACAGTCGATCACCATCT
CCTGCACTGGAACCAGCAGTGACGTTTTTAGGCCTCCTCTTGTCTCCTGGTACCAACAGCA
CCCAGGCAAAGCCCCCAAACATCATGATTTATTTTGCTAGTCTTCGGCCCTCAGGGGTTTCT
AATCGCTTCTCTGGCTCCAAGTCTGGCAACACGGCCTCCCTGACCATCTCTGGGCTCCAGG
CTGAGGACGAGGCTGATTATTACTGCAGCTCATGGGATCAGTCCGGGGACGGCCATGTGTT
CGGCGGAGGGACCAAGCTGACCGTCCTAGGTCAGCCCAAGGCTGCCCCCTCGGTCACTCTG
TTCCCGCCCTCCTCTGAGGAGCTTCAAGCCAACAAGGCCACACTGGTGTGTCTCATAAGTG
ACTTCTACCCGGGAGCCGTGACAGTGGCTTGGAAGCAGATAGCAGCCCCGTCAAGGCGGG
AGTGGAGACCACACACCTCCAAACAAAGCAACAACAAGTACGCGGCCAGCAGCTATCTG
AGCCTGACGCCTGAGCAGTGGAAAGTCCACAGAAGCTACAGCTGCCAGGTCACGCATGAAG
GGAGCACCGTGGAGAAGACAGTGGCCCCTACAGAATGTTCA

[00176] The “S71” or “S71_ Sc3_2C6_VLCL2 aPDL1 IGLV2-23” antibody has a heavy chain variable region comprising an amino acid sequence of SEQ ID NO: 6 which is encoded by a nucleic acid sequence of SEQ ID NO: 7 and a lambda light chain variable region comprising an amino acid sequence of SEQ ID NO: 83 which is encoded by a nucleic acid sequence of SEQ ID NO: 84.

>**S71_ Sc3_2C6_VLCL2 aPDL1 IGLV2-23-AA** (SEQ ID NO: 83)

QSALTQPASVSGSPGQSITISCTGTSSDVERPPLLSWYQQHPGKAPKLMIIYFASLRPSGVS
NRFSGSKSGNTASLTISGLQAEDEADYYCSSWDQSGDGHVFGGGTKLTVLGQPKAAPSVTL

>**S71_ Sc3_2C6_VLCL2 aPDL1 IGLV2-23-NT** (SEQ ID NO: 84)

CAGTCTGCCCTGACTCAGCCTGCCTCCGTGTCTGGGTCTCCTGGACAGTCGATCACCATCT
CCTGCACTGGAACCAGCAGTGACGTTTTTAGGCCTCCTCTTGTCTCCTGGTACCAACAGCA
CCCAGGCAAAGCCCCCAAACATCATGATTTATTTTGCTAGTCTTCGGCCCTCAGGGGTTTCT
AATCGCTTCTCTGGCTCCAAGTCTGGCAACACGGCCTCCCTGACCATCTCTGGGCTCCAGG
CTGAGGACGAGGCTGATTATTACTGCAGCTCATGGGATCAGTCCGGGGACGGCCATGTGTT
CGGCGGAGGGACCAAGCTGACCGTCCTAGGTCAGCCCAAGGCTGCCCCCTCGGTCACTCTG

[00177] The “S79” or “S79_ Sc3_1G7_VLCL2 aPDL1 IGLV2-23” antibody has a heavy chain variable region and heavy chain common region comprising an amino acid sequence of SEQ ID NO: 4, which is encoded by a nucleic acid sequence of SEQ ID NO: 5, and a lambda light chain comprising an amino acid sequence of SEQ ID NO: 85, which is encoded by a nucleic acid sequence of SEQ ID NO: 86.

>S79_ Sc3_1G7_VLCL2 aPDL1 IGLV2-23-AA (SEQ ID NO: 85)

QSALTQPASVSGSPGQSITISCTGTSSDVERPPLVSWYQQHPGKAPKLMIFYFASLRPSGVS
NRFSGSKSGNTASLTISGLQAEDEADYYCSSWDHTGDGHVFGGGTKLTVLGQPKAAPSVTL
 FPPSSEELQANKATLVCLISDFYPGAVTVAWKADSSPVKAGVETTTPSKQSNNKYAASSYL
 SLTPEQWKSHRSYSCQVTHEGSTVEKTVAPTECS

>S79_ Sc3_1G7_VLCL2 aPDL1 IGLV2-23-NT (SEQ ID NO: 86)

CAGTCTGCCCTGACTCAGCCTGCCTCCGTGTCTGGGTCTCCTGGACAGTCGATCACCATCT
 CCTGCACTGGAACCAGCAGTGACGTTTTTAGGCCTCCTCTTGTCTCCTGGTACCAACAGCA
 CCCAGGCAAAGCCCCAACTCATGATTTATTTTGCTAGTCTTCGGCCCTCAGGGGTTTCT
 AATCGCTTCTCTGGCTCCAAGTCTGGCAACACGGCCTCCCTGACCATCTCTGGGCTCCAGG
 CTGAGGACGAGGCTGATTATTACTGCAGCTCATGGGATCACACGGGCGATGGGCATGTCTT
 CGGCGGAGGGACCAAGCTGACCGTCCTAGGTCAGCCCAAGGCTGCCCCCTCGGTCACTCTG
 TTCCCGCCCTCCTCTGAGGAGCTTCAAGCCAACAAGGCCACACTGGTGTGTCTCATAAGTG
 ACTTCTACCCGGGAGCCGTGACAGTGGCTTGGAAGCAGATAGCAGCCCCGTCAAGGCGGG
 AGTGGAGACCACACCCCTCCAAACAAGCAACAACAAGTACGCGGCCAGCAGCTATCTG
 AGCCTGACGCCTGAGCAGTGGAAGTCCCACAGAAGCTACAGCTGCCAGGTCACGCATGAAG
 GGAGCACCGTGGAGAAGACAGTGGCCCCCTACAGAATGTTCA

[00178] The “S79” or “S79_ Sc3_1G7_VLCL2 aPDL1 IGLV2-23” antibody has a heavy chain variable region comprising an amino acid sequence of SEQ ID NO: 6 which is encoded by a nucleic acid sequence of SEQ ID NO: 7 and a lambda light chain variable region comprising an amino acid sequence of SEQ ID NO: 87 which is encoded by a nucleic acid sequence of SEQ ID NO: 88.

>S79_ Sc3_1G7_VLCL2 aPDL1 IGLV2-23-AA (SEQ ID NO: 87)

QSALTQPASVSGSPGQSITISCTGTSSDVERPPLVSWYQQHPGKAPKLMIFYFASLRPSGVS
 NRFSGSKSGNTASLTISGLQAEDEADYYCSSWDHTGDGHVFGGGTKLTVLGQPKAAPSVTL

>S79_ Sc3_1G7_VLCL2 aPDL1 IGLV2-23-NT (SEQ ID NO: 88)

CAGTCTGCCCTGACTCAGCCTGCCTCCGTGTCTGGGTCTCCTGGACAGTCGATCACCATCT
CCTGCACTGGAACCAGCAGTGACGTTTTTAGGCCTCCTCTTGTCTCCTGGTACCAACAGCA
CCCAGGCAAAGCCCCCAAACATCATGATTTATTTTGCTAGTCTTCGGCCCTCAGGGGTTTCT
AATCGCTTCTCTGGCTCCAAGTCTGGCAACACGGCCTCCCTGACCATCTCTGGGCTCCAGG
CTGAGGACGAGGCTGATTATTACTGCAGCTCATGGGATCACACGGGCGATGGGCATGTCTT
CGGCGGAGGGACCAAGCTGACCGTCCTAGGTCAGCCCAAGGCTGCCCCCTCGGTCACTCTG

[00179] The “S93” or “S93 IgG_Sa2_1F9” antibody has a heavy chain variable region and heavy chain common region comprising an amino acid sequence of SEQ ID NO: 4, which is encoded by a nucleic acid sequence of SEQ ID NO: 5, and a lambda light chain comprising an amino acid sequence of SEQ ID NO: 108, which is encoded by a nucleic acid sequence of SEQ ID NO: 90.

>S93 IgG_Sa2_1F9-AA (SEQ ID NO: 108)

QSVLTQPPSASGTPGQRVITISCVSTSDHIAHKPVNWIYQQLPGTAPKLLIYHDTSRPDGVPD
RFSGSKSGTSASLAISGLQSEDEADYYCAAWDFATWPATEVFVGGGTKLTVLGQPKAAPSVT
LFPPSSEELQANKATLVCLISDFYPGAVTVAWKADSSPVKAGVETTTPSKQSNKYAASSY
LSLTPEQWKSHRSYSCQVTHEGSTVEKTVAPTECS

>S93 IgG_Sa2_1F9-NT (SEQ ID NO: 90)

CAGTCTGTGCTGACTCAGCCACCCTCAGCGTCTGGGACCCCCGGGCAGAGGGTCACCATCT
CTTGTGTTTCTACTAGCGATCATATCGCTCATAAGCCTGTAAACTGGTACCAGCAGCTCCC
AGGAACGGCCCCCAAACCTCCTCATCTATCATGATACCTCTCGTCCTGATGGGGTCCCTGAC
CGATTCTCTGGCTCCAAGTCTGGCACCTCAGCCTCCCTGGCCATCAGTGGGCTCCAGTCTG
AGGATGAGGCTGATTATTACTGTGCAGCATGGGATTTTCGCGACGTGGCCGGCTACTGAGGT
GTTTCGGCGGAGGGACCAAGCTGACCGTCCTAGGTCAGCCCAAGGCTGCCCCCTCGGTCACT
CTGTTCCCGCCCTCCTCTGAGGAGCTTCAAGCCAACAAGGCCACACTGGTGTGTCTCATAA
GTGACTTCTACCCGGGAGCCGTGACAGTGGCTTGAAAGCAGATAGCAGCCCCGTCAAGGC
GGGAGTGGAGACCACACACCCTCCAAACAAAGCAACAACAAGTACGCGGCCAGCAGCTAT
CTGAGCCTGACGCCTGAGCAGTGGAAGTCCCACAGAAGCTACAGCTGCCAGGTCACGCATG
AAGGGAGCACCGTGGAGAAGACAGTGGCCCCTACAGAATGTTTCATAA

[00180] The “S93” or “S93 IgG_Sa2_1F9” antibody has a heavy chain variable region comprising an amino acid sequence of SEQ ID NO: 6 which is encoded by a nucleic acid sequence of SEQ ID NO: 7 and a lambda light chain variable region comprising an amino acid sequence of SEQ ID NO: 91 which is encoded by a nucleic acid sequence of SEQ ID NO: 109.

>S93 IgG_Sa2_1F9-AA (SEQ ID NO: 91)

QSVLTQPPSASGTPGQRTISCVSTSDHIAHKPVNWKYQQLPGTAPKLLIYHDTSRPDGVDP
RFSGSKSGTSASLAISGLQSEDEADYYCAAWDFATWPATEEVFGGKTLTVLGQPKAAPSVT
L

>S93 IgG_Sa2_1F9-NT (SEQ ID NO: 109)

CAGTCTGTGCTGACTCAGCCACCCTCAGCGTCTGGGACCCCCGGGCAGAGGGTCACCATCT
CTTGTGTTTCTACTAGCGATCATATCGCTCATAAGCCTGTAACTGGTACCAGCAGCTCCC
AGGAACGGCCCCCAAACCTCCTCATCTATCATGATACCTCTCGTCCTGATGGGGTCCCTGAC
CGATTCTCTGGCTCCAAGTCTGGCACCTCAGCCTCCCTGGCCATCAGTGGGCTCCAGTCTG
AGGATGAGGCTGATTATTACTGTGCAGCATGGGATTTGCGACGTGGCCGGCTACTGAGGT
GTTTCGGCGGAGGGACCAAGCTGACCGTCCTAGGTCAGCCCAAGGCTGCCCCCTCGGTCACT
CTG

[00181] The “S96” or “S96 IgG_Sa2_H10” antibody has a heavy chain variable region and heavy chain common region comprising an amino acid sequence of SEQ ID NO: 4, which is encoded by a nucleic acid sequence of SEQ ID NO: 5, and a lambda light chain comprising an amino acid sequence of SEQ ID NO: 93, which is encoded by a nucleic acid sequence of SEQ ID NO: 94.

>S96 IgG_Sa2_H10-AA (SEQ ID NO: 93)

QSVLTQPPSASGTPGQRTISCNLPSADIAHKPVNWKYQQLPGTAPKLLIYHDTSVVTGVDP
RFSGSKSGTSASLAISGLQSEDEADYYCAAWDFATWPATEEVFGGKTLTVLGQPKAAPSVT
LFPPSSEELQANKATLVCLISDFYPGAVTVAWKADSSPVKAGVETTTPSKQSNKYAASSY
LSLTPEQWKSHRSYSCQVTHEGSTVEKTVAPTECS

>S96 IgG_Sa2_H10-NT (SEQ ID NO: 94)

CAGTCTGTGCTGACTCAGCCACCCTCAGCGTCTGGGACCCCCGGGCAGAGGGTCACCATCT
 CTTGTTCTGGAAGCAGCTCCAACATCAGGGATAGTTTTGTAAACTGGTACCAGCAGCTCCC
 AGGAACGGCCCCCAAACCTCCTCATCTATGCTACGAATATTCGGCCCTCAGGGGTCCCTGAC
 CGATTCTCTGGCTCCAAGTCTGGCACCTCAGCCTCCCTGGCCATCAGTGGGCTCCAGTCTG
 AGGATGAGGCTGATTATTACTGTGCAGCATGGCACCCGTATTACACGTTGTTTCGGCGGAGG
 GACCAAGCTGACCGTCCTAGGTCAGCCCAAGGCTGCCCCCTCGGTCACTCTGTTCCCGCCC
 TCCTCTGAGGAGCTTCAAGCCAACAAGGCCACACTGGTGTGTCTCATAAGTGACTTCTACC
 CGGGAGCCGTGACAGTGGCTTGGAAAGCAGATAGCAGCCCCGTCAAGGCGGGAGTGGAGAC
 CACCACACCCTCCAAACAAAGCAACAACAAGTACGCGGCCAGCAGCTATCTGAGCCTGACG
 CCTGAGCAGTGGAAGTCCCACAGAAGCTACAGCTGCCAGGTCACGCATGAAGGGAGCACCG
 TGGAGAAGACAGTGGCCCCCTACAGAATGTTCA

[00182] The “S96” or “S96 IgG_Sa2_H10” antibody has a heavy chain variable region comprising an amino acid sequence of SEQ ID NO: 6 which is encoded by a nucleic acid sequence of SEQ ID NO: 7 and a lambda light chain variable region comprising an amino acid sequence of SEQ ID NO: 95 which is encoded by a nucleic acid sequence of SEQ ID NO: 110.

>S96 IgG_Sa2_H10-AA (SEQ ID NO: 95)

QSVLTQPPSASGTPGQRVITISCNLPSADIAHKPVNWFYQQLPGTAPKLLIYHDTSVVTGVPD
 RFGSKSGTSASLAISGLQSEDEADYYCAAWDFATWPATEVFVGGTKLTVLGQPKAAPSVT
 L

>S96 IgG_Sa2_H10-NT (SEQ ID NO: 110)

CAGTCTGTGCTGACTCAGCCACCCTCAGCGTCTGGGACCCCCGGGCAGAGGGTCACCATCT
 CTTGTTCTGGAAGCAGCTCCAACATCAGGGATAGTTTTGTAAACTGGTACCAGCAGCTCCC
 AGGAACGGCCCCCAAACCTCCTCATCTATGCTACGAATATTCGGCCCTCAGGGGTCCCTGAC
 CGATTCTCTGGCTCCAAGTCTGGCACCTCAGCCTCCCTGGCCATCAGTGGGCTCCAGTCTG
 AGGATGAGGCTGATTATTACTGTGCAGCATGGCACCCGTATTACACGTTGTTTCGGCGGAGG
 GACCAAGCTGACCGTCCTAGGTCAGCCCAAGGCTGCCCCCTCGGTCACTCTG

[00183] The “S100” or “S100 IgG_Sa2_1E5” antibody has a heavy chain variable region and heavy chain common region comprising an amino acid sequence of SEQ ID NO: 4, which is encoded by a nucleic acid sequence of SEQ ID NO: 5, and a lambda light chain comprising

an amino acid sequence of SEQ ID NO: 113, which is encoded by a nucleic acid sequence of SEQ ID NO: 114.

>**S100 IgG_Sa2_1E5-AA** (SEQ ID NO: 113)

QSVLTQPPSASGTPGQRTVISCSSSSNIENKPVNWWYQQLPGTAPKLLIYHDTTRPSGVDP
RFSGSKSGTSASLAISGLQSEDEADYYCAAWDFATWPATEVFEGGKLTVLGQPKAAPSVT
LFPPSSEELQANKATLVCLISDFYPGAVTVAWKADSSPVKAGVETTTPSKQSNNKYAASSY
LSLTPEQWKSHRSYSCQVTHEGSTVEKTVAPTECS

>**S100 IgG_Sa2_1E5-NT** (SEQ ID NO: 114)

CAGTCTGTGCTGACTCAGCCACCCTCAGCGTCTGGGACCCCCGGGCAGAGGGTCACCATCT
CTTGTTCTGGAAGCAGCTCCAACATCGAGAATAAGCCTGTAACTGGTACCAGCAGCTCCC
AGGAACGGCCCCCAAACCTCCTCATCTATCATGATACTACTCGGCCCTCAGGGGTCCCTGAC
CGATTCTCTGGCTCCAAGTCTGGCACCTCAGCCTCCCTGGCCATCAGTGGGCTCCAGTCTG
AGGATGAGGCTGATTATTACTGTGCAGCATGGGATTTTCGCGACGTGGCCGGCTACTGAGGT
GTTTCGGCGGAGGGACCAAGCTGACCGTCCCTAGGTCAGCCCAAGGCTGCCCCCTCGGTCACT
CTGTTCCCGCCCTCCTCTGAGGAGCTTCAAGCCAACAAGGCCACACTGGTGTGTCTCATAA
GTGACTTCTACCCGGGAGCCGTGACAGTGGCTTGGAAGCAGATAGCAGCCCCGTCAAGGC
GGGAGTGGAGACCACACCCCTCCAAACAAAGCAACAACAAGTACGCGGCCAGCAGCTAT
CTGAGCCTGACGCCTGAGCAGTGGAAAGTCCACAGAAGCTACAGCTGCCAGGTCACGCATG
AAGGGAGCACCGTGGAGAAGACAGTGGCCCCTACAGAATGTTTCATAA

[00184] The “S100” or “S100 IgG_Sa2_1E5” antibody has a heavy chain variable region comprising an amino acid sequence of SEQ ID NO: 6 which is encoded by a nucleic acid sequence of SEQ ID NO: 7 and a lambda light chain variable region comprising an amino acid sequence of SEQ ID NO: 99 which is encoded by a nucleic acid sequence of SEQ ID NO: 100.

>**S100 IgG_Sa2_1E5-AA** (SEQ ID NO: 99)

QSVLTQPPSASGTPGQRTVISCSSSSSNIENKPVNWWYQQLPGTAPKLLIYHDTTRPSGVDP
RFSGSKSGTSASLAISGLQSEDEADYYCAAWDFATWPATEVFEGGKLTVLGQPKAAPSVT
L

>**S100 IgG_Sa2_1E5-NT** (SEQ ID NO: 100)

CAGTCTGTGCTGACTCAGCCACCCTCAGCGTCTGGGACCCCCGGGCAGAGGGTCACCATCT
 CTTGTTCTGGAAGCAGCTCCAACATCGAGAATAAGCCTGTAAACTGGTACCAGCAGCTCCC
 AGGAACGGCCCCCAAACCTCCTCATCTATCATGATACTACTCGGCCCTCAGGGGTCCCTGAC
 CGATTCTCTGGCTCCAAGTCTGGCACCTCAGCCTCCCTGGCCATCAGTGGGCTCCAGTCTG
 AGGATGAGGCTGATTATTACTGTGCAGCATGGGATTTTCGCGACGTGGCCGGCTACTGAGGT
 GTTCGGCGGAGGGACCAAGCTGACCGTCCTAGGTGAGCCCAAGGCTGCCCCCTCGGTCACT
 CTG

DUMMY LIGHT CHAINS

[00185] The Dummy light chain 1 (SEQ ID NO: 112) is encoded by the nucleic acid sequence shown in SEQ ID NO: 111.

>DUMMY-LC1-NT (SEQ ID NO: 111)

CAGTCTGTGTTGACGCAGCCGCCCTCAGTGTCTGCGGCCCCAGGACAGAAGGTCACCATCTCCTGCT
 CTGGAAGCAGCTCCAATATTGAGACTGGTTCTGTATCCTGGTACCAGCAGCTCCCAGGAACAGCCCC
 CAAACTCCTCATTTATGACAATAATAAGCGACCCTCAGGGATTCTGACCGATTCTCTGGCTCCAAG
 TCTGGCACGTCAGCCACCCTGGGCATCACCGGACTCCAGACTGGGGACGAGGCCGATTATTACTGCG
 GAACATGGGATGACAGCCTGCCTGGATGGGTGTTTCGGCGGAGGGACCAAGCTGACCGTCCTAGGTCA
 GCCCAAGGCTGCCCCCTCGGTCACTCTGTTCCCGCCCTCCTCTGAGGAGCTTCAAGCCAACAAGGCC
 AACTGGTGTGTCTCATAAGTGACTTCTACCCGGGAGCCGTGACAGTGGCTTGGAAAGCAGATAGCA
 GCCCCGTCAAGGCGGGAGTGGAGACCACCACACCCTCCAAACAAAGCAACAACAAGTACGCGGCCAG
 CAGCTATCTGAGCCTGACGCCTGAGCAGTGGAAGTCCCACAGAAGCTACAGCTGCCAGGTCACGCAT
 GAAGGGAGCACCGTGGAGAAGACAGTGGCCCCTACAGAATGTTTCATAA

>DUMMY-LC1-AA (SEQ ID NO: 112)

QSVLTQPPSVSAAPGQKVTISCSGSSSNIETGSVSWYQQLPGTAPKLLIYDNNKRPSGIPDRFSGSK
 SGT SATLGITGLQTGDEADYYCGTWDDSLPGWVFGGGTKLTVLGQPKAAPSVTLFPPSSEELQANKA
 TLVCLISDFYPGAVTVAWKADSSPVKAGVETTTPSKQSNKYAASSYLSLTPEQWKSHRSYSCQVTH
 EGSTVEKTVAPTECS

[00186] The Dummy variable light domain 1 (SEQ ID NO: 206) is encoded by the nucleic acid sequence shown in SEQ ID NO: 205.

>DUMMY-VL1-NT (SEQ ID NO: 205)

CAGTCTGTGTTGACGCAGCCGCCCTCAGTGTCTGCGGCCCCAGGACAGAAGGTCACCATCTCCTGCT
 CTGGAAGCAGCTCCAATATTGAGACTGGTTCTGTATCCTGGTACCAGCAGCTCCCAGGAACAGCCCC
 CAAACTCCTCATTTATGACAATAATAAGCGACCCTCAGGGATTCTGACCGATTCTCTGGCTCCAAG
 TCTGGCACGTCAGCCACCCTGGGCATCACCGGACTCCAGACTGGGGACGAGGCCGATTATTACTGCG
 GAACATGGGATGACAGCCTGCCTGGATGGGTGTTTCGGCGGAGGGACCAAGCTGACCGTCCTA

>DUMMY-VL1-AA (SEQ ID NO: 206)

QSVLTQPPSVSAAPGQKVTISCSGSSSNIETGSVSWYQQLPGTAPKLLIYDNNKRPSGIPDRFSGSK
 SGT SATLGITGLQTGDEADYYCGTWDDSLPGWVFGGGTKLTVL

[00187] The Dummy light chain 2 (SEQ ID NO: 208) is encoded by the nucleic acid sequence shown in SEQ ID NO: 207.

>DUMMY-LC2-NT (SEQ ID NO: 207)

GAAATAGTGATGACGCAGTCTCCAGCCACCCTGTCTGTGTCTCCAGGGGAAAGAGCCACCCTCTCCT
 GCAGGGCCAGTCAGACGGTTAAGAATAATTTAGCCTGGTACCAGCAGAAACCTGGCCAGGCTCCCAG
 GCTCCTCATCTATGGTGCATCCACCAGGGCCACTGGTATCCCAGCCAGGTTTCAGTGGCAGTGGGTCT
 GGGACAGAGTTCACTCTCACCATCAGCAGCCTGCAGTCTGAAGATTTTGCAGTTTATTACTGTCAGC
 AGTATAACAACCTGGTTGCCCATCAACCCCTATACCTTCGGCCAAGGGACCAAGGTGGAAATCAAACG
 TACGGTGGCTGCACCATCTGTCTTCATCTTCCCGCCATCTGATGAGCAGTTGAAATCTGGAACCTGCC
 TCTGTTGTGTGCCTGCTGAATAACTTCTATCCCAGAGAGGCCAAAGTACAGTGGAAAGGTGGATAACG
 CCCTCCAATCGGGTAACTCCCAGGAGAGTGTCACAGAGCAGGACAGCAAGGACAGCACCTACAGCCT
 CAGCAGCACCCCTGACGCTGAGCAAAGCAGACTACGAGAAACACAAAGTCTACGCCTGCGAAGTCACC
 CATCAGGGCCTGAGCTCGCCCGTCACAAAGAGCTTCAACAGGGGAGAGTGTTAA

>DUMMY-LC2-AA (SEQ ID NO: 208)

EIVMTQSPATLSVSPGERATLSCRASQTVKNNLAWYQQKPGQAPRLLIYGASTRATGIPARFSGSGS
 GTEFTLTISSLQSEDFAVYYCQQYNNWLPINPYTFGQGTKVEIKRTVAAPSVFIFPPSDEQLKSGTA
 SVVCLLNNFYPREAKVQWKVDNALQSGNSQESVTEQDSKSTYSLSSLTLSKADYEKHKVYACEVT
 HQGLSSPVTKSFNRGEC

[00188] The Dummy variable light domain 2 (SEQ ID NO: 210) is encoded by the nucleic acid sequence shown in SEQ ID NO: 209.

>DUMMY-VL2-NT (SEQ ID NO: 209)

GAAATAGTGATGACGCAGTCTCCAGCCACCCTGTCTGTGTCTCCAGGGGAAAGAGCCACCCTCTCCT
 GCAGGGCCAGTCAGACGGTTAAGAATAATTTAGCCTGGTACCAGCAGAAACCTGGCCAGGCTCCCAG
 GCTCCTCATCTATGGTGCATCCACCAGGGCCACTGGTATCCCAGCCAGGTTCACTGGCAGTGGGTCT
 GGGACAGAGTTCACCTCTCACCATCAGCAGCCTGCAGTCTGAAGATTTTGCAGTTTATTACTGTCAGC
 AGTATAACAACCTGGTTGCCCATCAACCCCTATACCTTCGGCCAAGGGACCAAGGTGGAAATCAAA

>DUMMY-VL2-AA (SEQ ID NO: 210)

EIVMTQSPATLSVSPGERATLSCRASQTVKNNLAWYQQKPGQAPRLLIYGASTRATGIPARFSGSGS
 GTEFTLTISSLQSEDFAVYYCQQYNNWLPINPYTFGQGTKVEIK

Definitions:

[00189] Unless otherwise defined, scientific and technical terms used in connection with the present invention shall have the meanings that are commonly understood by those of ordinary skill in the art. Further, unless otherwise required by context, singular terms shall include pluralities and plural terms shall include the singular. Generally, nomenclatures utilized in connection with, and techniques of, cell and tissue culture, molecular biology, and protein and oligo- or polynucleotide chemistry and hybridization described herein are those well-known and commonly used in the art. Standard techniques are used for recombinant DNA, oligonucleotide synthesis, and tissue culture and transformation (*e.g.*, electroporation, lipofection). Enzymatic reactions and purification techniques are performed according to manufacturer's specifications or as commonly accomplished in the art or as described herein. The foregoing techniques and procedures are generally performed according to conventional methods well known in the art and as described in various general and more specific references that are cited and discussed throughout the present specification. *See e.g.*, Sambrook *et al.* Molecular Cloning: A Laboratory Manual (2d ed., Cold Spring Harbor Laboratory Press, Cold Spring Harbor, N.Y. (1989)). The nomenclatures utilized in connection with, and the laboratory procedures and techniques of, analytical chemistry, synthetic organic chemistry, and medicinal and pharmaceutical chemistry described herein are those well-known and commonly used in the art. Standard techniques are used for chemical syntheses, chemical analyses, pharmaceutical preparation, formulation, and delivery, and treatment of patients.

[00190] As utilized in accordance with the present disclosure, the following terms, unless otherwise indicated, shall be understood to have the following meanings:

[00191] As used herein, the term “antibody” refers to immunoglobulin molecules and immunologically active portions of immunoglobulin (Ig) molecules, *i.e.*, molecules that

contain an antigen binding site that specifically binds (immunoreacts with) an antigen. By “specifically bind” or “immunoreacts with” or “immunospecifically bind” is meant that the antibody reacts with one or more antigenic determinants of the desired antigen and does not react with other polypeptides or binds at much lower affinity ($K_d > 10^{-6}$). Antibodies include, but are not limited to, polyclonal, monoclonal, chimeric, dAb (domain antibody), single chain, F_{ab} , F_{ab}' and $F_{(ab')_2}$ fragments, scFvs, and an F_{ab} expression library.

[00192] The basic antibody structural unit is known to comprise a tetramer. Each tetramer is composed of two identical pairs of polypeptide chains, each pair having one “light” (about 25 kDa) and one “heavy” chain (about 50-70 kDa). The amino-terminal portion of each chain includes a variable region of about 100 to 110 or more amino acids primarily responsible for antigen recognition. The carboxy-terminal portion of each chain defines a constant region primarily responsible for effector function. In general, antibody molecules obtained from humans relate to any of the classes IgG, IgM, IgA, IgE and IgD, which differ from one another by the nature of the heavy chain present in the molecule. Certain classes have subclasses as well, such as IgG₁, IgG₂, and others. Furthermore, in humans, the light chain may be a kappa chain or a lambda chain.

[00193] The term “monoclonal antibody” (MAb) or “monoclonal antibody composition”, as used herein, refers to a population of antibody molecules that contain only one molecular species of antibody molecule consisting of a unique light chain gene product and a unique heavy chain gene product. In particular, the complementarity determining regions (CDRs) of the monoclonal antibody are identical in all the molecules of the population. MAbs contain an antigen binding site capable of immunoreacting with a particular epitope of the antigen characterized by a unique binding affinity for it.

[00194] The term “antigen binding region” or “antigen-binding site” or “binding portion” refers to the part of the immunoglobulin molecule that participates in antigen binding. The antigen binding site is formed by amino acid residues of the N-terminal variable (“V”) regions of the heavy (“H”) and light (“L”) chains. Three highly divergent stretches within the V regions of the heavy and light chains, referred to as “hypervariable regions,” are interposed between more conserved flanking stretches known as “framework regions,” or “FRs”. Thus, the term “FR” refers to amino acid sequences which are naturally found between, and adjacent to, hypervariable regions in immunoglobulins. In an antibody molecule, the three hypervariable regions of a light chain and the three hypervariable regions of a heavy chain are disposed relative to each other in three dimensional space to form an antigen-binding

surface. The antigen-binding surface is complementary to the three-dimensional surface of a bound antigen, and the three hypervariable regions of each of the heavy and light chains are referred to as “complementarity-determining regions,” or “CDRs.” Various methods are known in the art for numbering the amino acids sequences of antibodies and identification of the complementarity determining regions. For example, the Kabat numbering system (*See* Kabat, E.A., *et al.*, Sequences of Protein of immunological interest, Fifth Edition, US Department of Health and Human Services, US Government Printing Office (1991)) or the IMGT numbering system (*See* IMGT®, the international ImMunoGeneTics information system®. Available online: <http://www.imgt.org/>). The IMGT numbering system is routinely used and accepted as a reliable and accurate system in the art to determine amino acid positions in coding sequences, alignment of alleles, and to easily compare sequences in immunoglobulin (IG) and T-cell receptor (TR) from all vertebrate species. The accuracy and the consistency of the IMGT data are based on IMGT-ONTOLOGY, the first, and so far unique, ontology for immunogenetics and immunoinformatics (*See* Lefranc. M.P. *et al.*, *Biomolecules*, 2014 Dec; 4(4), 1102-1139). IMGT tools and databases run against IMGT reference directories built from a large repository of sequences. In the IMGT system the IG V-DOMAIN and IG C-DOMAIN are delimited taking into account the exon delimitation, whenever appropriate. Therefore, the availability of more sequences to the IMGT database, the IMGT exon numbering system can be and “is used” by those skilled in the art reliably to determine amino acid positions in coding sequences and for alignment of alleles. Additionally, correspondences between the IMGT unique numbering with other numberings (i.e., Kabat) are available in the IMGT Scientific chart (*See* Lefranc. M.P. *et al.*, *Biomolecules*, 2014 Dec; 4(4), 1102-1139).

[00195] The term “hypervariable region” or “variable region” refers to the amino acid residues of an antibody that are typically responsible for antigen-binding. The hypervariable region generally comprises amino acid residues from a “complementarity determining region” or “CDR” (e.g., around about residues 24-34 (L1), 50-56 (L2) and 89-97 (L3) in the V_L, and around about 31-35 (H1), 50-65 (H2) and 95-102 (H3) in the V_H when numbered in accordance with the Kabat numbering system; Kabat *et al.*, *Sequences of Proteins of Immunological Interest*, 5th Ed. Public Health Service, National Institutes of Health, Bethesda, Md. (1991)); and/or those residues from a “hypervariable loop” (e.g., residues 24-34 (L1), 50-56 (L2) and 89-97 (L3) in the V_L, and 26-32 (H1), 52-56 (H2) and 95-101 (H3) in the V_H when numbered in accordance with the Chothia numbering system; Chothia and Lesk,

J. Mol. Biol. 196:901-917 (1987)); and/or those residues from a "hypervariable loop" VCDR (e.g., residues 27-38 (L1), 56-65 (L2) and 105-120 (L3) in the V_L , and 27-38 (H1), 56-65 (H2) and 105-120 (H3) in the V_H when numbered in accordance with the IMGT numbering system; Lefranc, M.P. et al. Nucl. Acids Res. 27:209-212 (1999), Ruiz, M. et al. Nucl. Acids Res. 28:219-221 (2000)). Optionally, the antibody has symmetrical insertions at one or more of the following points 28, 36 (L1), 63, 74-75 (L2) and 123 (L3) in the V_L , and 28, 36 (H1), 63, 74-75 (H2) and 123 (H3) in the V_H when numbered in accordance with AHO; Honneger, A. and Plunkthun, A. J. Mol. Biol. 309:657-670 (2001)).

[00196] As used herein, the term “epitope” includes any protein determinant capable of specific binding to an immunoglobulin, an scFv, or a T-cell receptor. The term “epitope” includes any protein determinant capable of specific binding to an immunoglobulin or T-cell receptor. Epitopic determinants 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. For example, antibodies may be raised against N-terminal or C-terminal peptides of a polypeptide. An antibody is the to specifically bind an antigen when the dissociation constant is $\leq 1 \mu\text{M}$; e.g., $\leq 100 \text{ nM}$, preferably $\leq 10 \text{ nM}$ and more preferably $\leq 1 \text{ nM}$.

[00197] As used herein, the terms “immunological binding,” and “immunological binding properties” refer to the non-covalent interactions of the type which occur between an immunoglobulin molecule and an antigen for which the immunoglobulin is specific. The strength, or affinity of immunological binding interactions can be expressed in terms of the dissociation constant (K_d) of the interaction, wherein a smaller K_d represents a greater affinity. Immunological binding properties of selected polypeptides can be quantified using methods well known in the art. One such method entails measuring the rates of antigen-binding site/antigen complex formation and dissociation, wherein those rates depend on the concentrations of the complex partners, the affinity of the interaction, and geometric parameters that equally influence the rate in both directions. Thus, both the “on rate constant” (K_{on}) and the “off rate constant” (K_{off}) can be determined by calculation of the concentrations and the actual rates of association and dissociation. (See Nature 361:186-87 (1993)). The ratio of K_{off}/K_{on} enables the cancellation of all parameters not related to affinity, and is equal to the dissociation constant K_d . (See, generally, Davies et al. (1990) Annual Rev Biochem 59:439-473). An antibody of the present invention is the to specifically bind to its target, when the equilibrium binding constant (K_d) is $\leq 1 \mu\text{M}$, e.g., $\leq 100 \text{ nM}$, preferably $\leq 10 \text{ nM}$, and

more preferably ≤ 1 nM, as measured by assays such as radioligand binding assays or similar assays known to those skilled in the art.

[00198] The term “isolated polynucleotide” as used herein shall mean a polynucleotide of genomic, cDNA, or synthetic origin or some combination thereof, which by virtue of its origin the “isolated polynucleotide” (1) is not associated with all or a portion of a polynucleotide in which the “isolated polynucleotide” is found in nature, (2) is operably linked to a polynucleotide which it is not linked to in nature, or (3) does not occur in nature as part of a larger sequence. Polynucleotides in accordance with the invention include the nucleic acid molecules encoding the heavy chain immunoglobulin molecules, and nucleic acid molecules encoding the light chain immunoglobulin molecules described herein.

[00199] The term “isolated protein” referred to herein means a protein of cDNA, recombinant RNA, or synthetic origin or some combination thereof, which by virtue of its origin, or source of derivation, the “isolated protein” (1) is not associated with proteins found in nature, (2) is free of other proteins from the same source, *e.g.*, free of marine proteins, (3) is expressed by a cell from a different species, or (4) does not occur in nature.

[00200] The term “polypeptide” is used herein as a generic term to refer to native protein, fragments, or analogs of a polypeptide sequence. Hence, native protein fragments, and analogs are species of the polypeptide genus. Polypeptides in accordance with the invention comprise the heavy chain immunoglobulin molecules, and the light chain immunoglobulin molecules described herein, as well as antibody molecules formed by combinations comprising the heavy chain immunoglobulin molecules with light chain immunoglobulin molecules, such as kappa light chain immunoglobulin molecules, and vice versa, as well as fragments and analogs thereof.

[00201] The term “naturally-occurring” as used herein as applied to an object refers to the fact that an object can be found in nature. For example, a polypeptide or polynucleotide sequence that is present in an organism (including viruses) that can be isolated from a source in nature and which has not been intentionally modified by man in the laboratory or otherwise is naturally-occurring.

[00202] The term “operably linked” as used herein refers to positions of components so described are in a relationship permitting them to function in their intended manner. A control sequence “operably linked” to a coding sequence is ligated in such a way that expression of the coding sequence is achieved under conditions compatible with the control sequences.

[00203] The term “control sequence” as used herein refers to polynucleotide sequences which are necessary to effect the expression and processing of coding sequences to which they are ligated. The nature of such control sequences differs depending upon the host organism in prokaryotes, such control sequences generally include promoter, ribosomal binding site, and transcription termination sequence in eukaryotes, generally, such control sequences include promoters and transcription termination sequence. The term “control sequences” is intended to include, at a minimum, all components whose presence is essential for expression and processing, and can also include additional components whose presence is advantageous, for example, leader sequences and fusion partner sequences. The term “polynucleotide” as referred to herein means a polymeric boron of nucleotides of at least 10 bases in length, either ribonucleotides or deoxynucleotides or a modified form of either type of nucleotide. The term includes single and double stranded forms of DNA.

[00204] As used herein, the twenty conventional amino acids and their abbreviations follow conventional usage. *See Immunology - A Synthesis* (2nd Edition, E.S. Golub and D.R. Gren, Eds., Sinauer Associates, Sunderland Mass. (1991)). Stereoisomers (*e.g.*, D- amino acids) of the twenty conventional amino acids, unnatural amino acids such as α -, α -disubstituted amino acids, N-alkyl amino acids, lactic acid, and other unconventional amino acids may also be suitable components for polypeptides of the present invention. Examples of unconventional amino acids include: 4 hydroxyproline, γ -carboxyglutamate, ϵ -N,N,N-trimethyllysine, ϵ -N-acetyllysine, O-phosphoserine, N- acetylserine, N-formylmethionine, 3-methylhistidine, 5-hydroxylysine, σ -N-methylarginine, and other similar amino acids and imino acids (*e.g.*, 4-hydroxyproline). In the polypeptide notation used herein, the left-hand direction is the amino terminal direction and the right-hand direction is the carboxy-terminal direction, in accordance with standard usage and convention.

[00205] As applied to polypeptides, the term “substantial identity” means that two peptide sequences, when optimally aligned, such as by the programs GAP or BESTFIT using default gap weights, share at least 80 percent sequence identity, preferably at least 90 percent sequence identity, more preferably at least 95 percent sequence identity, and most preferably at least 99 percent sequence identity.

[00206] Preferably, residue positions which are not identical differ by conservative amino acid substitutions.

[00207] Conservative amino acid substitutions refer to the interchangeability of residues having similar side chains. For example, a group of amino acids having aliphatic side chains

is glycine, alanine, valine, leucine, and isoleucine; a group of amino acids having aliphatic-hydroxyl side chains is serine and threonine; a group of amino acids having amide-containing side chains is asparagine and glutamine; a group of amino acids having aromatic side chains is phenylalanine, tyrosine, and tryptophan; a group of amino acids having basic side chains is lysine, arginine, and histidine; and a group of amino acids having sulfur-containing side chains is cysteine and methionine. Preferred conservative amino acids substitution groups are: valine-leucine-isoleucine, phenylalanine-tyrosine, lysine-arginine, alanine valine, glutamic- aspartic, and asparagine-glutamine.

[00208] As discussed herein, minor variations in the amino acid sequences of antibodies or immunoglobulin molecules are contemplated as being encompassed by the present invention, providing that the variations in the amino acid sequence maintain at least 75%, more preferably at least 80%, 90%, 95%, and most preferably 99%. In particular, conservative amino acid replacements are contemplated. Conservative replacements are those that take place within a family of amino acids that are related in their side chains. Genetically encoded amino acids are generally divided into families: (1) acidic amino acids are aspartate, glutamate; (2) basic amino acids are lysine, arginine, histidine; (3) non-polar amino acids are alanine, valine, leucine, isoleucine, proline, phenylalanine, methionine, tryptophan, and (4) uncharged polar amino acids are glycine, asparagine, glutamine, cysteine, serine, threonine, tyrosine. The hydrophilic amino acids include arginine, asparagine, aspartate, glutamine, glutamate, histidine, lysine, serine, and threonine. The hydrophobic amino acids include alanine, cysteine, isoleucine, leucine, methionine, phenylalanine, proline, tryptophan, tyrosine and valine. Other families of amino acids include (i) serine and threonine, which are the aliphatic-hydroxy family; (ii) asparagine and glutamine, which are the amide containing family; (iii) alanine, valine, leucine and isoleucine, which are the aliphatic family; and (iv) phenylalanine, tryptophan, and tyrosine, which are the aromatic family. For example, it is reasonable to expect that an isolated replacement of a leucine with an isoleucine or valine, an aspartate with a glutamate, a threonine with a serine, or a similar replacement of an amino acid with a structurally related amino acid will not have a major effect on the binding or properties of the resulting molecule, especially if the replacement does not involve an amino acid within a framework site. Whether an amino acid change results in a functional peptide can readily be determined by assaying the specific activity of the polypeptide derivative. Assays are described in detail herein. Fragments or analogs of antibodies or immunoglobulin molecules can be readily prepared by those of ordinary skill in the art. Preferred amino- and

carboxy-termini of fragments or analogs occur near boundaries of functional domains. Structural and functional domains can be identified by comparison of the nucleotide and/or amino acid sequence data to public or proprietary sequence databases. Preferably, computerized comparison methods are used to identify sequence motifs or predicted protein conformation domains that occur in other proteins of known structure and/or function. Methods to identify protein sequences that fold into a known three-dimensional structure are known. Bowie *et al.* Science 253:164 (1991). Thus, the foregoing examples demonstrate that those of skill in the art can recognize sequence motifs and structural conformations that may be used to define structural and functional domains in accordance with the invention.

[00209] Preferred amino acid substitutions are those which: (1) reduce susceptibility to proteolysis, (2) reduce susceptibility to oxidation, (3) alter binding affinity for forming protein complexes, (4) alter binding affinities, and (4) confer or modify other physicochemical or functional properties of such analogs. Analogs can include various muteins of a sequence other than the naturally-occurring peptide sequence. For example, single or multiple amino acid substitutions (preferably conservative amino acid substitutions) may be made in the naturally- occurring sequence (preferably in the portion of the polypeptide outside the domain(s) forming intermolecular contacts. A conservative amino acid substitution should not substantially change the structural characteristics of the parent sequence (*e.g.*, a replacement amino acid should not tend to break a helix that occurs in the parent sequence, or disrupt other types of secondary structure that characterizes the parent sequence). Examples of art-recognized polypeptide secondary and tertiary structures are described in Proteins, Structures and Molecular Principles (Creighton, Ed., W. H. Freeman and Company, New York (1984)); Introduction to Protein Structure (C. Branden and J. Tooze, eds., Garland Publishing, New York, N.Y. (1991)); and Thornton et al. Nature 354:105 (1991).

[00210] As used herein, the terms “label” or “labeled” refers to incorporation of a detectable marker, *e.g.*, by incorporation of a radiolabeled amino acid or attachment to a polypeptide of biotinyl moieties that can be detected by marked avidin (*e.g.*, streptavidin containing a fluorescent marker or enzymatic activity that can be detected by optical or calorimetric methods). In certain situations, the label or marker can also be therapeutic. Various methods of labeling polypeptides and glycoproteins are known in the art and may be used. Examples of labels for polypeptides include, but are not limited to, the following: radioisotopes or radionuclides (*e.g.*, ^3H , ^{14}C , ^{15}N , ^{35}S , ^{90}Y , ^{99}Tc , ^{111}In , ^{125}I , ^{131}I), fluorescent labels (*e.g.*, FITC, rhodamine, lanthanide phosphors), enzymatic labels (*e.g.*, horseradish peroxidase, p-

galactosidase, luciferase, alkaline phosphatase), chemiluminescent, biotinyl groups, predetermined polypeptide epitopes recognized by a secondary reporter (*e.g.*, leucine zipper pair sequences, binding sites for secondary antibodies, metal binding domains, epitope tags). In some embodiments, labels are attached by spacer arms of various lengths to reduce potential steric hindrance. The term “pharmaceutical agent or drug” as used herein refers to a chemical compound or composition capable of inducing a desired therapeutic effect when properly administered to a patient.

[00211] Other chemistry terms herein are used according to conventional usage in the art, as exemplified by The McGraw-Hill Dictionary of Chemical Terms (Parker, S., Ed., McGraw-Hill, San Francisco (1985)).

[00212] As used herein, “substantially pure” means an object species is the predominant species present (*i.e.*, on a molar basis it is more abundant than any other individual species in the composition), and preferably a substantially purified fraction is a composition wherein the object species comprises at least about 50 percent (on a molar basis) of all macromolecular species present.

[00213] Generally, a substantially pure composition will comprise more than about 80 percent of all macromolecular species present in the composition, more preferably more than about 85%, 90%, 95%, and 99%. Most preferably, the object species is purified to essential homogeneity (contaminant species cannot be detected in the composition by conventional detection methods) wherein the composition consists essentially of a single macromolecular species.

[00214] The term patient includes human and veterinary subjects.

Antibodies

[00215] Various procedures known within the art may be used for the production of polyclonal or monoclonal antibodies directed against a given target, such as, for example, CD47, a tumor associated antigen or other target, or against derivatives, fragments, analogs homologs or orthologs thereof. (*See, for example*, Antibodies: A Laboratory Manual, Harlow E, and Lane D, 1988, Cold Spring Harbor Laboratory Press, Cold Spring Harbor, NY, incorporated herein by reference).

[00216] Antibodies are purified by well-known techniques, such as affinity chromatography using protein A or protein G, which provide primarily the IgG fraction of immune serum. Subsequently, or alternatively, the specific antigen which is the target of the immunoglobulin sought, or an epitope thereof, may be immobilized on a column to purify the immune specific

antibody by immunoaffinity chromatography. Purification of immunoglobulins is discussed, for example, by D. Wilkinson (The Scientist, published by The Scientist, Inc., Philadelphia PA, Vol. 14, No. 8 (April 17, 2000), pp. 25-28).

[00217] In some embodiments, the antibodies of the invention are monoclonal antibodies. Monoclonal antibodies are generated, for example, by using the procedures set forth in the Examples provided herein. Antibodies are also generated, *e.g.*, by immunizing BALB/c mice with combinations of cell transfectants expressing high levels of a given target on their surface. Hybridomas resulting from myeloma/B cell fusions are then screened for reactivity to the selected target.

[00218] Monoclonal antibodies are prepared, for example, using hybridoma methods, such as those described by Kohler and Milstein, *Nature*, 256:495 (1975). In a hybridoma method, a mouse, hamster, or other appropriate host animal, is typically immunized with an immunizing agent to elicit lymphocytes that produce or are capable of producing antibodies that will specifically bind to the immunizing agent. Alternatively, the lymphocytes can be immunized *in vitro*.

[00219] The immunizing agent will typically include the protein antigen, a fragment thereof or a fusion protein thereof. Generally, either peripheral blood lymphocytes are used if cells of human origin are desired, or spleen cells or lymph node cells are used if non-human mammalian sources are desired. The lymphocytes are then fused with an immortalized cell line using a suitable fusing agent, such as polyethylene glycol, to form a hybridoma cell (Goding, Monoclonal Antibodies: Principles and Practice, Academic Press, (1986) pp. 59-103). Immortalized cell lines are usually transformed mammalian cells, particularly myeloma cells of rodent, bovine and human origin. Usually, rat or mouse myeloma cell lines are employed. The hybridoma cells can be cultured in a suitable culture medium that preferably contains one or more substances that inhibit the growth or survival of the unfused, immortalized cells. For example, if the parental cells lack the enzyme hypoxanthine guanine phosphoribosyl transferase (HGPRT or HPRT), the culture medium for the hybridomas typically will include hypoxanthine, aminopterin, and thymidine ("HAT medium"), which substances prevent the growth of HGPRT-deficient cells.

[00220] Preferred immortalized cell lines are those that fuse efficiently, support stable high level expression of antibody by the selected antibody-producing cells, and are sensitive to a medium such as HAT medium. More preferred immortalized cell lines are murine myeloma lines, which can be obtained, for instance, from the Salk Institute Cell Distribution Center,

San Diego, California and the American Type Culture Collection, Manassas, Virginia. Human myeloma and mouse-human heteromyeloma cell lines also have been described for the production of monoclonal antibodies. (See Kozbor, J. Immunol., 133:3001 (1984); Brodeur et al., Monoclonal Antibody Production Techniques and Applications, Marcel Dekker, Inc., New York, (1987) pp. 51-63)).

[00221] The culture medium in which the hybridoma cells are cultured can then be assayed for the presence of monoclonal antibodies directed against the antigen. Preferably, the binding specificity of monoclonal antibodies produced by the hybridoma cells is determined by immunoprecipitation or by an *in vitro* binding assay, such as radioimmunoassay (RIA) or enzyme-linked immunoabsorbent assay (ELISA). Such techniques and assays are known in the art. The binding affinity of the monoclonal antibody can, for example, be determined by the Scatchard analysis of Munson and Pollard, Anal. Biochem., 107:220 (1980). Moreover, in therapeutic applications of monoclonal antibodies, it is important to identify antibodies having a high degree of specificity and a high binding affinity for the target antigen.

[00222] After the desired hybridoma cells are identified, the clones can be subcloned by limiting dilution procedures and grown by standard methods. (See Goding, Monoclonal Antibodies: Principles and Practice, Academic Press, (1986) pp. 59-103). Suitable culture media for this purpose include, for example, Dulbecco's Modified Eagle's Medium and RPMI-1640 medium. Alternatively, the hybridoma cells can be grown *in vivo* as ascites in a mammal.

[00223] The monoclonal antibodies secreted by the subclones can be isolated or purified from the culture medium or ascites fluid by conventional immunoglobulin purification procedures such as, for example, protein A-Sepharose, hydroxylapatite chromatography, gel electrophoresis, dialysis, or affinity chromatography.

[00224] Monoclonal antibodies can also be made by recombinant DNA methods, such as those described in U.S. Patent No. 4,816,567. DNA encoding the monoclonal antibodies of the invention can be 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 murine antibodies). The hybridoma cells of the invention serve as a preferred source of such DNA. Once isolated, the DNA can be placed into expression vectors, which are then transfected into host cells such as simian COS cells, Chinese hamster ovary (CHO) cells, or myeloma cells that do not otherwise produce immunoglobulin protein, to obtain the synthesis of monoclonal antibodies in the recombinant host cells. The DNA also

can be modified, for example, by substituting the coding sequence for human heavy and light chain constant domains in place of the homologous murine sequences (*see* U.S. Patent No. 4,816,567; Morrison, *Nature* 368, 812-13 (1994)) or by covalently joining to the immunoglobulin coding sequence all or part of the coding sequence for a non-immunoglobulin polypeptide. Such a non-immunoglobulin polypeptide can be substituted for the constant domains of an antibody of the invention, or can be substituted for the variable domains of one antigen-combining site of an antibody of the invention to create a chimeric bivalent antibody.

[00225] Monoclonal antibodies of the invention include humanized antibodies or human antibodies. These antibodies are suitable for administration to humans without engendering an immune response by the human against the administered immunoglobulin. Humanized forms of antibodies are chimeric immunoglobulins, immunoglobulin chains or fragments thereof (such as Fv, Fab, Fab', F(ab')₂ or other antigen-binding subsequences of antibodies) that are principally comprised of the sequence of a human immunoglobulin, and contain minimal sequence derived from a non-human immunoglobulin. Humanization is performed, *e.g.*, by following the method of Winter and co-workers (Jones et al., *Nature*, 321:522-525 (1986); Riechmann et al., *Nature*, 332:323-327 (1988); Verhoeyen et al., *Science*, 239:1534-1536 (1988)), by substituting rodent CDRs or CDR sequences for the corresponding sequences of a human antibody. (See also U.S. Patent No. 5,225,539). In some instances, Fv framework residues of the human immunoglobulin are replaced by corresponding non-human residues. Humanized antibodies also comprise, *e.g.*, residues which are found neither in the recipient antibody nor in the imported CDR or framework sequences. In general, the humanized antibody includes substantially all of at least one, and typically two, variable domains, in which all or substantially all of the CDR regions correspond to those of a non-human immunoglobulin and all or substantially all of the framework regions are those of a human immunoglobulin consensus sequence. The humanized antibody optimally also includes at least a portion of an immunoglobulin constant region (Fc), typically that of a human immunoglobulin (Jones et al., 1986; Riechmann et al., 1988; and Presta, *Curr. Op. Struct. Biol.*, 2:593-596 (1992)).

[00226] Fully human antibodies are antibody molecules in which the entire sequence of both the light chain and the heavy chain, including the CDRs, arise from human genes. Such antibodies are termed “human antibodies”, or “fully human antibodies” herein. Monoclonal antibodies can be prepared by using trioma technique; the human B-cell hybridoma technique

(see Kozbor, et al., 1983 Immunol Today 4: 72); and the EBV hybridoma technique to produce monoclonal antibodies (see Cole, et al., 1985 In: MONOCLONAL ANTIBODIES AND CANCER THERAPY, Alan R. Liss, Inc., pp. 77-96). Monoclonal antibodies may be utilized and may be produced by using human hybridomas (see Cote, et al., 1983. Proc Natl Acad Sci USA 80: 2026-2030) or by transforming human B-cells with Epstein Barr Virus *in vitro* (see Cole, et al., 1985 In: MONOCLONAL ANTIBODIES AND CANCER THERAPY, Alan R. Liss, Inc., pp. 77-96).

[00227] In addition, human antibodies can also be produced using additional techniques, including phage display libraries. (See Hoogenboom and Winter, J. Mol. Biol., 227:381 (1991); Marks et al., J. Mol. Biol., 222:581 (1991)). Similarly, human antibodies can be made by introducing human immunoglobulin loci into transgenic animals, *e.g.*, mice in which the endogenous immunoglobulin genes have been partially or completely inactivated. Upon challenge, human antibody production is observed, which closely resembles that seen in humans in all respects, including gene rearrangement, assembly, and antibody repertoire. This approach is described, for example, in U.S. Patent Nos. 5,545,807; 5,545,806; 5,569,825; 5,625,126; 5,633,425; 5,661,016, and in Marks et al., Bio/Technology 10, 779-783 (1992); Lonberg et al., Nature 368 856-859 (1994); Morrison, Nature 368, 812-13 (1994); Fishwild et al, Nature Biotechnology 14, 845-51 (1996); Neuberger, Nature Biotechnology 14, 826 (1996); and Lonberg and Huszar, Intern. Rev. Immunol. 13 65-93 (1995).

[00228] Human antibodies may additionally be produced using transgenic nonhuman animals which are modified so as to produce fully human antibodies rather than the animal's endogenous antibodies in response to challenge by an antigen. (See PCT publication WO94/02602). The endogenous genes encoding the heavy and light immunoglobulin chains in the nonhuman host have been incapacitated, and active loci encoding human heavy and light chain immunoglobulins are inserted into the host's genome. The human genes are incorporated, for example, using yeast artificial chromosomes containing the requisite human DNA segments. An animal which provides all the desired modifications is then obtained as progeny by crossbreeding intermediate transgenic animals containing fewer than the full complement of the modifications. An example of such a nonhuman animal is a mouse termed the XenomouseTM as disclosed in PCT publications WO 96/33735 and WO 96/34096. This animal produces B cells which secrete fully human immunoglobulins. The antibodies can be obtained directly from the animal after immunization with an immunogen of interest, as, for example, a preparation of a polyclonal antibody, or alternatively from immortalized B cells

derived from the animal, such as hybridomas producing monoclonal antibodies. Additionally, the genes encoding the immunoglobulins with human variable regions can be recovered and expressed to obtain the antibodies directly, or can be further modified to obtain analogs of antibodies such as, for example, single chain Fv (scFv) molecules.

[00229] An example of a method of producing a nonhuman host, exemplified as a mouse, lacking expression of an endogenous immunoglobulin heavy chain is disclosed in U.S. Patent No. 5,939,598. It can be obtained by a method, which includes deleting the J segment genes from at least one endogenous heavy chain locus in an embryonic stem cell to prevent rearrangement of the locus and to prevent formation of a transcript of a rearranged immunoglobulin heavy chain locus, the deletion being effected by a targeting vector containing a gene encoding a selectable marker; and producing from the embryonic stem cell a transgenic mouse whose somatic and germ cells contain the gene encoding the selectable marker.

[00230] One method for producing an antibody of interest, such as a human antibody, is disclosed in U.S. Patent No. 5,916,771. This method includes introducing an expression vector that contains a nucleotide sequence encoding a heavy chain into one mammalian host cell in culture, introducing an expression vector containing a nucleotide sequence encoding a light chain into another mammalian host cell, and fusing the two cells to form a hybrid cell. The hybrid cell expresses an antibody containing the heavy chain and the light chain.

[00231] In a further improvement on this procedure, a method for identifying a clinically relevant epitope on an immunogen and a correlative method for selecting an antibody that binds specifically to the relevant epitope with high affinity are disclosed in PCT publication WO 99/53049.

[00232] The antibody can be expressed by a vector containing a DNA segment encoding the single chain antibody described above.

[00233] These can include vectors, liposomes, naked DNA, adjuvant-assisted DNA, gene gun, catheters, *etc.* Vectors include chemical conjugates such as described in WO 93/64701, which has targeting moiety (*e.g.*, a ligand to a cellular surface receptor), and a nucleic acid binding moiety (*e.g.*, polylysine), viral vector (*e.g.*, a DNA or RNA viral vector), fusion proteins such as described in PCT/US 95/02140 (WO 95/22618) which is a fusion protein containing a target moiety (*e.g.*, an antibody specific for a target cell) and a nucleic acid binding moiety (*e.g.*, a protamine), plasmids, phage, *etc.* The vectors can be chromosomal, non-chromosomal or synthetic.

[00234] Preferred vectors include viral vectors, fusion proteins and chemical conjugates. Retroviral vectors include moloney murine leukemia viruses. DNA viral vectors are preferred. These vectors include pox vectors such as orthopox or avipox vectors, herpesvirus vectors such as a herpes simplex I virus (HSV) vector (*see* Geller, A. I. et al., J. Neurochem, 64:487 (1995); Lim, F., et al., in DNA Cloning: Mammalian Systems, D. Glover, Ed. (Oxford Univ. Press, Oxford England) (1995); Geller, A. I. et al., Proc Natl. Acad. Sci.: U.S.A. 90:7603 (1993); Geller, A. I., et al., Proc Natl. Acad. Sci USA 87:1149 (1990), Adenovirus Vectors (*see* LeGal LaSalle et al., Science, 259:988 (1993); Davidson, et al., Nat. Genet 3:219 (1993); Yang, et al., J. Virol. 69:2004 (1995) and Adeno-associated Virus Vectors (*see* Kaplitt, M. G. et al., Nat. Genet. 8:148 (1994).

[00235] Pox viral vectors introduce the gene into the cells cytoplasm. Avipox virus vectors result in only a short term expression of the nucleic acid. Adenovirus vectors, adeno-associated virus vectors and herpes simplex virus (HSV) vectors are preferred for introducing the nucleic acid into neural cells. The adenovirus vector results in a shorter term expression (about 2 months) than adeno-associated virus (about 4 months), which in turn is shorter than HSV vectors. The particular vector chosen will depend upon the target cell and the condition being treated. The introduction can be by standard techniques, *e.g.*, infection, transfection, transduction or transformation. Examples of modes of gene transfer include *e.g.*, naked DNA, CaPO₄ precipitation, DEAE dextran, electroporation, protoplast fusion, lipofection, cell microinjection, and viral vectors.

[00236] The vector can be employed to target essentially any desired target cell. For example, stereotaxic injection can be used to direct the vectors (*e.g.*, adenovirus, HSV) to a desired location. Additionally, the particles can be delivered by intracerebroventricular (icv) infusion using a minipump infusion system, such as a SynchroMed Infusion System. A method based on bulk flow, termed convection, has also proven effective at delivering large molecules to extended areas of the brain and may be useful in delivering the vector to the target cell. (*See* Bobo et al., Proc. Natl. Acad. Sci. USA 91:2076-2080 (1994); Morrison et al., Am. J. Physiol. 266:292-305 (1994)). Other methods that can be used include catheters, intravenous, parenteral, intraperitoneal and subcutaneous injection, and oral or other known routes of administration.

[00237] Bispecific antibodies are antibodies that have binding specificities for at least two different antigens. In the present case, one of the binding specificities is for a target such as

CD47 or any fragment thereof. The second binding target is any other antigen, and advantageously is a cell-surface protein or receptor or receptor subunit.

[00238] Methods for making bispecific antibodies are known in the art. 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).

[00239] Bispecific and/or monovalent antibodies of the invention can be made using any of a variety of art-recognized techniques, including those disclosed in co-pending application WO 2012/023053, filed August 16, 2011, the contents of which are hereby incorporated by reference in their entirety. The methods described in WO 2012/023053 generate bispecific antibodies that are identical in structure to a human immunoglobulin. This type of molecule is composed of two copies of a unique heavy chain polypeptide, a first light chain variable region fused to a constant Kappa domain and second light chain variable region fused to a constant Lambda domain. Each combining site displays a different antigen specificity to which both the heavy and light chain contribute. The light chain variable regions can be of the Lambda or Kappa family and are preferably fused to a Lambda and Kappa constant domains, respectively. This is preferred in order to avoid the generation of non-natural polypeptide junctions. However it is also possible to obtain bispecific antibodies of the invention by fusing a Kappa light chain variable domain to a constant Lambda domain for a first specificity and fusing a Lambda light chain variable domain to a constant Kappa domain for the second specificity. The bispecific antibodies described in WO 2012/023053 are referred to as IgG $\kappa\lambda$ antibodies or “ $\kappa\lambda$ bodies,” a new fully human bispecific IgG format. This $\kappa\lambda$ -body format allows the affinity purification of a bispecific antibody that is undistinguishable from a standard IgG molecule with characteristics that are undistinguishable from a standard monoclonal antibody and, therefore, favorable as compared to previous formats.

[00240] An essential step of the method is the identification of two antibody Fv regions (each composed by a variable light chain and variable heavy chain domain) having different antigen

specificities that share the same heavy chain variable domain. Numerous methods have been described for the generation of monoclonal antibodies and fragments thereof. (*See, e.g.,* Antibodies: A Laboratory Manual, Harlow E, and Lane D, 1988, Cold Spring Harbor Laboratory Press, Cold Spring Harbor, NY, incorporated herein by reference). Fully human antibodies are antibody molecules in which the sequence of both the light chain and the heavy chain, including the CDRs 1 and 2, arise from human genes. The CDR3 region can be of human origin or designed by synthetic means. Such antibodies are termed “human antibodies”, or “fully human antibodies” herein. Human monoclonal antibodies can be prepared by using the trioma technique; the human B-cell hybridoma technique (*see* Kozbor, et al., 1983 Immunol Today 4: 72); and the EBV hybridoma technique to produce human monoclonal antibodies (*see* Cole, et al., 1985 In: MONOCLONAL ANTIBODIES AND CANCER THERAPY, Alan R. Liss, Inc., pp. 77-96). Human monoclonal antibodies may be utilized and may be produced by using human hybridomas (*see* Cote, et al., 1983. Proc Natl Acad Sci USA 80: 2026-2030) or by transforming human B-cells with Epstein Barr Virus *in vitro* (*see* Cole, et al., 1985 In: MONOCLONAL ANTIBODIES AND CANCER THERAPY, Alan R. Liss, Inc., pp. 77-96).

[00241] Monoclonal antibodies are generated, *e.g.*, by immunizing an animal with a target antigen or an immunogenic fragment, derivative or variant thereof. Alternatively, the animal is immunized with cells transfected with a vector containing a nucleic acid molecule encoding the target antigen, such that the target antigen is expressed and associated with the surface of the transfected cells. A variety of techniques are well-known in the art for producing xenogenic non-human animals. For example, see U.S. Pat. No. 6,075,181 and No. 6,150,584, which is hereby incorporated by reference in its entirety.

[00242] Alternatively, the antibodies are obtained by screening a library that contains antibody or antigen binding domain sequences for binding to the target antigen. This library is prepared, *e.g.*, in bacteriophage as protein or peptide fusions to a bacteriophage coat protein that is expressed on the surface of assembled phage particles and the encoding DNA sequences contained within the phage particles (*i.e.*, “phage displayed library”).

[00243] Hybridomas resulting from myeloma/B cell fusions are then screened for reactivity to the target antigen. Monoclonal antibodies are prepared, for example, using hybridoma methods, such as those described by Kohler and Milstein, Nature, 256:495 (1975). In a hybridoma method, a mouse, hamster, or other appropriate host animal, is typically immunized with an immunizing agent to elicit lymphocytes that produce or are capable of

producing antibodies that will specifically bind to the immunizing agent. Alternatively, the lymphocytes can be immunized *in vitro*.

[00244] Although not strictly impossible, the serendipitous identification of different antibodies having the same heavy chain variable domain but directed against different antigens is highly unlikely. Indeed, in most cases the heavy chain contributes largely to the antigen binding surface and is also the most variable in sequence. In particular the CDR3 on the heavy chain is the most diverse CDR in sequence, length and structure. Thus, two antibodies specific for different antigens will almost invariably carry different heavy chain variable domains.

[00245] The methods disclosed in co-pending application WO 2012/023053 overcomes this limitation and greatly facilitates the isolation of antibodies having the same heavy chain variable domain by the use of antibody libraries in which the heavy chain variable domain is the same for all the library members and thus the diversity is confined to the light chain variable domain. Such libraries are described, for example, in co-pending applications WO 2010/135558 and WO 2011/084255, each of which is hereby incorporated by reference in its entirety. However, as the light chain variable domain is expressed in conjunction with the heavy variable domain, both domains can contribute to antigen binding. To further facilitate the process, antibody libraries containing the same heavy chain variable domain and either a diversity of Lambda variable light chains or Kappa variable light chains can be used in parallel for *in vitro* selection of antibodies against different antigens. This approach enables the identification of two antibodies having a common heavy chain but one carrying a Lambda light chain variable domain and the other a Kappa light chain variable domain that can be used as building blocks for the generation of a bispecific antibody in the full immunoglobulin format of the invention. The bispecific antibodies of the invention can be of different Isotypes and their Fc portion can be modified in order to alter the bind properties to different Fc receptors and in this way modify the effectors functions of the antibody as well as its pharmacokinetic properties. Numerous methods for the modification of the Fc portion have been described and are applicable to antibodies of the invention. (see for example Strohl, WR Curr Opin Biotechnol 2009 (6):685-91; U.S. Pat. No. 6,528,624; PCT/US2009/0191199 filed Jan 9, 2009). The methods of the invention can also be used to generate bispecific antibodies and antibody mixtures in a F(ab')₂ format that lacks the Fc portion.

[00246] The common heavy chain and two different light chains are co-expressed into a single cell to allow for the assembly of a bispecific antibody of the invention. If all the

polypeptides get expressed at the same level and get assembled equally well to form an immunoglobulin molecule then the ratio of monospecific (same light chains) and bispecific (two different light chains) should be 50%. However, it is likely that different light chains are expressed at different levels and/or do not assemble with the same efficiency. Therefore, a means to modulate the relative expression of the different polypeptides is used to compensate for their intrinsic expression characteristics or different propensities to assemble with the common heavy chain. This modulation can be achieved via promoter strength, the use of internal ribosome entry sites (IRES) featuring different efficiencies or other types of regulatory elements that can act at transcriptional or translational levels as well as acting on mRNA stability. Different promoters of different strength could include CMV (Immediate-early Cytomegalovirus virus promoter); EF1-1 α (Human elongation factor 1 α -subunit promoter); Ubc (Human ubiquitin C promoter); SV40 (Simian virus 40 promoter). Different IRES have also been described from mammalian and viral origin. (See *e.g.*, Hellen CU and Sarnow P. *Genes Dev* 2001 **15**: 1593–612). These IRES can greatly differ in their length and ribosome recruiting efficiency. Furthermore, it is possible to further tune the activity by introducing multiple copies of an IRES (Stephen et al. 2000 *Proc Natl Acad Sci USA* 97: 1536-1541). The modulation of the expression can also be achieved by multiple sequential transfections of cells to increase the copy number of individual genes expressing one or the other light chain and thus modify their relative expressions. The Examples provided herein demonstrate that controlling the relative expression of the different chains is critical for maximizing the assembly and overall yield of the bispecific antibody.

[00247] The co-expression of the heavy chain and two light chains generates a mixture of three different antibodies into the cell culture supernatant: two monospecific bivalent antibodies and one bispecific bivalent antibody. The latter has to be purified from the mixture to obtain the molecule of interest. The method described herein greatly facilitates this purification procedure by the use of affinity chromatography media that specifically interact with the Kappa or Lambda light chain constant domains such as the CaptureSelect Fab Kappa and CaptureSelect Fab Lambda affinity matrices (BAC BV, Holland). This multi-step affinity chromatography purification approach is efficient and generally applicable to antibodies of the invention. This is in sharp contrast to specific purification methods that have to be developed and optimized for each bispecific antibodies derived from quadromas or other cell lines expressing antibody mixtures. Indeed, if the biochemical characteristics of the different antibodies in the mixtures are similar, their separation using standard chromatography

technique such as ion exchange chromatography can be challenging or not possible at all.

[00248] Other suitable purification methods include those disclosed in co-pending application PCT/IB2012/003028, filed on October 19, 2012, published as WO2013/088259, the contents of which are hereby incorporated by reference in their entirety.

[00249] In other embodiments of producing bispecific antibodies, 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).

[00250] 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 includes 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.

[00251] Techniques for generating bispecific antibodies from antibody fragments have been described in the literature. For example, bispecific antibodies can be prepared using chemical linkage. The bispecific antibodies produced can be used as agents for the selective immobilization of enzymes.

[00252] 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 for the production of 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 (V_H) connected to a light-chain variable domain (V_L) 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 by the use of single-chain Fv (sFv) dimers has also been reported. See, Gruber et al., J. Immunol. 152:5368 (1994). [00253] Antibodies with more than two valencies are contemplated. For example, trispecific antibodies can be prepared. Tutt et al., J. Immunol. 147:60 (1991).

[00254] Exemplary bispecific antibodies can bind to two different epitopes, at least one of which originates in the protein antigen of the invention. Alternatively, an anti-antigenic arm of an immunoglobulin molecule can be combined with an arm which binds to a triggering molecule on a leukocyte such as a T-cell receptor molecule (*e.g.*, CD2, CD3, CD28, or B7), or Fc receptors for IgG (Fc γ R), such as Fc γ RI (CD64), Fc γ RII (CD32) and Fc γ RIII (CD16) so as to focus cellular defense mechanisms to the cell expressing the particular antigen. Bispecific antibodies can also be used to direct cytotoxic agents to cells which express a particular antigen. These antibodies possess an antigen-binding arm and an arm which binds a cytotoxic agent or a radionuclide chelator, such as EOTUBE, DPTA, DOTA, or TETA. Another bispecific antibody of interest binds the protein antigen described herein and further binds tissue factor (TF).

[00255] Heteroconjugate antibodies are also within the scope of the present invention. Heteroconjugate antibodies are composed of two covalently joined antibodies. Such antibodies have, for example, been proposed to target immune system cells to unwanted cells (*see* U.S. Patent No. 4,676,980), and for treatment of HIV infection (*see* WO 91/00360; WO 92/200373; EP 03089). It is contemplated that the antibodies can be prepared *in vitro* using known methods in synthetic protein chemistry, including those involving crosslinking agents. For example, immunotoxins can be constructed using a disulfide exchange reaction or by forming a thioether bond. Examples of suitable reagents for this purpose include iminothiolate and methyl-4-mercaptobutyrimidate and those disclosed, for example, in U.S. Patent No. 4,676,980.

[00256] It can be desirable to modify the antibody of the invention with respect to effector function, so as to enhance, *e.g.*, the effectiveness of the antibody in treating cancer and/or other diseases and disorders associated with aberrant CD47 expression and/or activity. For example, cysteine residue(s) can be introduced into the Fc region, thereby allowing interchain disulfide bond formation in this region. The homodimeric antibody thus generated can have improved internalization capability and/or increased complement-mediated cell killing and antibody-dependent cellular cytotoxicity (ADCC). (*See* Caron et al., J. Exp Med., 176: 1191-1195 (1992) and Shopes, J. Immunol., 148: 2918-2922 (1992)). Alternatively, an antibody can be engineered that has dual Fc regions and can thereby have enhanced complement lysis and ADCC capabilities. (*See* Stevenson et al., Anti-Cancer Drug Design, 3: 219-230 (1989)).

[00257] The invention also pertains to immunoconjugates comprising an antibody conjugated to a cytotoxic agent such as a toxin (*e.g.*, an enzymatically active toxin of bacterial, fungal, plant, or animal origin, or fragments thereof), or a radioactive isotope (*i.e.*, a radioconjugate).

[00258] Enzymatically active toxins and fragments thereof that can be used include diphtheria A chain, nonbinding active fragments of diphtheria toxin, exotoxin A chain (from *Pseudomonas aeruginosa*), ricin A chain, abrin A chain, modeccin A chain, alpha-sarcin, Aleurites fordii proteins, dianthin proteins, Phytolaca americana proteins (PAPI, PAPII, and PAP-S), momordica charantia inhibitor, curcin, crotin, sapaonaria officinalis inhibitor, gelonin, mitogellin, restrictocin, phenomycin, enomycin, and the tricothecenes. A variety of radionuclides are available for the production of radioconjugated antibodies. Examples include ^{212}Bi , ^{131}I , ^{131}In , ^{90}Y , and ^{186}Re .

[00259] Conjugates of the antibody and cytotoxic agent are made using a variety of bifunctional protein-coupling agents such as N-succinimidyl-3-(2-pyridyldithiol) propionate (SPDP), iminothiolane (IT), bifunctional derivatives of imidoesters (such as dimethyl adipimidate HCL), active esters (such as disuccinimidyl suberate), aldehydes (such as glutaraldehyde), bis-azido compounds (such as bis (p-azidobenzoyl) hexanediamine), bis-diazonium derivatives (such as bis-(p-diazoniumbenzoyl)-ethylenediamine), diisocyanates (such as tolyene 2,6-diisocyanate), and bis-active fluorine compounds (such as 1,5-difluoro-2,4-dinitrobenzene). For example, a ricin immunotoxin can be prepared as described in Vitetta et al., Science 238: 1098 (1987). Carbon-14-labeled 1-isothiocyanatobenzyl-3-methyldiethylene triaminepentaacetic acid (MX-DTPA) is an exemplary chelating agent for conjugation of radionucleotide to the antibody. (*See* WO94/11026).

[00260] Those of ordinary skill in the art will recognize that a large variety of possible moieties can be coupled to the resultant antibodies of the invention. (*See, for example*, “Conjugate Vaccines”, Contributions to Microbiology and Immunology, J. M. Cruse and R. E. Lewis, Jr (eds), Carger Press, New York, (1989), the entire contents of which are incorporated herein by reference).

[00261] Coupling may be accomplished by any chemical reaction that will bind the two molecules so long as the antibody and the other moiety retain their respective activities. This linkage can include many chemical mechanisms, for instance covalent binding, affinity binding, intercalation, coordinate binding and complexation. The preferred binding is, however, covalent binding. Covalent binding can be achieved either by direct condensation of existing side chains or by the incorporation of external bridging molecules. Many bivalent or polyvalent linking agents are useful in coupling protein molecules, such as the antibodies of the present invention, to other molecules. For example, representative coupling agents can include organic compounds such as thioesters, carbodiimides, succinimide esters, diisocyanates, glutaraldehyde, diazobenzenes and hexamethylene diamines. This listing is not intended to be exhaustive of the various classes of coupling agents known in the art but, rather, is exemplary of the more common coupling agents. (*See* Killen and Lindstrom, Jour. Immun. 133:1335-2549 (1984); Jansen et al., Immunological Reviews 62:185-216 (1982); and Vitetta et al., Science 238:1098 (1987)).

[00262] Preferred linkers are described in the literature. (*See, for example*, Ramakrishnan, S. et al., Cancer Res. 44:201-208 (1984) describing use of MBS (M-maleimidobenzoyl-N-hydroxysuccinimide ester). *See also*, U.S. Patent No. 5,030,719, describing use of halogenated acetyl hydrazide derivative coupled to an antibody by way of an oligopeptide linker. Particularly preferred linkers include: (i) EDC (1-ethyl-3-(3-dimethylamino-propyl) carbodiimide hydrochloride; (ii) SMPT (4-succinimidylloxycarbonyl-alpha-methyl-alpha-(2-pyridyl-dithio)-toluene (Pierce Chem. Co., Cat. (21558G); (iii) SPDP (succinimidyl-6 [3-(2-pyridyldithio) propionamido]hexanoate (Pierce Chem. Co., Cat #21651G); (iv) Sulfo-LC-SPDP (sulfosuccinimidyl 6 [3-(2-pyridyldithio)-propionamide] hexanoate (Pierce Chem. Co. Cat. #2165-G); and (v) sulfo-NHS (N-hydroxysulfo-succinimide: Pierce Chem. Co., Cat. #24510) conjugated to EDC.

[00263] The linkers described above contain components that have different attributes, thus leading to conjugates with differing physio-chemical properties. For example, sulfo-NHS esters of alkyl carboxylates are more stable than sulfo-NHS esters of aromatic carboxylates.

NHS-ester containing linkers are less soluble than sulfo-NHS esters. Further, the linker SMPT contains a sterically hindered disulfide bond, and can form conjugates with increased stability. Disulfide linkages, are in general, less stable than other linkages because the disulfide linkage is cleaved *in vitro*, resulting in less conjugate available. Sulfo-NHS, in particular, can enhance the stability of carbodimide couplings. Carbodimide couplings (such as EDC) when used in conjunction with sulfo-NHS, forms esters that are more resistant to hydrolysis than the carbodimide coupling reaction alone.

[00264] The antibodies disclosed herein can also be formulated as immunoliposomes. Liposomes containing the antibody are prepared by methods known in the art, such as described in Epstein et al., Proc. Natl. Acad. Sci. USA, 82: 3688 (1985); Hwang et al., Proc. Natl. Acad. Sci. USA, 77: 4030 (1980); and U.S. Pat. Nos. 4,485,045 and 4,544,545. Liposomes with enhanced circulation time are disclosed in U.S. Patent No. 5,013,556.

[00265] Particularly useful liposomes can be generated by the reverse-phase evaporation method with a lipid composition comprising phosphatidylcholine, cholesterol, and PEG-derivatized phosphatidylethanolamine (PEG-PE). Liposomes are extruded through filters of defined pore size to yield liposomes with the desired diameter. Fab' fragments of the antibody of the present invention can be conjugated to the liposomes as described in Martin et al., J. Biol. Chem., 257: 286-288 (1982) via a disulfide-interchange reaction.

[00266] METHODS OF USE

[00267] It will be appreciated that administration of therapeutic entities in accordance with the invention will be administered with suitable carriers, excipients, and other agents that are incorporated into formulations to provide improved transfer, delivery, tolerance, and the like. A multitude of appropriate formulations can be found in the formulary known to all pharmaceutical chemists: Remington's Pharmaceutical Sciences (15th ed, Mack Publishing Company, Easton, PA (1975)), particularly Chapter 87 by Blaug, Seymour, therein. These formulations include, for example, powders, pastes, ointments, jellies, waxes, oils, lipids, lipid (cationic or anionic) containing vesicles (such as Lipofectin™), DNA conjugates, anhydrous absorption pastes, oil-in-water and water-in-oil emulsions, emulsions carbowax (polyethylene glycols of various molecular weights), semi-solid gels, and semi-solid mixtures containing carbowax. Any of the foregoing mixtures may be appropriate in treatments and therapies in accordance with the present invention, provided that the active ingredient in the formulation is not inactivated by the formulation and the formulation is physiologically compatible and tolerable with the route of administration. See also Baldrick P.

“Pharmaceutical excipient development: the need for preclinical guidance.” Regul. Toxicol Pharmacol. 32(2):210-8 (2000), Wang W. “Lyophilization and development of solid protein pharmaceuticals.” Int. J. Pharm. 203(1-2):1-60 (2000), Charman WN “Lipids, lipophilic drugs, and oral drug delivery-some emerging concepts.” J Pharm Sci. 89(8):967-78 (2000), Powell *et al.* “Compendium of excipients for parenteral formulations” PDA J Pharm Sci Technol. 52:238-311 (1998) and the citations therein for additional information related to formulations, excipients and carriers well known to pharmaceutical chemists.

[00268] Therapeutic formulations of the invention, which include an antibody of the invention, are used to treat or alleviate a symptom associated with a cancer, such as, by way of non-limiting example, leukemias, lymphomas, breast cancer, colon cancer, ovarian cancer, bladder cancer, prostate cancer, glioma, lung & bronchial cancer, colorectal cancer, pancreatic cancer, esophageal cancer, liver cancer, urinary bladder cancer, kidney and renal pelvis cancer, oral cavity & pharynx cancer, uterine corpus cancer, and/or melanoma The present invention also provides methods of treating or alleviating a symptom associated with a cancer. A therapeutic regimen is carried out by identifying a subject, *e.g.*, a human patient suffering from (or at risk of developing) a cancer, using standard methods.

[00269] Efficaciousness of treatment is determined in association with any known method for diagnosing or treating the particular immune-related disorder. Alleviation of one or more symptoms of the immune-related disorder indicates that the antibody confers a clinical benefit.

[00270] Methods for the screening of antibodies 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.

[00271] Antibodies directed against a target such as CD47, PD-L1, or a combination thereof (or a fragment thereof), may be used in methods known within the art relating to the localization and/or quantitation of these targets, *e.g.*, for use in measuring levels of these targets within appropriate physiological samples, for use in diagnostic methods, for use in imaging the protein, and the like). In a given embodiment, antibodies specific any of these targets, or derivative, fragment, analog or homolog thereof, that contain the antibody derived antigen binding domain, are utilized as pharmacologically active compounds (referred to hereinafter as “Therapeutics”).

[00272] An antibody of the invention can be used to isolate a particular target using standard techniques, such as immunoaffinity, chromatography or immunoprecipitation. Antibodies of

the invention (or a fragment thereof) can be used diagnostically to monitor protein levels in tissue as part of a clinical testing procedure, *e.g.*, to determine the efficacy of a given treatment regimen. Detection can be facilitated by coupling (*i.e.*, physically linking) the antibody 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 .

[00273] Antibodies of the invention, including polyclonal, monoclonal, humanized and fully human antibodies, may be used as therapeutic agents. Such agents will generally be employed to treat or prevent a disease or pathology associated with aberrant expression or activation of a given target in a subject. An antibody preparation, preferably one having high specificity and high affinity for its target antigen, is administered to the subject and will generally have an effect due to its binding with the target. Administration of the antibody may abrogate or inhibit or interfere with the signaling function of the target. Administration of the antibody may abrogate or inhibit or interfere with the binding of the target with an endogenous ligand to which it naturally binds. For example, the antibody binds to the target and neutralizes or otherwise inhibits the interaction between CD47 and SIRP α .

[00274] A therapeutically effective amount of an antibody of the invention relates generally to the amount needed to achieve a therapeutic objective. As noted above, this may be a binding interaction between the antibody and its target antigen that, in certain cases, interferes with the functioning of the target. The amount required to be administered will furthermore depend on the binding affinity of the antibody for its specific antigen, and will also depend on the rate at which an administered antibody is depleted from the free volume other subject to which it is administered. Common ranges for therapeutically effective dosing of an antibody or antibody fragment of the invention may be, by way of nonlimiting example, from about 0.1 mg/kg body weight to about 50 mg/kg body weight. Common dosing frequencies may range, for example, from twice daily to once a week.

[00275] Antibodies or a fragment thereof of the invention can be administered for the treatment of a variety of diseases and disorders in the form of pharmaceutical compositions. Principles and considerations involved in preparing such compositions, as well as guidance in the choice of components are provided, for example, in Remington: The Science And Practice Of Pharmacy 19th ed. (Alfonso R. Gennaro, et al., editors) Mack Pub. Co., Easton, Pa.: 1995; Drug Absorption Enhancement: Concepts, Possibilities, Limitations, And Trends, Harwood Academic Publishers, Langhorne, Pa., 1994; and Peptide And Protein Drug Delivery (Advances In Parenteral Sciences, Vol. 4), 1991, M. Dekker, New York.

[00276] Where antibody fragments are used, the smallest inhibitory fragment that specifically binds to the binding domain of the target protein is preferred. For example, based upon the variable-region sequences of an antibody, peptide molecules can be designed that retain the ability to bind the target protein sequence. Such peptides can be synthesized chemically and/or produced by recombinant DNA technology. (See, e.g., Marasco et al., Proc. Natl. Acad. Sci. USA, 90: 7889-7893 (1993)). The formulation can 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 can comprise an agent that enhances its function, such as, for example, a cytotoxic agent, cytokine, chemotherapeutic agent, or growth-inhibitory agent. Such molecules are suitably present in combination in amounts that are effective for the purpose intended.

[00277] The active ingredients can 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.

[00278] The formulations to be used for *in vivo* administration must be sterile. This is readily accomplished by filtration through sterile filtration membranes.

[00279] Sustained-release preparations can be prepared. Suitable examples of sustained-release preparations include semipermeable matrices of solid hydrophobic polymers containing the antibody, which matrices are in the form of shaped articles, e.g., films, or microcapsules. Examples of sustained-release matrices include polyesters, hydrogels (for example, poly(2-hydroxyethyl-methacrylate), or poly(vinylalcohol)), polylactides (U.S. Pat.

No. 3,773,919), copolymers of L-glutamic acid and γ ethyl-L-glutamate, non-degradable ethylene-vinyl acetate, degradable lactic acid-glycolic acid copolymers such as the LUPRON DEPOTTM (injectable microspheres composed of lactic acid-glycolic acid copolymer and leuprolide acetate), and poly-D-(-)-3-hydroxybutyric acid. While polymers such as ethylene-vinyl acetate and lactic acid-glycolic acid enable release of molecules for over 100 days, certain hydrogels release proteins for shorter time periods.

[00280] An antibody according to the invention can be used as an agent for detecting the presence of a given target (or a protein fragment thereof) in a sample. In some embodiments, the antibody contains a detectable label. Antibodies are polyclonal, or more preferably, monoclonal. An intact antibody, or a fragment thereof (*e.g.*, F_{ab}, scFv, or F_(ab)₂) is used. The term “labeled”, with regard to the probe or antibody, is intended to encompass direct labeling of the probe or antibody by coupling (*i.e.*, physically linking) a detectable substance to the probe or antibody, as well as indirect labeling of the probe or antibody by reactivity with another reagent that is directly labeled. Examples of indirect labeling include detection of a primary antibody 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. 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 of the invention 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.

[00281] Pharmaceutical compositions

[00282] The antibodies of the invention (also referred to herein as “active compounds”), and derivatives, fragments, analogs and homologs thereof, can be incorporated into pharmaceutical compositions suitable for administration. Such compositions typically comprise the antibody and a pharmaceutically acceptable carrier. As used herein, the term “pharmaceutically acceptable carrier” is intended to include any and all solvents, dispersion media, coatings, antibacterial and antifungal agents, isotonic and absorption delaying agents, and the like, compatible with pharmaceutical administration. Suitable carriers are described in the most recent edition of Remington’s Pharmaceutical Sciences, a standard reference text in the field, which is incorporated herein by reference. Preferred examples of such carriers or diluents include, but are not limited to, water, saline, ringer’s solutions, dextrose solution, and 5% human serum albumin. Liposomes and non-aqueous vehicles such as fixed oils may also be used. The use of such media and agents for pharmaceutically active substances is well known in the art. Except insofar as any conventional media or agent is incompatible with the active compound, use thereof in the compositions is contemplated. Supplementary active compounds can also be incorporated into the compositions.

[00283] A pharmaceutical composition of the invention is formulated to be compatible with its intended route of administration. Examples of routes of administration include parenteral, *e.g.*, intravenous, intradermal, subcutaneous, oral (*e.g.*, inhalation), transdermal (*i.e.*, topical), transmucosal, and rectal administration. Solutions or suspensions used for parenteral, intradermal, or subcutaneous application can include the following components: a sterile diluent such as water for injection, saline solution, fixed oils, polyethylene glycols, glycerine, propylene glycol or other synthetic solvents; antibacterial agents such as benzyl alcohol or methyl parabens; antioxidants such as ascorbic acid or sodium bisulfite; chelating agents such as ethylenediaminetetraacetic acid (EDTA); buffers such as acetates, citrates or phosphates, and agents for the adjustment of tonicity such as sodium chloride or dextrose. The pH can be adjusted with acids or bases, such as hydrochloric acid or sodium hydroxide. The parenteral preparation can be enclosed in ampoules, disposable syringes or multiple dose vials made of glass or plastic.

[00284] Pharmaceutical compositions suitable for injectable use include sterile aqueous solutions (where water soluble) or dispersions and sterile powders for the extemporaneous

preparation of sterile injectable solutions or dispersion. For intravenous administration, suitable carriers include physiological saline, bacteriostatic water, Cremophor EL™ (BASF, Parsippany, N.J.) or phosphate buffered saline (PBS). In all cases, the composition must be sterile and should be fluid to the extent that easy syringeability exists. It must be stable under the conditions of manufacture and storage and must be preserved against the contaminating action of microorganisms such as bacteria and fungi. The carrier can be a solvent or dispersion medium containing, for example, water, ethanol, polyol (for example, glycerol, propylene glycol, and liquid polyethylene glycol, and the like), and suitable mixtures thereof. The proper fluidity can be maintained, for example, by the use of a coating such as lecithin, by the maintenance of the required particle size in the case of dispersion and by the use of surfactants. Prevention of the action of microorganisms can be achieved by various antibacterial and antifungal agents, for example, parabens, chlorobutanol, phenol, ascorbic acid, thimerosal, and the like. In many cases, it will be preferable to include isotonic agents, for example, sugars, polyalcohols such as manitol, sorbitol, sodium chloride in the composition. Prolonged absorption of the injectable compositions can be brought about by including in the composition an agent which delays absorption, for example, aluminum monostearate and gelatin.

[00285] Sterile injectable solutions can be prepared by incorporating the active compound in the required amount in an appropriate solvent with one or a combination of ingredients enumerated above, as required, followed by filtered sterilization. Generally, dispersions are prepared by incorporating the active compound into a sterile vehicle that contains a basic dispersion medium and the required other ingredients from those enumerated above. In the case of sterile powders for the preparation of sterile injectable solutions, methods of preparation are vacuum drying and freeze-drying that yields a powder of the active ingredient plus any additional desired ingredient from a previously sterile-filtered solution thereof.

[00286] Oral compositions generally include an inert diluent or an edible carrier. They can be enclosed in gelatin capsules or compressed into tablets. For the purpose of oral therapeutic administration, the active compound can be incorporated with excipients and used in the form of tablets, troches, or capsules. Oral compositions can also be prepared using a fluid carrier for use as a mouthwash, wherein the compound in the fluid carrier is applied orally and swished and expectorated or swallowed. Pharmaceutically compatible binding agents, and/or adjuvant materials can be included as part of the composition. The tablets, pills, capsules, troches and the like can contain any of the following ingredients, or compounds of a similar

nature: a binder such as microcrystalline cellulose, gum tragacanth or gelatin; an excipient such as starch or lactose, a disintegrating agent such as alginic acid, Primogel, or corn starch; a lubricant such as magnesium stearate or Sterotes; a glidant such as colloidal silicon dioxide; a sweetening agent such as sucrose or saccharin; or a flavoring agent such as peppermint, methyl salicylate, or orange flavoring.

[00287] For administration by inhalation, the compounds are delivered in the form of an aerosol spray from pressured container or dispenser which contains a suitable propellant, *e.g.*, a gas such as carbon dioxide, or a nebulizer.

[00288] Systemic administration can also be by transmucosal or transdermal means. For transmucosal or transdermal administration, penetrants appropriate to the barrier to be permeated are used in the formulation. Such penetrants are generally known in the art, and include, for example, for transmucosal administration, detergents, bile salts, and fusidic acid derivatives. Transmucosal administration can be accomplished through the use of nasal sprays or suppositories. For transdermal administration, the active compounds are formulated into ointments, salves, gels, or creams as generally known in the art.

[00289] The compounds can also be prepared in the form of suppositories (*e.g.*, with conventional suppository bases such as cocoa butter and other glycerides) or retention enemas for rectal delivery.

[00290] In one embodiment, the active compounds are prepared with carriers that will protect the compound against rapid elimination from the body, such as a controlled release formulation, including implants and microencapsulated delivery systems. Biodegradable, biocompatible polymers can be used, such as ethylene vinyl acetate, polyanhydrides, polyglycolic acid, collagen, polyorthoesters, and polylactic acid. Methods for preparation of such formulations will be apparent to those skilled in the art. The materials can also be obtained commercially from Alza Corporation and Nova Pharmaceuticals, Inc. Liposomal suspensions (including liposomes targeted to infected cells with monoclonal antibodies to viral antigens) can also be used as pharmaceutically acceptable carriers. These can be prepared according to methods known to those skilled in the art, for example, as described in U.S. Patent No. 4,522,811.

[00291] It is especially advantageous to formulate oral or parenteral compositions in dosage unit form for ease of administration and uniformity of dosage. Dosage unit form as used herein refers to physically discrete units suited as unitary dosages for the subject to be treated; each unit containing a predetermined quantity of active compound calculated to produce the

desired therapeutic effect in association with the required pharmaceutical carrier. The specification for the dosage unit forms of the invention are dictated by and directly dependent on the unique characteristics of the active compound and the particular therapeutic effect to be achieved, and the limitations inherent in the art of compounding such an active compound for the treatment of individuals.

[00292] The pharmaceutical compositions can be included in a container, pack, or dispenser together with instructions for administration.

[00293] The invention will be further described in the following examples, which do not limit the scope of the invention described in the claims.

[00294] EXAMPLES

[00295] EXAMPLE 1: Phage Display Selection of PD-L1 Fvs Using Human scFv Libraries Containing Fixed Variable Heavy Chain

[00296] General procedures for construction and handling of human scFv libraries displayed on M13 bacteriophage are described in Vaughan et al., (Nat. Biotech. 1996, 14:309-314), hereby incorporated by reference in its entirety. The libraries for selection and screening encode scFv that all share the same VH domain and are solely diversified in the VL domain. Methods for the generation of fixed VH libraries and their use for the identification and assembly of bispecific antibodies are described in US 2012/0184716 and WO 2012/023053, each of which is hereby incorporated by reference in its entirety. The procedures to identify scFv binding to human PD-L1 are described below.

[00297] A. Protein Selections

[00298] Aliquots of scFv phage libraries (10^{12} Pfu) are blocked with PBS containing 3% (w/v) skimmed milk for one hour at room temperature on a rotary mixer. Blocked phage is deselected on streptavidin magnetic beads (Dynabeads™ M-280) for one hour at room temperature on a rotary mixer. For selections against CD47, in some cases, 10^8 purified red blood cells were added to the beads for deselection. Deselected phage is incubated with 100 nM of biotinylated human PD-L1 extracellular domain captured on streptavidin magnetic beads for two hours at room temperature on a rotary mixer. For improving the binding affinity decreasing concentrations of PD-L1 are used at each round of selection (from 10 nM to 0.1 nM). Beads are captured using a magnetic stand followed by five washes with PBS/0.1% Tween 20 and two washes with PBS. Phage is eluted with 100 nM TEA for 30 minutes at room temperature on a rotary mixer. Eluted phage and beads are neutralized with Tris-HCl 1M pH 7.4 and directly added to 10 ml of exponentially growing TG1 cells and incubated for

one hour at 37°C with slow shaking (90 rpm). An aliquot of the infected TG1 is serially diluted to titer the selection output. The remaining infected TG1 are spun at 3800 rpm for 10 minutes and resuspended in 2 ml 2xTY and spread on 2xTYAG (2xTY medium containing 100 µg/ml ampicillin and 2% glucose) agar Bioassay plates. After overnight incubation at 30°C, 10 ml of 2xTY is added to the plates and the cells are scraped from the surface and transferred to a 50 ml polypropylene tube. 50% glycerol solution is added to the cell suspension to obtain a final concentration of 17% glycerol. Aliquots of the selection rounds are kept at -80°C.

[00299] B. Phage Rescue

[00300] 50 µl of cell suspension obtained from previous selection rounds are added to 50 ml of 2xTYAG and grown at 37°C with agitation (240 rpm) until an OD₆₀₀ of 0.3 to 0.5 is reached. The culture is then super-infected with 1.2x10¹¹ M13K07 helper phage and incubated for one hour at 37°C (90 rpm). The medium is changed by centrifuging the cells at 3800 rpm for 10 minutes, removing the medium and resuspending the pellet in 50 ml of 2xTYAK (2xTY medium containing 100 µg/ml ampicillin; 50 µg/ml kanamycin). The culture is then grown overnight at 30°C (240 rpm). The next day, the phage-containing supernatant is used for the next round of selection.

[00301] C. Cell Surface Selections

[00302] Phage-containing supernatants are blocked with PBS containing 3% (w/v) skimmed milk for one hour at room temperature on a rotary mixer. Blocked phage is then deselected for one hour on 1x10⁷ MKN-45 cells that do not express human PD-L1. Deselected phage is incubated with 1x10⁷ A431 or THP-1 cells pre-incubated for 24h with IFNγ to boost PD-L1 expression (blocked in PBS 3% BSA, 0.1% NaN₃) for two hours at room temperature with gentle shaking. Cells are pelleted and washed six times with PBS. Bound phage is eluted with 76 mM citric acid and shaking for 10 minutes. After neutralization with Tris-HCl 1M pH 8, the eluates with the cells are added directly to 10 ml of exponentially growing TG1 and incubated for one hour at 37°C with slow shaking. An aliquot of the infected TG1 is serially diluted to titer the selection output. Infected TG1 are spun at 3800 rpm for 10 minutes and resuspended in 2 ml 2xTY medium and spread on a 2xTYAG agar Bioassay plate. After overnight incubation at 30°C 10 ml of 2xTY is added to the plate and the cells are scraped from the surface and transferred to a 50 ml polypropylene tube. 50% glycerol solution is added to the cell suspension to obtain a final concentration of 17% glycerol. Aliquots of the selection rounds are kept at -80°C.

[00303] **EXAMPLE 2: Screening for scFv Binding to PD-L1**

[00304] A. scFv Periplasmic Preparation for Binding and Functional Tests

[00305] Individual infected TG1 clones are inoculated into a deep-well 96-well plate containing 0.9 ml per well of 2xTYAG medium (2xTY medium containing 100 µg/ml ampicillin, 0.1% glucose) and grown at 37°C for 5-6 hours (240 rpm). IPTG 0.2 mM in 2xTY medium is added to give a final concentration of 0.02 mM. The plate is incubated overnight at 30°C with shaking at 240 rpm. The deep-well plate is centrifuged at 3200 rpm for 10 minutes at 4°C and the supernatant carefully removed. The pellets are resuspended in 150 µl TES buffer (50 mM Tris-HCl (pH 8), 1 mM EDTA (pH 8), 20% sucrose). A hypotonic shock is produced by adding 150 µl of diluted TES buffer (1:5 TES:water dilution) and incubation on ice for 30 minutes. The plate is centrifuged at 4000 rpm for 10 minutes at 4°C to pellet cells and debris. The supernatants are carefully transferred into a 96-well microtiter plate and kept on ice for immediate testing in functional assays or binding assays.

[00306] B. Binding

[00307] Screening of scFv for binding to PD-L1 is tested in a homogenous assay using CellInsight™ technology. The following reagents are mixed in each well of a 384 clear bottom well plate (Corning): 30 µl of a streptavidin polystyrene bead suspension (Polysciences; 3000 beads/well) coated with biotinylated PD-L1 or a biotinylated irrelevant protein for a control protein; 60 µl of blocked scFv periplasmic preparation; 10 µl of detection buffer (PBS containing human anti-c-myc antibody at 2 µg/ml; anti-human IgG Fc AlexaFluor® 647 diluted 1:500). After shaking at 600 rpm for 5 minutes, the 384-well plate is incubated at room temperature and read after 2 hours on a CellInsight™ CX5 High-Content Screening platform (ThermoFisher Scientific). Clones expressing scFv giving a specific signal on PD-L1 and not on the control protein are selected for further analysis or sequencing.

[00308] C. Inhibition of PD-1/PD-L1 interaction

[00309] ScFv targeting PD-L1 were screened for their capacity to inhibit the interaction between PD-1 and PD-L1 in a bead based homogenous assay using the CellInsight™ technology. The following reagents were mixed in each well of a 384 clear bottom well plate (Corning): 30 µl of a streptavidin polystyrene bead suspension (Polysciences; 3000 beads/well) coated with biotinylated PD-L1, 0.1 µg/ml PD-1-huFc (ACROBiosystems), anti-human IgG Fc AlexaFluor® 647 diluted 1:2000 and 50 µl of scFv periplasmic preparation. After shaking at 600 rpm for 5 minutes, the 384-well plate was incubated at room temperature and read after 2 hours on a CellInsight™ CX5 High-Content Screening platform (ThermoFisher Scientific). Control wells containing an irrelevant scFv not binding to PD-L1

were included in each plate so that clones expressing scFv leading to a reduction of the PD-1/PD-L1 signal measured in controls were selected for further analysis or sequencing.

[00310] EXAMPLE 3: Expression and purification of bispecific antibodies carrying a Lambda and a Kappa light chain.

[00311] The simultaneous expression of one heavy chain and two light chains in the same cell can lead to the assembly of three different antibodies. Simultaneous expression can be achieved in different ways such as the transfection of multiple vectors expressing one of the chains to be co-expressed or by using vectors that drive multiple gene expression. A vector pNovi κ H λ was previously generated to allow for the co-expression of one heavy chain, one kappa light chain and one lambda light chain as described in US 2012/0184716 and WO 2012/023053, each of which is hereby incorporated by reference in its entirety. The expression of the three genes is driven by human cytomegalovirus promoters (hCMV), and the vector also contains a glutamine synthetase gene (GS) that enables the selection and establishment of stable cell lines. The VL genes of the anti-hPD-L1 IgG λ or the anti-hCD47 IgG κ were cloned in the vector pNovi κ H λ , for transient expression in mammalian cells. Expi293 cells (Gibco) were amplified and split in Erlenmeyer flask at a concentration of 3×10^6 cells per mL in 50mL of Expi293 culture medium (Gibco). 62.5 μ g of plasmid DNA were transfected into the cells using polyethylenimine transfection reagent (PEI, Polyscience) according to manufacturer's instructions. IgG concentration in supernatant of transfected cells was measured during the production using the Bio-Layer Interferometry (BLI) technology. An OctetRED96 instrument and Protein A biosensors were used for quantitation (Sartorius). Biosensors were pre-conditioned and regenerated using 10 mM glycine pH 1.7 and IgG calibrators diluted in conditioned cell medium were prepared for standard curve generation. Concentrations were determined using the dose response 5PL unweighted Y standard curve equation and an initial slope binding rate equation. According to antibody concentration, supernatants were harvested 7 to 10 days after transfection and clarified by centrifugation at 2000 g for 10 min and filtration on 0.22 μ m. The purification process was composed of three steps using affinity resins from Thermo Fisher Scientific. First, the CaptureSelect FcXL resin was washed with PBS and then added to the clarified supernatant. After incubation overnight at +4°C and 15rpm, supernatants were centrifuged at 600 g for 10 min, flow through was stored until the end of the purification process and resin washed twice with PBS. Then, the resin was transferred on Amicon Pro columns (Merck Millipore) and a solution containing 50 mM glycine at pH 3 was used for elution. Several elution fractions were generated,

neutralized with 1/10 Tris HCl pH7.4 (Invitrogen) and pooled. The purified product, containing total human IgGs, was quantified using a Nanodrop spectrophotometer (NanoDrop Technologies) and incubated for 30 min at RT and 15 rpm with the appropriate volume of CaptureSelect Kappa XL affinity matrix. Incubation, resin recovery, elution and neutralization steps were performed as described previously. The last affinity purification step was performed using the CaptureSelect LC-lambda (Hu) affinity matrix applying the same process as for the two previous purifications. The pool of elution fractions was desalted against 25mM histidine/125mM NaCl pH6.0 using 50kDa Amicon centrifugal units (Merck Millipore). Purified $\kappa\lambda$ bodies were quantified using the Nanodrop and analyzed by capillary electrophoresis in denaturing and reducing conditions using the Agilent 2100 Bioanalyzer and Protein 80 kit as described by the manufacturer (Agilent Technologies). An aliquot from the first purification step (containing the bispecific antibody and both monospecific mAbs) and an aliquot of the final product (containing the purified $\kappa\lambda$ body) were loaded on an IsoElectric Focusing (IEF) gel to evaluate the purity of the final purified bispecific antibody (absence of mAb contamination). The aggregate level was determined by SEC-UPLC. Finally, all samples were tested for endotoxin contamination using the Limulus Amebocyte Lysate test (LAL; Charles River Laboratories).

[00312] EXAMPLE 4: Characterization of exemplary CD47xPD-L1 bispecific antibodies

[00313] The CD47xPD-L1 bispecific antibodies (bsAbs) were generated by pairing previously described CD47 arm K2 (=Ka3 arm, WO2014087248A2) with various anti-PD-L1 arms from this invention. All the bsAb were reformatted with a human IgG1 Fc domain.

[00314] Binding to recombinant human PD-L1 and cross-reactivity

[00315] The ability of selected CD47xPD-L1 bsAbs to bind recombinant human PD-L1 (ACROBiosystems), cynomolgus monkey PD-L1 (Sino Biological) and mouse PD-L1 (in-house production) soluble proteins was assessed by a sandwich ELISA assay. Briefly, a goat anti-human Fc capture antibody (Jackson ImmunoResearch), diluted in PBS at 5 $\mu\text{g/ml}$, was coated O/N at 4°C in a MaxiSorp 96-well black plate (Nunc). The plate was blocked with blocking reagent (PBS Buffer/BSA 3%/Tween 0.05%) for one hour at room temperature. After 3 washes with PBS Buffer-Tween 0.05%, a fixed concentration of the bsAbs was added and incubated for one hour at room temperature, and 3 more washes were performed. After washing, increasing concentrations of biotinylated human, cynomolgus or mouse recombinant PD-L1 proteins were added and incubated for one hour at room temperature.

Finally, after one hour incubation with Streptavidin-HRP, Amplex red detection reagent was added and incubated at room temperature for 20 minutes in the dark and the fluorescence signal was detected using a plate reader. **FIG. 1A** shows various monovalent binding to human PD-L1 of selected bsAbs and mAbs as compared to anti-PD-L1 benchmarks atezolizumab and avelumab. **FIG. 1B** highlights that all the PD-L1 arms tested are cross-reactive to cynomolgus PD-L1 while **FIG. 1C** shows that only some PD-L1 of them are cross-reactive with mouse PD-L1.

[00316] B. PD-L1 Specificity

[00317] The specificity of exemplary CD47xPD-L1 bsAbs to PD-L1 was determined by evaluating their absence of binding to human PD-L2 by ELISA. Human PD-L2 has 34% of sequence identity with human PD-L1. The ability of the bsAbs to bind recombinant human PD-L2 soluble protein (ACROBiosystems) was assessed by a sandwich ELISA assay. Briefly, a goat anti-mouse Fc capture antibody (Jackson ImmunoResearch), diluted in PBS at 5 µg/ml, was coated O/N at 4°C in a MaxiSorp 96-well black plate (Nunc). The plate was blocked with blocking reagent (PBS Buffer/BSA 3%/Tween 0.05%) for one hour at room temperature. After 3 washes with PBS Buffer-Tween 0.05%, a fixed concentration of the bsAbs was added and incubated for one hour at room temperature. After washing, increasing concentrations of biotinylated human recombinant PD-L2 protein were added and incubated for one hour at room temperature. Finally, after one hour incubation with Streptavidin-HRP, Amplex red detection reagent was added and incubated at room temperature for 20 minutes in the dark and the fluorescence signal was detected using a plate reader. An isotype control antibody was used as a negative control, and a commercially available mouse anti-human PD-L2 IgG (R&D system) served as a positive control.

[00318] As shown in **FIG. 1D**, none of the CD47xPD-L1 bsAbs tested cross-react with human PD-L2.

[00319] C. PD-1/PD-L1 blocking activity on PD-L1 transfected CHO cells

[00320] The PD-1 blocking activity of the CD47xPD-L1 bsAbs was evaluated in the PD-1/PD-L1 competitive binding cell-based assay. Human PD-L1-transfected CHO cells (negative for human CD47), pre-stained with Cell Trace Violet (Invitrogen), were incubated with various concentrations of bsAbs for 1 hour at room temperature. As detection reagent, a mix of a human PD-1-moFc protein (ACROBiosystem, final concentration 100 ng/ml) and an anti-mouse Fc AF647 (Jackson ImmunoResearch) were added for 3 hours at room temperature. Finally, the plate was read using the CellInsight™ CX5 High Content Screening

Platform. **FIG. 1E** shows that the selected bsAbs block monovalently (i.e. without CD47 co engagement) the interaction between PD-1 and PD-L1 with various potencies (**Table 4**). The bivalent anti-PD-L1 atezolizumab was used as a reference.

[00321] Table 4: PD-1 blocking potency of selected CD47xPD-L1 bispecific antibodies and anti-PD-L1 atezolizumab on human PD-L1 transfected CHO cells

Antibody name	PD-1 inhibition potency (IC ₅₀ in nM)
Atezolizumab (bivalent anti-PD-L1)	0.02
S37 bsAb	0.14
S79 bsAb	0.03
S94 bsAb	0.05
S100 bsAb	0.07
S28 bsAb	0.12

[00322] E. Binding to CD47-positive tumor cells

[00323] The CD47 binding on cells of an exemplary CD47xPD-L1 bsAb was studied by flow cytometry using human Raji (ATCC; CCL-86) and Nalm-6 (ATCC; CRL-3273) tumor cell lines and CHO cells as a negative control. Both Raji and Nalm-6 cell lines express very low levels or do not express PD-L1 (**Table 5**) allowing the evaluation of monovalent CD47 binding of the bsAb.

[00324] Antibodies were incubated for 15 minutes at 4°C at various concentrations with the cells previously resuspended in PBS/BSA 2%. After two washes, bound Abs were detected using a AF647 conjugated anti-human Fc F(ab')₂ (Jackson ImmunoResearch). After incubation for 15 minutes at 4°C, followed by 2 washing steps, cells were analyzed by flow cytometry.

[00325] Table 5: Target density of PD-L1 and CD47 at the cell surface of Raji and Nalm-6 human tumor cell lines

Cell line	Origin	PD-L1 binding sites	CD47 binding sites
Raji	Burkitt's lymphoma	700	44'000
Nalm-6	Acute lymphoblastic leukemia	<100	53'000

[00326] FIG. 2D and 2E show the binding profile of the CD47 arm of the CD47xPD-L1 bsAb and bivalent anti-CD47 5F9 analog on Raji and Nalm-6 tumor cells, respectively. The binding profiles are consistent between both tumor cell lines. The CD47 arm K2 shows as

expected low binding to tumor cells as compared to the high affinity anti-CD47 5F9 analog. No binding of any of the molecules tested was observed on CHO cells (data not shown).

[00327] F. CD47/SIRPa blocking activity on CD47-positive tumor cells

[00328] The SIRPa blocking activity of an exemplary CD47xPD-L1 bsAb was determined in the CD47/SIRPa cell-based competitive binding assay. PD-L1⁺CD47⁺ Nalm-6 tumor cells (**Table 5**), pre-stained with Cell Trace Violet (Invitrogen), were incubated with various concentrations of bsAbs and controls for 1 hour at room temperature. As detection reagent, a mix of human SIRPa-mouse Fc protein (in-house) and anti-mouse Fc AF647 (Jackson ImmunoResearch) was added for 3 hours at room temperature. Finally, the plate was read using the CellInsight™ CX5 High Content Screening Platform.

[00329] Following monovalent engagement of CD47, the bsAb induces SIRPa blockade with low potency as compared to anti-CD47 5F9 analog (**Table 6**), consistent with their CD47 binding properties (**FIG. 2F**).

[00330] **Table 6: SIRPa blocking potency of an exemplary CD47xPD-L1 bispecific antibody and anti-CD47 5F9 analog on human PD-L1⁺CD47⁺ Nalm-6 tumor cells**

Antibody name	SIRPα inhibition potency (IC ₅₀ in nM)
5F9 mAb analog	0.025
K2 bsAb	84

[00331] G. Binding to human red blood cells (RBC)

[00332] Human RBC express CD47 target at their cell surface, but not PD-L1, and represent a significant antigen sink for CD47-targeting antibodies, impacting on their safety and pharmacokinetic properties. Therefore, the binding of selected CD47xPD-L1 bsAbs, bearing the same low-affinity CD47 K2 arm, was assessed on human RBC by flow cytometry and compared to the anti-CD47 5F9 analog used as a clinical benchmark molecule.

[00333] RBC were isolated from whole blood of healthy donors, resuspended in PBS/BSA 2%, and incubated with antibodies at various concentrations for 15 minutes at 4°C. After two washes, bound bsAbs were detected using an AF647 conjugated anti-human Fc F(ab')₂ (Jackson ImmunoResearch). After 15 minutes incubation at 4°C and 2 washing steps, cells were analyzed by flow cytometry.

[00334] **FIG. 3** shows representative binding profiles of K2xS100 and K2XS23 CD47xPD-L1 bsAbs and 5F9 analog to human red blood cells. As expected, based on their low affinity CD47 arm, the bsAbs show very weak binding to red blood cells.

[00335] H. Binding affinity of selected bispecific antibodies to PD-L1

[00336] The affinity of selected CD47xPD-L1 bsAbs to PD-L1 recombinant proteins was determined at 30°C using the Bio-Layer Interferometry technology. An OctetRED96 instrument was used. After hydration and a baseline step in kinetic buffer (Sartorius, #18-1105; PBS, 0.02% Tween20, 0.1% BSA, 0.05% sodium azide), streptavidin biosensors (Sartorius, #18-5019) were loaded for 5 min with the biotinylated human, cynomolgus or mouse PD-L1 recombinant protein at 1 µg/mL (Acrobiosystems, #PD1-H82E5, #PD1-C52H4 and #PD1-M5220 respectively) in kinetic buffer. Then, biosensors were dipped into a serial dilution of bsAbs starting from 28.6 nM with a 2-fold dilution factor. The association and the dissociation steps were monitored for 600secs and 900secs, respectively. The affinity was measured applying a 1:1 global fitting model on double referenced curves, on the full association and dissociation steps. The affinity results are shown in **Table 7**.

[00337] **Table 7: Binding Affinity of selected bsAbs to recombinant PD-L1 soluble proteins**

CD47xPD-L1 bsAb	Human PD-L1 K_D (in nM $\pm$ SD)	Cyno PD-L1 K_D (in nM $\pm$ SD)	Mouse PD-L1 K_D (in nM $\pm$ SD)
K2xS8	1.23 $\pm$ 0.03	ND	ND
K2xS15	3 $\pm$ 0.6		
K2xS23	0.92 $\pm$ 0.06		
K2xS28	0.7 $\pm$ 0.2		
K2xS58	0.83 $\pm$ 0.007		
K2xS93	0.7 $\pm$ 0.5		
K2xS94	0.7 $\pm$ 0.4		
K2xS96	0.8 $\pm$ 0.5		
K2xS100	0.31 $\pm$ 0.008		
K2xS79	0.5 $\pm$ 0.3	1.35 $\pm$ 0.02	0.52 $\pm$ 0.01

ND: not determined

[00338] I. Binding to CD47/PD-L1 double-positive tumor cells

[00339] The binding of selected CD47xPD-L1 bsAbs to PD-L1⁺CD47⁺ human tumor cells was studied by flow cytometry using the HT-1080 tumor cell line (ATCC; CCL-121) pre-activated with IFN γ (**Table 8**). CHO cell line was used as a negative cell line.

[00340] Antibodies were incubated at various concentrations with the tumor cells, previously resuspended in PBS/BSA 2%, for 15 minutes at 4°C. After two washes, bound Abs were detected using a AF647 conjugated anti-human Fc F(ab')₂ (Jackson ImmunoResearch). After incubation for 15 minutes at 4°C, followed by 2 washing steps, cells were analyzed by flow cytometry.

[00341] **Table 8: Target density of PD-L1 and CD47 at the cell surface of HT-1080 tumor cells after IFN γ induction for 24h**

Cell line	Origin	PD-L1 binding sites	CD47 binding sites	Ratio CD47:PD-L1
HT-1080	Fibrosarcoma	55'950	159'800	2.85

[00342] **FIG. 4A** shows that the binding profiles of anti-PD-L1 mAbs S79 and S100 are similar to anti-PD-L1 benchmarks atezolizumab and avelumab. As expected, based on CD47 and PD-L1 co-engagement, the bsAbs demonstrate higher binding than the anti-PD-L1 mAbs, that is dependent on their affinity to PD-L1 (K2xS23 bsAb has a lower affinity to PD-L1 than K2xS100, as illustrated in table 7 and Figure 1A).

[00343] **FIG. 4B** shows the binding of the bsAb K2xS100 as compared to the CD47 and PD-L1 monovalent controls K2 and S100, highlighting the contribution of the co-engagement of both target in the binding of the molecule. No binding of any of the molecules tested was observed on CHO cells (data not shown).

[00344] J. CD47/SIRPa and PD-1/PD-L1 blocking activity on CD47 and PD-L1 positive tumor cells

[00345] The SIRPa and PD-1 blocking activity of selected CD47xPD-L1 bsAbs and anti-PD-L1 mAbs was assessed in the CD47/SIRPa and PD-1/PD-L1 cell-based competitive binding assay, as compared to various controls. Briefly, PD-L1⁺CD47⁺ HT-1080 tumor cells induced with IFN γ for 24h (**Table 8**), and stained with Cell Trace Violet (Invitrogen), were incubated with various concentrations of antibodies for 1 hour at room temperature. As detection reagent, a mix of human SIRPa-mouse Fc protein (in-house) or human PD-1-moFc protein (ACROBiosystem,) and anti-mouse Fc AF647 (Jackson ImmunoResearch) was added for 3 hours at room temperature. Finally, the plate was read using the CellInsight™ CX5 High Content Screening Platform.

[00346] As depicted in **FIG. 5A and Table 9**, the anti-PD-L1 mAb S100 and S79 as well as the K2xS100 bsAb demonstrate improved PD-1 blockade as compared to the anti-PD-L1

clinical benchmarks. The significantly lower PD-1 blockade of the monovalent PD-L1 control S100 shows the contribution of the K2 CD47 arm in the potent blockade of K2xS100 bsAb. On the other hand, thanks to PD-L1 co-engagement, K2xS100 bsAb can induce SIRPa blocking activity that is superior to the monovalent CD47 control K2 (**FIG. 5B**). The bsAb presents a biphasic SIRPa blocking curve that likely rely on the co-engagement with PD-L1 at low bsAb concentrations, and mostly on monovalent CD47 blockade at the highest concentrations, once PD-L1 targets are saturated. Due to this curve profile, the SIRPa blocking potency was not determined.

[00347] Table 9: PD-1 blocking potency of selected CD47xPD-L1 bispecific antibodies and anti PD-L1 mAbs on human PD-L1⁺CD47⁺ HT-1080 tumor cells

Antibody name	PD-1 inhibition potency (IC50 in pM)
Atezolizumab	2.96
Avelumab	9.11
K2xS100 bsAb	2.19
Monovalent PD-L1 control S100	15.05
S100 anti-PD-L1 mAb	0.84
S79 anti-PD-L1 mAb	2.52

[00348] EXAMPLE 5: Antibody Dependent Cellular Phagocytosis (ADCP) and Antibody Dependent Cellular Cytotoxicity (ADCC) Induced by Bispecific Antibodies Targeting PD-L1 and CD47

[00349] The *in vitro* killing activity through ADCP or ADCC of selected anti-CD47xPD-L1 bispecific antibodies of the invention was assessed against various tumor cell lines obtained from ATCC, that were pre-exposed to IFN γ for 24h to induce PD-L1 expression (**Table 10**).

[00350] Table 10: Target density of PD-L1 and CD47 at the cell surface of tumor cells after IFN γ induction for 24h used in ADCP and ADCC assays

Cell line	Disease	PD-L1 binding sites	CD47 binding sites	ADCP	ADCC
HT-1080	Fibrosarcoma	55'950	159'800	X	
NCI-N87	Gastric carcinoma	15'000	84'000	X	X

NCI-H226	Mesothelioma	36'000	105'000		X
A375	Melanoma	23'000	133'000		X

[00351] A. Activity in ADCP assay

[00352] The assay relies on an imaging-based method, which makes use of the CellInsight™ CX5 High Content Screening Platform. The phagocytosis index obtained is defined as the average number of target cells engulfed by 100 macrophages.

[00353] 1. Preparation of the macrophages:

[00354] Human peripheral blood mononuclear cells (PBMCs) are isolated from buffy coats of healthy donors by Ficoll gradient. Macrophages are generated by culturing PBMCs for 7 to 9 days in complete medium (RPMI 1640, 10% heat-inactivated fetal calf serum, Invitrogen), 2 mM L-glutamine, 1 mM sodium pyruvate, 10 mM HEPES buffer, 25 mg/mL gentamicin (all from Sigma-Aldrich), and 50 mM 2-mercaptoethanol (Thermo Fisher Scientific) in the presence of 20 ng/mL of human macrophage colony-stimulating factor (M-CSF) (PeproTech). Non-adherent cells are subsequently eliminated in the differentiation phase (day+1) by exchanging the cell culture medium, and adherent cells representing macrophages are detached using cell dissociation buffer at day 6 and seeded at 30'000 per well in 96-well optical plate (Costar).

[00355] 2. Assessment of the phagocytosis activity

[00356] Macrophages (stained with calcein red orange) adhering to microplate wells are co-incubated with Calcein AM-labeled target at an effector:target cell ratio of 1:3 for 2.5 hours at 37°C in the presence of different concentrations of the tested antibodies. At the end of the incubation period, supernatants are replaced by complete culture medium and the microplates are imaged with the CellInsight™ CX5 High Content Screening Platform. 1500 macrophages are acquired and analyzed per well. Phagocytosis is evidenced as double-positive events (macrophage + target tumor cell) and the phagocytosis indexes are calculated by the CellInsight™ manufacturer's software.

[00357] FIG. 6 shows that selected bsAbs of the invention induce phagocytosis of NCI-N87 (A) and HT-1080 (B) tumor cells in a dose-dependent manner with an activity similar to or better than the IgG1 anti-PD-L1 benchmark avelumab. In addition, as shown in FIG.6B, K2xS100 bsAb is more effective than either the monovalent PD-L1 control S100 or the monovalent control CD47 K2 at potentiating phagocytosis of HT-1080 tumor cells.

[00358] B. Activity in ADCC assays

Peripheral Blood Mononuclear Cells (PBMCs) from healthy donors were activated overnight at 37°C with RPMI/10% heat inactivated FCS supplemented with 10 ng/mL of recombinant hIL-2. The next day, target cancer cells NCI-H226, NCI-N87 or A375 were opsonized with different concentrations of tested antibodies. The PBMCs and the opsonized target cells were co-incubated at a ratio Effector/Target of 50/1 or 25/1 in round bottom plates for 6 hours at 37°C. Supernatants were then transferred into optical flat bottom plate and the LDH release was quantified with a commercial kit from Roche by measuring OD with a microplate reader. The percentage of specific lysis was calculated with the following formula:

$$\text{Specific lysis} = \left(\frac{\text{LDH Sample} - (\text{LDH Effector} + \text{Target cells})}{\text{Maximum LDH} - \text{LDH Target cells alone}} \right) \times 100$$

[00359] FIG. 7 shows that selected bsAbs of the invention induce various killing of NCI-H226 (A), NCI-N87 (B) and A375 (C) tumor cells, in a dose-dependent manner, with an improved activity of K2xS94, K2xS96 and K2xS100 bsAb over the IgG1 anti-PD-L1 benchmark avelumab.

[00360] EXAMPLE 6: Enhancement of T-cell activation by CD47xPD-L1 bispecific antibodies

[00361] The ability of the CD47xPD-L1 bsAbs to enhance T-cell activation was evaluated by incubating serial dilutions of the bsAbs and anti-PD-L1 clinical benchmarks with human PBMCs from healthy donors in the presence of *Staphylococcal enterotoxin A* (200 ng/mL; SEA) for 96 hours. Human IL-2 production in the supernatant was measured by ELISA (DuoSET ELISA R&D system DY2020) and use to determine T-cell activation.

[00362] Results show that selected anti-CD47xPD-L1 bispecific antibodies of the invention effectively enhanced T cell activation in comparable range to anti-PD-L1 atezolizumab and avelumab (**FIG. 8**).

[00363] EXAMPLE 7: In vivo antitumor activity of the anti-PD-L1 mAb S79 in the MC38 colon carcinoma model implanted in C57BL/6 mice

[00364] 8- to 10-week-old female C57BL/6 mice were engrafted subcutaneously (s.c.) with 5×10^5 MC38 tumor cells. Eight days after implantation, mice bearing MC38 tumors were treated intraperitoneally every 3 days with 10mg/kg of the IgG1 anti-PD-L1 mAb S79 or an irrelevant IgG1 for a total of 3 doses.

[00365] As observed in **FIG.9** the IgG1 anti-PD-L1 mAb S79 significantly delays tumor progression, inducing inhibition of tumor growth few days after the 1st administration and during the treatment period. Then, tumors relapse around one week after stopping treatment.

Other Embodiments

[00366] While the invention has been described in conjunction with the detailed description thereof, the foregoing description is intended to illustrate and not limit the scope of the invention, which is defined by the scope of the appended claims. Other aspects, advantages, and modifications are within the scope of the following claims.

What is claimed is:

1. A bispecific antibody comprising:
 - i) a heavy chain comprising
 - a heavy chain complementarity determining region 1 (CDRH1) comprising an amino acid sequence of SEQ ID NO: 1;
 - a heavy chain complementarity determining region 2 (CDRH2) comprising an amino acid sequence of SEQ ID NO: 2; and
 - a heavy chain complementarity determining region 3 (CDRH3) comprising an amino acid sequence of SEQ ID NO: 3;
 - ii) a first light chain comprising
 - a light chain complementarity determining region 1 (CDRL1) comprising an amino acid sequence of SEQ ID NO: 89; or
 - a light chain complementarity determining region 2 (CDRL2) comprising an amino acid sequence of SEQ ID NO: 92; and
 - a light chain complementarity determining region 3 (CDRL3) comprising an amino acid sequence of SEQ ID NO: 96; and
 - iii) a second light chain comprising:
 - a) a CDRL1 comprising an amino acid sequence of SEQ ID NO: 8;
a CDRL2 comprising an amino acid sequence of SEQ ID NO: 15; and
a CDRL3 comprising an amino acid sequence of SEQ ID NO: 20; or
 - b) a CDRL1 comprising an amino acid sequence of SEQ ID NO: 8;
a CDRL2 comprising an amino acid sequence of SEQ ID NO: 15; and
a CDRL3 comprising an amino acid sequence of SEQ ID NO: 21; or
 - c) a CDRL1 comprising an amino acid sequence of SEQ ID NO: 8;
a CDRL2 comprising an amino acid sequence of SEQ ID NO: 15; and
a CDRL3 comprising an amino acid sequence of SEQ ID NO: 22; or
 - d) a CDRL1 comprising an amino acid sequence of SEQ ID NO: 9;
a CDRL2 comprising an amino acid sequence of SEQ ID NO: 16; and
a CDRL3 comprising an amino acid sequence of SEQ ID NO: 23; or

- e) a CDRL1 comprising an amino acid sequence of SEQ ID NO: 9;
a CDRL2 comprising an amino acid sequence of SEQ ID NO: 16; and
a CDRL3 comprising an amino acid sequence of SEQ ID NO: 24; or
- f) a CDRL1 comprising an amino acid sequence of SEQ ID NO: 9;
a CDRL2 comprising an amino acid sequence of SEQ ID NO: 16; and
a CDRL3 comprising an amino acid sequence of SEQ ID NO: 25; or
- g) a CDRL1 comprising an amino acid sequence of SEQ ID NO: 10;
a CDRL2 comprising an amino acid sequence of SEQ ID NO: 17; and
a CDRL3 comprising an amino acid sequence of SEQ ID NO: 97; or
- h) a CDRL1 comprising an amino acid sequence of SEQ ID NO: 10;
a CDRL2 comprising an amino acid sequence of SEQ ID NO: 17; and
a CDRL3 comprising an amino acid sequence of SEQ ID NO: 98; or
- i) a CDRL1 comprising an amino acid sequence of SEQ ID NO: 11;
a CDRL2 comprising an amino acid sequence of SEQ ID NO: 18; and
a CDRL3 comprising an amino acid sequence of SEQ ID NO: 97; or
- j) a CDRL1 comprising an amino acid sequence of SEQ ID NO: 12;
a CDRL2 comprising an amino acid sequence of SEQ ID NO: 19; and
a CDRL3 comprising an amino acid sequence of SEQ ID NO: 26; or
- k) a CDRL1 comprising an amino acid sequence of SEQ ID NO: 13;
a CDRL2 comprising an amino acid sequence of SEQ ID NO: 19; and
a CDRL3 comprising an amino acid sequence of SEQ ID NO: 26; or
- l) a CDRL1 comprising an amino acid sequence of SEQ ID NO: 14;
a CDRL2 comprising an amino acid sequence of SEQ ID NO: 19; and
a CDRL3 comprising an amino acid sequence of SEQ ID NO: 27; or

- m) a CDRL1 comprising an amino acid sequence of SEQ ID NO: 14;
a CDRL2 comprising an amino acid sequence of SEQ ID NO: 19; and
a CDRL3 comprising an amino acid sequence of SEQ ID NO: 28; or
- n) a CDRL1 comprising the amino acid sequence of SEQ ID NO: 101;
a CDRL2 comprising the amino acid sequence of SEQ ID NO: 18; and
a CDRL3 comprising the amino acid sequence of SEQ ID NO: 97; or
- o) a CDRL1 comprising the amino acid sequence of SEQ ID NO: 102;
a CDRL2 comprising the amino acid sequence of SEQ ID NO: 18; and
a CDRL3 comprising the amino acid sequence of SEQ ID NO: 97; or
- p) a CDRL1 comprising the amino acid sequence of SEQ ID NO: 103;
a CDRL2 comprising the amino acid sequence of SEQ ID NO: 18; and
a CDRL3 comprising the amino acid sequence of SEQ ID NO: 97; and

wherein the bispecific antibody comprises a first antigen binding region comprising i) and ii) that specifically binds to CD47 and a second antigen binding region comprising i) and iii) that specifically binds to Programmed Death- ligand 1 (PD-L1).

2. The isolated bispecific antibody of claim 1, wherein at least a portion of the first light chain is of the kappa type and at least a portion of the second light chain is of the lambda type.
3. The isolated bispecific antibody of claim 2, wherein the first light chain comprises at least a Kappa constant region.
4. The isolated bispecific antibody of claim 3, wherein the first light chain further comprises a Kappa variable region.
5. The isolated bispecific antibody of claim 3, wherein the first light chain further comprises a Lambda variable region.
6. The isolated bispecific antibody of claim 2, wherein the second light chain comprises at least a Lambda constant region.

7. The isolated bispecific antibody of claim 6, wherein the second light chain further comprises a Lambda variable region.
8. The isolated bispecific antibody of claim 5, wherein the second light chain further comprises a Kappa variable region.
9. The isolated bispecific antibody of claim 2, wherein the first light chain comprises a Kappa constant region and a Kappa variable region, and wherein the second light chain comprises a Lambda constant region and a Lambda variable region.
10. The bispecific antibody of any one of claims 1 to 9, wherein the bispecific antibody is human antibody.
11. The bispecific antibody of any one of claims 1 to 10, wherein the bispecific antibody is an IgG1 antibody.
12. A composition comprising the bispecific antibody of any one of claims 1-11 and a pharmaceutically acceptable carrier.
13. A method of reducing the proliferation of and/or killing a tumor cell comprising contacting the cell with the composition of claim 12.
14. A method of treating a cancer in a subject comprising administering to the subject the composition of claim 12.
15. Use of the composition of claim 12, for treating, preventing, or delaying the progression of pathologies associated with aberrant CD47 expression or activity, or associated with aberrant CD47-SIRP α expression or activity.
16. The use of claim 15, wherein the pathology is cancer.
17. The use of claim 15, wherein the cancer is a solid tumor.
18. The use of claim 15, wherein the solid tumor is or is derived from breast cancer, ovarian cancer, head and neck cancer, bladder cancer, melanoma, mesothelioma, colorectal cancer, cholangiocarcinoma, pancreatic cancer, lung cancer, leiomyoma, leiomyosarcoma,

kidney cancer, glioma, glioblastoma, endometrial cancer, esophageal cancer, biliary gastric cancer, prostate cancer, or combinations thereof.

19. An antibody comprising:

i) a heavy chain comprising:

- a CDRH1 comprising an amino acid sequence of SEQ ID NO: 1;
- a CDRH2 comprising an amino acid sequence of SEQ ID NO: 2; and
- a CDRH3 comprising an amino acid sequence of SEQ ID NO: 3; and

ii) light chain comprising:

- a) a CDRL1 comprising an amino acid sequence of SEQ ID NO: 8;
a CDRL2 comprising an amino acid sequence of SEQ ID NO: 15; and
a CDRL3 comprising an amino acid sequence of SEQ ID NO: 20; or
- b) a CDRL1 comprising an amino acid sequence of SEQ ID NO: 8;
a CDRL2 comprising an amino acid sequence of SEQ ID NO: 15; and
a CDRL3 comprising an amino acid sequence of SEQ ID NO: 21; or
- c) a CDRL1 comprising an amino acid sequence of SEQ ID NO: 8;
a CDRL2 comprising an amino acid sequence of SEQ ID NO: 15; and
a CDRL3 comprising an amino acid sequence of SEQ ID NO: 22; or
- d) a CDRL1 comprising an amino acid sequence of SEQ ID NO: 9;
a CDRL2 comprising an amino acid sequence of SEQ ID NO: 16; and
a CDRL3 comprising an amino acid sequence of SEQ ID NO: 23; or
- e) a CDRL1 comprising an amino acid sequence of SEQ ID NO: 9;
a CDRL2 comprising an amino acid sequence of SEQ ID NO: 16; and
a CDRL3 comprising an amino acid sequence of SEQ ID NO: 24; or
- f) a CDRL1 comprising an amino acid sequence of SEQ ID NO: 9;
a CDRL2 comprising an amino acid sequence of SEQ ID NO: 16; and
a CDRL3 comprising an amino acid sequence of SEQ ID NO: 25; or

- g) a CDRL1 comprising an amino acid sequence of SEQ ID NO: 10;
a CDRL2 comprising an amino acid sequence of SEQ ID NO: 17; and
a CDRL3 comprising an amino acid sequence of SEQ ID NO: 97; or
- h) a CDRL1 comprising an amino acid sequence of SEQ ID NO: 10;
a CDRL2 comprising an amino acid sequence of SEQ ID NO: 17; and
a CDRL3 comprising an amino acid sequence of SEQ ID NO: 98; or
- i) a CDRL1 comprising an amino acid sequence of SEQ ID NO: 11;
a CDRL2 comprising an amino acid sequence of SEQ ID NO: 18; and
a CDRL3 comprising an amino acid sequence of SEQ ID NO: 97; or
- j) a CDRL1 comprising an amino acid sequence of SEQ ID NO: 12;
a CDRL2 comprising an amino acid sequence of SEQ ID NO: 19; and
a CDRL3 comprising an amino acid sequence of SEQ ID NO: 26; or
- k) a CDRL1 comprising an amino acid sequence of SEQ ID NO: 13;
a CDRL2 comprising an amino acid sequence of SEQ ID NO: 19; and
a CDRL3 comprising an amino acid sequence of SEQ ID NO: 26; or
- l) a CDRL1 comprising an amino acid sequence of SEQ ID NO: 14;
a CDRL2 comprising an amino acid sequence of SEQ ID NO: 19; and
a CDRL3 comprising an amino acid sequence of SEQ ID NO: 27; or
- m) a CDRL1 comprising an amino acid sequence of SEQ ID NO: 14;
a CDRL2 comprising an amino acid sequence of SEQ ID NO: 19; and
a CDRL3 comprising an amino acid sequence of SEQ ID NO: 28; or
- n) a CDRL1 comprising the amino acid sequence of SEQ ID NO: 101;
a CDRL2 comprising the amino acid sequence of SEQ ID NO: 18; and
a CDRL3 comprising the amino acid sequence of SEQ ID NO: 97; or

- o) a CDRL1 comprising the amino acid sequence of SEQ ID NO: 102;
a CDRL2 comprising the amino acid sequence of SEQ ID NO: 18; and
a CDRL3 comprising the amino acid sequence of SEQ ID NO: 97; or
- p) a CDRL1 comprising the amino acid sequence of SEQ ID NO: 103;
a CDRL2 comprising the amino acid sequence of SEQ ID NO: 18; and
a CDRL3 comprising the amino acid sequence of SEQ ID NO: 97; and

wherein the antibody specifically binds to Programmed Death-ligand 1 (PD-L1).

- 20. The antibody of claim 19, wherein the antibody is human antibody.
- 21. The antibody of claim 19, wherein the antibody is an IgG1 antibody.
- 22. The antibody of claim 19, wherein the antibody is a F(ab) fragment, a F(ab')₂ fragment, and Fv fragment or a single chain Fv fragment.
- 23. The antibody of claim 19, wherein the antibody is monospecific.
- 24. The antibody of claim 19, wherein the antibody is monovalent.
- 25. A composition comprising the antibody of any one of claims 19-24 and a pharmaceutically acceptable carrier.
- 26. A method of reducing the proliferation of and/or killing a tumor cell comprising contacting the cell with the composition of claim 25.
- 27. A method of treating a cancer in a subject comprising administering to the subject the composition of claim 25.
- 28. Use of the composition of claim 25, for treating, preventing, or delaying the progression of pathologies associated with aberrant PD-L1 expression or activity, or associated with aberrant PD-L1 expression or activity.
- 29. The use of claim 28, wherein the pathology is cancer.

30. The use of claim 29, wherein the cancer is a solid tumor.
31. The use of claim 30, wherein the solid tumor is or is derived from breast cancer, ovarian cancer, head and neck cancer, bladder cancer, melanoma, mesothelioma, colorectal cancer, cholangiocarcinoma, pancreatic cancer, lung cancer, leiomyoma, leiomyosarcoma, kidney cancer, glioma, glioblastoma, endometrial cancer, esophageal cancer, biliary gastric cancer, prostate cancer, or combinations thereof.

FIG. 1A

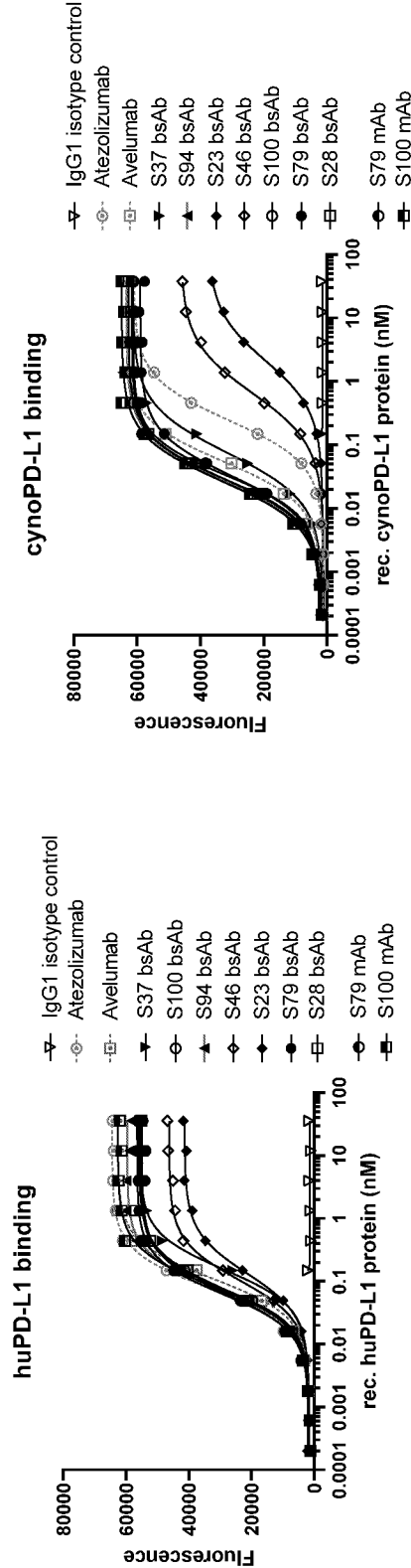


FIG. 1B

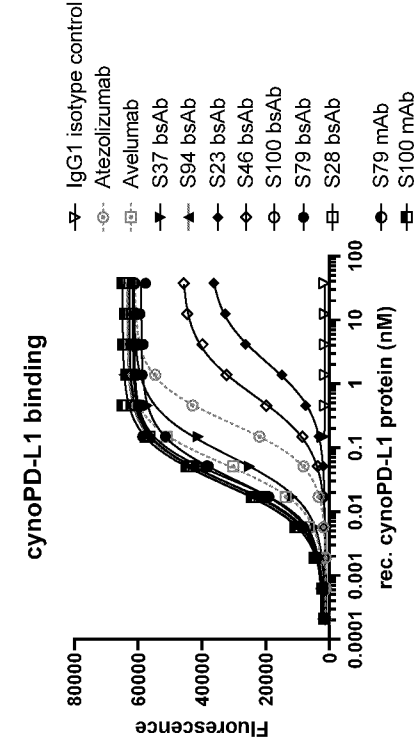


FIG. 1C

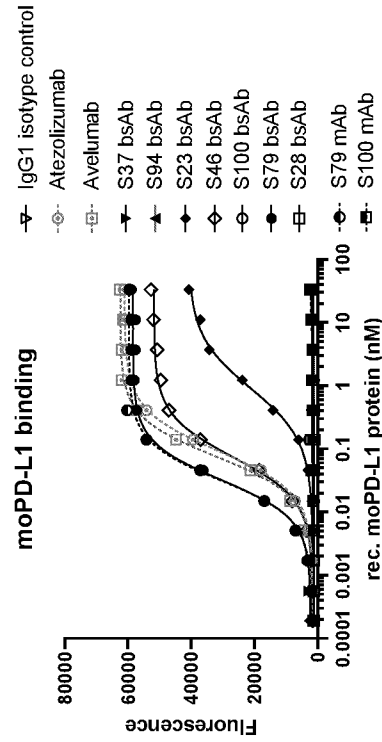


FIG. 1D

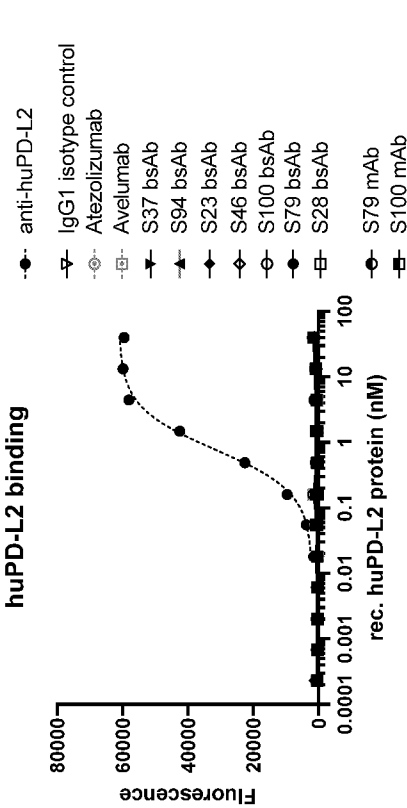


FIG. 1E

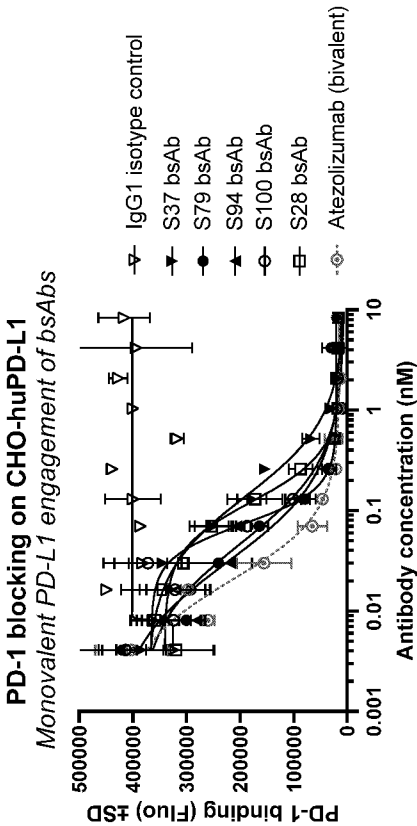


FIG. 2A

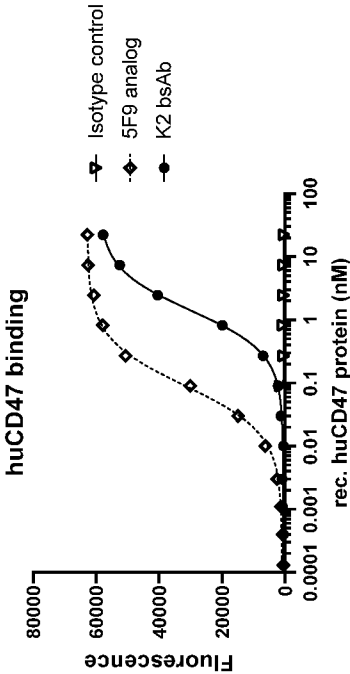


FIG. 2B

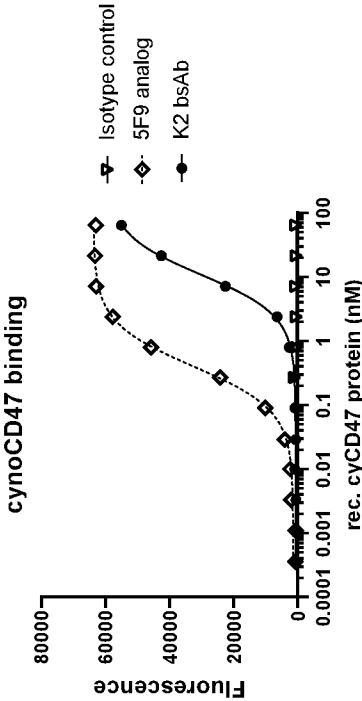


FIG. 2C

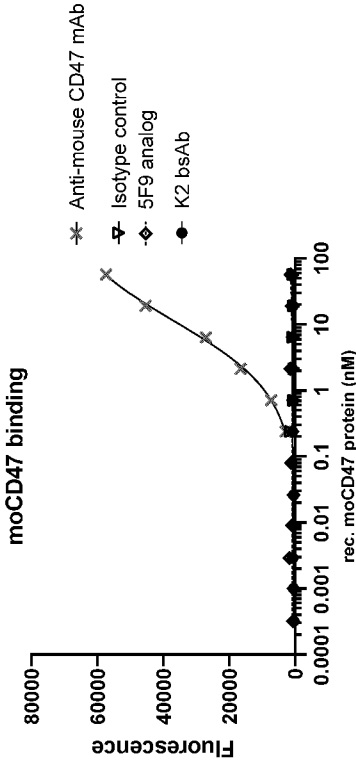


FIG. 2D

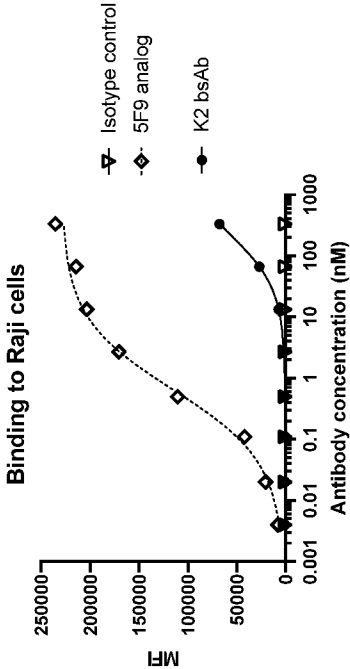


FIG. 2E

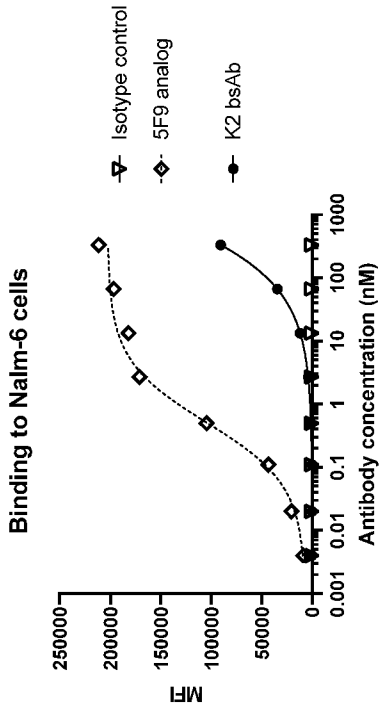


FIG. 2F

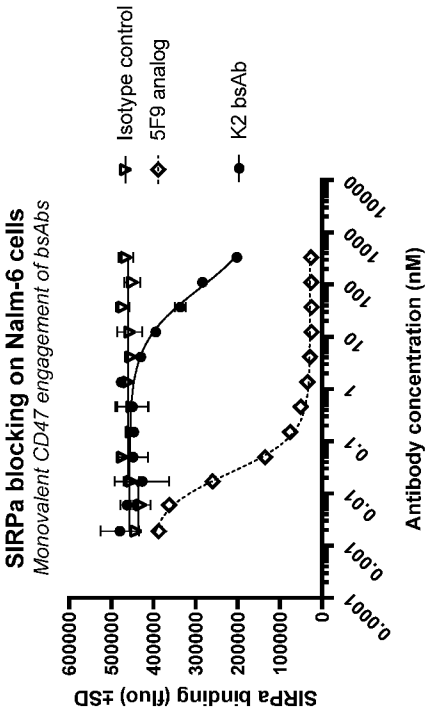


FIG. 3
Binding to human red blood cells

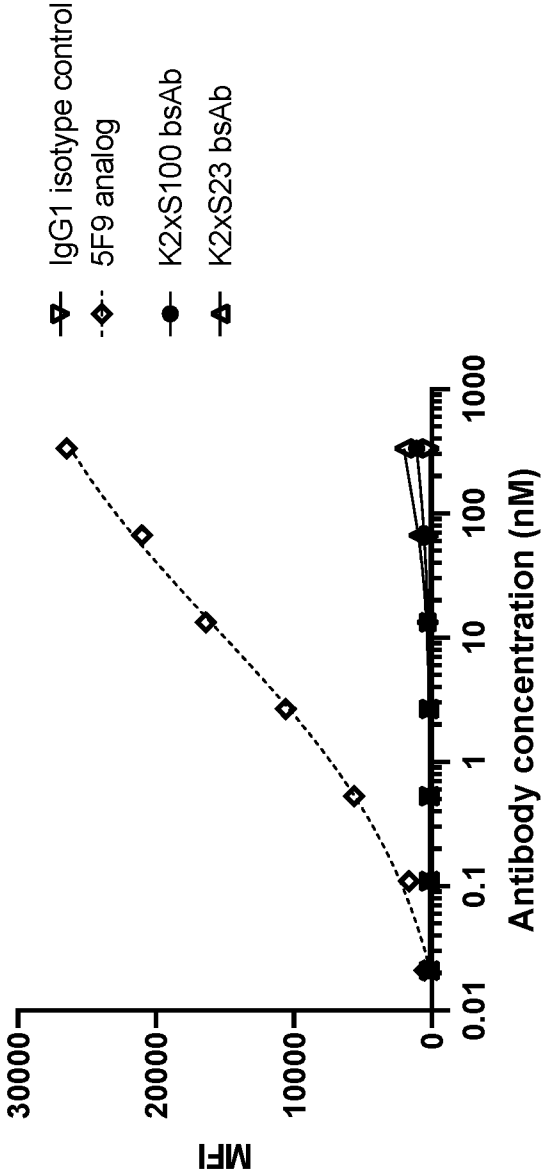


FIG. 4A

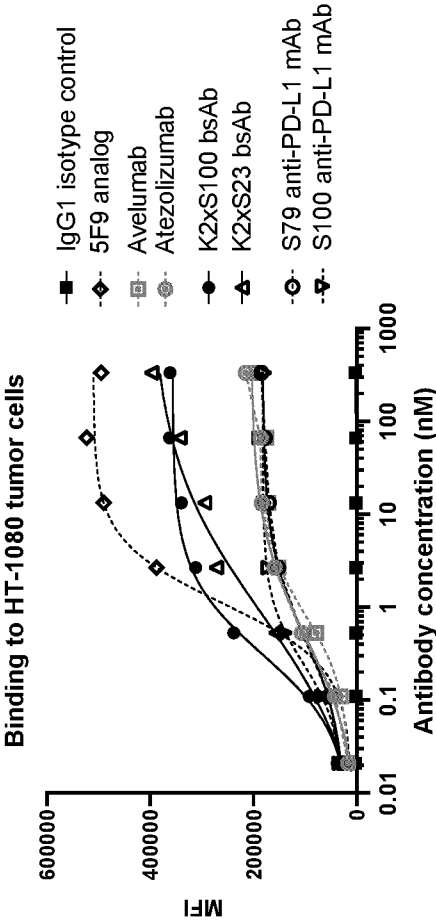


FIG. 4B

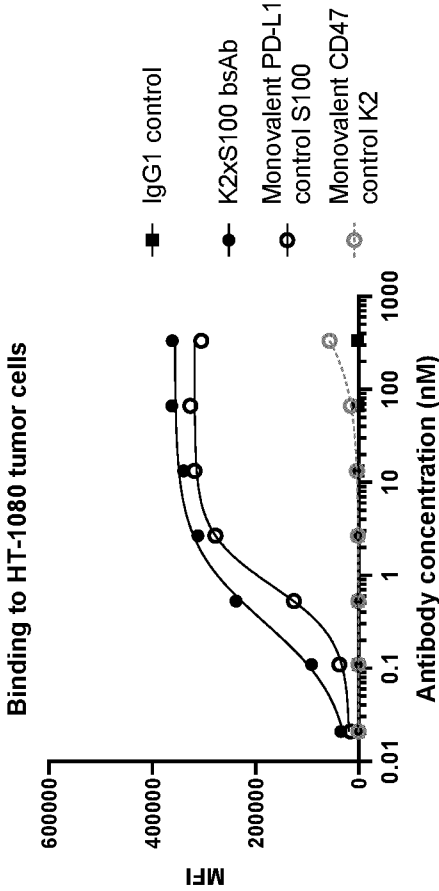


FIG. 5A

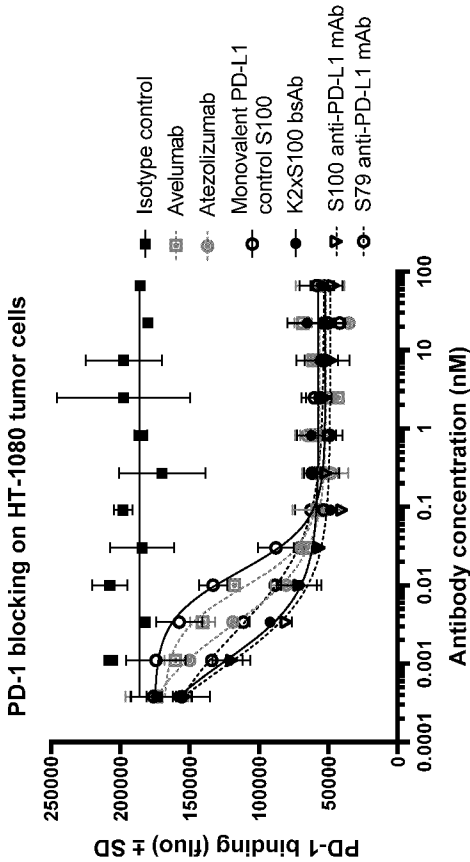


FIG. 5B

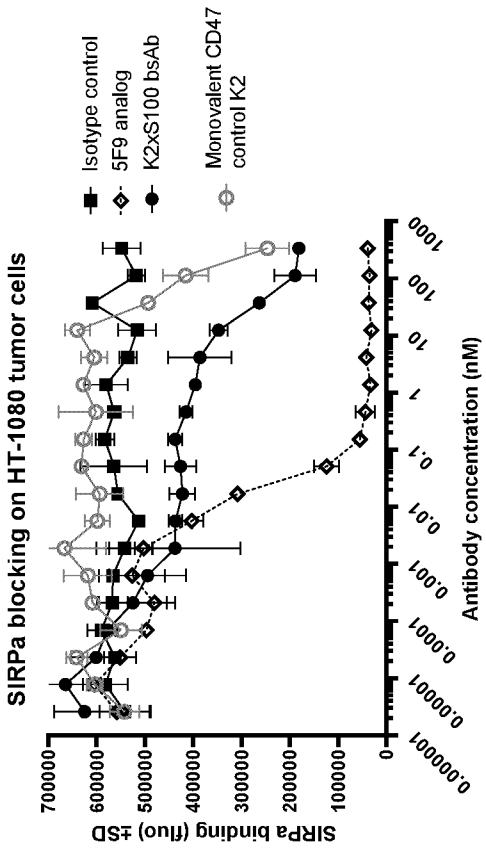


FIG. 6A

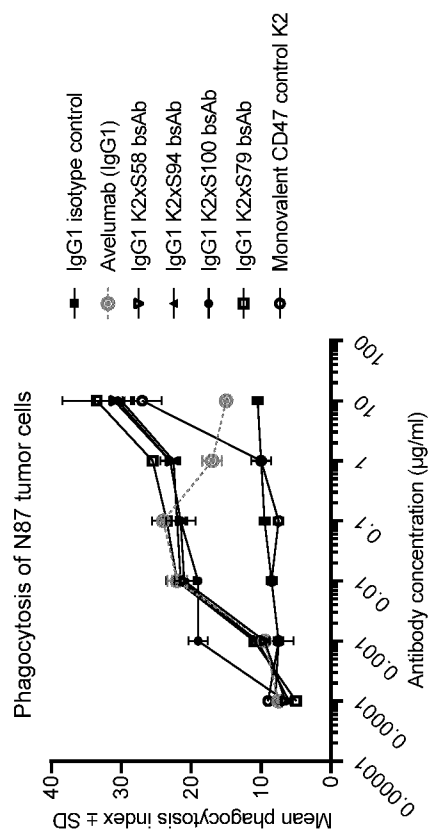


FIG. 6B

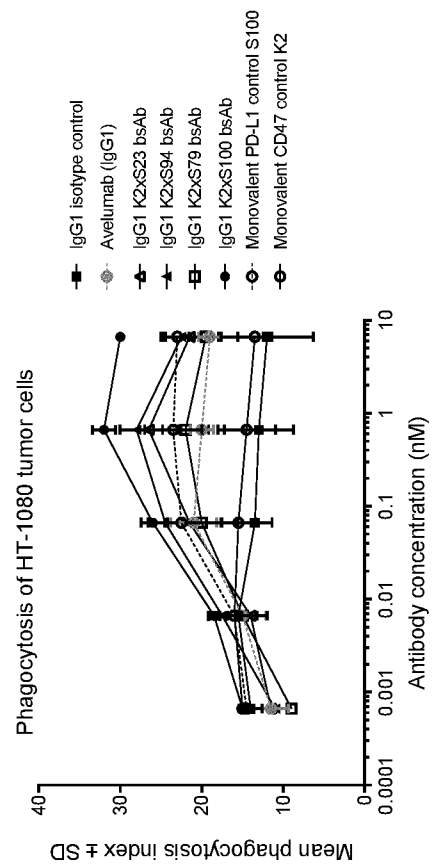


FIG. 7A

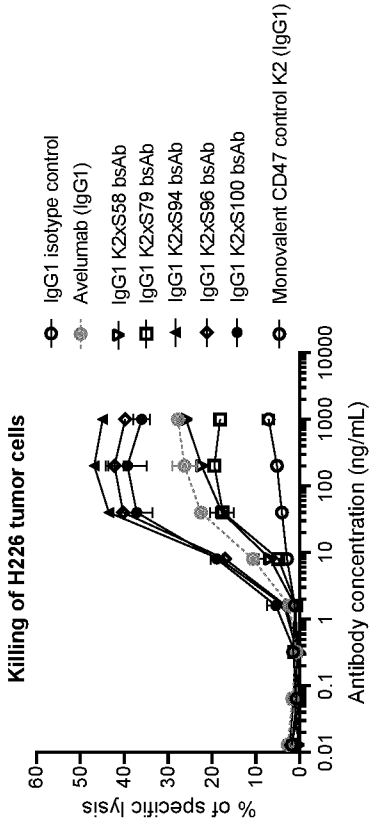


FIG. 7B

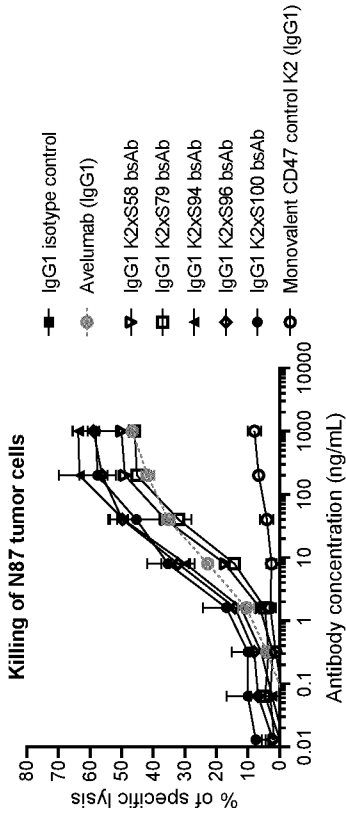


FIG. 7C

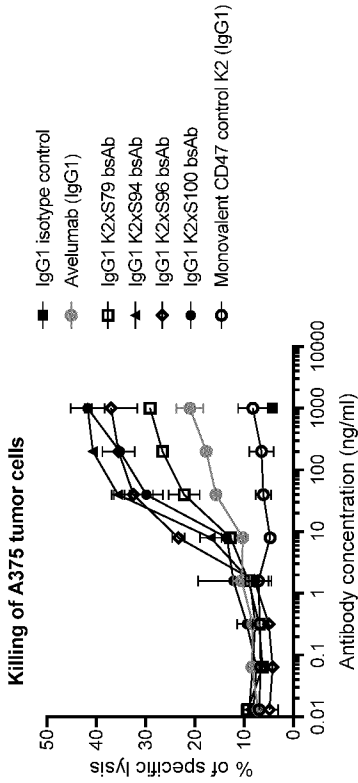


FIG. 9

