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(54) Title: COMPOSITIONS AND USES THEREOF FOR TREATING DISEASE OR CONDITION

(57) Abstract: The present application provides methods, imaging agents and kits for determination of the distribution and expression levels of an immune checkpoint ligand (such as PD-L1 or a PD-L1 like ligand) in an individual having a disease or condition. Anti-PD-L1 antibody agents, and methods for treating diseases or disorders by administering anti-PD-L1 antibody agents are also provided.

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COMPOSITIONS AND USES THEREOF FOR TREATING DISEASE OR CONDITION

CROSS-REFERENCE TO RELATED APPLICATIONS

[1] The present application claims the benefit of, and priority to PCT Application Nos. PCT/CN2018/089672 filed June 1, 2018 and PCT/CN2018/099556 filed August 9, 2018. The contents of the applications are incorporated herein by reference in their entirety.

SUBMISSION OF SEQUENCE LISTING ON ASCII TEXT FILE

[2] The content of the following submission on ASCII text file is incorporated herein by reference in its entirety: a computer readable form (CRF) of the Sequence Listing (file name: 792572000341SEQLIST.TXT, date recorded: May 28, 2019, size: 64 KB).

FIELD OF THE INVENTION

[3] The present invention relates to antibodies, imaging agents, methods of imaging an immune checkpoint ligand and methods of treating a disease or condition.

BACKGROUND OF THE INVENTION

[4] The Programmed Death (PD) network involves at least five interacting molecules: PD-1 (Programmed Cell Death 1), two PD-1 ligands (PD-L1 and PD-L2), and two inhibitory receptors (PD-1 and CD80) of PD-L1. The crucial function of the PD pathway in modulating the activity of T cells in the peripheral tissues in an inflammatory response to infection and in limiting autoimmunity appears to be hijacked by tumor cells and by viruses during chronic viral infections. PD-L1 is overexpressed on many freshly isolated human tumors from multiple tissue origins (Dong *et al. Nature Medicine* 2002; 8:793-800; Romano *et al. Journal for Immunotherapy of Cancer* 2015; 3:15; Hirano *et al. Cancer Research* 2005; 65:1089-1096). The expression of PD-L1 has been correlated with the progression and poor prognosis of certain types of human cancers (Wang *et al. European journal of surgical oncology: the journal of the European Society of Surgical Oncology and the British Association of Surgical Oncology* 2015; 41:450-456; Cierna *et al. Annals of oncology : official journal of the European Society for Medical Oncology / ESMO* 2016; 27:300-305; Gandini *et al. Critical reviews in oncology/hematology* 2016; 100:88-98; Thierauf *et al. Melanoma research* 2015; 25:503-509; Taube *et al. Clinical cancer research : an official journal of the American Association for Cancer Research* 2014; 20:5064-5074). During chronic viral infections, PD-L1 is persistently expressed on many tissues, while PD-1 is up-regulated on virus-specific CTLs (Yao *et al. Trends in molecular medicine* 2006; 12:244-246). Tumor- or virus-induced PD-L1 may utilize multiple mechanisms to facilitate the evasion of host immune surveillance,

including T cell anergy, apoptosis, exhaustion, IL-10 production, DC suppression, as well as Treg induction and expansion (Zou *et al. Nature reviews Immunology* 2008; 8:467-477).

[5] The PD-L1 expression level determined using immunohistochemistry (IHC) has been assessed as a predictive biomarker in clinical trials of PD-1/PD-L1-directed therapy on multiple cancer types, including melanoma, renal cell carcinoma (RCC), non-small cell lung cancer (NSCLC), metastatic colorectal cancer (mCRC), and metastatic castration-resistant prostate cancer (mCRPC). Patients with higher levels of PD-L1 determined by IHC appeared to have superior responses to PD-1/PD-L1–directed therapy. However, PD-L1–negative patients with melanoma can still obtain durable response to anti-PD-1/PD-L1 therapy, while response rates in PD-L1–negative NSCLC patients are rare.

[6] The accuracy of PD-L1 detection by IHC in human tumor specimens is confounded by multiple factors. A multitude of PD-L1 antibodies for IHC detection have been utilized, including 28-8, 22C3, 5H1, MIH1, and 405.9A11. In addition, a number of proprietary companion diagnostics are being developed in this area, such as Ventana SP142 and Ventana SP263 assay. Comparative performance characteristics of these assays are not well known. In addition to the existing issue of heterogeneous PD-L1 expression within the tumor microenvironment, there's also a lack of a clear definition of "positive" PD-L1 staining by IHC in tumor samples. Cut-off points for a positive result could range from >1% to >50%, based on percent tumor cells stained. Furthermore, PD-L1 has limited binding sites for IHC detection antibodies, as it contains only two small hydrophilic regions, which makes immunohistochemical approaches classically used in formalin-fixed, paraffin-embedded (FFPE) specimens less effective. Due to the lack of binding sites on PD-L1, IHC antibodies typically bind PD-L1 at structurally unique sites compared with therapeutic PD-L1 antibodies.

[7] Additionally, PD-L1 is biologically active only when expressed on the cell membrane, either through dynamic IFNγ expression or through constitutive oncogene activation. Oncogene-driven PD-L1 expression represents a histopathologically and biologically distinct entity compared to inflammation driven PD-L1 expression. While the latter occurs focally at sites of IFNγ-mediated immunologic attack, oncogene-driven PD-L1 expression is constitutive and diffuse. IFNγ induced PD-L1 expression represents a dynamic biomarker and is present at sites of active inflammation, and biopsy samples represent a snapshot of the tumor immune microenvironment in space and time. Other factors in the tumor metabolic microenvironment, including hypoxia, can result in PD-L1 upregulation and are dependent on signaling via HIF1a. Smaller tumor biopsies may miss the pertinent tumor–immune interface, or the biopsy may be performed after the biologically relevant PD-L1 overexpression has already taken place. PD-L1 itself is expressed at two potentially clinically relevant immunologic synapses—the tumor/T-cell interface, as well as the APC/T-cell interface. For the tumor/T-cell interface, biopsy capture of the tumor/immune interface is a key determinant in PD-L1 detection by IHC in melanoma. In a study assessing PD-L1 expression in patients with metastatic melanoma, 96% of PD-L1–

overexpressing melanomas had lymphocytic infiltrate (TIL), while the remaining 4% of PD-L1–overexpressing lacked TILs, possibly representing oncogene- driven PD-L1 expression. In addition, 22% of PD-L1 negative samples were associated with TIL, indicating alternative mechanisms of tumor immune interference.

[8] The majority of PD-L1 expression occurs at the tumor interface, with immune cells secreting IFNγ, leading to the counterintuitive hypothesis that PD-L1 overexpression may be an initially protective response to successful tumor killing by TILs, which over time becomes co-opted into an immunosuppressive tumor environment. In addition, selection of the appropriate site for biopsy for PD-L1 detection remains enigmatic. While pretreatment FFPE primary tumor samples may be most readily available, these samples may not reflect the overall immunologic state that currently exists in a given patient, particularly if interim treatment has been administered. The absence of PD-L1 expression in a biopsied lesion may not reflect the systemic immunologic landscape, and may not capture the beneficial effect of the therapy at other sites of the disease that are dependent on PD-L1 signaling. In summary, there is an unmet need for accurate and alternative PD-L1 detection agents and methods.

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

BRIEF SUMMARY OF THE INVENTION

[10] The present application provides anti-PD-L1 antibodies, imaging agents comprising a labeled antibody moiety that specifically recognizing an immune checkpoint ligand (such as PD-L1 or a PD-L1 like ligand), methods of preparing the imaging agents, and methods of imaging and diagnosis using the imaging agents. The present application also provides anti-PD-L1 antibody agents, uses of anti-PD-L1 antibody agents and methods for treating diseases or disorders by administering the anti-PD-L1 antibody agents into individuals.

[11] One aspect of this application provides methods of treating a disease or condition in an individual, comprising administering to the individual an effective amount of an anti-PD-L1 antibody agent, wherein the anti-PD-L1 antibody agent comprises an antibody moiety comprising a heavy chain variable region (V_H) and a light chain variable region (V_L), wherein: a) the V_H comprises a heavy chain complementarity determination region 1 (HC-CDR1) comprising the amino acid sequence of SEQ ID NO: 41, an HC-CDR2 comprising the amino acid sequence of SEQ ID NO: 42, and an HC-CDR3 comprising the amino acid sequence of SEQ ID NO: 43, or a variant thereof comprising up to a total of about 5 amino acid substitutions in the HC-CDRs; and b) the V_L comprises a light chain complementarity determination region 1 (LC-CDR1) comprising the amino acid sequence of SEQ ID NO: 44, an LC-CDR2 comprising the amino acid sequence of SEQ ID NO: 45, and an LC-CDR3 comprising the amino acid sequence of SEQ ID NO: 46, or a variant thereof comprising up to a total of about 5 amino acid substitutions in the LC-CDRs. In some embodiments, the V_H comprises an HC-CDR1

comprising the amino acid sequence of SEQ ID NO: 41, an HC-CDR2 comprising the amino acid sequence of SEQ ID NO: 42, and an HC-CDR3 comprising the amino acid sequence of SEQ ID NO: 43; and the V_L comprises an LC-CDR1 comprising the amino acid sequence of SEQ ID NO: 44, an LC-CDR2 comprising the amino acid sequence of SEQ ID NO: 45, and an LC-CDR3 comprising the amino acid sequence of SEQ ID NO: 46. In some embodiments, the antibody moiety comprises an amino acid sequence having at least about 80% sequence identity to the amino acid sequence of SEQ ID NO: 21 or 23.

[12] In some embodiments according to any of the methods described above, the V_H comprises an amino acid sequence having at least about 80% sequence identity to the amino acid sequence of any one of SEQ ID NOs: 1, 5, 9, 11, and 13; and/or the V_L comprises an amino acid sequence having at least about 80% sequence identity to the amino acid sequence of any one of SEQ ID NOs: 3, 7, 15, 17 and 19. In some embodiments, the antibody moiety comprises: (a) a V_H comprising the amino acid sequence of SEQ ID NO: 9, and a V_L comprising the amino acid sequence of SEQ ID NO: 15; (b) a V_H comprising the amino acid sequence of SEQ ID NO: 9, and a V_L comprising the amino acid sequence of SEQ ID NO: 17; (c) a V_H comprising the amino acid sequence of SEQ ID NO: 9, and a V_L comprising the amino acid sequence of SEQ ID NO: 19; (d) a V_H comprising the amino acid sequence of SEQ ID NO: 11, and a V_L comprising the amino acid sequence of SEQ ID NO: 15; (e) a V_H comprising the amino acid sequence of SEQ ID NO: 11, and a V_L comprising the amino acid sequence of SEQ ID NO: 17; (f) a V_H comprising the amino acid sequence of SEQ ID NO: 11, and a V_L comprising the amino acid sequence of SEQ ID NO: 19; (g) a V_H comprising the amino acid sequence of SEQ ID NO: 13, and a V_L comprising the amino acid sequence of SEQ ID NO: 15; (h) a V_H comprising the amino acid sequence of SEQ ID NO: 13, and a V_L comprising the amino acid sequence of SEQ ID NO: 17; or (i) a V_H comprising the amino acid sequence of SEQ ID NO: 13, and a V_L comprising the amino acid sequence of SEQ ID NO: 19.

[13] Another aspect of this application provides methods of treating a disease or condition in an individual, comprising administering to the individual an effective amount of an anti-PD-L1 antibody agent, wherein the antibody agent comprises an antibody moiety comprising: a) a HC-CDR1, a HC-CDR2, and a HC-CDR3, respectively comprising the amino acid sequences of a CDR1, a CDR2, and a CDR3 within a heavy chain variable region (V_H) having the sequence set forth in any of SEQ ID NOs: 1, 5, 9, 11, and 13; and b) a LC-CDR1, a LC-CDR2, and a LC-CDR3, respectively comprising the amino acid sequences of a CDR1, a CDR2, and a CDR3 within a light chain variable region (V_L) having the sequence set forth in any of SEQ ID NOs: 3, 7, 15, 17 and 19.

[14] In some embodiments according to any of the methods described above, the antibody moiety is chimeric or humanized.

[15] In some embodiments according to any of the methods described above, the antibody moiety is selected from the group consisting of a single-chain Fv (scFv), a Fab, a Fab', a F(ab')2, an Fv fragment, a disulfide stabilized Fv fragment (dsFv), a $(dsFv)_2$, a V_HH, a Fv-Fc fusion, an scFv-Fc fusion, an scFv-Fv fusion, a diabody, a tribody, and a tetrabody.

[16] In some embodiments according to any of the methods described above, the antibody moiety is a single-chain antibody. In some embodiments, the antibody moiety is an scFv.

[17] In some embodiments according to any of the methods described above, the antibody moiety comprises an Fc fragment. In some embodiments, the antibody moiety is a full-length antibody. In some embodiments, the antibody moiety has an isotype selected from the group consisting of an IgG, an IgM, an IgA, an IgD, and an IgE. In some embodiments, the Fc fragment is an Fc fragment of IgG. In some embodiments, the Fc fragment is an Fc fragment of IgG1 or IgG4. In some embodiments, the Fc fragment comprises H310A and H435Q mutations, wherein the amino acid positions are based on the Kabat numbering system.

[18] In some embodiments according to any of the methods described above, the individual is a human.

[19] In some embodiments according to any of the methods described above, the disease or condition is a cancer. In some embodiments, the cancer is selected from the group consisting of melanoma, renal cell carcinoma, colorectal cancer, urothelial carcinoma, Hodgkin's lymphoma, small cell lung cancer, non-small cell lung cancer, head and neck tumors, stomach cancer, B cell lymphoma, Merkel cell carcinoma, liver cancer, and cervical cancer.

[20] In some embodiments according to any of the methods described above, the antibody agent is suitable for intravenous, intraperitoneal, intramuscular, subcutaneous, or oral administration.

[21] In some embodiments according to any of the methods described above, the antibody agent is adminsitered intravenously, intraperitoneally, intramuscularly, subcutaneously, or orally.

[22] In some embodiments according to any of the methods described above, the method further comprises administering an effective amount of a second agent. In some embodiments, the second agent is a chemotherapeutic agent.

[23] In some embodiments according to any of the methods described above, the effective amount of the anti-PD-L1 antibody agent is about 0.005 μg/kg to about 5g/kg of total body weight of the individual.

[24] Another aspect of this application provides a pharmaceutical composition comprising an anti-PD-L1 antibody agent and a pharmaceutical acceptable carrier, wherein the antibody agent comprises an antibody moiety comprising a heavy chain variable region (V_H) and a light chain variable region (V_L), wherein: a) the V_H comprises an HC-CDR1 comprising the amino acid sequence of SEQ ID NO: 41, an HC-CDR2 comprising the amino acid sequence of SEQ ID NO: 42, and an HC-CDR3 comprising the amino acid sequence of SEQ ID NO: 43, or a variant thereof comprising up to a total of about 5 amino acid substitutions in the HC-CDRs; and b) the

V_L comprises an LC-CDR1 comprising the amino acid sequence of SEQ ID NO: 44, an LC-CDR2 comprising the amino acid sequence of SEQ ID NO: 45, and an LC-CDR3 comprising the amino acid sequence of SEQ ID NO: 46, or a variant thereof comprising up to a total of about 5 amino acid substitutions in the LC-CDRs. In some embodiments, the pharmaceutical composition is lyophilized. In some embodiments, the pharmaceutical composition is a solution. In some embodiments, the pharmaceutical composition comprises about 0.001 μg to about 10 g of the antibody moiety.

[25] Another aspect of this application provides a kit for treating a disease or condition in an individual, comprising any one of the pharmaceutical composition described above and an instruction.

[26] Another aspect of the present application provides a method of determining the distribution of an immune checkpoint ligand in an individual, comprising: (a) administering to the individual an imaging agent comprising an antibody moiety labeled with a radionuclide, wherein the antibody fragment specifically binds the immune checkpoint ligand; and (b) imaging the imaging agent in the individual with a non-invasive imaging technique. In some embodiments, the method further comprises determining the expression level of the immune checkpoint ligand in a tissue of interest in the individual based on signals emitted by the imaging agent from the tissue. In some embodiments, the method comprises determining the distribution of two or more immune checkpoint ligands in the individual.

[27] In some embodiments according to any one of the methods described above, the imaging agent is cleared from the individual within about 10 minutes to about 48 hours (*e.g.*, about 2 hours to about 4 hours, about 4 hours to about 8 hours, or about 8 hours to about 24 hours) in serum. In some embodiments, the half-life of the antibody moiety is between about 10 minutes to about 24 hours (*e.g.*, about 1 hour to about 2 hours, about 2 hours to about 4 hours, about 4 hours to about 12 hours, or about 12 hours to about 24 hours).

[28] In some embodiments according to any one of the methods described above, the molecular weight of the antibody moiety is no more than about 120 kDa (*e.g.*, about any one of 30-50kDa, 50-100kDa, or 30-80kDa).

[29] In some embodiments according to any one of the methods described above, the antibody moiety has a K_D between about $9x10^{-10}$ M to about $1x10^{-8}$ M (*e.g.*, about 9×10^{-10} to 1×10^{-9}, about 1×10^{-9} to 2×10^{-9}, about 2×10^{-10} to 5×10^{-9}, or about 5×10^{-10} to 1×10^{-8}) with the immune checkpoint ligand.

[30] In some embodiments according to any one of the methods described above, the antibody moiety cross-reacts with the immune checkpoint ligand from a non-human mammal. In some embodiments, the antibody moiety cross-reacts with the immune checkpoint ligand from a cynomolgus monkey. In some embodiments, the antibody moiety cross-reacts with the immune checkpoint ligand from a mouse.

[31] In some embodiments according to any one of the methods described above, the antibody moiety is humanized. In some embodiments, the antibody moiety is human. In some embodiments, the antibody moiety is chimeric.

[32] In some embodiments according to any one of the methods described above, the antibody moiety is stable at acidic or neutral pH. In some embodiments, the antibody moiety has a melting temperature of about 55-70 °C (*e.g.*, about 55-60°C, about 60-65°C, or about 65-70°C).

[33] In some embodiments according to any one of the methods described above, the antibody moiety is selected from the group consisting of a single-chain Fv (scFv), a diabody, a Fab, a Fab', a $F(ab')_2$, an Fv fragment, a disulfide stabilized Fv fragment (dsFv), a $(dsFv)_2$, and a V_HH. In some embodiments, the antibody moiety is an scFv. In some embodiments, the scFv comprises one or more engineered disulfide bonds. In some embodiments, the scFv comprises a first engineered cysteine residue at position 44 of V_H and a second engineered cysteine residue at position 100 of V_L, or a first engineered cysteine residue at position 105 of V_H and a second engineered cysteine residue at position 43 of V_L, wherein the first engineered cysteine residue and the second engineered cysteine residue form a disulfide bond, and wherein the amino acid positions are based on the Kabat numbering system. In some embodiments, the antibody moiety is an scFv fused to an Fc fragment. In some embodiments, the Fc fragment is a human IgG1 Fc fragment. In some embodiments, the Fc fragment has H310A and H435Q mutations, wherein the amino acid positions are based on the Kabat numbering system. In some embodiments, the scFv comprises from the N-terminus to the C-terminus: a heavy chain variable region (V_H), an optional peptide linker, and a light chain variable region (V_L). In some embodiments, the scFv comprises from the N-terminus to the C-terminus: a V_L, an optional peptide linker, and a V_H. In some embodiments, the scFv comprises a peptide linker comprising the amino acid sequence of SEQ ID NO: 47 or 48.

[34] In some embodiments according to any one of the methods described above, the immune checkpoint ligand is PD-L1 or a PD-L1 like ligand. In some embodiments, the immune checkpoint ligand is PD-L1. In some embodiments, the antibody moiety comprises: a V_H comprising a HC-CDR1 comprising the amino acid sequence of SEQ ID NO: 41, a HC-CDR2 comprising the amino acid sequence of SEQ ID NO: 42, and a HC-CDR3 comprising the amino acid sequence of SEQ ID NO: 43; and a V_L comprising a LC-CDR1 comprising the amino acid sequence of SEQ ID NO: 44, a LC-CDR2 comprising the amino acid sequence of SEQ ID NO: 45, and a LC-CDR3 comprising the amino acid sequence of SEQ ID NO: 46; or the antibody moiety specifically binds PD-L1 competitively with an anti-PD-L1 antibody comprising: a V_H comprising a HC-CDR1 comprising the amino acid sequence of SEQ ID NO: 41, a HC-CDR2 comprising the amino acid sequence of SEQ ID NO: 42, and a HC-CDR3 comprising the amino acid sequence of SEQ ID NO: 43; and a V_L comprising a LC-CDR1 comprising the amino acid sequence of SEQ ID NO: 44, a LC-CDR2 comprising the amino acid sequence of SEQ ID NO: 45, and a LC-CDR3 comprising the amino acid sequence of SEQ ID NO: 46.

[35] In some embodiments according to any one of the methods described above, the tissue of interest is negative for the immune checkpoint ligand based on an immunohistochemistry (IHC) assay or another assay. In some embodiments, the tissue of interest has a low expression level of the immune checkpoint ligand. In some

embodiments, the tissue of interest only expresses the immune checkpoint ligand upon infiltration of immune cells.

[36] In some embodiments according to any one of the methods described above, the method comprises imaging the individual over a period of time.

[37] In some embodiments according to any one of the methods described above, the radionuclide is selected from the group consisting of ^{64}Cu, ^{18}F, ^{67}Ga, ^{68}Ga, ^{111}In, ^{177}Lu, ^{90}Y, ^{89}Zr, ^{61}Cu, ^{62}Cu, ^{67}Cu, ^{19}F, ^{66}Ga, ^{72}Ga, ^{44}Sc, ^{47}Sc, ^{86}Y, ^{88}Y and ^{45}Ti. In some embodiments, the radionuclide is ^{68}Ga. In some embodiments, the antibody moiety is conjugated to a chelating compound that chelates the radionuclide. In some embodiments, the chelating compound is 1,4,7- triazacyclononane-1,4,7-trisacetic acid (NOTA), 1,4,7,10-tetraazacyclododecane-1,4,7,10-tetraacetic acid (DOTA) or derivatives thereof. In some embodiments, the chelating compound is NOTA.

[38] In some embodiments according to any one of the methods described above, the method further comprises preparing the imaging agent by labeling the antibody moiety with the radionuclide.

[39] In some embodiments according to any one of the methods described above, the non-invasive imaging technique comprises single photon emission computed tomography (SPECT) imaging or positron emission tomography (PET) imaging. In some embodiments, the non-invasive imaging technique comprises computed tomography imaging, magnetic resonance imaging, chemical luminescence imaging, or electrochemical luminescence imaging.

[40] In some embodiments according to any one of the methods described above, the imaging agent is administered intravenously, intraperitoneally, intramuscularly, subcutaneously, or orally.

[41] In some embodiments according to any one of the methods described above, the imaging is carried out between about 10 minutes to about 24 hours (*e.g.*, about 10 minutes to 1 hour, about 1 hour to 2 hours, about 2 hours to 4 hours, about 4 hours to 8 hours, or about 8 hours to 24 hours) after the administration of the imaging agent.

[42] In some embodiments according to any one of the methods described above, the method further comprises administering to the individual an antibody moiety not labeled with a radionuclide prior to the administration of the imaging agent.

[43] In some embodiments according to any one of the methods described above, the individual has a solid tumor. In some embodiments, the solid tumor is selected from the group consisting of colon tumor, melanoma, kidney tumor, ovarian tumor, lung tumor, breast tumor, and pancreatic tumor. In some embodiments, the individual has a hematological malignancy. In some embodiments, the hematological malignancy is selected from the group consisting of leukemia, lymphoma, acute lymphoblastic leukemia (ALL), acute non-lymphoblastic leukemia (ANLL), chronic lymphocytic leukemia (CLL), chronic myeloid leukemia (CML), non-

Hodgkin lymphoma, and Hodgkin lymphoma. In some embodiments, the individual has an infectious disease, autoimmune disease, or metabolic disease.

[44] Another aspect of the present application provides a method of diagnosing an individual having a disease or condition, comprising: (a) determining the distribution of an immune checkpoint ligand in the individual using the method according to any one of the methods described above; and (b) diagnosing the individual as positive for the immune checkpoint ligand if signal of the imaging agent is detected at a tissue of interest, or diagnosing the individual as negative for the immune checkpoint ligand if signal of the imaging agent is not detected at a tissue of interest.

[45] Another aspect of the present application provides a method of treating an individual having a disease or condition, comprising: (a) diagnosing the individual using the method according to any one of the methods of diagnosis described above; and (b) administering to the individual an effective amount of a therapeutic agent targeting the immune checkpoint ligand or receptor thereof, if the individual is diagnosed as positive for the immune checkpoint ligand. In some embodiments, the therapeutic agent is an inhibitor of the immune checkpoint ligand or receptor thereof. In some embodiments, the therapeutic agent is a radiolabeled molecule specifically binding the immune checkpoint ligand or receptor thereof. In some embodiments, wherein the immune checkpoint ligand is PD-L1, the individual is administered with an antibody specifically binding PD-1 or PD-L1. In some embodiments, the immune checkpoint ligand is a PD-L1 like ligand.

[46] Another aspect of the present application provides an isolated anti-PD-L1 antibody agent comprising an antibody moiety comprising a heavy chain variable region (V_H) comprising a heavy chain complementarity determining region (HC-CDR)1 comprising the amino acid sequence of SEQ ID NO: 41, a HC-CDR2 comprising the amino acid sequence of SEQ ID NO: 42, and a HC-CDR3 comprising the amino acid sequence of SEQ ID NO: 43, or a variant thereof comprising up to about 5 (*e.g.*, 1, 2, 3, 4, or 5) amino acid substitutions; and a light chain variable region (V_L) comprising a light chain complementarity determining region (LC-CDR)1 comprising the amino acid sequence of SEQ ID NO: 44, a LC-CDR2 comprising the amino acid sequence of SEQ ID NO: 45, and a LC-CDR3 comprising the amino acid sequence of SEQ ID NO: 46, or a variant thereof comprising up to about 5 (*e.g.*, 1, 2, 3, 4, or 5) amino acid substitutions. In some embodiments, the antibody moiety comprises: a V_H comprising a HC-CDR1 comprising the amino acid sequence of SEQ ID NO: 41, a HC-CDR2 comprising the amino acid sequence of SEQ ID NO: 42, and a HC-CDR3 comprising the amino acid sequence of SEQ ID NO: 43; and a V_L comprising a LC-CDR1 comprising the amino acid sequence of SEQ ID NO: 44, a LC-CDR2 comprising the amino acid sequence of SEQ ID NO: 45, and a LC-CDR3 comprising the amino acid sequence of SEQ ID NO: 46.

[47] In some embodiments, there is provided an isolated anti-PD-L1 antibody agent comprising an antibody moiety comprising a V_H comprising a HC-CDR1, a HC-CDR2, and a HC-CDR3 of SEQ ID NO: 1;

and a V_L comprising a LC-CDR1, a LC-CDR2, and a LC-CDR3 of SEQ ID NO: 3. In some embodiments, the antibody moiety comprises: a V_H comprising an amino acid sequence having at least about 80% (*e.g.*, at least about any one of 80%, 85%, 90%, 95%, 98%, 99% or higher) sequence identity to the amino acid sequence of any one of SEQ ID NOs: 1, 5, 9, 11, and 13; and a V_L comprising an amino acid sequence having at least about 80% (*e.g.*, at least about any one of 80%, 85%, 90%, 95%, 98%, 99% or higher) sequence identity to the amino acid sequence of any one of SEQ ID NOs: 3, 7, 15, 17 and 19. In some embodiments, the antibody moiety comprises: (a) a V_H comprising the amino acid sequence of SEQ ID NO: 9, and a V_L comprising the amino acid sequence of SEQ ID NO: 15; (b) a V_H comprising the amino acid sequence of SEQ ID NO: 9, and a V_L comprising the amino acid sequence of SEQ ID NO: 17; (c) a V_H comprising the amino acid sequence of SEQ ID NO: 9, and a V_L comprising the amino acid sequence of SEQ ID NO: 19; (d) a V_H comprising the amino acid sequence of SEQ ID NO: 11, and a V_L comprising the amino acid sequence of SEQ ID NO: 15; (e) a V_H comprising the amino acid sequence of SEQ ID NO: 11, and a V_L comprising the amino acid sequence of SEQ ID NO: 17; (f) a V_H comprising the amino acid sequence of SEQ ID NO: 11, and a V_L comprising the amino acid sequence of SEQ ID NO: 19; (g) a V_H comprising the amino acid sequence of SEQ ID NO: 13, and a V_L comprising the amino acid sequence of SEQ ID NO: 15; (h) a V_H comprising the amino acid sequence of SEQ ID NO: 13, and a V_L comprising the amino acid sequence of SEQ ID NO: 17; or (i) a V_H comprising the amino acid sequence of SEQ ID NO: 13, and a V_L comprising the amino acid sequence of SEQ ID NO: 19.

[48] In some embodiments according to any one of the isolated anti-PD-L1 antibody agents described above, the antibody moiety is humanized. In some embodiments, the antibody moiety is human. In some embodiments, the antibody moiety is chimeric.

[49] In some embodiments according to any one of the isolated anti-PD-L1 antibody agents described above, the antibody moiety comprises an scFv. In some embodiments, the scFv comprises a first engineered cysteine residue at position 44 of V_H and a second engineered cysteine residue at position 100 of V_L, or a first engineered cysteine residue at position 105 of V_H and a second engineered cysteine residue at position 43 of V_L, wherein the first engineered cysteine residue and the second engineered cysteine residue form a disulfide bond, and wherein the amino acid positions are based on the Kabat numbering system. In some embodiments, the scFv comprises an amino acid sequence having at least about 80% (*e.g.*, at least about any one of 80%, 85%, 90%, 95%, 98%, 99% or higher) sequence identity to the amino acid sequence of any one of SEQ ID NOs: 25, 27, 29, 31, 33, 35, 37 and 39. In some embodiments, the scFv comprises the amino acid sequence of any one of SEQ ID NOs: 25, 27, 29, 31, 33, 35, 37 and 39. In some embodiments, the antibody moiety is an scFv. In some embodiments, the antibody moiety is an scFv fused to an Fc fragment. In some embodiments, the Fc fragment is a human IgG1 Fc fragment. In some embodiments, the Fc fragment has H310A and H435Q mutations, wherein the amino acid positions are based on the Kabat numbering system.

[50] In some embodiments, there is provided an anti-PD-L1 antibody agent comprising an antibody moiety that specifically binds PD-L1 competitively with the antibody moiety in the anti-PD-L1 antibody agent according to any one of the isolated anti-PD-L1 antibody agents described above.

[51] Another aspect of the present application provides an imaging agent comprising an antibody moiety labeled with a radionuclide, wherein the antibody moiety specifically binds an immune checkpoint ligand. In some embodiments, the immune checkpoint ligand is PD-L1 or a PD-L1 like ligand. In some embodiments, there is provided an imaging agent comprising the isolated anti-PD-L1 antibody agent of according to any one of the isolated anti-PD-L1 antibody agents described herein, wherein the antibody moiety is labeled with a radionuclide.

[52] In some embodiments according to any one of the imaging agents described herein, the radionuclide is selected from the group consisting of ^{64}Cu, ^{18}F, ^{67}Ga, ^{68}Ga, ^{111}In, ^{177}Lu, ^{90}Y, ^{89}Zr, ^{61}Cu, ^{62}Cu, ^{67}Cu, ^{19}F, ^{66}Ga, ^{72}Ga, ^{44}Sc, ^{47}Sc, ^{86}Y, ^{88}Y and ^{45}Ti. In some embodiments, the radionuclide is ^{68}Ga. In some embodiments, the antibody moiety is conjugated to a chelating compound that chelates the radionuclide. In some embodiments, the chelating compound is NOTA, DOTA, or derivatives thereof. In some embodiments, the chelating compound is NOTA.

[53] Also provided is an isolated nucleic acid encoding the isolated anti-PD-L1 antibody agent according to any one of the isolated anti-PD-L1 antibody agents described herein, a vector comprising the isolated nucleic acid, and an isolated host cell comprising the isolated anti-PD-L1 antibody agent, the isolated nucleic acid, or the vector.

[54] Another aspect of the present application provides a method of preparing an imaging agent targeting an immune checkpoint ligand, comprising: (a) conjugating a chelating compound to an antibody moiety specifically binding the immune checkpoint ligand to provide an antibody moiety conjugate; (b) contacting a radionuclide with the antibody moiety conjugate, thereby providing the imaging agent. In some embodiments, the immune checkpoint ligand is PD-L1 or a PD-L1 like ligand.

[55] In some embodiments, there is provided a method of preparing an imaging agent targeting PD-L1, comprising: (a) conjugating a chelating compound to the antibody moiety in the isolated anti-PD-L1 antibody agent according to any one of the isolated anti-PD-L1 antibody agents described above to provide an anti-PD-L1 conjugate; and (b) contacting a radionuclide with the anti-PD-L1 antibody conjugate, thereby providing the imaging agent.

[56] In some embodiments according to any one of the methods of preparation described herein, the chelating compound is conjugated to a lysine of the antibody moiety.

[57] Further provided is a kit comprising: (a) an antibody moiety specifically binding an immune checkpoint ligand; and (b) a chelating compound. In some embodiments, the kit further comprises a radionuclide.

[58] In some embodiments, there is provided a kit comprising: (a) an imaging agent comprising an antibody moiety labeled with a radionuclide, wherein the antibody moiety specifically binds an immune checkpoint ligand; and (b) an antibody moiety not labeled with a radionuclide.

[59] Also provided are compositions, kits and articles of manufacture comprising the any one of the anti-PD-L1 antibody agents and imaging agents described herein.

BRIEF DESCRIPTION OF THE DRAWINGS

[60] FIGs. 1A and 1B show the binding affinity of anti hPD-L1 monoclonal antibody 5B7 to hPD-L1 protein. FIG. 1A shows histograms demonstrating the binding affinity of anti hPD-L1 monoclonal antibody 5B7 to hPD-L1 protein at different concentrations. FIG. 1B shows the mean fluorescence intensity at the respective concentrations.

[61] FIGs. 2A and 2B show the binding affinity of the anti-hPD-L1 monoclonal antibody to CHO cells expressing hPD-L1 protein or mPD-L1 protein.

[62] FIGs. 3A and 3B show the blocking activities of mouse anti-hPD-L1 antibodies on the binding of hPD-L1 to hPD-1 (A) and hB7-1 (B).

[63] FIG. 4 shows the blocking activities of humanized anti-hPD-L1 antibodies on hPD-1/hPD-L1 binding as compared to the parental chimeric antibody.

[64] FIG. 5 shows a schematic diagram of the construct design for scFv-HuFc(Wt) and scFv-HuFc(Mt).

[65] FIG. 6 shows the SDS-PAGE results for scFv-HuFc(Wt) and scFv-HuFc(Mt) in both the reduced and non-reduced conditions.

[66] FIG. 7 shows histograms demonstrating the binding affinities of scFv-HuFc(Wt) and scFv-HuFc(Mt) to PD-L1 as compared to that of the parental antibody.

[67] FIGs. 8A and 8B show serum titers of anti-hPD-L1 antibodies and hIgG following intravenous injections of anti-hPD-L1 scFv-HuFc fusion proteins scFv-hFc Wt and scFv-hFc Mt. FIG. 8A shows serum titers of anti-hPD-L1 antibodies. FIG. 8B shows serum titers of hIgG.

[68] FIG. 9 shows schematic diagrams of the construct designs for anti-hPD-L1 scFvs, the parental humanized IgG1 positive control antibody and a negative control scFv.

[69] FIG. 10 shows SDS-PAGE results for scFv (PD-L1), the parental humanized IgG1 positive control antibody, and the negative control scFv under both reduced and non-reduced conditions.

[70] FIG. 11 shows the binding affinity of the parental humanized IgG1 positive control antibody and scFv (PD-L1) to PD-L1 at various concentrations.

[71] FIG. 12 shows the temperatures of hydrophobic exposure of scFv (PD-L1), the parental humanized IgG1 positive control antibody, and the negative control scFv, as measured by Differential Scanning Fluorimetry (DSF).

[72] FIGs. 13A-13D show the binding affinities of full-length anti-PD-L1 antibody and scFv (PD-L1) to PD-L1 under a high temperature (40°C) and high pH (pH=7.4) condition (FIGs. 13A and 13B) as compared to a low temperature (4°C) and acidic pH (pH=5.5) condition (FIGs. 13C and 13D).

[73] FIG. 14 shows the yield of ^{68}Ga-NOTA-scFv as measured using instant thin layer chromatography on Silica Gel.

[74] FIG. 15 shows the purity of ^{68}Ga-NOTA-scFv as measured using instant thin layer chromatography on Silica Gel.

[75] FIG. 16 shows the binding affinity of ^{68}Ga-NOTA-scFv to MC38-PD-L1 cells as compared to MC38 cells.

[76] FIG. 17 shows the blocking effect of anti-PD-L1 IgG1 on the binding of ^{68}Ga-NOTA- anti-PD-L1 scFv to MC38-PD-L1cells at various concentrations.

[77] FIG. 18 shows *in vivo* imaging of tumors induced by injections of MC38-PD-L1 and MC38 cells using ^{68}Ga-NOTA-anti-PD-L1 scFv.

[78] FIG. 19 shows *in vivo* imaging of tumors induced by injections of MC38-PD-L1 and MC38 cells using ^{68}Ga-NOTA-anti-PD-L1 scFv.

[79] FIG. 20 shows *in vivo* imaging results demonstrating the competition between unlabeled anti-PD-L1 IgG1 and ^{68}Ga-NOTA-anti-PD-L1 scFv for binding to PD-L1.

[80] FIG. 21 shows *in vivo* imaging results of tumors in mice 30 minutes after injection of ^{68}Ga-NOTA-anti-PD-L1 scFv, unlabeled anti-PD-L1 scFv and ^{68}Ga-NOTA-anti-PD-L1 scFv, ^{68}Ga-NOTA-anti-PD-L1 scFv-Fc(wt) at normal camera sensitivity setting, and ^{68}Ga-NOTA-anti-PD-L1 scFv-Fc(wt) at ¼ of normal camera sensitivity setting.

[81] FIG. 22 shows *in vivo* imaging results of tumors in mice 1 hour (top panel) or 2 hours (bottom panel) after injection of ^{68}Ga-NOTA-anti-PD-L1 scFv, unlabeled anti-PD-L1 scFv and ^{68}Ga-NOTA-anti-PD-L1 scFv, ^{68}Ga-NOTA-anti-PD-L1 scFv-Fc(wt) at normal camera sensitivity setting, and ^{68}Ga-NOTA-anti-PD-L1 scFv-Fc(wt) at ¼ of normal camera sensitivity setting.

[82] FIG. 23 shows exemplary NOTA compounds that can be used to chelate a radionuclide and to conjugate to an antibody moiety.

DETAILED DESCRIPTION OF THE INVENTION

[83] The present application provides imaging agents and methods for detection of an immune checkpoint ligand in an individual. The imaging agents described herein comprise an antibody moiety labeled with a radionuclide, wherein the antibody moiety specifically binds the immune checkpoint ligand, such as PD-L1 or a PD-L1 like ligand. The antibody moiety (such as Fab, scFv or scFv fused to an Fc) is characterized by a small size and rapid clearance from blood and normal organs. In some embodiments, the antibody moiety is engineered to have enhanced thermal stability. Imaging agents comprising such antibody moieties labeled with short-lived radionuclides allow effective targeting and penetration of diseased tissues expressing the immune checkpoint ligand. Distribution and expression levels of the immune checkpoint ligand can be determined by *in vivo* live imaging of an individual administered with the imaging agent. Prior to the present invention, accurate diagnosis based on PD-L1 and other immune checkpoint ligands as biomarkers remain a challenge in the field of cancer immunotherapy.

[84] Accordingly, one aspect of the present application provides a method of determining the distribution of an immune checkpoint ligand (such as PD-L1 or a PD-L1 like ligand) in an individual, comprising: (a) administering to the individual an imaging agent comprising an antibody moiety labeled with a radionuclide, wherein the antibody moiety specifically binds the immune checkpoint ligand; and (b) imaging the imaging agent in the individual with a non-invasive imaging technique.

[85] Another aspect of the present application provides an imaging agent comprising an antibody moiety labeled with a radionuclide, wherein the antibody moiety specifically binds an immune checkpoint ligand (such as PD-L1 or a PD-L1 like ligand).

[86] Another aspect of the presentation provides an anti-PD-L1 antibody agent comprising: a V_H comprising a HC-CDR1, a HC-CDR2, and a HC-CDR3 of SEQ ID NO: 1; and a V_L comprising a LC-CDR1, a LC-CDR2, and a LC-CDR3 of SEQ ID NO: 3.

[87] Also provided are compositions, kits and articles of manufacture comprising the imaging agents and anti-PD-L1 antibody agents described herein, methods of making thereof, and methods of diagnosing or treating an individual having a disease or condition (such as cancer, infectious disease, autoimmune disease or metabolic disease).

I. Definitions

[88] As used herein, "immune system checkpoints," or "immune checkpoints" refer to inhibitory pathways in the immune system that generally act to maintain self-tolerance or modulate the duration and amplitude of physiological immune responses to minimize collateral tissue damage. Stimulatory checkpoint molecules are molecules, such as proteins, that stimulate or positively regulate the immune system. Inhibitory checkpoint

molecules are molecules, such as proteins, that inhibit or negatively regulate the immune system. Immune system checkpoint molecules include, but are not limited to, cytotoxic T-lymphocyte antigen 4 (CTLA-4), programmed cell death 1 protein (PD-1), PD-L1, PD-L2, lymphocyte activation gene 3 (LAG3), B7-1, B7-H3, B7-H4, T cell membrane protein 3 (TIM3), B- and T-lymphocyte attenuator (BTLA), V-domain immunoglobulin (Ig)-containing suppressor of T-cell activation (VISTA), Killer-cell immunoglobulin-like receptor (KIR), and A2A adenosine receptor (A2aR).

[89] "Immune checkpoint receptors" are immune checkpoint molecules that are expressed on immune cells, such as T cells.

[90] As used herein, the term "immune checkpoint ligand" refers to a naturally-occurring or non-naturally occurring ligand that is specifically recognized by an immune checkpoint receptor. Naturally occurring immune checkpoint ligands are immune checkpoint molecules that may be expressed by diseased tissue, such as tumor cells, infected cells, or inflamed tissue, which can regulate immune cells that express immune checkpoint receptors that specifically recognize the immune checkpoint ligands. Non-naturally occurring immune checkpoint ligands include synthetic and recombinant molecules, such as therapeutic inhibitors, ligands, and antibodies of immune checkpoint receptors. Non-naturally occurring immune checkpoint ligands may be introduced to the individual, *e.g.*, by administration to the individual. An immune checkpoint ligand can inhibit an immune checkpoint by stimulating the activity of a stimulatory checkpoint receptor, or inhibiting the activity of an inhibitory checkpoint receptor in the pathway. Exemplary naturally-occurring immune checkpoint ligands include, but are not limited to, PD-L1, PD-L2, B7-H3 (also known as CD276), galectin-9, CD80, CD86 and ICOSL. In some embodiments, the immune checkpoint ligand is PD-L1. In some embodiments, the immune checkpoint ligand is a PD-L1 like ligand. "PD-L1 like ligand" refers to a naturally occurring or non-naturally occurring ligand of PD-1.

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

[92] A full-length antibody comprises two heavy chains and two light chains. The variable regions of the light and heavy chains are responsible for antigen binding. The variable domains of the heavy chain and light chain may be referred to as "V_H" and "V_L", respectively. The variable regions in both chains generally contain three highly variable loops called the complementarity determining regions (CDRs) (light chain (LC) CDRs including LC-CDR1, LC-CDR2, and LC-CDR3, heavy chain (HC) CDRs including HC-CDR1, HC-CDR2, and HC-CDR3). CDR boundaries for the antibodies and antigen-binding fragments disclosed herein may be defined

or identified by the conventions of Kabat, Chothia, or Al-Lazikani (Al-Lazikani 1997; Chothia 1985; Chothia 1987; Chothia 1989; Kabat 1987; Kabat 1991). The three CDRs of the heavy or light chains are interposed between flanking stretches known as framework regions (FRs), which are more highly conserved than the CDRs and form a scaffold to support the hypervariable loops. The constant regions of the heavy and light chains are not involved in antigen binding, but exhibit various effector functions. Antibodies are assigned to classes based on the amino acid sequence of the constant region of their heavy chain. The five major classes or isotypes of antibodies are IgA, IgD, IgE, IgG, and IgM, which are characterized by the presence of α, δ, ε, γ, and μ heavy chains, respectively. Several of the major antibody classes are divided into subclasses such as lgG1 (γ1 heavy chain), lgG2 (γ2 heavy chain), lgG3 (γ3 heavy chain), lgG4 (γ4 heavy chain), lgA1 (α1 heavy chain), or lgA2 (α2 heavy chain).

[93] The term "antigen-binding fragment" as used herein refers to an antibody fragment including, for example, a diabody, a Fab, a Fab', a F(ab')2, an Fv fragment, a disulfide stabilized Fv fragment (dsFv), a (dsFv)2, a bispecific dsFv (dsFv-dsFv'), a disulfide stabilized diabody (ds diabody), a single-chain Fv (scFv), an scFv dimer (bivalent diabody), a multispecific antibody formed from a portion of an antibody comprising one or more CDRs, a camelized single domain antibody, a nanobody, a domain antibody, a bivalent domain antibody, or any other antibody fragment that binds to an antigen but does not comprise a complete antibody structure. An antigen-binding fragment is capable of binding to the same antigen to which the parent antibody or a parent antibody fragment (*e.g.*, a parent scFv) binds. In some embodiments, an antigen-binding fragment may comprise one or more CDRs from a particular human antibody grafted to a framework region from one or more different human antibodies.

[94] "Fv" is the minimum antibody fragment which contains a complete antigen-recognition and -binding site. This fragment consists of a dimer of one heavy- and one light-chain variable region domain in tight, non-covalent association. From the folding of these two domains emanate six hypervariable loops (3 loops each from the heavy and light chain) that contribute the amino acid residues for antigen binding and confer antigen binding specificity to the antibody. However, even a single variable domain (or half of an Fv comprising only three CDRs specific for an antigen) has the ability to recognize and bind antigen, although at a lower affinity than the entire binding site.

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

[96] The term "diabodies" refers to small antibody fragments prepared by constructing scFv fragments (see preceding paragraph) typically with short linkers (such as about 5 to about 10 residues) between the V_H and V_L domains such that inter-chain but not intra-chain pairing of the V domains is achieved, resulting in a bivalent fragment, *i.e.*, fragment having two antigen-binding sites. Bispecific diabodies are heterodimers of two "crossover" scFv fragments in which the V_H and V_L domains of the two antibodies are present on different polypeptide chains. Diabodies are described more fully in, for example, EP 404,097; WO 93/11161; and Hollinger *et al.*, *Proc. Natl. Acad. Sci. USA*, 90:6444-6448 (1993).

[97] As used herein, the term "CDR" or "complementarity determining region" is intended to mean the non-contiguous antigen combining sites found within the variable region of both heavy and light chain polypeptides. These particular regions have been described by Kabat *et al.*, J. Biol. Chem. 252:6609-6616 (1977); Kabat *et al.*, U.S. Dept. of Health and Human Services, "Sequences of proteins of immunological interest" (1991); Chothia *et al.*, J. Mol. Biol. 196:901-917 (1987); Al-Lazikani B. *et al.*, *J. Mol. Biol.*, 273: 927-948 (1997); MacCallum *et al.*, J. Mol. Biol. 262:732-745 (1996); Abhinandan and Martin, *Mol. Immunol.*, 45: 3832-3839 (2008); Lefranc M.P. *et al.*, *Dev. Comp. Immunol.*, 27: 55-77 (2003); and Honegger and Plückthun, *J. Mol. Biol.*, 309:657-670 (2001), where the definitions include overlapping or subsets of amino acid residues when compared against each other. Nevertheless, application of either definition to refer to a CDR of an antibody or grafted antibodies or variants thereof is intended to be within the scope of the term as defined and used herein. The amino acid residues which encompass the CDRs as defined by each of the above cited references are set forth below in Table 1 as a comparison. CDR prediction algorithms and interfaces are known in the art, including, for example, Abhinandan and Martin, *Mol. Immunol.*, 45: 3832-3839 (2008); Ehrenmann F. *et al.*, *Nucleic Acids Res.*, 38: D301-D307 (2010); and Adolf-Bryfogle J. *et al.*, *Nucleic Acids Res.*, 43: D432-D438 (2015). The contents of the references cited in this paragraph are incorporated herein by reference in their entireties for use in the present invention and for possible inclusion in one or more claims herein.

TABLE 1: CDR DEFINITIONS

	Kabat[1]	**Chothia[2]**	**MacCallum[3]**	**IMGT[4]**	**AHo[5]**
V_H CDR1	31-35	26-32	30-35	27-38	25-40
V_H CDR2	50-65	53-55	47-58	56-65	58-77
V_H CDR3	95-102	96-101	93-101	105-117	109-137
V_L CDR1	24-34	26-32	30-36	27-38	25-40
V_L CDR2	50-56	50-52	46-55	56-65	58-77
V_L CDR3	89-97	91-96	89-96	105-117	109-137

[1]Residue numbering follows the nomenclature of Kabat *et al.*, *supra*

[2]Residue numbering follows the nomenclature of Chothia *et al.*, *supra*

[3]Residue numbering follows the nomenclature of MacCallum *et al.*, *supra*

[4]Residue numbering follows the nomenclature of Lefranc *et al.*, *supra*

[5]Residue numbering follows the nomenclature of Honegger and Plückthun, *supra*

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

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

[100] "Framework" or "FR" residues are those variable-domain residues other than the CDR residues as herein defined.

[101] The term "chimeric antibodies" refer to antibodies in which a portion of the heavy and/or light chain is identical with or homologous to corresponding sequences in antibodies derived from a particular species or belonging to a particular antibody class or subclass, while the remainder of the chain(s) is identical with or homologous to corresponding sequences in antibodies derived from another species or belonging to another antibody class or subclass, as well as fragments of such antibodies, so long as they exhibit a biological activity of this invention (*see* U.S. Patent No. 4,816,567; and Morrison *et al.*, Proc. Natl. Acad. Sci. USA, 81:6851-6855 (1984)).

[102] The term "semi-synthetic" in reference to an antibody or antibody moiety means that the antibody or antibody moiety has one or more naturally occurring sequences and one or more non-naturally occurring (*i.e.*, synthetic) sequences.

[103] "Humanized" forms of non-human (*e.g.*, rodent) antibodies are chimeric antibodies that contain minimal sequence derived from the non-human antibody. For the most part, humanized antibodies are human immunoglobulins (recipient antibody) in which residues from a hypervariable region (HVR) of the recipient are replaced by residues from a hypervariable region of a non-human species (donor antibody) such as mouse, rat, rabbit or non-human primate having the desired antibody specificity, affinity, and capability. In some instances, framework region (FR) residues of the human immunoglobulin are replaced by corresponding non-human

residues. Furthermore, humanized antibodies can comprise residues that are not found in the recipient antibody or in the donor antibody. These modifications are made to further refine antibody performance. In general, the humanized antibody will comprise substantially all of at least one, and typically two, variable domains, in which all or substantially all of the hypervariable loops correspond to those of a non-human immunoglobulin and all or substantially all of the FRs are those of a human immunoglobulin sequence. The humanized antibody optionally also will comprise at least a portion of an immunoglobulin constant region (Fc), typically that of a human immunoglobulin. For further details, *see* Jones *et al.*, *Nature* 321:522-525 (1986); Riechmann *et al.*, *Nature* 332:323-329 (1988); and Presta, *Curr. Op. Struct. Biol.* 2:593-596 (1992).

[104] A "human antibody" is an antibody that possesses an amino-acid sequence corresponding to that of an antibody produced by a human and/or has been made using any of the techniques for making human antibodies as disclosed herein. This definition of a human antibody specifically excludes a humanized antibody comprising non-human antigen-binding residues. Human antibodies can be produced using various techniques known in the art, including phage-display libraries. Hoogenboom and Winter, *J. Mol. Biol.*, 227:381 (1991); Marks *et al.*, *J. Mol. Biol.*, 222:581 (1991). Also available for the preparation of human monoclonal antibodies are methods described in Cole *et al.*, *Monoclonal Antibodies and Cancer Therapy*, Alan R. Liss, p. 77 (1985); Boerner *et al.*, *J. Immunol.*, 147(1):86-95 (1991). See also van Dijk and van de Winkel, *Curr. Opin. Pharmacol.*, 5: 368-74 (2001). Human antibodies can be prepared by administering the antigen to a transgenic animal that has been modified to produce such antibodies in response to antigenic challenge, but whose endogenous loci have been disabled, *e.g.*, immunized xenomice (see, *e.g.*, U.S. Pat. Nos. 6,075,181 and 6,150,584 regarding XENOMOUSE™ technology). See also, for example, Li *et al.*, *Proc. Natl. Acad. Sci. USA*, 103:3557-3562 (2006) regarding human antibodies generated via a human B-cell hybridoma technology.

[105] "Percent (%) amino acid sequence identity" or "homology" with respect to the polypeptide and antibody sequences identified herein is defined as the percentage of amino acid residues in a candidate sequence that are identical with the amino acid residues in the polypeptide being compared, after aligning the sequences considering any conservative substitutions as part of the sequence identity. Alignment for purposes of determining percent amino acid sequence identity can be achieved in various ways that are within the skill in the art, for instance, using publicly available computer software such as BLAST, BLAST-2, ALIGN, Megalign (DNASTAR), or MUSCLE software. Those skilled in the art can determine appropriate parameters for measuring alignment, including any algorithms needed to achieve maximal alignment over the full-length of the sequences being compared. For purposes herein, however, % amino acid sequence identity values are generated using the sequence comparison computer program MUSCLE (Edgar, R.C., *Nucleic Acids Research* 32(5):1792-1797, 2004; Edgar, R.C., *BMC Bioinformatics* 5(1):113, 2004).

[106] "Homologous" refers to the sequence similarity or sequence identity between two polypeptides or between two nucleic acid molecules. When a position in both of the two compared sequences is occupied by the same base or amino acid monomer subunit, *e.g.*, if a position in each of two DNA molecules is occupied by adenine, then the molecules are homologous at that position. The percent of homology between two sequences is a function of the number of matching or homologous positions shared by the two sequences divided by the number of positions compared times 100. For example, if 6 of 10 of the positions in two sequences are matched or homologous then the two sequences are 60% homologous. By way of example, the DNA sequences ATTGCC and TATGGC share 50% homology. Generally, a comparison is made when two sequences are aligned to give maximum homology.

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

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

[109] The "CH1 domain" of a human IgG Fc region (also referred to as "C1" of "H1" domain) usually extends from about amino acid 118 to about amino acid 215 (EU numbering system).

[110] "Hinge region" is generally defined as stretching from Glu216 to Pro230 of human IgG1 (Burton, *Molec. Immunol.*22:161-206 (1985)). Hinge regions of other IgG isotypes may be aligned with the IgG1 sequence by placing the first and last cysteine residues forming inter-heavy chain S-S bonds in the same positions.

[111] The "CH2 domain" of a human IgG Fc region (also referred to as "C2" of "H2" domain) usually extends from about amino acid 231 to about amino acid 340. The CH2 domain is unique in that it is not closely paired with another domain. Rather, two N-linked branched carbohydrate chains are interposed between the two CH2 domains of an intact native IgG molecule. It has been speculated that the carbohydrate may provide a substitute for the domain-domain pairing and help stabilize the CH2 domain. Burton, *Molec Immunol.* 22:161-206 (1985).

[112] The "CH3 domain" (also referred to as "C2" or "H3" domain) comprises the stretch of residues C-terminal to a CH2 domain in an Fc region (*i.e.* from about amino acid residue 341 to the C-terminal end of an antibody sequence, typically at amino acid residue 446 or 447 of an IgG).

[113] The term "Fc region" or "fragment crystallizable region" herein is used to define a C-terminal region of an immunoglobulin heavy chain, including native-sequence Fc regions and variant Fc regions. Although the

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

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

[115] The term "epitope" as used herein refers to the specific group of atoms or amino acids on an antigen to which an antibody or antibody moiety binds. Two antibodies or antibody moieties may bind the same epitope within an antigen if they exhibit competitive binding for the antigen.

[116] As used herein, a first antibody moiety "competes" for binding to a target antigen with a second antibody moiety when the first antibody moiety inhibits the target antigen binding of the second antibody moiety by at least about 50% (such as at least about any one of 55%, 60%, 65%, 70%, 75%, 80%, 85%, 90%, 95%, 98% or 99%) in the presence of an equimolar concentration of the first antibody moiety, or *vice versa*. A high throughput process for "binning" antibodies based upon their cross-competition is described in PCT Publication No. WO 03/48731.

[117] As use herein, the terms "specifically binds," "specifically recognizing," and "is specific for" refer to measurable and reproducible interactions, such as binding between a target and an antibody or antibody moiety, which is determinative of the presence of the target in the presence of a heterogeneous population of molecules, including biological molecules. For example, an antibody or antibody moiety that specifically recognizes a

target (which can be an epitope) is an antibody or antibody moiety that binds this target with greater affinity, avidity, more readily, and/or with greater duration than its bindings to other targets. In some embodiments, the extent of binding of an antibody to an unrelated target is less than about 10% of the binding of the antibody to the target as measured, *e.g.*, by a radioimmunoassay (RIA). In some embodiments, an antibody that specifically binds a target has a dissociation constant (K_D) of $\leq 10^{-5}$ M, $\leq 10^{-6}$ M, $\leq 10^{-7}$ M, $\leq 10^{-8}$ M, $\leq 10^{-9}$ M, $\leq 10^{-10}$ M, $\leq 10^{-11}$ M, or $\leq 10^{-12}$ M. In some embodiments, an antibody specifically binds an epitope on a protein that is conserved among the protein from different species. In some embodiments, specific binding can include, but does not require exclusive binding. Binding specificity of the antibody or antigen-binding domain can be determined experimentally by methods known in the art. Such methods comprise, but are not limited to Western blots, ELISA-, RIA-, ECL-, IRMA-, EIA-, BIACORE™ -tests and peptide scans.

[118] An "isolated" antibody (or construct) is one that has been identified, separated and/or recovered from a component of its production environment (*e.g.*, natural or recombinant). Preferably, the isolated polypeptide is free of association with all other components from its production environment.

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

[120] The term "control sequences" refers to DNA sequences necessary for the expression of an operably linked coding sequence in a particular host organism. The control sequences that are suitable for prokaryotes, for example, include a promoter, optionally an operator sequence, and a ribosome binding site. Eukaryotic cells are known to utilize promoters, polyadenylation signals, and enhancers.

[121] Nucleic acid is "operably linked" when it is placed into a functional relationship with another nucleic acid sequence. For example, DNA for a presequence or secretory leader is operably linked to DNA for a polypeptide if it is expressed as a preprotein that participates in the secretion of the polypeptide; a promoter or enhancer is operably linked to a coding sequence if it affects the transcription of the sequence; or a ribosome binding site is operably linked to a coding sequence if it is positioned so as to facilitate translation. Generally, "operably linked" means that the DNA sequences being linked are contiguous, and, in the case of a secretory

leader, contiguous and in reading phase. However, enhancers do not have to be contiguous. Linking is accomplished by ligation at convenient restriction sites. If such sites do not exist, the synthetic oligonucleotide adaptors or linkers are used in accordance with conventional practice.

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

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

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

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

[126] In the context of cancer, the term "treating" includes any or all of: inhibiting growth of cancer cells, inhibiting replication of cancer cells, lessening of overall tumor burden and ameliorating one or more symptoms associated with the disease.

[127] In the context of an autoimmune disease, the term "treating" includes any or all of: preventing replication of cells associated with an autoimmune disease state including, but not limited to, cells capable of producing an autoimmune antibody, lessening the autoimmune-antibody burden and ameliorating one or more symptoms of an autoimmune disease. In the context of an infectious disease, the term "treating" includes any or all of preventing the growth, multiplication or replication of the pathogen that causes the infectious disease and ameliorating one or more symptoms of an infectious disease. In the context of an ischemic disease, the term "treating" includes any or all of preventing the growth, multiplication or replication of the pathogen that causes the ischemic disease and ameliorating one or more symptoms of an ischemic disease.

[128] The terms "inhibition" or "inhibit" refer to a decrease or cessation of any phenotypic characteristic or to the decrease or cessation in the incidence, degree, or likelihood of that characteristic. To "reduce" or "inhibit" is to decrease, reduce or arrest an activity, function, and/or amount as compared to a reference. In certain embodiments, by "reduce" or "inhibit" is meant the ability to cause an overall decrease of 20% or greater. In another embodiment, by "reduce" or "inhibit" is meant the ability to cause an overall decrease of 50% or greater. In yet another embodiment, by "reduce" or "inhibit" is meant the ability to cause an overall decrease of 75%, 85%, 90%, 95%, or greater.

[129] A "reference" as used herein, refers to any sample, standard, or level that is used for comparison purposes. A reference may be obtained from a healthy and/or non-diseased sample. In some examples, a reference may be obtained from an untreated sample. In some examples, a reference is obtained from a non-diseased on non-treated sample of a subject individual. In some examples, a reference is obtained from one or more healthy individuals who are not the subject or patient.

[130] As used herein, "delaying development of a disease" means to defer, hinder, slow, retard, stabilize, suppress and/or postpone development of the disease (such as cancer). This delay can be of varying lengths of time, depending on the history of the disease and/or individual being treated. As is evident to one skilled in the art, a sufficient or significant delay can, in effect, encompass prevention, in that the individual does not develop the disease. For example, a late stage cancer, such as development of metastasis, may be delayed.

[131] "Preventing," as used herein, includes providing prophylaxis with respect to the occurrence or recurrence of a disease in a subject that may be predisposed to the disease but has not yet been diagnosed with the disease.

[132] As used herein, to "suppress" a function or activity is to reduce the function or activity when compared to otherwise same conditions except for a condition or parameter of interest, or alternatively, as compared to another condition. For example, an antibody which suppresses tumor growth reduces the rate of growth of the tumor compared to the rate of growth of the tumor in the absence of the antibody.

[133] The terms "subject," "individual," and "patient" are used interchangeably herein to refer to a mammal, including, but not limited to, human, bovine, horse, feline, canine, rodent, or primate. In some embodiments, the individual is a human.

[134] An "effective amount" of an agent refers to an amount effective, at dosages and for periods of time necessary, to achieve the desired therapeutic or prophylactic result. The term also applies to a dose that will provide an image for detection by any one of the imaging methods described herein. The specific dose may vary depending on one or more of: the particular agent chosen, the dosing regimen to be followed, whether it is administered in combination with other compounds, timing of administration, the tissue to be imaged, and the physical delivery system in which it is carried.

[135] A "therapeutically effective amount" of a substance/molecule of the invention, agonist or antagonist may vary according to factors such as the disease state, age, sex, and weight of the individual, and the ability of the substance/molecule, agonist or antagonist to elicit a desired response in the individual. A therapeutically effective amount is also one in which any toxic or detrimental effects of the substance/molecule, agonist or antagonist are outweighed by the therapeutically beneficial effects. A therapeutically effective amount may be delivered in one or more administrations.

[136] A "prophylactically effective amount" refers to an amount effective, at dosages and for periods of time necessary, to achieve the desired prophylactic result. Typically, but not necessarily, since a prophylactic dose is used in subjects prior to or at an earlier stage of disease, the prophylactically effective amount will be less than the therapeutically effective amount.

[137] The terms "pharmaceutical formulation" and "pharmaceutical composition" refer to a preparation which is in such form as to permit the biological activity of the active ingredient(s) to be effective, and which contains no additional components which are unacceptably toxic to a subject to which the formulation would be administered. Such formulations may be sterile.

[138] A "pharmaceutically acceptable carrier" refers to a non-toxic solid, semisolid, or liquid filler, diluent, encapsulating material, formulation auxiliary, or carrier conventional in the art for use with a therapeutic agent that together comprise a "pharmaceutical composition" for administration to a subject. A pharmaceutically acceptable carrier is non-toxic to recipients at the dosages and concentrations employed and is compatible with other ingredients of the formulation. The pharmaceutically acceptable carrier is appropriate for the formulation employed.

[139] A "sterile" formulation is aseptic or essentially free from living microorganisms and their spores.

[140] Administration "in combination with" one or more further therapeutic agents includes simultaneous (concurrent) and consecutive or sequential administration in any order.

[141] The term "concurrently" is used herein to refer to administration of two or more therapeutic agents, where at least part of the administration overlaps in time or where the administration of one therapeutic agent falls within a short period of time relative to administration of the other therapeutic agent. For example, the two or more therapeutic agents are administered with a time separation of no more than about 60 minutes, such as no more than about any of 30, 15, 10, 5, or 1 minutes.

[142] The term "sequentially" is used herein to refer to administration of two or more therapeutic agents where the administration of one or more agent(s) continues after discontinuing the administration of one or more other agent(s). For example, administration of the two or more therapeutic agents are administered with a time separation of more than about 15 minutes, such as about any of 20, 30, 40, 50, or 60 minutes, 1 day, 2 days, 3 days, 1 week, 2 weeks, or 1 month, or longer.

[143] As used herein, "in conjunction with" refers to administration of one treatment modality in addition to another treatment modality. As such, "in conjunction with" refers to administration of one treatment modality before, during or after administration of the other treatment modality to the individual.

[144] The term "package insert" is used to refer to instructions customarily included in commercial packages of therapeutic products, that contain information about the indications, usage, dosage, administration, combination therapy, contraindications and/or warnings concerning the use of such therapeutic products.

[145] An "article of manufacture" is any manufacture (*e.g.*, a package or container) or kit comprising at least one reagent, *e.g.*, a medicament for treatment of a disease or disorder (*e.g.*, cancer), or a probe for specifically detecting a biomarker described herein. In certain embodiments, the manufacture or kit is promoted, distributed, or sold as a unit for performing the methods described herein.

[146] It is understood that embodiments of the invention described herein include "consisting" and/or "consisting essentially of" embodiments.

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

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

[149] The term "about X-Y" used herein has the same meaning as "about X to about Y."

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

II. Methods of imaging

[151] One aspect of the present application provides a method of determining the distribution and/or expression level of an immune checkpoint ligand in an individual using an imaging agent comprising an antibody moiety labeled with a radionuclide, wherein the antibody moiety specifically binds the immune checkpoint ligand. In some embodiments, the method comprises determination of the distribution and/or expression level of two or more immune checkpoint ligands in the individual.

[152] In some embodiments, there is provided a method of determining the distribution of an immune checkpoint ligand in an individual, comprising: (a) administering to the individual an imaging agent comprising an antibody moiety labeled with a radionuclide, wherein the antibody fragment specifically binds the immune checkpoint ligand; and (b) imaging the imaging agent in the individual with a non-invasive imaging technique. In some embodiments, the method further comprises determining the expression level of the immune checkpoint ligand in a tissue of interest in the individual based on signals emitted by the imaging agent from the tissue. In some embodiments, the method further comprises preparing the imaging agent by labeling the antibody moiety with the radionuclide. In some embodiments, the non-invasive imaging technique comprises single photon emission computed tomography (SPECT) imaging or positron emission tomography (PET) imaging. In some embodiments, the non-invasive imaging technique further comprises computed tomography imaging, magnetic resonance imaging, chemical luminescence imaging, or electrochemical luminescence imaging. In some embodiments, the imaging agent is administered intravenously, intraperitoneally, intramuscularly, subcutaneously, or orally. In some embodiments, the imaging is carried out between about 10 minutes to about 24 hours after the administration of the imaging agent. In some embodiments, the method further comprises administering to the individual an antibody moiety not labeled with a radioisotope prior to the administration of the imaging agent. In some embodiments, the method comprises imaging the individual over a period of time. In some embodiments, the immune checkpoint ligand is selected from the group consisting of PD-L1, PD-L2, B7-H3, galectin-9, CD80, CD86 and ICOSL. In some embodiments, the immune checkpoint ligand is a PD-L1 like ligand. In some embodiments, the radionuclide is selected from the group consisting of ^{64}Cu, ^{18}F, ^{67}Ga, ^{68}Ga, ^{111}In, ^{177}Lu, ^{90}Y, ^{89}Zr, ^{61}Cu, ^{62}Cu, ^{67}Cu, ^{19}F, ^{66}Ga, ^{72}Ga, ^{44}Sc, ^{47}Sc, ^{86}Y, ^{88}Y and ^{45}Ti. In some embodiments, the radionuclide is ^{68}Ga. In some embodiments, the antibody moiety is conjugated to a chelating compound that chelates the radionuclide. In some embodiments, the chelating compound is 1,4,7-triazacyclononane-l,4,7-trisacetic acid (NOTA), 1,4,7,10-tetraazacyclododecane-1,4,7,10-tetraacetic acid (DOTA) or derivatives thereof. In some embodiments, the antibody moiety has a half-life of about 10 minutes to about 24 hours (such as about any one of 10 minutes to 2 hours, 1 hour to 4 hours, 4 hours to 8 hours, 8 hours to 12 hours or 12 hours to 24 hours) in serum. In some embodiments, the antibody moiety is no more than about 120 kDa (such as no more than about 30 kDa, 50 kDa, 80 kDa, or 100 kDa, or about any one of 30-50kDa, 50-

100kDa, or 30-80kDa). In some embodiments, the antibody moiety has a K_D between about 9×10^{-10} M to about 1×10^{-8} M (such as about 9×10^{-10} to 1×10^{-9}, about 1×10^{-9} to 2×10^{-9}, about 2×10^{-10} to 5×10^{-9}, or about 5×10^{-10} to 1×10^{-8}) with the immune checkpoint ligand. In some embodiments, the antibody moiety cross-reacts with the immune checkpoint ligand from a non-human mammal (*e.g.*, mouse, rat or monkey). In some embodiments, the antibody moiety is humanized. In some embodiments, the antibody moiety is stable at acidic pH (*e.g.*, at a pH lower than about 6.5, 6.0, 5.5, or 5.0). In some embodiments, the antibody moiety has a melting temperature (Tm) of about 55-70°C (such as about any one of 55-60, 60-65, or 65-70°C). In some embodiments, the antibody moiety is selected from the group consisting of a single-chain Fv (scFv), a diabody, a Fab, a Fab', a $F(ab')_2$, an Fv fragment, a disulfide stabilized Fv fragment (dsFv), a $(dsFv)_2$, and a V_HH.

[153] In some embodiments, there is provided a method of determining the distribution of an immune checkpoint ligand in an individual, comprising: (a) administering to the individual an imaging agent comprising an scFv labeled with a radionuclide, wherein the scFv specifically binds the immune checkpoint ligand; and (b) imaging the imaging agent in the individual with a non-invasive imaging technique. In some embodiments, the method further comprises determining the expression level of the immune checkpoint ligand in a tissue of interest in the individual based on signals emitted by the imaging agent from the tissue. In some embodiments, the method further comprises preparing the imaging agent by labeling the scFv with the radionuclide. In some embodiments, the non-invasive imaging technique comprises single photon emission computed tomography (SPECT) imaging or positron emission tomography (PET) imaging. In some embodiments, the non-invasive imaging technique further comprises computed tomography imaging, magnetic resonance imaging, chemical luminescence imaging, or electrochemical luminescence imaging. In some embodiments, the imaging agent is administered intravenously, intraperitoneally, intramuscularly, subcutaneously, or orally. In some embodiments, the imaging is carried out between about 10 minutes to about 24 hours after the administration of the imaging agent. In some embodiments, the method further comprises administering to the individual the scFv not labeled with a radioisotope prior to the administration of the imaging agent. In some embodiments, the method comprises imaging the individual over a period of time. In some embodiments, the immune checkpoint ligand is selected from the group consisting of PD-L1, PD-L2, B7-H3, galectin-9, CD80, CD86 and ICOSL. In some embodiments, the immune checkpoint ligand is a PD-L1 like ligand. In some embodiments, the radionuclide is selected from the group consisting of ^{64}Cu, ^{18}F, ^{67}Ga, ^{68}Ga, ^{111}In, ^{177}Lu, ^{90}Y, ^{89}Zr, ^{61}Cu, ^{62}Cu, ^{67}Cu, ^{19}F, ^{66}Ga, ^{72}Ga, ^{44}Sc, ^{47}Sc, ^{86}Y, ^{88}Y and ^{45}Ti. In some embodiments, the radionuclide is ^{68}Ga. In some embodiments, the scFv is conjugated to a chelating compound that chelates the radionuclide. In some embodiments, the chelating compound is NOTA, DOTA or derivatives thereof. In some embodiments, the scFv has a K_D between about 9×10^{-10} M to about 1×10^{-8} M (such as about 9×10^{-10} to 1×10^{-9}, about 1×10^{-9} to 2×10^{-9}, about 2×10^{-10} to 5×10^{-9}, or about 5×10^{-10} to 1×10^{-8}) with the immune checkpoint ligand. In some embodiments, the scFv cross-reacts

with the immune checkpoint ligand from a non-human mammal (*e.g.*, mouse, rat or monkey). In some embodiments, the scFv is humanized. In some embodiments, the scFv is stable at acidic pH (*e.g.*, at a pH lower than about 6.5, 6.0, 5.5, or 5.0). In some embodiments, the scFv has a melting temperature (Tm) of about 55-70°C (such as about any one of 55-60, 60-65, or 65-70°C). In some embodiments, the scFv comprises one or more engineered disulfide bonds. In some embodiments, the scFv comprises from the N-terminus to the C-terminus: a V_H, an optional peptide linker, and a V_L. In some embodiments, the scFv comprises from the N-terminus to the C-terminus: a V_L, an optional peptide linker, and a V_H. In some embodiments, the scFv comprises a peptide linker comprising the amino acid sequence of SEQ ID NO: 47 or 48. In some embodiments, the scFv comprises one or more (such as 1, 2, 3, or more) engineered disulfide bonds. In some embodiments, the scFv comprises a first engineered cysteine residue at position 44 of V_H and a second engineered cysteine residue at position 100 of V_L, and/or a first engineered cysteine residue at position 105 of V_H and a second engineered cysteine residue at position 43 of V_L, wherein the first engineered cysteine residue and the second engineered cysteine residue form a disulfide bond, and wherein the amino acid positions are based on the Kabat numbering system.

[154] In some embodiments, there is provided a method of determining the distribution of an immune checkpoint ligand in an individual, comprising: (a) administering to the individual an imaging agent comprising an antibody moiety labeled with a radionuclide, wherein the antibody moiety is an scFv fused to an Fc fragment, wherein the antibody fragment specifically binds the immune checkpoint ligand; and (b) imaging the imaging agent in the individual with a non-invasive imaging technique. In some embodiments, the method further comprises determining the expression level of the immune checkpoint ligand in a tissue of interest in the individual based on signals emitted by the imaging agent from the tissue. In some embodiments, the method further comprises preparing the imaging agent by labeling the antibody moiety with the radionuclide. In some embodiments, the non-invasive imaging technique comprises single photon emission computed tomography (SPECT) imaging or positron emission tomography (PET) imaging. In some embodiments, the non-invasive imaging technique further comprises computed tomography imaging, magnetic resonance imaging, chemical luminescence imaging, or electrochemical luminescence imaging. In some embodiments, the imaging agent is administered intravenously, intraperitoneally, intramuscularly, subcutaneously, or orally. In some embodiments, the imaging is carried out between about 10 minutes to about 24 hours after the administration of the imaging agent. In some embodiments, the method further comprises administering to the individual an antibody moiety not labeled with a radioisotope prior to the administration of the imaging agent. In some embodiments, the method comprises imaging the individual over a period of time. In some embodiments, the immune checkpoint ligand is selected from the group consisting of PD-L1, PD-L2, B7-H3, galectin-9, CD80, CD86 and ICOSL. In some embodiments, the immune checkpoint ligand is a PD-L1 like ligand. In some embodiments, the

radionuclide is selected from the group consisting of ^{64}Cu, ^{18}F, ^{67}Ga, ^{68}Ga, ^{111}In, ^{177}Lu, ^{90}Y, ^{89}Zr, ^{61}Cu, ^{62}Cu, ^{67}Cu, ^{19}F, ^{66}Ga, ^{72}Ga, ^{44}Sc, ^{47}Sc, ^{86}Y, ^{88}Y and ^{45}Ti. In some embodiments, the radionuclide is ^{68}Ga. In some embodiments, the antibody moiety is conjugated to a chelating compound that chelates the radionuclide. In some embodiments, the chelating compound is NOTA, DOTA or derivatives thereof. In some embodiments, the antibody moiety has a K_D between about 9×10^{-10} M to about 1×10^{-8} M (such as about 9×10^{-10} to 1×10^{-9}, about 1×10^{-9} to 2×10^{-9}, about 2×10^{-10} to 5×10^{-9}, or about 5×10^{-10} to 1×10^{-8}) with the immune checkpoint ligand. In some embodiments, the antibody moiety cross-reacts with the immune checkpoint ligand from a non-human mammal (*e.g.*, mouse, rat or monkey). In some embodiments, the antibody moiety is humanized. In some embodiments, the antibody moiety is stable at acidic pH (*e.g.*, at a pH lower than about 6.5, 6.0, 5.5, or 5.0). In some embodiments, the antibody moiety has a melting temperature (Tm) of about 55-70°C (such as about any one of 55-60, 60-65, or 65-70°C). In some embodiments, the scFv comprises one or more engineered disulfide bonds. In some embodiments, the scFv comprises from the N-terminus to the C-terminus: a V_H, an optional peptide linker, and a V_L. In some embodiments, the scFv comprises from the N-terminus to the C-terminus: a V_L, an optional peptide linker, and a V_H. In some embodiments, the scFv comprises a peptide linker comprising the amino acid sequence of SEQ ID NO: 47 or 48. In some embodiments, the scFv comprises one or more (such as 1, 2, 3, or more) engineered disulfide bonds. In some embodiments, the scFv comprises a first engineered cysteine residue at position 44 of V_H and a second engineered cysteine residue at position 100 of V_L, and/or a first engineered cysteine residue at position 105 of V_H and a second engineered cysteine residue at position 43 of V_L, wherein the first engineered cysteine residue and the second engineered cysteine residue form a disulfide bond, and wherein the amino acid positions are based on the Kabat numbering system. In some embodiments, the Fc fragment is a human IgG1 Fc fragment. In some embodiments, the Fc fragment has H310A and H435Q mutations, wherein the amino acid positions are based on the Kabat numbering system.

[155] In some embodiments, there is provided a method of determining the distribution of PD-L1 in an individual, comprising: (a) administering to the individual an imaging agent comprising an antibody moiety (*e.g.*, an scFv) labeled with a radionuclide, wherein the antibody fragment specifically binds PD-L1; and (b) imaging the imaging agent in the individual with a non-invasive imaging technique. In some embodiments, the method further comprises determining the expression level of PD-L1 in a tissue of interest in the individual based on signals emitted by the imaging agent from the tissue. In some embodiments, the method further comprises preparing the imaging agent by labeling the antibody moiety with the radionuclide. In some embodiments, the non-invasive imaging technique comprises single photon emission computed tomography (SPECT) imaging or positron emission tomography (PET) imaging. In some embodiments, the non-invasive imaging technique further comprises computed tomography imaging, magnetic resonance imaging, chemical luminescence imaging, or electrochemical luminescence imaging. In some embodiments, the imaging agent is

administered intravenously, intraperitoneally, intramuscularly, subcutaneously, or orally. In some embodiments, the imaging is carried out between about 10 minutes to about 24 hours after the administration of the imaging agent. In some embodiments, the method further comprises administering to the individual an antibody moiety not labeled with a radioisotope prior to the administration of the imaging agent. In some embodiments, the method comprises imaging the individual over a period of time. In some embodiments, the radionuclide is selected from the group consisting of ^{64}Cu, ^{18}F, ^{67}Ga, ^{68}Ga, ^{111}In, ^{177}Lu, ^{90}Y, ^{89}Zr, ^{61}Cu, ^{62}Cu, ^{67}Cu, ^{19}F, ^{66}Ga, ^{72}Ga, ^{44}Sc, ^{47}Sc, ^{86}Y, ^{88}Y and ^{45}Ti. In some embodiments, the radionuclide is ^{68}Ga. In some embodiments, the anti-PD-L1 antibody moiety is conjugated to a chelating compound that chelates the radionuclide. In some embodiments, the chelating compound is NOTA, DOTA or derivatives thereof. In some embodiments, the anti-PD-L1 antibody moiety has a half-life of about 10 minutes to about 24 hours (such as about any one of 10 minutes to 2 hours, 1 hour to 4 hours, 4 hours to 8 hours, 8 hours to 12 hours or 12 hours to 24 hours) in serum. In some embodiments, the anti-PD-L1 antibody moiety is no more than about 120 kDa (such as no more than about 30 kDa, 50 kDa, 80 kDa, or 100 kDa, or about any one of 30-50kDa, 50-100kDa, or 30-80kDa). In some embodiments, the anti-PD-L1 antibody moiety has a K_D between about 9×10^{-10} M to about 1×10^{-8} M (such as about 9×10^{-10} to 1×10^{-9}, about 1×10^{-9} to 2×10^{-9}, about 2×10^{-10} to 5×10^{-9}, or about 5×10^{-10} to 1×10^{-8}) with the immune checkpoint ligand. In some embodiments, the anti-PD-L1 antibody moiety cross-reacts with the immune checkpoint ligand from a non-human mammal (*e.g.*, mouse, rat or monkey). In some embodiments, the anti-PD-L1 antibody moiety is humanized. In some embodiments, the anti-PD-L1 antibody moiety is stable at acidic pH (*e.g.*, at a pH lower than about 6.5, 6.0, 5.5, or 5.0). In some embodiments, the anti-PD-L1 antibody moiety has a melting temperature (Tm) of about 55-70°C (such as about any one of 55-60, 60-65, or 65-70°C). In some embodiments, the anti-PD-L1 antibody moiety is selected from the group consisting of a single-chain Fv (scFv), a diabody, a Fab, a Fab', a $F(ab')_2$, an Fv fragment, a disulfide stabilized Fv fragment (dsFv), a $(dsFv)_2$, and a V_HH. In some embodiments, the anti-PD-L1 antibody moiety is an scFv. In some embodiments, the anti-PD-L1 antibody moiety is an scFv fused to an Fc.

[156] In some embodiments, there is provided a method of determining the distribution of a PD-L1 like ligand in an individual, comprising: (a) administering to the individual an imaging agent comprising an antibody moiety (*e.g.*, an scFv) labeled with a radionuclide, wherein the antibody fragment specifically binds the PD-L1 like ligand; and (b) imaging the imaging agent in the individual with a non-invasive imaging technique. In some embodiments, the PD-L1 like ligand is a naturally occurring ligand of PD-1. In some embodiments, the PD-L1 like ligand is a non-naturally occurring ligand of PD-1, and wherein the PD-L1 like ligand has been administered to the individual. In some embodiments, the method further comprises determining the expression level of the PD-L1 like ligand in a tissue of interest in the individual based on signals emitted by the imaging agent from the tissue. In some embodiments, the method further comprises

preparing the imaging agent by labeling the antibody moiety with the radionuclide. In some embodiments, the non-invasive imaging technique comprises single photon emission computed tomography (SPECT) imaging or positron emission tomography (PET) imaging. In some embodiments, the non-invasive imaging technique further comprises computed tomography imaging, magnetic resonance imaging, chemical luminescence imaging, or electrochemical luminescence imaging. In some embodiments, the imaging agent is administered intravenously, intraperitoneally, intramuscularly, subcutaneously, or orally. In some embodiments, the imaging is carried out between about 10 minutes to about 24 hours after the administration of the imaging agent. In some embodiments, the method further comprises administering to the individual an antibody moiety not labeled with a radioisotope prior to the administration of the imaging agent. In some embodiments, the method comprises imaging the individual over a period of time. In some embodiments, the radionuclide is selected from the group consisting of ^{64}Cu, ^{18}F, ^{67}Ga, ^{68}Ga, ^{111}In, ^{177}Lu, ^{90}Y, ^{89}Zr, ^{61}Cu, ^{62}Cu, ^{67}Cu, ^{19}F, ^{66}Ga, ^{72}Ga, ^{44}Sc, ^{47}Sc, ^{86}Y, ^{88}Y and ^{45}Ti. In some embodiments, the radionuclide is ^{68}Ga. In some embodiments, the antibody moiety is conjugated to a chelating compound that chelates the radionuclide. In some embodiments, the chelating compound is NOTA, DOTA or derivatives thereof. In some embodiments, the antibody moiety has a half-life of about 10 minutes to about 24 hours (such as about any one of 10 minutes to 2 hours, 1 hour to 4 hours, 4 hours to 8 hours, 8 hours to 12 hours or 12 hours to 24 hours) in serum. In some embodiments, the antibody moiety is no more than about 120 kDa (such as no more than about 30 kDa, 50 kDa, 80 kDa, or 100 kDa, or about any one of 30-50kDa, 50-100kDa, or 30-80kDa). In some embodiments, the antibody moiety has a K_D between about 9×10^{-10} M to about 1×10^{-8} M (such as about 9×10^{-10} to 1×10^{-9}, about 1×10^{-9} to 2×10^{-9}, about 2×10^{-10} to 5×10^{-9}, or about 5×10^{-10} to 1×10^{-8}) with the immune checkpoint ligand. In some embodiments, the antibody moiety cross-reacts with the immune checkpoint ligand from a non-human mammal (*e.g.*, mouse, rat or monkey). In some embodiments, the antibody moiety is humanized. In some embodiments, the antibody moiety is stable at acidic pH (*e.g.*, at a pH lower than about 6.5, 6.0, 5.5, or 5.0). In some embodiments, the antibody moiety has a melting temperature (Tm) of about 55-70°C (such as about any one of 55-60, 60-65, or 65-70°C). In some embodiments, the anti- antibody moiety is selected from the group consisting of a single-chain Fv (scFv), a diabody, a Fab, a Fab', a $F(ab')_2$, an Fv fragment, a disulfide stabilized Fv fragment (dsFv), a $(dsFv)_2$, and a V_HH. In some embodiments, the antibody moiety is an scFv. In some embodiments, the anti-PD-L1 antibody moiety is an scFv fused to an Fc.

[157] In some embodiments, there is provided a method of determining the distribution of PD-L1 in an individual, comprising: (a) administering to the individual an imaging agent comprising an anti-PD-L1 antibody moiety labeled with a radionuclide; and (b) imaging the imaging agent in the individual with a non-invasive imaging technique, wherein the anti-PD-L1 antibody moiety comprises: a V_H comprising a HC-CDR1 comprising the amino acid sequence of SEQ ID NO: 41, a HC-CDR2 comprising the amino acid sequence of

SEQ ID NO: 42, and a HC-CDR3 comprising the amino acid sequence of SEQ ID NO: 43, or a variant thereof comprising up to about 5 amino acid substitutions; and a V_L comprising a LC-CDR1 comprising the amino acid sequence of SEQ ID NO: 44, a LC-CDR2 comprising the amino acid sequence of SEQ ID NO: 45, and a LC-CDR3 comprising the amino acid sequence of SEQ ID NO: 46, or a variant thereof comprising up to about 5 amino acid substitutions. In some embodiments, the method further comprises determining the expression level of PD-L1 in a tissue of interest in the individual based on signals emitted by the imaging agent from the tissue. In some embodiments, the method further comprises preparing the imaging agent by labeling the antibody moiety with the radionuclide. In some embodiments, the non-invasive imaging technique comprises single photon emission computed tomography (SPECT) imaging or positron emission tomography (PET) imaging. In some embodiments, the non-invasive imaging technique further comprises computed tomography imaging, magnetic resonance imaging, chemical luminescence imaging, or electrochemical luminescence imaging. In some embodiments, the imaging agent is administered intravenously, intraperitoneally, intramuscularly, subcutaneously, or orally. In some embodiments, the imaging is carried out between about 10 minutes to about 24 hours after the administration of the imaging agent. In some embodiments, the method further comprises administering to the individual an antibody moiety not labeled with a radioisotope prior to the administration of the imaging agent. In some embodiments, the method comprises imaging the individual over a period of time. In some embodiments, the anti-PD-L1 antibody moiety comprises: a V_H comprising the amino acid sequence of any one of SEQ ID NOs: 1, 5, 9, 11, and 13, or a variant thereof having at least about 80% (such as at least about any one of 80%, 85%, 90%, 95%, 96%, 97%, 98%, or 99%) sequence identity to the amino acid sequence of any one of SEQ ID NOs: 1, 5, 9, 11, and 13; and a V_L comprising the amino acid sequence of any one of SEQ ID NOs: 3, 7, 15, 17 and 19, or a variant thereof having at least about 80% (such as at least about any one of 80%, 85%, 90%, 95%, 96%, 97%, 98%, or 99%) sequence identity to the amino acid sequence of any one of SEQ ID NOs: 3, 7, 15, 17 and 19. In some embodiments, the anti-PD-L1 antibody moiety is humanized. In some embodiments, the radionuclide is selected from the group consisting of ^{64}Cu, ^{18}F, ^{67}Ga, ^{68}Ga, ^{111}In, ^{177}Lu, ^{90}Y, ^{89}Zr, ^{61}Cu, ^{62}Cu, ^{67}Cu, ^{19}F, ^{66}Ga, ^{72}Ga, ^{44}Sc, ^{47}Sc, ^{86}Y, ^{88}Y and ^{45}Ti. In some embodiments, the radionuclide is ^{68}Ga. In some embodiments, the anti-PD-L1 antibody moiety is conjugated to a chelating compound that chelates the radionuclide. In some embodiments, the chelating compound is NOTA, DOTA or derivatives thereof.

[158] In some embodiments, there is provided a method of determining the distribution of PD-L1 in an individual, comprising: (a) administering to the individual an imaging agent comprising an anti-PD-L1 scFv labeled with a radionuclide; and (b) imaging the imaging agent in the individual with a non-invasive imaging technique, wherein the anti-PD-L1 scFv comprises: a V_H comprising a HC-CDR1 comprising the amino acid sequence of SEQ ID NO: 41, a HC-CDR2 comprising the amino acid sequence of SEQ ID NO: 42, and a HC-

CDR3 comprising the amino acid sequence of SEQ ID NO: 43, or a variant thereof comprising up to about 5 amino acid substitutions; and a V_L comprising a LC-CDR1 comprising the amino acid sequence of SEQ ID NO: 44, a LC-CDR2 comprising the amino acid sequence of SEQ ID NO: 45, and a LC-CDR3 comprising the amino acid sequence of SEQ ID NO: 46, or a variant thereof comprising up to about 5 amino acid substitutions. In some embodiments, the method further comprises determining the expression level of PD-L1 in a tissue of interest in the individual based on signals emitted by the imaging agent from the tissue. In some embodiments, the method further comprises preparing the imaging agent by labeling the antibody moiety with the radionuclide. In some embodiments, the non-invasive imaging technique comprises single photon emission computed tomography (SPECT) imaging or positron emission tomography (PET) imaging. In some embodiments, the non-invasive imaging technique further comprises computed tomography imaging, magnetic resonance imaging, chemical luminescence imaging, or electrochemical luminescence imaging. In some embodiments, the imaging agent is administered intravenously, intraperitoneally, intramuscularly, subcutaneously, or orally. In some embodiments, the imaging is carried out between about 10 minutes to about 24 hours after the administration of the imaging agent. In some embodiments, the method further comprises administering to the individual an antibody moiety not labeled with a radioisotope prior to the administration of the imaging agent. In some embodiments, the method comprises imaging the individual over a period of time. In some embodiments, the anti-PD-L1 scFv comprises: a V_H comprising the amino acid sequence of any one of SEQ ID NOs: 1, 5, 9, 11, and 13, or a variant thereof having at least about 80% (such as at least about any one of 80%, 85%, 90%, 95%, 96%, 97%, 98%, or 99%) sequence identity to the amino acid sequence of any one of SEQ ID NOs: 1, 5, 9, 11, and 13; and a V_L comprising the amino acid sequence of any one of SEQ ID NOs: 3, 7, 15, 17 and 19, or a variant thereof having at least about 80% (such as at least about any one of 80%, 85%, 90%, 95%, 96%, 97%, 98%, or 99%) sequence identity to the amino acid sequence of any one of SEQ ID NOs: 3, 7, 15, 17 and 19. In some embodiments, the anti-PD-L1 scFv is humanized. In some embodiments, the anti-PD-L1 scFv comprises a first engineered cysteine residue at position 44 of V_H and a second engineered cysteine residue at position 100 of V_L, or a first engineered cysteine residue at position 105 of V_H and a second engineered cysteine residue at position 43 of V_L, wherein the first engineered cysteine residue and the second engineered cysteine residue form a disulfide bond, and wherein the amino acid positions are based on the Kabat numbering system. In some embodiments, the anti-PD-L1 scFv comprises the amino acid sequence of any one of SEQ ID NOs: 25, 27, 29, 31, 33, 35, 37 and 39, or a variant thereof having at least about 80% (such as at least about any one of 80%, 85%, 90%, 95%, 96%, 97%, 98%, or 99%) sequence identity to the amino acid sequence of any one of SEQ ID NOs: 25, 27, 29, 31, 33, 35, 37 and 39. In some embodiments, the radionuclide is selected from the group consisting of ^{64}Cu, ^{18}F, ^{67}Ga, ^{68}Ga, ^{111}In, ^{177}Lu, ^{90}Y, ^{89}Zr, ^{61}Cu, ^{62}Cu, ^{67}Cu, ^{19}F, ^{66}Ga, ^{72}Ga, ^{44}Sc, ^{47}Sc, ^{86}Y, ^{88}Y and ^{45}Ti. In some embodiments, the radionuclide is ^{68}Ga. In some embodiments, the anti-PD-L1 antibody

moiety is conjugated to a chelating compound that chelates the radionuclide. In some embodiments, the chelating compound is NOTA, DOTA or derivatives thereof.

[159] In some embodiments, there is provided a method of determining the distribution of PD-L1 in an individual, comprising: (a) administering to the individual an imaging agent comprising an anti-PD-L1 antibody moiety labeled with a radionuclide; and (b) imaging the imaging agent in the individual with a non-invasive imaging technique, wherein the anti-PD-L1 antibody moiety comprises an anti-PD-L1 scFv fused to an Fc fragment, and wherein the anti-PD-L1 scFv comprises: a V_H comprising a HC-CDR1 comprising the amino acid sequence of SEQ ID NO: 41, a HC-CDR2 comprising the amino acid sequence of SEQ ID NO: 42, and a HC-CDR3 comprising the amino acid sequence of SEQ ID NO: 43, or a variant thereof comprising up to about 5 amino acid substitutions; and a V_L comprising a LC-CDR1 comprising the amino acid sequence of SEQ ID NO: 44, a LC-CDR2 comprising the amino acid sequence of SEQ ID NO: 45, and a LC-CDR3 comprising the amino acid sequence of SEQ ID NO: 46, or a variant thereof comprising up to about 5 amino acid substitutions. In some embodiments, the method further comprises determining the expression level of PD-L1 in a tissue of interest in the individual based on signals emitted by the imaging agent from the tissue. In some embodiments, the method further comprises preparing the imaging agent by labeling the antibody moiety with the radionuclide. In some embodiments, the non-invasive imaging technique comprises single photon emission computed tomography (SPECT) imaging or positron emission tomography (PET) imaging. In some embodiments, the non-invasive imaging technique further comprises computed tomography imaging, magnetic resonance imaging, chemical luminescence imaging, or electrochemical luminescence imaging. In some embodiments, the imaging agent is administered intravenously, intraperitoneally, intramuscularly, subcutaneously, or orally. In some embodiments, the imaging is carried out between about 10 minutes to about 24 hours after the administration of the imaging agent. In some embodiments, the method further comprises administering to the individual an antibody moiety not labeled with a radioisotope prior to the administration of the imaging agent. In some embodiments, the method comprises imaging the individual over a period of time. In some embodiments, the anti-PD-L1 antibody moiety comprises: a V_H comprising the amino acid sequence of any one of SEQ ID NOs: 1, 5, 9, 11, and 13, or a variant thereof having at least about 80% (such as at least about any one of 80%, 85%, 90%, 95%, 96%, 97%, 98%, or 99%) sequence identity to the amino acid sequence of any one of SEQ ID NOs: 1, 5, 9, 11, and 13; and a V_L comprising the amino acid sequence of any one of SEQ ID NOs: 3, 7, 15, 17 and 19, or a variant thereof having at least about 80% (such as at least about any one of 80%, 85%, 90%, 95%, 96%, 97%, 98%, or 99%) sequence identity to the amino acid sequence of any one of SEQ ID NOs: 3, 7, 15, 17 and 19. In some embodiments, the anti-PD-L1 antibody moiety is humanized. In some embodiments, the anti-PD-L1 scFv comprises a first engineered cysteine residue at position 44 of V_H and a second engineered cysteine residue at position 100 of V_L, or a first engineered cysteine residue at position 105 of V_H and a second

engineered cysteine residue at position 43 of V_L, wherein the first engineered cysteine residue and the second engineered cysteine residue form a disulfide bond, and wherein the amino acid positions are based on the Kabat numbering system. In some embodiments, the Fc fragment is an IgG1 Fc fragment. In some embodiments, the Fc fragment has H310A and H435Q mutations, wherein the amino acid positions are based on the Kabat numbering system. In some embodiments, the anti-PD-L1 scFv comprises the amino acid sequence of any one of SEQ ID NOs: 25, 27, 29, 31, 33, 35, 37 and 39, or a variant thereof having at least about 80% (such as at least about any one of 80%, 85%, 90%, 95%, 96%, 97%, 98%, or 99%) sequence identity to the amino acid sequence of any one of SEQ ID NOs: 25, 27, 29, 31, 33, 35, 37 and 39. In some embodiments, the radionuclide is selected from the group consisting of ^{64}Cu, ^{18}F, ^{67}Ga, ^{68}Ga, ^{111}In, ^{177}Lu, ^{90}Y, ^{89}Zr, ^{61}Cu, ^{62}Cu, ^{67}Cu, ^{19}F, ^{66}Ga, ^{72}Ga, ^{44}Sc, ^{47}Sc, ^{86}Y, ^{88}Y and ^{45}Ti. In some embodiments, the radionuclide is ^{68}Ga. In some embodiments, the anti-PD-L1 antibody moiety is conjugated to a chelating compound that chelates the radionuclide. In some embodiments, the chelating compound is NOTA, DOTA or derivatives thereof.

[160] The methods described herein can be used to determine the distribution of an immune checkpoint ligand (*e.g.*, PD-L1 or PD-L1 like ligands) in an individual or a tissue of interest in an individual. The method may also provide qualitative or quantitative information on the expression level of the immune checkpoint ligand in one or more tissues or organ of an individual. Additionally, the methods described herein can allow imaging of an individual over a period of time, for example, by providing a plurality of sets of imaging results at different time points after the administration of the imaging agent to the individual. In some embodiments, the imaging is carried out for at least 2, 3, 4, 5, 6, 7, 8, 9, 10 or more times over a period between about 10 minutes to about 24 hours (such as about any one of 10 minutes to 1 hour, 1 hour to 2 hours, 2 hours to 4 hours, 4 hours to 8 hours, 8 hours to 12 hours, 12 hours to 24 hours, 1 hour to 4 hours or 1 hour to 8 hours). In some embodiments, the imaging is carried out between about 10 minutes to about 24 hours after the administration of the imaging agent, for example, between about any one of 10 minutes to 1 hour, 1 hour to 2 hours, 2 hours to 4 hours, 4 hours to 8 hours, 8 hours to 12 hours, 12 hours to 24 hours, 1 hour to 4 hours or 1 hour to 8 hours.

[161] Methods of imaging using labeled polypeptides are well known in the art, and any such known methods may be used with the imaging agents disclosed herein. See, for example, Srivastava (ed.), Radiolabeled Monoclonal Antibodies for Imaging and Therapy (Plenum Press 1988), Chase, "Medical Applications of Radioisotopes," in Remington's Pharmaceutical Sciences, 18th Edition, Gennaro et al. (eds.), pp. 624-652 (Mack Publishing Co., 1990), and Brown, "Clinical Use of Monoclonal Antibodies," in Biotechnology and Pharmacy 227-49, Pezzuto et al. (eds.) (Chapman & Hall 1993). In some embodiments, the non-invasive imaging technique uses positron-emitting radionuclides (PET isotopes), such as with an energy of about 511 keV, such as ^{18}F, ^{68}Ga, ^{64}Cu, and ^{124}I. Such radionuclides may be imaged by well-known PET scanning techniques. See, also, U.S Patent Nos. 6,953,567; 9,884,131 and international patent application publication No.

WO2016149188A1, and Kim HY. *et al.*, (2018) PLoS ONE 13(3): e0192821, which are incorporated herein by reference.

[162] In some embodiments, the non-invasive imaging technique comprises single photon emission computed tomography (SPECT) imaging. In some embodiments, the non-invasive imaging technique comprises positron emission tomography (PET) imaging. In some embodiments, SPEC or PET imaging is combined with one or more other non-invasive imaging method, which may or may not be based on the signals from the imaging agent. For example, PET may be combined with computed tomography (CT) imaging, magnetic resonance imaging (MRI), chemical luminescence imaging, or electrochemical luminescence imaging.

[163] The imaging methods described herein are suitable for detecting immune checkpoint ligands at low, moderate, or high expression levels. In some embodiments, the imaging method provides dynamic information on the expression level and distribution of the immune checkpoint ligand. In some embodiments, the imaging method is capable of detecting the immune checkpoint ligand in situations that might be challenging for other methods of detection, such as immunohistochemistry (IHC). For example, in some embodiments, the tissue of interest is negative for the immune checkpoint ligand based on an immunohistochemistry (IHC) assay or another assay. Molecular assays that may be used for detecting the presence or absence of an immune checkpoint ligand include, but are not limited to, polymerase chain reaction (PCR)-based assays, next-generation sequencing (NGS) assays, hybridization assays, and ELISA. In some embodiments, the tissue of interest has a low expression level of the immune checkpoint ligand. In some embodiments, the tissue of interest only expresses the immune checkpoint ligand upon infiltration of immune cells.

[164] The imaging agent may be administered to the individual using any suitable dosage and routes of administration. The route of administration is in accordance with known and accepted methods, such as by single or multiple bolus or infusion over a period of time in a suitable manner, *e.g.*, injection or infusion by subcutaneous, intravenous, intraperitoneal, intramuscular, intra-arterial, intralesional, intraarticular, intratumoral, or oral routes. The determination of the appropriate dosage or route of administration is well within the skill of an ordinary artisan. Animal experiments provide reliable guidance for the determination of effective doses for human diagnostic applications. Interspecies scaling of effective doses can be performed following the principles laid down by Mordenti, J. and Chappell, W. "The Use of Interspecies Scaling in Toxicokinetics," In *Toxicokinetics and New Drug Development*, Yacobi *et al.*, Eds, Pergamon Press, New York 1989, pp. 42-46.

Diagnosis and treatment

[165] The methods described herein are useful for diagnosis and as a companion diagnostic method for treatment of a variety of diseases and conditions that are associated with abnormal immune response. In some embodiments, the disease or condition is associated with immune deficiency. In some embodiments, the disease or condition is cancer, infectious disease, autoimmune disease, or a metabolic disease.

[166] In some embodiments, there is provided a method of diagnosing an individual having a disease or condition, comprising: (a) determining the distribution of an immune checkpoint ligand in the individual using any one of the methods for determining distribution of an immune checkpoint ligand described herein; and (b) diagnosing the individual as positive for the immune checkpoint ligand if signal of the imaging agent is detected at a tissue of interest, or diagnosing the individual as negative for the immune checkpoint ligand if signal of the imaging agent is not detected at a tissue of interest. In some embodiments, the disease or condition is cancer, infection, autoimmune disease, or metabolic disease. In some embodiments, the immune checkpoint ligand is PD-L1. In some embodiments, the immune checkpoint ligand is a PD-L1 like ligand.

[167] In some embodiments, there is provided a method of diagnosing an individual having a disease or condition, comprising: (a) administering to the individual an imaging agent comprising an antibody moiety labeled with a radionuclide, wherein the antibody fragment specifically binds the immune checkpoint ligand; (b) imaging the imaging agent in the individual with a non-invasive imaging technique; and (c) diagnosing the individual as positive for the immune checkpoint ligand if signal of the imaging agent is detected at a tissue of interest, or diagnosing the individual as negative for the immune checkpoint ligand if signal of the imaging agent is not detected at a tissue of interest. In some embodiments, the method further comprises determining the expression level of the immune checkpoint ligand in a tissue of interest in the individual based on signals emitted by the imaging agent from the tissue. In some embodiments, the method further comprises preparing the imaging agent by labeling the antibody moiety with the radionuclide. In some embodiments, the non-invasive imaging technique comprises single photon emission computed tomography (SPECT) imaging or positron emission tomography (PET) imaging. In some embodiments, the non-invasive imaging technique further comprises computed tomography imaging, magnetic resonance imaging, chemical luminescence imaging, or electrochemical luminescence imaging. In some embodiments, the imaging agent is administered intravenously, intraperitoneally, intramuscularly, subcutaneously, or orally. In some embodiments, the imaging is carried out between about 10 minutes to about 24 hours after the administration of the imaging agent. In some embodiments, the method further comprises administering to the individual an antibody moiety not labeled with a radioisotope prior to the administration of the imaging agent. In some embodiments, the method comprises imaging the individual over a period of time. In some embodiments, the immune checkpoint ligand is selected from the group consisting of PD-L1, PD-L2, B7-H3, galectin-9, CD80, CD86 and ICOSL. In some embodiments, the radionuclide is selected from the group consisting of ^{664}Cu, ^{18}F, ^{67}Ga, ^{68}Ga, ^{111}In, ^{177}Lu, ^{90}Y, ^{89}Zr, ^{61}Cu, ^{62}Cu, ^{67}Cu, ^{19}F, ^{66}Ga, ^{72}Ga, ^{44}Sc, ^{47}Sc, ^{86}Y, ^{88}Y and ^{45}Ti. In some embodiments, the radionuclide is ^{68}Ga. In some embodiments, the antibody moiety is conjugated to a chelating compound that chelates the radionuclide. In some embodiments, the chelating compound is NOTA, DOTA or derivatives thereof. In some embodiments, the antibody moiety has a half-life of about 10 minutes to about 24 hours (such as about any one

of 10 minutes to 2 hours, 1 hour to 4 hours, 4 hours to 8 hours, 8 hours to 12 hours or 12 hours to 24 hours) in serum. In some embodiments, the antibody moiety is no more than about 120 kDa (such as no more than about 30 kDa, 50 kDa, 80 kDa, or 100 kDa, or about any one of 30-50kDa, 50-100kDa, or 30-80kDa). In some embodiments, the antibody moiety has a K_D between about 9×10^{-10} M to about 1×10^{-8} M (such as about 9×10^{-10} to 1×10^{-9}, about 1×10^{-9} to 2×10^{-9}, about 2×10^{-10} to 5×10^{-9}, or about 5×10^{-10} to 1×10^{-8}) with the immune checkpoint ligand. In some embodiments, the antibody moiety cross-reacts with the immune checkpoint ligand from a non-human mammal (*e.g.*, mouse, rat or monkey). In some embodiments, the antibody moiety is humanized. In some embodiments, the antibody moiety is stable at acidic pH (*e.g.*, at a pH lower than about 6.5, 6.0, 5.5, or 5.0). In some embodiments, the antibody moiety has a melting temperature (Tm) of about 55-70oC (such as about any one of 55-60, 60-65, or 65-70°C). In some embodiments, the antibody moiety is selected from the group consisting of a single-chain Fv (scFv), a diabody, a Fab, a Fab', a $F(ab')_2$, an Fv fragment, a disulfide stabilized Fv fragment (dsFv), a $(dsFv)_2$, and a V_HH. In some embodiments, the disease or condition is cancer, infection, autoimmune disease, or metabolic disease. In some embodiments, the immune checkpoint ligand is PD-L1. In some embodiments, the immune checkpoint ligand is a PD-L1 like ligand. In some embodiments, the antibody moiety is an scFv. In some embodiments, the antibody moiety is an scFv fused to an Fc fragment (such as a human IgG1 Fc).

[168] In some embodiments, there is provided a method of diagnosing an individual having a disease or condition, comprising: (a) administering to the individual an imaging agent comprising an anti-PD-L1 antibody moiety labeled with a radionuclide; (b) imaging the imaging agent in the individual with a non-invasive imaging technique; and (c) diagnosing the individual as positive for PD-L1 if signal of the imaging agent is detected at a tissue of interest, or diagnosing the individual as negative for the immune checkpoint ligand if signal of the imaging agent is not detected at a tissue of interest; wherein the anti-PD-L1 antibody moiety comprises: a V_H comprising a HC-CDR1 comprising the amino acid sequence of SEQ ID NO: 41, a HC-CDR2 comprising the amino acid sequence of SEQ ID NO: 42, and a HC-CDR3 comprising the amino acid sequence of SEQ ID NO: 43, or a variant thereof comprising up to about 5 amino acid substitutions; and a V_L comprising a LC-CDR1 comprising the amino acid sequence of SEQ ID NO: 44, a LC-CDR2 comprising the amino acid sequence of SEQ ID NO: 45, and a LC-CDR3 comprising the amino acid sequence of SEQ ID NO: 46, or a variant thereof comprising up to about 5 amino acid substitutions. In some embodiments, the method further comprises determining the expression level of PD-L1 in a tissue of interest in the individual based on signals emitted by the imaging agent from the tissue. In some embodiments, the method further comprises preparing the imaging agent by labeling the antibody moiety with the radionuclide. In some embodiments, the non-invasive imaging technique comprises single photon emission computed tomography (SPECT) imaging or positron emission tomography (PET) imaging. In some embodiments, the non-invasive imaging technique further comprises

computed tomography imaging, magnetic resonance imaging, chemical luminescence imaging, or electrochemical luminescence imaging. In some embodiments, the imaging agent is administered intravenously, intraperitoneally, intramuscularly, subcutaneously, or orally. In some embodiments, the imaging is carried out between about 10 minutes to about 24 hours after the administration of the imaging agent. In some embodiments, the method further comprises administering to the individual an antibody moiety not labeled with a radioisotope prior to the administration of the imaging agent. In some embodiments, the method comprises imaging the individual over a period of time. In some embodiments, the anti-PD-L1 antibody moiety comprises: a V_H comprising the amino acid sequence of any one of SEQ ID NOs: 1, 5, 9, 11, and 13, or a variant thereof having at least about 80% (such as at least about any one of 80%, 85%, 90%, 95%, 96%, 97%, 98%, or 99%) sequence identity to the amino acid sequence of any one of SEQ ID NOs: 1, 5, 9, 11, and 13; and a V_L comprising the amino acid sequence of any one of SEQ ID NOs: 3, 7, 15, 17 and 19, or a variant thereof having at least about 80% (such as at least about any one of 80%, 85%, 90%, 95%, 96%, 97%, 98%, or 99%) sequence identity to the amino acid sequence of any one of SEQ ID NOs: 3, 7, 15, 17 and 19. In some embodiments, the anti-PD-L1 antibody moiety is humanized. In some embodiments, the radionuclide is selected from the group consisting of ^{64}Cu, ^{18}F, ^{67}Ga, ^{68}Ga, ^{111}In, ^{177}Lu, ^{90}Y, ^{89}Zr, ^{61}Cu, ^{62}Cu, ^{67}Cu, ^{19}F, ^{66}Ga, ^{72}Ga, ^{44}Sc, ^{47}Sc, ^{86}Y, ^{88}Y and ^{45}Ti. In some embodiments, the radionuclide is ^{68}Ga. In some embodiments, the anti-PD-L1 antibody moiety is conjugated to a chelating compound that chelates the radionuclide. In some embodiments, the chelating compound is NOTA, DOTA or derivatives thereof. In some embodiments, the disease or condition is cancer, infection, autoimmune disease, or metabolic disease. In some embodiments, the antibody moiety is an scFv. In some embodiments, the antibody moiety is an scFv fused to an Fc fragment (such as a human IgG1 Fc). In some embodiments, the scFv comprises one or more (such as 1, 2, 3, or more) engineered disulfide bonds. In some embodiments, the scFv comprises a first engineered cysteine residue at position 44 of V_H and a second engineered cysteine residue at position 100 of V_L, and/or a first engineered cysteine residue at position 105 of V_H and a second engineered cysteine residue at position 43 of V_L, wherein the first engineered cysteine residue and the second engineered cysteine residue form a disulfide bond, and wherein the amino acid positions are based on the Kabat numbering system. In some embodiments, the anti-PD-L1 antibody moiety comprises the amino acid sequence of any one of SEQ ID NOs: 25, 27, 29, 31, 33, 35, 37 and 39, or a variant thereof having at least about 80% (such as at least about any one of 80%, 85%, 90%, 95%, 96%, 97%, 98%, or 99%) sequence identity to the amino acid sequence of any one of SEQ ID NOs: 25, 27, 29, 31, 33, 35, 37 and 39.

[169] In some embodiments, there is provided a method of treating an individual having a disease or condition, comprising: (a) diagnosing the individual using any method of diagnosis described herein; and (b) administering to the individual an effective amount of a therapeutic agent targeting the immune checkpoint ligand or receptor thereof, if the individual is diagnosed as positive for the immune checkpoint ligand. In some

embodiments, the therapeutic agent is an inhibitor of the immune checkpoint ligand or receptor thereof. In some embodiments, the therapeutic agent is a radiolabeled molecule specifically binding the immune checkpoint ligand or receptor thereof. In some embodiments, the disease or condition is cancer, infection, autoimmune disease, or metabolic disease. In some embodiments, the immune checkpoint ligand is PD-L1. In some embodiments, the immune checkpoint ligand is a PD-L1 like ligand.

[170] In some embodiments, there is provided a method of treating an individual having a disease or condition, comprising: (a) administering to the individual an imaging agent comprising an antibody moiety labeled with a radionuclide, wherein the antibody fragment specifically binds the immune checkpoint ligand; (b) imaging the imaging agent in the individual with a non-invasive imaging technique; (c) diagnosing the individual as positive for the immune checkpoint ligand if signal of the imaging agent is detected at a tissue of interest, or diagnosing the individual as negative for the immune checkpoint ligand if signal of the imaging agent is not detected at a tissue of interest; and (d) administering to the individual an effective amount of a therapeutic agent targeting the immune checkpoint ligand or receptor thereof (*e.g.*, an inhibitor of the immune checkpoint ligand or receptor thereof, or a radiolabeled molecule specifically binding the immune checkpoint ligand or receptor thereof), if the individual is diagnosed as positive for the immune checkpoint ligand. In some embodiments, the method further comprises determining the expression level of the immune checkpoint ligand in a tissue of interest in the individual based on signals emitted by the imaging agent from the tissue. In some embodiments, the method further comprises preparing the imaging agent by labeling the antibody moiety with the radionuclide. In some embodiments, the non-invasive imaging technique comprises single photon emission computed tomography (SPECT) imaging or positron emission tomography (PET) imaging. In some embodiments, the non-invasive imaging technique further comprises computed tomography imaging, magnetic resonance imaging, chemical luminescence imaging, or electrochemical luminescence imaging. In some embodiments, the imaging agent is administered intravenously, intraperitoneally, intramuscularly, subcutaneously, or orally. In some embodiments, the imaging is carried out between about 10 minutes to about 24 hours after the administration of the imaging agent. In some embodiments, the method further comprises administering to the individual an antibody moiety not labeled with a radioisotope prior to the administration of the imaging agent. In some embodiments, the method comprises imaging the individual over a period of time. In some embodiments, the immune checkpoint ligand is selected from the group consisting of PD-L1, PD-L2, B7-H3, galectin-9, CD80, CD86 and ICOSL. In some embodiments, the radionuclide is selected from the group consisting of ^{64}Cu, ^{18}F, ^{67}Ga, ^{68}Ga, ^{111}In, ^{177}Lu, ^{90}Y, ^{89}Zr, ^{61}Cu, ^{62}Cu, ^{67}Cu, ^{19}F, ^{66}Ga, ^{72}Ga, ^{44}Sc, ^{47}Sc, ^{86}Y, ^{88}Y and ^{45}Ti. In some embodiments, the radionuclide is ^{68}Ga. In some embodiments, the antibody moiety is conjugated to a chelating compound that chelates the radionuclide. In some embodiments, the chelating compound is NOTA, DOTA or derivatives thereof. In some embodiments, the antibody moiety has a half-life of

about 10 minutes to about 24 hours (such as about any one of 10 minutes to 2 hours, 1 hour to 4 hours, 4 hours to 8 hours, 8 hours to 12 hours or 12 hours to 24 hours) in serum. In some embodiments, the antibody moiety is no more than about 120 kDa (such as no more than about 30 kDa, 50 kDa, 80 kDa, or 100 kDa, or about any one of 30-50kDa, 50-100kDa, or 30-80kDa). In some embodiments, the antibody moiety has a K_D between about 9×10^{-10} M to about 1×10^{-8} M (such as about 9×10^{-10} to 1×10^{-9}, about 1×10^{-9} to 2×10^{-9}, about 2×10^{-10} to 5×10^{-9}, or about 5×10^{-10} to 1×10^{-8}) with the immune checkpoint ligand. In some embodiments, the antibody moiety cross-reacts with the immune checkpoint ligand from a non-human mammal (*e.g.*, mouse, rat or monkey). In some embodiments, the antibody moiety is humanized. In some embodiments, the antibody moiety is stable at acidic pH (*e.g.*, at a pH lower than about 6.5, 6.0, 5.5, or 5.0). In some embodiments, the antibody moiety has a melting temperature (Tm) of about 55-70oC (such as about any one of 55-60, 60-65, or 65-70°C). In some embodiments, the antibody moiety is selected from the group consisting of a single-chain Fv (scFv), a diabody, a Fab, a Fab', a $F(ab')_2$, an Fv fragment, a disulfide stabilized Fv fragment (dsFv), a $(dsFv)_2$, and a V_HH. In some embodiments, the disease or condition is cancer, infection, autoimmune disease, or metabolic disease. In some embodiments, the immune checkpoint ligand is PD-L1. In some embodiments, the immune checkpoint ligand is a PD-L1 like ligand. In some embodiments, the antibody moiety is an scFv. In some embodiments, the antibody moiety is an scFv fused to an Fc fragment (such as a human IgG1 Fc).

[171] In some embodiments, there is provided a method of treating an individual having a disease or condition, comprising: (a) administering to the individual an imaging agent comprising an anti-PD-L1 antibody moiety labeled with a radionuclide; (b) imaging the imaging agent in the individual with a non-invasive imaging technique; (c) diagnosing the individual as positive for PD-L1 if signal of the imaging agent is detected at a tissue of interest, or diagnosing the individual as negative for the immune checkpoint ligand if signal of the imaging agent is not detected at a tissue of interest; and (d) administering to the individual an effective amount of a therapeutic agent targeting PD-L1 or PD-1 (*e.g.*, an inhibitor of PD-L1 or PD-1, such as an anti-PD-L1 antibody or anti-PD-1 antibody; or a radiolabeled molecule specifically binding PD-L1 or PD-1), if the individual is diagnosed as positive for PD-L1, wherein the anti-PD-L1 antibody moiety comprises: a V_H comprising a HC-CDR1 comprising the amino acid sequence of SEQ ID NO: 41, a HC-CDR2 comprising the amino acid sequence of SEQ ID NO: 42, and a HC-CDR3 comprising the amino acid sequence of SEQ ID NO: 43, or a variant thereof comprising up to about 5 amino acid substitutions; and a V_L comprising a LC-CDR1 comprising the amino acid sequence of SEQ ID NO: 44, a LC-CDR2 comprising the amino acid sequence of SEQ ID NO: 45, and a LC-CDR3 comprising the amino acid sequence of SEQ ID NO: 46, or a variant thereof comprising up to about 5 amino acid substitutions. In some embodiments, the method further comprises determining the expression level of PD-L1 in a tissue of interest in the individual based on signals emitted by the imaging agent from the tissue. In some embodiments, the method further comprises preparing the imaging

agent by labeling the antibody moiety with the radionuclide. In some embodiments, the non-invasive imaging technique comprises single photon emission computed tomography (SPECT) imaging or positron emission tomography (PET) imaging. In some embodiments, the non-invasive imaging technique further comprises computed tomography imaging, magnetic resonance imaging, chemical luminescence imaging, or electrochemical luminescence imaging. In some embodiments, the imaging agent is administered intravenously, intraperitoneally, intramuscularly, subcutaneously, or orally. In some embodiments, the imaging is carried out between about 10 minutes to about 24 hours after the administration of the imaging agent. In some embodiments, the method further comprises administering to the individual an antibody moiety not labeled with a radioisotope prior to the administration of the imaging agent. In some embodiments, the method comprises imaging the individual over a period of time. In some embodiments, the anti-PD-L1 antibody moiety comprises: a V_H comprising the amino acid sequence of any one of SEQ ID NOs: 1, 5, 9, 11, and 13, or a variant thereof having at least about 80% (such as at least about any one of 80%, 85%, 90%, 95%, 96%, 97%, 98%, or 99%) sequence identity to the amino acid sequence of any one of SEQ ID NOs: 1, 5, 9, 11, and 13; and a V_L comprising the amino acid sequence of any one of SEQ ID NOs: 3, 7, 15, 17 and 19, or a variant thereof having at least about 80% (such as at least about any one of 80%, 85%, 90%, 95%, 96%, 97%, 98%, or 99%) sequence identity to the amino acid sequence of any one of SEQ ID NOs: 3, 7, 15, 17 and 19. In some embodiments, the anti-PD-L1 antibody moiety is humanized. In some embodiments, the radionuclide is selected from the group consisting of ^{64}Cu, ^{18}F, ^{67}Ga, ^{68}Ga, ^{111}In, ^{177}Lu, ^{90}Y, ^{89}Zr, ^{61}Cu, ^{62}Cu, ^{67}Cu, ^{19}F, ^{66}Ga, ^{72}Ga, ^{44}Sc, ^{47}Sc, ^{86}Y, ^{88}Y and ^{45}Ti. In some embodiments, the radionuclide is ^{68}Ga. In some embodiments, the anti-PD-L1 antibody moiety is conjugated to a chelating compound that chelates the radionuclide. In some embodiments, the chelating compound is NOTA, DOTA or derivatives thereof. In some embodiments, the disease or condition is cancer, infection, autoimmune disease, or metabolic disease. In some embodiments, the antibody moiety is an scFv. In some embodiments, the antibody moiety is an scFv fused to an Fc fragment (such as a human IgG1 Fc). In some embodiments, the scFv comprises one or more (such as 1, 2, 3, or more) engineered disulfide bonds. In some embodiments, the scFv comprises a first engineered cysteine residue at position 44 of V_H and a second engineered cysteine residue at position 100 of V_L, and/or a first engineered cysteine residue at position 105 of V_H and a second engineered cysteine residue at position 43 of V_L, wherein the first engineered cysteine residue and the second engineered cysteine residue form a disulfide bond, and wherein the amino acid positions are based on the Kabat numbering system. In some embodiments, the anti-PD-L1 antibody moiety comprises the amino acid sequence of any one of SEQ ID NOs: 25, 27, 29, 31, 33, 35, 37 and 39, or a variant thereof having at least about 80% (such as at least about any one of 80%, 85%, 90%, 95%, 96%, 97%, 98%, or 99%) sequence identity to the amino acid sequence of any one of SEQ ID NOs: 25, 27, 29, 31, 33, 35, 37 and 39.

[172] In some embodiments, the individual has cancer. The cancer may comprise non-solid tumors (such as hematological tumors, for example, leukemias and lymphomas) or may comprise solid tumors. Exemplary cancers that may be diagnosed using the methods described herein, include, but are not limited to, carcinoma, blastoma, and sarcoma, and certain leukemia or lymphoid malignancies, benign and malignant tumors, and malignancies *e.g.*, sarcomas, carcinomas, and melanomas. Adult tumors/cancers and pediatric tumors/cancers are also included. Solid or hematologic cancers discussed herein include, but is not limited to, Hodgkin lymphoma, non-Hodgkin lymphoma, sarcomas and carcinomas such as fibrosarcoma, myxosarcoma, liposarcoma, chondrosarcoma, osteogenic sarcoma, chordoma, angiosarcoma, endotheliosarcoma, lymphangiosarcoma, lymphangioendotheliosarcoma, Kaposi's sarcoma, soft tissue sarcoma, uterine sacronomasynovioma, mesothelioma, Ewing's tumor, leiomyosarcoma, rhabdomyosarcoma, colon carcinoma, pancreatic cancer, breast cancer, ovarian cancer, prostate cancer, squamous cell carcinoma, basal cell carcinoma, adenocarcinoma, sweat gland carcinoma, sebaceous gland carcinoma, papillary carcinoma, papillary adenocarcinomas, cystadenocarcinoma, medullary carcinoma, bronchogenic carcinoma, renal cell carcinoma, hepatoma, bile duct carcinoma, choriocarcinoma, seminoma, embryonal carcinoma, Wilm's tumor, cervical cancer, testicular tumor, lung carcinoma, small cell lung carcinoma, bladder carcinoma, epithelial carcinoma, and melanoma.

[173] The methods described herein are applicable to solid or hematologic cancers of all stages, including stages, I, II, III, and IV, according to the American Joint Committee on Cancer (AJCC) staging groups. In some embodiments, the solid or hematologic cancer is an/a: early stage cancer, non-metastatic cancer, primary cancer, advanced cancer, locally advanced cancer, metastatic cancer, or cancer in remission.

[174] In some embodiments, the individual has a hematologic cancer. Exemplary hematologic cancers that can be diagnosed using the methods described herein include, but are not limited to, leukemia, lymphoma, acute lymphoblastic leukemia (ALL), acute non-lymphoblastic leukemia (ANLL), chronic lymphocytic leukemia (CLL), chronic myeloid leukemia (CML), non-Hodgkin lymphoma, and Hodgkin lymphoma.

[175] In some embodiments, the individual has a solid tumor. Exemplary solid tumors that can be diagnosed using the methods described herein include, but are not limited to, colon tumor, melanoma, kidney tumor, ovarian tumor, lung tumor, breast tumor, and pancreatic tumor.

[176] Cancer treatments can be evaluated, for example, by tumor regression, tumor weight or size shrinkage, time to progression, duration of survival, progression free survival, overall response rate, duration of response, quality of life, protein expression and/or activity. Approaches to determining efficacy of the therapy can be employed, including for example, measurement of response through radiological imaging.

[177] In some embodiments, the individual has an infectious disease. The infection may be caused by a virus, bacteria, protozoa, or parasite. Exemplary pathogens include, but are not limited to, Acinetobacter baumannii,

Anaplasma genus, Anaplasma phagocytophilum, Ancylostoma braziliense, Ancylostoma duodenale, Arcanobacterium haemolyticum, Ascaris lumbricoides, Aspergillus genus, Astroviridae, Babesia genus, Bacillus anthracis, Bacillus cereus, Bartonella henselae, BK virus, Blastocystis hominis, Blastomyces dermatitidis, Bordetella pertussis, Borrelia burgdorferi, Borrelia genus, Borrelia spp, Brucella genus, Brugia malayi, Bunyaviridae family, Burkholderia cepacia and other Burkholderia species, Burkholderia mallei, Burkholderia pseudomallei, Caliciviridae family, Campylobacter genus, Candida albicans, Candida spp, Chlamydia trachomatis, Chlamydophila pneumoniae, Chlamydophila psittaci, CJD prion, Clonorchis sinensis, Clostridium botulinum, Clostridium difficile, Clostridium perfringens, Clostridium perfringens, Clostridium spp, Clostridium tetani, Coccidioides spp, coronaviruses, Corynebacterium diphtheriae, Coxiella burnetii, Crimean-Congo hemorrhagic fever virus, Cryptococcus neoformans, Cryptosporidium genus, Cytomegalovirus (CMV), Dengue viruses (DEN-1, DEN-2, DEN-3 and DEN-4), Dientamoeba fragilis, Ebolavirus (EBOV), Echinococcus genus, Ehrlichia chaffeensis, Ehrlichia ewingii, Ehrlichia genus, Entamoeba histolytica, Enterococcus genus, Enterovirus genus, Enteroviruses, mainly Coxsackie A virus and Enterovirus 71 (EV71), Epidermophyton spp, Epstein-Barr Virus (EBV), Escherichia coli O157:H7, O111 and O104:H4, Fasciola hepatica and Fasciola gigantica, FFI prion, Filarioidea superfamily, Flaviviruses, Francisella tularensis, Fusobacterium genus, Geotrichum candidum, Giardia intestinalis, Gnathostoma spp, GSS prion, Guanarito virus, Haemophilus ducreyi, Haemophilus influenzae, Helicobacter pylori, Henipavirus (Hendra virus Nipah virus), Hepatitis A Virus, Hepatitis B Virus (HBV), Hepatitis C Virus (HCV), Hepatitis D Virus, Hepatitis E Virus, Herpes simplex virus 1 and 2 (HSV-1 and HSV-2), Histoplasma capsulatum, HIV (Human immunodeficiency virus), Hortaea werneckii, Human bocavirus (HBoV), Human herpesvirus 6 (HHV-6) and Human herpesvirus 7 (HHV-7), Human metapneumovirus (hMPV), Human papillomavirus (HPV), Human parainfluenza viruses (HPIV), Human T cell leukemia virus 1 (HTLV-1), Japanese encephalitis virus, JC virus, Junin virus, Kaposi's Sarcoma associated herpesvirus (KSHV), Kingella kingae, Klebsiella granulomatis, Kuru prion, Lassa virus, Legionella pneumophila, Leishmania genus, Leptospira genus, Listeria monocytogenes, Lymphocytic choriomeningitis virus (LCMV), Machupo virus, Malassezia spp, Marburg virus, Measles virus, Metagonimus yokagawai, Microsporidia phylum, Molluscum contagiosum virus (MCV), Mumps virus, Mycobacterium leprae and Mycobacterium lepromatosis, Mycobacterium tuberculosis, Mycobacterium ulcerans, Mycoplasma pneumoniae, Naegleria fowleri, Necator americanus, Neisseria gonorrhoeae, Neisseria meningitidis, Nocardia asteroides, Nocardia spp, Onchocerca volvulus, Orientia tsutsugamushi, Orthomyxoviridae family (Influenza), Paracoccidioides brasiliensis, Paragonimus spp, Paragonimus westermani, Parvovirus B19, Pasteurella genus, Plasmodium genus, Pneumocystis jirovecii, Poliovirus, Rabies virus, Respiratory syncytial virus (RSV), Rhinovirus, rhinoviruses, Rickettsia akari, Rickettsia genus, Rickettsia prowazekii, Rickettsia rickettsii, Rickettsia typhi, Rift Valley fever virus, Rotavirus, Rubella virus, Sabia virus, Salmonella genus, Sarcoptes

scabiei, SARS coronavirus, Schistosoma genus, Shigella genus, Sin Nombre virus, Hantavirus, Sporothrix schenckii, Staphylococcus genus, Staphylococcus genus, Streptococcus agalactiae, Streptococcus pneumoniae, Streptococcus pyogenes, Strongyloides stercoralis, Taenia genus, Taenia solium, Tick-borne encephalitis virus (TBEV), Toxocara canis or Toxocara cati, Toxoplasma gondii, Treponema pallidum, Trichinella spiralis, Trichomonas vaginalis, Trichophyton spp, Trichuris trichiura, Trypanosoma brucei, Trypanosoma cruzi, Ureaplasma urealyticum, Varicella zoster virus (VZV), Varicella zoster virus (VZV), Variola major or Variola minor, vCJD prion, Venezuelan equine encephalitis virus, Vibrio cholerae, West Nile virus, Western equine encephalitis virus, Wuchereria bancrofti, Yellow fever virus, Yersinia enterocolitica, Yersinia pestis, and Yersinia pseudotuberculosis.

[178] In some embodiments, the individual has an autoimmune disease. Exemplary autoimmune disease include, but are not limited to, Behcet disease, systemic lupus erythematosus, multiple sclerosis (systemic scleroderma and progressive systemic scleroderma), scleroderma, polymyositis, dermatomyositis, periarteritis nodosa (polyarteritis nodosa and microscopic polyangiitis), aortitis syndrome (Takayasu arteritis), malignant rheumatoid arthritis, rheumatoid arthritis, Wegner's granulomatosis, mixed connective tissue disease, Sjogren syndrome, adult-onset Still's disease, allergic granulomatous angiitis, hypersensitivity angiitis, Cogan's syndrome, RS3PE, temporal arteritis, polymyalgia rheumatica, fibromyalgia syndrome, antiphospholipid antibody syndrome, eosinophilic fasciitis, IgG4-related diseases (e.g., primary sclerosing cholangitis and autoimmune pancreatitis), Guillain-Barre syndrome, myasthenia gravis, chronic atrophic gastritis, autoimmune hepatitis, primary biliary cirrhosis, aortitis syndrome, Goodpasture's syndrome, rapidly progressive glomerulonephritis, megaloblastic anemia, autoimmune hemolytic anemia, autoimmune neutropenia, idiopathic thrombocytopenic purpura, Graves' disease (hyperthyroidism), Hashimoto's thyroiditis, autoimmune adrenal insufficiency, primary hypothyroidism, idiopathic Addison's disease (chronic adrenal insufficiency), type I diabetes mellitus, chronic discoid lupus erythematosus, localized scleroderma, psoriasis, psoriatic arthritis, pemphigus, pemphigoid, herpes gestationis, linear IgA bullous skin disease, epidermolysis bullosa acquisita, alopecia areata, vitiligo, Harada disease, autoimmune optic neuropathy, idiopathic azoospermia, recurrent fetal loss, and inflammatory bowel diseases (ulcerative colitis and Crohn's disease).

[179] In some embodiments, the individual has a metabolic disease associated with abnormal immune response. Exemplary metabolic diseases include, but are not limited to, inflammatory bowel disease, multiple sclerosis, psoriasis, rheumatoid arthritis, and systemic lupus erythematosus.

III. Imaging agents

[180] One aspect of the present application provides an imaging agent comprising an antibody moiety labeled with a radionuclide, wherein the antibody moiety specifically binds an immune checkpoint ligand. Any

one of the imaging agents described in this section may be used in the methods of determining the distribution and/or expression level of an immune checkpoint ligand, or methods of diagnosis or treatment described herein.

[181] In some embodiments, there is provided an imaging agent comprising an antibody moiety labeled with a radionuclide, wherein the antibody moiety specifically binds an immune checkpoint ligand. In some embodiments, the immune checkpoint ligand is selected from the group consisting of PD-L1, PD-L2, B7-H3, galectin-9, CD80, CD86 and ICOSL. In some embodiments, the immune checkpoint ligand is a PD-L1 like ligand. In some embodiments, the radionuclide is selected from the group consisting of ^{64}Cu, ^{18}F, ^{67}Ga, ^{68}Ga, ^{111}In, ^{177}Lu, ^{90}Y, ^{89}Zr, ^{61}Cu, ^{62}Cu, ^{67}Cu, ^{19}F, ^{66}Ga, ^{72}Ga, ^{44}Sc, ^{47}Sc, ^{86}Y, ^{88}Y and ^{45}Ti. In some embodiments, the radionuclide is ^{68}Ga. In some embodiments, the antibody moiety is conjugated to a chelating compound that chelates the radionuclide. In some embodiments, the chelating compound is 1,4,7- triazacyclononane-l,4,7-trisacetic acid (NOTA), 1, 4, 7, 10-tetraazacyclododecane-1,4,7,10-tetraacetic acid (DOTA) or derivatives thereof. In some embodiments, the antibody moiety has a half-life of about 10 minutes to about 24 hours (such as about any one of 10 minutes to 2 hours, 1 hour to 4 hours, 4 hours to 8 hours, 8 hours to 12 hours or 12 hours to 24 hours) in serum. In some embodiments, the antibody moiety is no more than about 120 kDa (such as no more than about 30 kDa, 50 kDa, 80 kDa, or 100 kDa, or about any one of 30-50kDa, 50-100kDa, or 30-80kDa). In some embodiments, the antibody moiety has a K_D between about 9×10^{-10} M to about 1×10^{-8} M (such as about 9×10^{-10} to 1×10^{-9}, about 1×10^{-9} to 2×10^{-9}, about 2×10^{-10} to 5×10^{-9}, or about 5×10^{-10} to 1×10^{-8}) with the immune checkpoint ligand. In some embodiments, the antibody moiety cross-reacts with the immune checkpoint ligand from a non-human mammal (*e.g.*, mouse, rat or monkey). In some embodiments, the antibody moiety is humanized. In some embodiments, the antibody moiety is stable at acidic pH (*e.g.*, at a pH lower than about 6.5, 6.0, 5.5, or 5.0). In some embodiments, the antibody moiety has a melting temperature (Tm) of about 55-70oC (such as about any one of 55-60, 60-65, or 65-70°C). In some embodiments, the antibody moiety is selected from the group consisting of a single-chain Fv (scFv), a diabody, a Fab, a Fab', a $F(ab')_2$, an Fv fragment, a disulfide stabilized Fv fragment (dsFv), a $(dsFv)_2$, and a V_HH.

[182] In some embodiments, there is provided an imaging agent comprising an antibody moiety conjugated to a chelating compound that chelates a radionuclide, wherein the antibody moiety specifically binds an immune checkpoint ligand. In some embodiments, the immune checkpoint ligand is selected from the group consisting of PD-L1, PD-L2, B7-H3, galectin-9, CD80, CD86 and ICOSL. In some embodiments, the immune checkpoint ligand is a PD-L1 like ligand. In some embodiments, the radionuclide is selected from the group consisting of ^{64}Cu, ^{18}F, ^{67}Ga, ^{68}Ga, ^{111}In, ^{177}Lu, ^{90}Y, ^{89}Zr, ^{61}Cu, ^{62}Cu, ^{67}Cu, ^{19}F, ^{66}Ga, ^{72}Ga, ^{44}Sc, ^{47}Sc, ^{86}Y, ^{88}Y and ^{45}Ti. In some embodiments, the radionuclide is ^{68}Ga. In some embodiments, the chelating compound is NOTA, DOTA or derivatives thereof. In some embodiments, the antibody moiety has a half-life of about 10 minutes to about 24 hours (such as about any one of 10 minutes to 2 hours, 1 hour to 4 hours, 4 hours to 8 hours, 8 hours to 12

hours or 12 hours to 24 hours) in serum. In some embodiments, the antibody moiety is no more than about 120 kDa (such as no more than about 30 kDa, 50 kDa, 80 kDa, or 100 kDa, or about any one of 30-50kDa, 50-100kDa, or 30-80kDa). In some embodiments, the antibody moiety has a K_D between about 9×10^{-10} M to about 1×10^{-8} M (such as about 9×10^{-10} to 1×10^{-9}, about 1×10^{-9} to 2×10^{-9}, about 2×10^{-10} to 5×10^{-9}, or about 5×10^{-10} to 1×10^{-8}) with the immune checkpoint ligand. In some embodiments, the antibody moiety cross-reacts with the immune checkpoint ligand from a non-human mammal (*e.g.*, mouse, rat or monkey). In some embodiments, the antibody moiety is humanized. In some embodiments, the antibody moiety is stable at acidic pH (*e.g.*, at a pH lower than about 6.5, 6.0, 5.5, or 5.0). In some embodiments, the antibody moiety has a melting temperature (Tm) of about 55-70oC (such as about any one of 55-60, 60-65, or 65-70°C). In some embodiments, the antibody moiety is selected from the group consisting of a single-chain Fv (scFv), a diabody, a Fab, a Fab', a $F(ab')_2$, an Fv fragment, a disulfide stabilized Fv fragment (dsFv), a $(dsFv)_2$, and a V_HH.

[183] In some embodiments, the antibody moiety described herein has a half-life in the serum suitable for rapid clearance rate from the body, which is amenable for *in vivo* imaging. In some embodiments, the antibody moiety has a half-life in the serum of no more than about any one of 24 hours, 20 hours, 16 hours, 12 hours, 8 hours, 4 hours, 2 hours, 1 hour, 30 minutes or less. In some embodiments, the antibody moiety has a half-life in the serum of about 10 minutes to about 24 hours, including, for example, any one of about 10 minutes to about 30 minutes, about 30 minutes to about 1 hour, about 1 hour to about 2 hours, about 2 hours to about 3 hours, about 3 hours to about 4 hours, about 4 hours to about 6 hours, about 6 hours to about 8 hours, about 8 hours to about 12 hours, about 12 hours to about 16 hours, about 16 hours to about 20 hours, about 20 hours to about 24 hours, about 10 minutes to about 2 hours, about 1 hour to about 4 hours, about 4 hours to about 8 hours, about 8 hours to about 12 hours, or about 12 hours to about 24 hours. In some embodiments, the antibody moiety is cleared from the body no more than about any one of 48 hours, 36 hours, 30 hours, 24 hours, 20 hours, 16 hours, 12 hours, 8 hours, 4 hours, 2 hours, 1 hour, 30 minutes or less. In some embodiments, the antibody moiety is cleared from the body between about 10 minutes to about 48 hours, including, for example, any one of about 10 minutes to about 30 minutes, about 30 minutes to about 1 hour, about 1 hour to about 2 hours, about 2 hours to about 3 hours, about 3 hours to about 4 hours, about 4 hours to about 6 hours, about 6 hours to about 8 hours, about 8 hours to about 12 hours, about 12 hours to about 16 hours, about 16 hours to about 20 hours, about 20 hours to about 24 hours, about 24 hours to about 36 hours, about 36 hours to about 48 hours, about 10 minutes to about 2 hours, about 1 hour to about 4 hours, about 4 hours to about 8 hours, about 8 hours to about 12 hours, or about 12 hours to about 48 hours.

[184] In some embodiments, the antibody moiety has a low molecular weight that enables its rapid clearance from the body. In some embodiments, the antibody moiety has a molecular weight of no more than about any one of 120 kDa, 110 kDa, 100 kDa, 90 kDa, 80 kDa, 70 kDa, 60 kDa, 50 kDa, 40 kDa or 30 kDa. In some

embodiments, the antibody moiety has a molecular weight of about 15 kDa to about 30 kDa, about 30 kDa to about 50 kDa, about 50 kDa to about 100 kDa, about 80 kDa to about 120 kDa or about 15 kDa to about 120 kDa. For example, an scFv has a molecular weight of about 27 kDa, an Fc has a molecular weight of about 26 kDa, and an scFv-Fc has a molecular weight of about 80 kDa.

[185] In some embodiments, the antibody moiety has a suitable affinity to the immune checkpoint ligand. In some embodiments, the antibody moiety has a K_D to the immune checkpoint ligand that is stronger than about any one of 10^{-8} M, 9×10^{-9} M, 8×10^{-9} M, 7×10^{-9} M, 6×10^{-9} M, 5×10^{-9} M, 4×10^{-9} M, 3×10^{-9} M, 2×10^{-9} M, 1×10^{-9} M, or 9×10^{-10} M. In some embodiments, the antibody moiety has a K_D the immune checkpoint ligand that is weaker than about any one of 9×10^{-10} M, 1×10^{-9} M, 2×10^{-9} M, 3×10^{-9} M, 4×10^{-9} M, 5×10^{-9} M, 6×10^{-9} M, 7×10^{-9} M, 8×10^{-9} M, 9×10^{-9} M, or 10^{-8} M. In some embodiments, the antibody moiety has a K_D to the immune checkpoint ligand that is about any one of 9×10^{-10} M to 1×10^{-8} M, 9×10^{-10} M to 1×10^{-9} M, 1×10^{-9} M to 2×10^{-9} M, 2×10^{-9} M to 3×10^{-9} M, 3×10^{-9} M to 4×10^{-9} M, 4×10^{-9} M to 5×10^{-9} M, 5×10^{-9} M to 6×10^{-9} M, 6×10^{-9} M to 7×10^{-9} M, 7×10^{-9} M to 8×10^{-9} M, 8×10^{-9} M to 9×10^{-9} M, 9×10^{-9} M to 1×10^{-8} M, 2×10^{-10} to 5×10^{-9}, or 5×10^{-10} to 1×10^{-8}.

[186] In some embodiments, the antibody moiety is stable at acidic pH or neutral pH. In some embodiments, the antibody moiety is stable at a pH lower than about 7.0, 6.9, 6.8, 6.7, 6.6, 6.5, 6.4, 6.3, 6.2, 6.1, 6.0, 5.9, 5.8, 5.7, 5.6, 5.5, 5.4, 5.3, 5.2, 5.1, 5.0 or less. In some embodiments, the antibody moiety is stable at an acidic pH or neutral pH for at least about any one of 4 hours, 6 hours, 8 hours, 10 hours, 12 hours, 24 hours, 48 hours, 3 days, 4 days, 5 days, 6 days, 7 days or more. In some embodiments, the antibody moiety is stable at basic pH, for example at a pH higher than about 7.0, 7.5, 8.0, 8.5 or higher. In some embodiments, the antibody moiety is stable at a basic pH for at least about any one of 4 hours, 8 hours, 12 hours, 24 hours, 48 hours, 3 days, 5 days, 7 days or more. Stability can be measured by incubating the imaging agent or antibody moiety in a buffer having the corresponding pH over a period of time (such as 12 hours, 24 hours, or longer), and assessing the integrity of the imaging agent or antibody moiety using known methods in the art, including SDS-PAGE, dynamic light scattering, chromatography, NMR, *etc.*

[187] In some embodiments, the antibody moiety is stable at an elevated temperature, *e.g.*, at room temperature or physiological temperature. In some embodiments, the antibody moiety has a melting temperature of at least about any one of 50°C, 55°C, 60°C, 61°C, 62°C, 63°C, 64°C, 65°C, 66°C, 67°C, 68°C, 69°C, 70°C or higher. In some embodiments, the antibody moiety has a melting temperature of about 55° to about 70°C, including, for example, about any one of 55 °C -60 °C, 60 °C -65 °C, 50 °C -65 °C, 64 °C -68 °C, or 65 °C -70°C. Melting temperature of an antibody moiety can be measured using any known methods in the art, including, for example, Differential Scanning Fluorimetry (DSF).

[0100] In some embodiments, the antibody moiety is engineered with one or more disulfide bonds to increase the melting temperature or stability of the antibody moiety. In some embodiments, wherein the antibody moiety comprises an scFv, the scFv comprises one or more (such as 1, 2, 3, or more) engineered disulfide bonds. In some embodiments, the scFv comprises a first engineered cysteine residue at position 44 of V_H and a second engineered cysteine residue at position 100 of V_L, and/or a first engineered cysteine residue at position 105 of V_H and a second engineered cysteine residue at position 43 of V_L, wherein the first engineered cysteine residue and the second engineered cysteine residue form a disulfide bond, and wherein the amino acid positions are based on the Kabat numbering system. Other engineered disulfide bonds may be introduced into the scFv by engineering a cysteine in the VH and a cysteine in the VL at suitable positions based on the structure and sequences of the scFv.

[188] Contemplated antibody moieties include, but are not limited to, humanized antibodies, partially humanized antibodies, fully humanized antibodies, semi-synthetic antibodies, chimeric antibodies, mouse antibodies, human antibodies, and antibodies comprising the heavy chain and/or light chain CDRs discussed herein, *e.g.*, in the "anti-PD-L1 antibody agents" section.

[189] In some embodiments, the antibody moiety specifically recognizes the immune checkpoint ligand from human. In some embodiments, the antibody moiety cross-reacts with the immune checkpoint ligand from two or more species. Cross-reactivity of the antibody moiety with model animals and human facilities clinical studies of the imaging agent. In some embodiments, the antibody moiety cross-reacts with the immune checkpoint ligand from a non-human animal, such as mammal. In some embodiments, the antibody moiety cross-reacts with the immune checkpoint ligand from a rodent, such as mouse or rat. In some embodiments, the antibody moiety cross-reacts with the immune checkpoint ligand from a non-human primate, such as a cynomolgus monkey.

[190] In some embodiments, the antibody moiety is an antigen-binding fragment. In some embodiments, the antibody moiety is not a full-length antibody. Suitable antibody moieties include, but are not limited to, scFv, Fab, Fab', $F(ab')_2$, Fv, disulfide stabilized Fv fragment (dsFv), a $(dsFv)_2$, V_HH, and Fc fusions thereof. In some embodiments, the antibody moiety is an scFv. Antibody fragments and variants that are suitable for the imaging agents described herein are further described in the section "Antibody moieties." In some embodiments, the antibody moiety is a Fab. In some embodiments, the antibody moiety is an scFv fused to an Fc fragment.

[191] Thus, in some embodiments, there is provided an imaging agent comprising a scFv labeled with a radionuclide, wherein the scFv specifically binds an immune checkpoint ligand. In some embodiments, the immune checkpoint ligand is selected from the group consisting of PD-L1, PD-L2, B7-H3, galectin-9, CD80, CD86 and ICOSL. In some embodiments, the immune checkpoint ligand is a PD-L1 like ligand. In some embodiments, the radionuclide is selected from the group consisting of ^{64}Cu, ^{18}F, ^{67}Ga, ^{68}Ga, ^{111}In, ^{177}Lu, ^{90}Y,

^{89}Zr, ^{61}Cu, ^{62}Cu, ^{67}Cu, ^{19}F, ^{66}Ga, ^{72}Ga, ^{44}Sc, ^{47}Sc, ^{86}Y, ^{88}Y and ^{45}Ti. In some embodiments, the radionuclide is ^{68}Ga. In some embodiments, the scFv is conjugated to a chelating compound that chelates the radionuclide. In some embodiments, the chelating compound is NOTA, DOTA or derivatives thereof. In some embodiments, the scFv has a K_D between about 9×10^{-10} M to about 1×10^{-8} M (such as about 9×10^{-10} to 1×10^{-9}, about 1×10^{-9} to 2×10^{-9}, about 2×10^{-10} to 5×10^{-9}, or about 5×10^{-10} to 1×10^{-8}) with the immune checkpoint ligand. In some embodiments, the scFv cross-reacts with the immune checkpoint ligand from a non-human mammal (*e.g.*, mouse, rat or monkey). In some embodiments, the scFv is humanized. In some embodiments, the scFv is stable at acidic pH (*e.g.*, at a pH lower than about 6.5, 6.0, 5.5, or 5.0). In some embodiments, the scFv has a melting temperature (Tm) of about 55-70°C (such as about any one of 55-60, 60-65, or 65-70°C). In some embodiments, the scFv comprises one or more engineered disulfide bonds. In some embodiments, the scFv comprises from the N-terminus to the C-terminus: a V_H, an optional peptide linker, and a V_L. In some embodiments, the scFv comprises from the N-terminus to the C-terminus: a V_L, an optional peptide linker, and a V_H. In some embodiments, the scFv comprises a peptide linker comprising the amino acid sequence of SEQ ID NO: 47 or 48. In some embodiments, the scFv comprises one or more (such as 1, 2, 3, or more) engineered disulfide bonds. In some embodiments, the scFv comprises a first engineered cysteine residue at position 44 of V_H and a second engineered cysteine residue at position 100 of V_L, and/or a first engineered cysteine residue at position 105 of V_H and a second engineered cysteine residue at position 43 of V_L, wherein the first engineered cysteine residue and the second engineered cysteine residue form a disulfide bond, and wherein the amino acid positions are based on the Kabat numbering system.

[192] In some embodiments, there is provided an imaging agent comprising an antibody moiety labeled with a radionuclide, wherein the antibody moiety specifically binds an immune checkpoint ligand, and wherein the antibody moiety is an scFv fused to an Fc fragment. In some embodiments, the immune checkpoint ligand is selected from the group consisting of PD-L1, PD-L2, B7-H3, galectin-9, CD80, CD86 and ICOSL. In some embodiments, the immune checkpoint ligand is a PD-L1 like ligand. In some embodiments, the radionuclide is selected from the group consisting of ^{64}Cu, ^{18}F, ^{67}Ga, ^{68}Ga, ^{111}In, ^{177}Lu, ^{90}Y, ^{89}Zr, ^{61}Cu, ^{62}Cu, ^{67}Cu, ^{19}F, ^{66}Ga, ^{72}Ga, ^{44}Sc, ^{47}Sc, ^{86}Y, ^{88}Y and ^{45}Ti. In some embodiments, the radionuclide is ^{68}Ga. In some embodiments, the antibody moiety is conjugated to a chelating compound that chelates the radionuclide. In some embodiments, the chelating compound is NOTA, DOTA or derivatives thereof. In some embodiments, the antibody moiety has a K_D between about 9×10^{-10} M to about 1×10^{-8} M (such as about 9×10^{-10} to 1×10^{-9}, about 1×10^{-9} to 2×10^{-9}, about 2×10^{-10} to 5×10^{-9}, or about 5×10^{-10} to 1×10^{-8}) with the immune checkpoint ligand. In some embodiments, the antibody moiety cross-reacts with the immune checkpoint ligand from a non-human mammal (*e.g.*, mouse, rat or monkey). In some embodiments, the antibody moiety is humanized. In some embodiments, the antibody moiety is stable at acidic pH (*e.g.*, at a pH lower than about 6.5, 6.0, 5.5, or 5.0). In some embodiments, the

antibody moiety has a melting temperature (Tm) of about 55-70°C (such as about any one of 55-60, 60-65, or 65-70°C). In some embodiments, the scFv comprises one or more engineered disulfide bonds. In some embodiments, the scFv comprises from the N-terminus to the C-terminus: a V_H, an optional peptide linker, and a V_L. In some embodiments, the scFv comprises from the N-terminus to the C-terminus: a V_L, an optional peptide linker, and a V_H. In some embodiments, the scFv comprises a peptide linker comprising the amino acid sequence of SEQ ID NO: 47 or 48. In some embodiments, the scFv comprises one or more (such as 1, 2, 3, or more) engineered disulfide bonds. In some embodiments, the scFv comprises a first engineered cysteine residue at position 44 of V_H and a second engineered cysteine residue at position 100 of V_L, and/or a first engineered cysteine residue at position 105 of V_H and a second engineered cysteine residue at position 43 of V_L, wherein the first engineered cysteine residue and the second engineered cysteine residue form a disulfide bond, and wherein the amino acid positions are based on the Kabat numbering system. In some embodiments, the Fc fragment is a human IgG1 Fc fragment. In some embodiments, the Fc fragment has H310A and H435Q mutations, wherein the amino acid positions are based on the Kabat numbering system.

[193] In some embodiments, there is provided an imaging agent comprising an antibody moiety labeled conjugated to a chelating compound (*e.g.*, NOTA, DOTA, or derivatives thereof) that chelates a radionuclide (*e.g.*, ^{68}Ga), wherein the antibody moiety specifically binds an immune checkpoint ligand, and wherein the antibody moiety is an scFv fused to an Fc fragment. In some embodiments, there is provided an imaging agent comprising an antibody moiety conjugated to NOTA that chelates a radionuclide (*e.g.*, ^{68}Ga), wherein the antibody moiety specifically binds an immune checkpoint ligand. In some embodiments, the immune checkpoint ligand is selected from the group consisting of PD-L1, PD-L2, B7-H3, galectin-9, CD80, CD86 and ICOSL. In some embodiments, the immune checkpoint ligand is a PD-L1 like ligand. In some embodiments, the antibody moiety has a half-life of about 10 minutes to about 24 hours (such as about any one of 10 minutes to 2 hours, 1 hour to 4 hours, 4 hours to 8 hours, 8 hours to 12 hours or 12 hours to 24 hours) in serum. In some embodiments, the antibody moiety is no more than about 120 kDa (such as no more than about 30 kDa, 50 kDa, 80 kDa, or 100 kDa, or about any one of 30-50kDa, 50-100kDa, or 30-80kDa). In some embodiments, the antibody moiety has a K_D between about 9×10^{-10} M to about 1×10^{-8} M (such as about 9×10^{-10} to 1×10^{-9}, about 1×10^{-9} to 2×10^{-9}, about 2×10^{-10} to 5×10^{-9}, or about 5×10^{-10} to 1×10^{-8}) with the immune checkpoint ligand. In some embodiments, the antibody moiety cross-reacts with the immune checkpoint ligand from a non-human mammal (*e.g.*, mouse, rat or monkey). In some embodiments, the antibody moiety is humanized. In some embodiments, the antibody moiety is stable at acidic pH (*e.g.*, at a pH lower than about 6.5, 6.0, 5.5, or 5.0). In some embodiments, the antibody moiety has a melting temperature (Tm) of about 55-70oC (such as about any one of 55-60, 60-65, or 65-70°C). In some embodiments, the antibody moiety is selected from the group consisting of a single-chain Fv (scFv), a diabody, a Fab, a Fab', a $F(ab')_2$, an Fv fragment, a disulfide stabilized

Fv fragment (dsFv), a $(dsFv)_2$, and a V_HH. In some embodiments, the radionuclide is selected from the group consisting of ^{64}Cu, ^{18}F, ^{67}Ga, ^{68}Ga, ^{111}In, ^{177}Lu, ^{90}Y, ^{89}Zr, ^{61}Cu, ^{62}Cu, ^{67}Cu, ^{19}F, ^{66}Ga, ^{72}Ga, ^{44}Sc, ^{47}Sc, ^{86}Y, ^{88}Y and ^{45}Ti.

[194] In some embodiments, there is provided an imaging agent comprising a scFv conjugated to a chelating compound (*e.g.*, NOTA, DOTA, or derivative thereof) that chelates a radionuclide (*e.g.*, ^{68}Ga), wherein the scFv specifically binds an immune checkpoint ligand. In some embodiments, there is provided an imaging agent comprising a scFv conjugated to NOTA that chelates a radionuclide (*e.g.*, ^{68}Ga), wherein the scFv specifically binds an immune checkpoint ligand. In some embodiments, the immune checkpoint ligand is selected from the group consisting of PD-L1, PD-L2, B7-H3, galectin-9, CD80, CD86 and ICOSL. In some embodiments, the immune checkpoint ligand is a PD-L1 like ligand. In some embodiments, the scFv has a K_D between about 9×10^{-10} M to about 1×10^{-8} M (such as about 9×10^{-10} to 1×10^{-9}, about 1×10^{-9} to 2×10^{-9}, about 2×10^{-10} to 5×10^{-9}, or about 5×10^{-10} to 1×10^{-8}) with the immune checkpoint ligand. In some embodiments, the scFv cross-reacts with the immune checkpoint ligand from a non-human mammal (*e.g.*, mouse, rat or monkey). In some embodiments, the scFv is humanized. In some embodiments, the scFv is stable at acidic pH (*e.g.*, at a pH lower than about 6.5, 6.0, 5.5, or 5.0). In some embodiments, the scFv has a melting temperature (Tm) of about 55-70°C (such as about any one of 55-60, 60-65, or 65-70°C). In some embodiments, the scFv comprises one or more engineered disulfide bonds. In some embodiments, the scFv comprises from the N-terminus to the C-terminus: a V_H, an optional peptide linker, and a V_L. In some embodiments, the scFv comprises from the N-terminus to the C-terminus: a V_L, an optional peptide linker, and a V_H. In some embodiments, the scFv comprises a peptide linker comprising the amino acid sequence of SEQ ID NO: 47 or 48. In some embodiments, the scFv comprises one or more (such as 1, 2, 3, or more) engineered disulfide bonds. In some embodiments, the scFv comprises a first engineered cysteine residue at position 44 of V_H and a second engineered cysteine residue at position 100 of V_L, and/or a first engineered cysteine residue at position 105 of V_H and a second engineered cysteine residue at position 43 of V_L, wherein the first engineered cysteine residue and the second engineered cysteine residue form a disulfide bond, and wherein the amino acid positions are based on the Kabat numbering system. In some embodiments, the radionuclide is selected from the group consisting of ^{64}Cu, ^{18}F, ^{67}Ga, ^{68}Ga, ^{111}In, ^{177}Lu, ^{90}Y, ^{89}Zr, ^{61}Cu, ^{62}Cu, ^{67}Cu, ^{19}F, ^{66}Ga, ^{72}Ga, ^{44}Sc, ^{47}Sc, ^{86}Y, ^{88}Y and ^{45}Ti.

[195] In some embodiments, there is provided an imaging agent comprising an antibody moiety conjugated to NOTA that chelates a radionuclide (*e.g.*, ^{68}Ga), wherein the antibody moiety specifically binds an immune checkpoint ligand, and wherein the antibody moiety is an scFv fused to an Fc fragment. In some embodiments, the immune checkpoint ligand is selected from the group consisting of PD-L1, PD-L2, B7-H3, galectin-9, CD80, CD86 and ICOSL. In some embodiments, the immune checkpoint ligand is a PD-L1 like ligand. In some embodiments, the antibody moiety has a K_D between about 9×10^{-10} M to about 1×10^{-8} M (such as about 9×10^{-10}

to 1×10^{-9}, about 1×10^{-9} to 2×10^{-9}, about 2×10^{-10} to 5×10^{-9}, or about 5×10^{-10} to 1×10^{-8}) with the immune checkpoint ligand. In some embodiments, the antibody moiety cross-reacts with the immune checkpoint ligand from a non-human mammal (*e.g.*, mouse, rat or monkey). In some embodiments, the antibody moiety is humanized. In some embodiments, the antibody moiety is stable at acidic pH (*e.g.*, at a pH lower than about 6.5, 6.0, 5.5, or 5.0). In some embodiments, the antibody moiety has a melting temperature (Tm) of about 55-70°C (such as about any one of 55-60, 60-65, or 65-70°C). In some embodiments, the scFv comprises one or more engineered disulfide bonds. In some embodiments, the scFv comprises from the N-terminus to the C-terminus: a V_H, an optional peptide linker, and a V_L. In some embodiments, the scFv comprises from the N-terminus to the C-terminus: a V_L, an optional peptide linker, and a V_H. In some embodiments, the scFv comprises a peptide linker comprising the amino acid sequence of SEQ ID NO: 47 or 48. In some embodiments, the scFv comprises one or more (such as 1, 2, 3, or more) engineered disulfide bonds. In some embodiments, the scFv comprises a first engineered cysteine residue at position 44 of V_H and a second engineered cysteine residue at position 100 of V_L, and/or a first engineered cysteine residue at position 105 of V_H and a second engineered cysteine residue at position 43 of V_L, wherein the first engineered cysteine residue and the second engineered cysteine residue form a disulfide bond, and wherein the amino acid positions are based on the Kabat numbering system. In some embodiments, the Fc fragment is a human IgG1 Fc fragment. In some embodiments, the Fc fragment has H310A and H435Q mutations, wherein the amino acid positions are based on the Kabat numbering system. In some embodiments, the radionuclide is selected from the group consisting of ^{64}Cu, ^{18}F, ^{67}Ga, ^{68}Ga, ^{111}In, ^{177}Lu, ^{90}Y, ^{89}Zr, ^{61}Cu, ^{62}Cu, ^{67}Cu, ^{19}F, ^{66}Ga, ^{72}Ga, ^{44}Sc, ^{47}Sc, ^{86}Y, ^{88}Y and ^{45}Ti.

[196] In some embodiments, there is provided an imaging agent comprising an anti-PD-L1 antibody moiety labeled with a radionuclide, wherein the anti-PD-L1 antibody moiety specifically binds PD-L1. In some embodiments, the radionuclide is selected from the group consisting of ^{64}Cu, ^{18}F, ^{67}Ga, ^{68}Ga, ^{111}In, ^{177}Lu, ^{90}Y, ^{89}Zr, ^{61}Cu, ^{62}Cu, ^{67}Cu, ^{19}F, ^{66}Ga, ^{72}Ga, ^{44}Sc, ^{47}Sc, ^{86}Y, ^{88}Y and ^{45}Ti. In some embodiments, the radionuclide is ^{68}Ga. In some embodiments, the anti-PD-L1 antibody moiety is conjugated to a chelating compound that chelates the radionuclide. In some embodiments, the chelating compound is NOTA, DOTA or derivatives thereof. In some embodiments, the anti-PD-L1 antibody moiety has a half-life of about 10 minutes to about 24 hours (such as about any one of 10 minutes to 2 hours, 1 hour to 4 hours, 4 hours to 8 hours, 8 hours to 12 hours or 12 hours to 24 hours) in serum. In some embodiments, the anti-PD-L1 antibody moiety is no more than about 120 kDa (such as no more than about 30 kDa, 50 kDa, 80 kDa, or 100 kDa, or about any one of 30-50kDa, 50-100kDa, or 30-80kDa). In some embodiments, the anti-PD-L1 antibody moiety has a K_D between about 9×10^{-10} M to about 1×10^{-8} M (such as about 9×10^{-10} to 1×10^{-9}, about 1×10^{-9} to 2×10^{-9}, about 2×10^{-10} to 5×10^{-9}, or about 5×10^{-10} to 1×10^{-8}) with the immune checkpoint ligand. In some embodiments, the anti-PD-L1 antibody moiety cross-reacts with the immune checkpoint ligand from a non-human mammal (*e.g.*, mouse, rat or monkey). In

some embodiments, the anti-PD-L1 antibody moiety is humanized. In some embodiments, the anti-PD-L1 antibody moiety is stable at acidic pH (*e.g.*, at a pH lower than about 6.5, 6.0, 5.5, or 5.0). In some embodiments, the anti-PD-L1 antibody moiety has a melting temperature (Tm) of about 55-70°C (such as about any one of 55-60, 60-65, or 65-70°C). In some embodiments, the anti-PD-L1 antibody moiety is selected from the group consisting of a single-chain Fv (scFv), a diabody, a Fab, a Fab', a $F(ab')_2$, an Fv fragment, a disulfide stabilized Fv fragment (dsFv), a $(dsFv)_2$, and a V_HH. In some embodiments, the anti-PD-L1 antibody moiety is an scFv. In some embodiments, the anti-PD-L1 antibody moiety is an scFv fused to an Fc. Exemplary anti-PD-L1 antibody moieties are discussed in detail in the "Anti-PD-L1 antibody agents" section.

Radionuclide

[197] The imaging agents described herein comprise a label. For diagnostic purposes, the label may be a radionuclide, a radiological contrast agent, a paramagnetic ion, a metal, a fluorescent label, a chemiluminescent label, an ultrasound contrast agent and a photoactive agent. Such diagnostic labels are well known and any such known labels may be used.

[198] In some embodiments, the imaging agent comprises a radionuclide. "Radionuclides" are often referred to as "radioactive isotopes" or "radioisotopes." Exemplary radionuclides or stable isotopes that may be attached to the antibody moieties described herein include, but are not limited to, ^{110}In, ^{111}In, ^{177}Lu, ^{18}F, ^{52}Fe, ^{62}Cu, ^{64}Cu, ^{67}Cu, ^{67}Ga, ^{68}Ga, ^{86}Y, ^{90}Y, ^{89}Zr, ^{94m}Tc, ^{94}Tc, ^{99m}Tc, ^{120}I, ^{123}I, ^{124}I, ^{125}I, ^{131}I, $^{154\text{-}158}Gd$, ^{32}P, ^{11}C, ^{13}N, ^{15}O, ^{186}Re, ^{188}Re, ^{51}Mn, ^{52m}Mn, ^{55}Co, ^{72}As, ^{75}Br, ^{76}Br, ^{82m}Rb, ^{83}Sr, or other gamma-, beta-, or positron-emitters. In some embodiments, the radionuclide is selected from the group consisting of ^{64}Cu, ^{18}F, ^{67}Ga, ^{68}Ga, ^{111}In, ^{177}Lu, ^{90}Y, ^{89}Zr, ^{61}Cu, ^{62}Cu, ^{67}Cu, ^{19}F, ^{66}Ga, ^{72}Ga, ^{44}Sc, ^{47}Sc, ^{86}Y, ^{88}Y and ^{45}Ti. In some embodiments, the radionuclide is ^{68}Ga.

[199] Paramagnetic ions of use may include chromium (III), manganese (II), iron (III), iron (II), cobalt (II), nickel (II), copper (II), neodymium (III), samarium (III), ytterbium (III), gadolinium (III), vanadium (II), terbium (III), dysprosium (III), holmium (III) or erbium (III). Metal contrast agents may include lanthanum (III), gold (III), lead (II) or bismuth (III). Radiopaque diagnostic agents may be selected from compounds, barium compounds, gallium compounds, and thallium compounds. A wide variety of fluorescent labels are known in the art, including but not limited to fluorescein isothiocyanate, rhodamine, phycoelytherin, phycocyanin, allophycocyanin, ophthaldehyde and fluorescamine. Chemiluminescent labels of use may include luminol, isoluminol, an aromatic acridinium ester, an imidazole, an acridinium salt or an oxalate ester.

[200] Radioimmunodetection (RAID) has emerged as a clinically useful field over the last 35 years. Almost 1000 clinical trials using RAID have been conducted during this time, with some clear and important findings. The greater facility of this technique to detect lesions deemed "occult" by conventional imaging was recognized

even in early studies and has repeatedly been confirmed by studies, regardless of antibody, tumor or radionuclide type.

[201] Many radionuclides, such as ^{68}Ga, ^{99}Tc, ^{64}Cu and ^{18}F are good imaging agent of choice. They usually have a gamma or beta energy that is ideal for safe imaging, and are inexpensive and are readily available, being generator-produced and carrier-free. Their short half-life (less than 6 hrs) readily lends themselves to coupling with antibody fragments for early imaging studies.

[202] In some embodiments, the imaging agent comprises a chelating compound that chelates the radionuclide. In some embodiments, the chelating compound chelates a radioactive metal. In some embodiments, the chelating compound chelates a metal ^{18}F. In some embodiments, the chelating compound is a hydrophilic chelating compound, which can bind metal ions and help to ensure rapid *in vivo* clearance. Suitable chelating compounds may be selected for their particular metal-binding properties, and substitution by known chemical cross-linking techniques or by use of chelators with side-chain reactive groups (such as bifunctional chelating compounds) may be performed with only routine experimentation.

[203] Particularly useful metal-chelating compound combinations include 2-benzyl-DTPA (diethylenetriamine pentaacetic acid) and its monomethyl and cyclohexyl analogs, used with diagnostic isotopes in the general energy range of 60 to 4,000 keV, such as ^{125}I, ^{131}I, ^{123}I, ^{124}I , ^{62}Cu, ^{64}Cu, ^{18}F, ^{111}In, ^{67}Ga, ^{68}Ga, ^{99}Tc, ^{94}Tc, ^{11}C, ^{13}N, ^{15}O, ^{76}Br, for radio-imaging. The same chelating compounds, when complexed with nonradioactive metals, such as manganese, iron and gadolinium are useful for MRI. Macrocyclic chelating compounds such as NOTA (1,4,7-triazacyclononane-1,4,7-triacetic acid), DOTA (1,4,7,10-Tetraazacyclododecane-*N,N',N'',N'''*-tetraacetic acid), TETA (bromoacetamido-benzyl-tetraethylaminetetraacetic acid) and NETA ({4-[2-(bis-carboxymethyl-amino)-ethyl]-7-carboxymethyl-[1,4,7]triazonan-1-yl}-acetic acid) are of use with a variety of diagnostic radiometals, such as gallium, yttrium and copper. Such metal-chelating complexes can be made very stable by tailoring the ring size to the metal of interest. The person of ordinary skill will understand that, by varying the groups attached to a macrocyclic ring structure such as NOTA, the binding characteristics and affinity for different metals and/or radionuclides may change and such derivatives or analogs of, e.g. NOTA, may therefore be designed to bind any of the metals or radionuclides discussed herein.

[204] DTPA and DOTA-type chelators, where the ligand includes hard base chelating functions such as carboxylate or amine groups, are most effective for chelating hard acid cations, especially Group IIa and Group IIIa metal cations. Such metal-chelate complexes can be made very stable by tailoring the ring size to the metal of interest. Other ring-type chelators such as macrocyclic polyethers are of interest for stably binding nuclides. Porphyrin chelators may be used with numerous metal complexes. More than one type of chelator may be conjugated to a peptide to bind multiple metal ions. Chelators such as those disclosed in U.S. Pat. No. 5,753,206,

especially thiosemicarbazonylglyoxylcysteine (Tscg-Cys) and thiosemicarbazinyl-acetylcysteine (Tsca-Cys) chelators are advantageously used to bind soft acid cations of Tc, Re, Bi and other transition metals, lanthanides and actinides that are tightly bound to soft base ligands. Other hard acid chelators such as DOTA, TETA and the like can be substituted for the DTPA and/or TscgCys groups.

[205] In some embodiments, the chelating compound comprises a functional group that can be conjugated to the antibody moiety. In some embodiments, the chelating compound comprises a functional group that is reactive with a primary amine ($-NH_2$) group in the antibody moiety. Primary amines exist at the N-terminus of each polypeptide chain and in the side-chain of lysine (Lys) amino acid residues. Exemplary functional groups that can be conjugated to a primary amine, *e.g.*, a lysine side chain, of the antibody moiety, include, but are not limited to, isothiocyanates, isocyanates, acyl azides, N-hydroxysuccinimide (NHS) esters, sulfonyl chlorides, aldehydes, glyoxals, epoxides, oxiranes, carbonates, aryl halides, imidoesters, carbodiimides, anhydrides, and fluorophenyl esters. Most of these functional groups conjugate to amines by either acylation or alkylation.

[206] In some embodiments, the chelating compound comprises a functional group that is reactive with a cysteine side chain (*i.e.*, sulfhydryl group) in the antibody moiety. Exemplary sulfhydryl reactive groups include, but are not limited to, haloacetyls, maleimides, aziridines, acryloyls, arylating agents, vinylsulfones, pyridyl disulfides, TNB-thiols and disulfide reducing agents. Most of these groups conjugate to sulfhydryls by either alkylation (usually the formation of a thioether bond) or disulfide exchange (formation of a disulfide bond).

[207] In some embodiments, the chelating compound is NOTA, including NOTA derivatives. Exemplary NOTA compounds with functional groups suitable for conjugation to antibody moieties, *e.g.*, via amino acid side chains such as lysines and cysteines, are shown in FIG. 23. In some embodiments, the imaging agent comprises NOTA conjugated to the antibody moiety. In some embodiments, the NOTA compound comprises an isothiocyanate (-SCN) group. In some embodiments, the NOTA compound is p-SCN-Bn-NOTA. In some embodiments, the chelating compound comprises a NOTA conjugated to a lysine residue in the antibody moiety, and the NOTA chelates ^{68}Ga. In some embodiments, the NOTA compound is first labeled with a radioactive metal, such as ^{68}Ga, or ^{18}F-metal, and then conjugated to the antibody moiety.

IV. Anti-PD-L1 antibody agents

[208] One aspect of the present application provides an isolated anti-PD-L1 antibody agent and an anti-PD-L1 imaging agent. The isolated anti-PD-L1 antibody agent may be unlabeled or labeled with a radionuclide. The isolated anti-PD-L1 antibody agents described herein do not encompass anti-PD-L1 therapeutic agents.

[209] In some embodiments, there is provided an isolated anti-PD-L1 antibody agent comprising any one of the anti-PD-L1 antibody moieties described herein. In some embodiments, the anti-PD-L1 antibody moiety is

humanized. In some embodiments, the anti-PD-L1 antibody moiety comprises an scFv. In some embodiments, the anti-PD-L1 antibody moiety is an scFv. In some embodiments, the scFv comprises a first engineered cysteine residue at position 44 of V_H and a second engineered cysteine residue at position 100 of V_L, or a first engineered cysteine residue at position 105 of V_H and a second engineered cysteine residue at position 43 of V_L, wherein the first engineered cysteine residue and the second engineered cysteine residue form a disulfide bond, and wherein the amino acid positions are based on the Kabat numbering system. In some embodiments, the anti-PD-L1 antibody moiety is an scFv fused to an Fc fragment (such as IgG1 Fc fragment). In some embodiments, the Fc fragment has H310A and H435Q mutations, wherein the amino acid positions are based on the Kabat numbering system.

[210] In some embodiments, there is provided an isolated anti-PD-L1 antibody agent comprising an anti-PD-L1 antibody moiety comprising: a V_H comprising a HC-CDR1 comprising the amino acid sequence of SEQ ID NO: 41, a HC-CDR2 comprising the amino acid sequence of SEQ ID NO: 42, and a HC-CDR3 comprising the amino acid sequence of SEQ ID NO: 43, or a variant thereof comprising up to about 5 amino acid substitutions; and a V_L comprising a LC-CDR1 comprising the amino acid sequence of SEQ ID NO: 44, a LC-CDR2 comprising the amino acid sequence of SEQ ID NO: 45, and a LC-CDR3 comprising the amino acid sequence of SEQ ID NO: 46, or a variant thereof comprising up to about 5 amino acid substitutions. In some embodiments, the anti-PD-L1 antibody moiety comprises: a V_H comprising the amino acid sequence of any one of SEQ ID NOs: 1, 5, 9, 11, and 13, or a variant thereof having at least about 80% (such as at least about any one of 80%, 85%, 90%, 95%, 96%, 97%, 98%, or 99%) sequence identity to the amino acid sequence of any one of SEQ ID NOs: 1, 5, 9, 11, and 13; and a V_L comprising the amino acid sequence of any one of SEQ ID NOs: 3, 7, 15, 17 and 19, or a variant thereof having at least about 80% (such as at least about any one of 80%, 85%, 90%, 95%, 96%, 97%, 98%, or 99%) sequence identity to the amino acid sequence of any one of SEQ ID NOs: 3, 7, 15, 17 and 19. In some embodiments, the anti-PD-L1 antibody moiety is humanized.

[211] In some embodiments, there is provided an isolated anti-PD-L1 antibody agent comprising an anti-PD-L1 scFv comprising: a V_H comprising a HC-CDR1 comprising the amino acid sequence of SEQ ID NO: 41, a HC-CDR2 comprising the amino acid sequence of SEQ ID NO: 42, and a HC-CDR3 comprising the amino acid sequence of SEQ ID NO: 43, or a variant thereof comprising up to about 5 amino acid substitutions; and a V_L comprising a LC-CDR1 comprising the amino acid sequence of SEQ ID NO: 44, a LC-CDR2 comprising the amino acid sequence of SEQ ID NO: 45, and a LC-CDR3 comprising the amino acid sequence of SEQ ID NO: 46, or a variant thereof comprising up to about 5 amino acid substitutions. In some embodiments, the anti-PD-L1 antibody moiety comprises: a V_H comprising the amino acid sequence of any one of SEQ ID NOs: 1, 5, 9, 11, and 13, or a variant thereof having at least about 80% (such as at least about any one of 80%, 85%, 90%, 95%, 96%, 97%, 98%, or 99%) sequence identity to the amino acid sequence of any one of SEQ ID NOs: 1, 5, 9,

11, and 13; and a V_L comprising the amino acid sequence of any one of SEQ ID NOs: 3, 7, 15, 17 and 19, or a variant thereof having at least about 80% (such as at least about any one of 80%, 85%, 90%, 95%, 96%, 97%, 98%, or 99%) sequence identity to the amino acid sequence of any one of SEQ ID NOs: 3, 7, 15, 17 and 19. In some embodiments, the anti-PD-L1 antibody moiety is humanized. In some embodiments, the anti-PD-L1 scFv comprises a first engineered cysteine residue at position 44 of V_H and a second engineered cysteine residue at position 100 of V_L, or a first engineered cysteine residue at position 105 of V_H and a second engineered cysteine residue at position 43 of V_L, wherein the first engineered cysteine residue and the second engineered cysteine residue form a disulfide bond, and wherein the amino acid positions are based on the Kabat numbering system. In some embodiments, the anti-PD-L1 scFv comprises the amino acid sequence of any one of SEQ ID NOs: 25, 27, 29, 31, 33, 35, 37 and 39, or a variant thereof having at least about 80% (such as at least about any one of 80%, 85%, 90%, 95%, 96%, 97%, 98%, or 99%) sequence identity to the amino acid sequence of any one of SEQ ID NOs: 25, 27, 29, 31, 33, 35, 37 and 39.

[212] In some embodiments, there is provided an isolated anti-PD-L1 antibody agent comprising an anti-PD-L1 scFv fused to an Fc fragment, wherein the anti-PD-L1 scFv comprises: a V_H comprising a HC-CDR1 comprising the amino acid sequence of SEQ ID NO: 41, a HC-CDR2 comprising the amino acid sequence of SEQ ID NO: 42, and a HC-CDR3 comprising the amino acid sequence of SEQ ID NO: 43, or a variant thereof comprising up to about 5 amino acid substitutions; and a V_L comprising a LC-CDR1 comprising the amino acid sequence of SEQ ID NO: 44, a LC-CDR2 comprising the amino acid sequence of SEQ ID NO: 45, and a LC-CDR3 comprising the amino acid sequence of SEQ ID NO: 46, or a variant thereof comprising up to about 5 amino acid substitutions. In some embodiments, the anti-PD-L1 antibody moiety comprises: a V_H comprising the amino acid sequence of any one of SEQ ID NOs: 1, 5, 9, 11, and 13, or a variant thereof having at least about 80% (such as at least about any one of 80%, 85%, 90%, 95%, 96%, 97%, 98%, or 99%) sequence identity to the amino acid sequence of any one of SEQ ID NOs: 1, 5, 9, 11, and 13; and a V_L comprising the amino acid sequence of any one of SEQ ID NOs: 3, 7, 15, 17 and 19, or a variant thereof having at least about 80% (such as at least about any one of 80%, 85%, 90%, 95%, 96%, 97%, 98%, or 99%) sequence identity to the amino acid sequence of any one of SEQ ID NOs: 3, 7, 15, 17 and 19. In some embodiments, the anti-PD-L1 antibody moiety is humanized. In some embodiments, the anti-PD-L1 scFv comprises a first engineered cysteine residue at position 44 of V_H and a second engineered cysteine residue at position 100 of V_L, or a first engineered cysteine residue at position 105 of V_H and a second engineered cysteine residue at position 43 of V_L, wherein the first engineered cysteine residue and the second engineered cysteine residue form a disulfide bond, and wherein the amino acid positions are based on the Kabat numbering system. In some embodiments, the Fc fragment is an IgG1 Fc fragment. In some embodiments, the Fc fragment has H310A and H435Q mutations, wherein the amino acid positions are based on the Kabat numbering system. In some embodiments, the anti-PD-

L1 scFv comprises the amino acid sequence of any one of SEQ ID NOs: 25, 27, 29, 31, 33, 35, 37 and 39, or a variant thereof having at least about 80% (such as at least about any one of 80%, 85%, 90%, 95%, 96%, 97%, 98%, or 99%) sequence identity to the amino acid sequence of any one of SEQ ID NOs: 25, 27, 29, 31, 33, 35, 37 and 39.

Anti-PD-L1 antibody moieties

[213] The isolated anti-PD-L1 antibody agents described herein comprise an antibody moiety that specifically binds to PD-L1. Contemplated anti-PD-L1 antibody moieties include, for example, anti-PD-L1 scFv, anti-PD-L1 Fab, anti-PD-L1 Fc fusion protein (*e.g.*, anti-PD-L1 scFv fused to an Fc). The anti-PD-L1 antibody moieties described herein include, but are not limited to, humanized antibodies, partially humanized antibodies, fully humanized antibodies, semi-synthetic antibodies, chimeric antibodies, mouse antibodies, human antibodies, and antibodies comprising the heavy chain and/or light chain CDRs discussed herein.

[214] In some embodiments, the anti-PD-L1 antibody moiety specifically recognizes PD-L1. In some embodiments, the anti-PD-L1 antibody moiety specifically recognizes human PD-L1. In some embodiments, the anti-PD-L1 antibody moiety specifically recognizes the extracellular domain of PD-L1. In some embodiments, the anti-PD-L1 antibody moiety specifically recognizes an epitope within the amino acid sequence of amino acids 19-238 of SEQ ID NO: 49.

SEQ ID NO: 49 human PD-L1 sequence

MRIFAVFIFMTYWHLLNAFTVTVPKDLYVVEYGSNMTIECKFPVEKQLDLAALIVYWEMEDKNIIQFV
HGEEDLKVQHSSYRQRARLLKDQLSLGNAALQITDVKLQDAGVYRCMISYGGADYKRITVKVNAPYN
KINQRILVVDPVTSEHELTCQAEGYPKAEVIWTSSDHQVLSGKTTTTNSKREEKLFNVTSTLRINTTTNE
IFYCTFRRLDPEENHTAELVIPELPLAHPPNERTHLVILGAILLCLGVALTFIFRLRKGRMMDVKKCGIQ
DTNSKKQSDTHLEET

[215] In some embodiments, the anti-PD-L1 antibody moiety comprises: a heavy chain variable domain (V_H) comprising an HC-CDR3 comprising the amino acid sequence of SEQ ID NO: 43, or a variant thereof comprising up to about 5 (such as about any of 1, 2, 3, 4, or 5) amino acid substitutions; and ii) a light chain variable domain (V_L) comprising an LC-CDR3 comprising the amino acid sequence of SEQ ID NO: 46, or a variant thereof comprising up to about 5 (such as about any of 1, 2, 3, 4, or 5) amino acid substitutions.

[216] In some embodiments, the anti-PD-L1 antibody moiety comprises: i) a V_H comprising an HC-CDR3 comprising the amino acid sequence of SEQ ID NO: 43; and ii) a V_L comprising an LC-CDR3 comprising the amino acid sequence of SEQ ID NO: 46.

[217] In some embodiments, the anti-PD-L1 antibody moiety comprises: i) a V_H comprising an HC-CDR1 comprising the amino acid sequence of SEQ ID NO: 41, or a variant thereof comprising up to about 5 (such as

about any of 1, 2, 3, 4, or 5) amino acid substitutions, an HC-CDR2 comprising the amino acid sequence of SEQ ID NO: 42, or a variant thereof comprising up to about 5 (such as about any of 1, 2, 3, 4, or 5) amino acid substitutions, and an HC-CDR3 comprising the amino acid sequence of a SEQ ID NO: 43, or a variant thereof comprising up to about 5 (such as about any of 1, 2, 3, 4, or 5) amino acid substitutions; and ii) a V_L comprising an LC-CDR1 comprising the amino acid sequence of SEQ ID NO: 44, or a variant thereof comprising up to about 5 (such as about any of 1, 2, 3, 4, or 5) amino acid substitutions, an LC-CDR2 comprising the amino acid sequence of SEQ ID NO: 45, or a variant thereof comprising up to about 5 (such as about any of 1, 2, 3, 4, or 5) amino acid substitutions, and an LC-CDR3 comprising the amino acid sequence of SEQ ID NO: 46, or a variant thereof comprising up to about 5 (such as about any of 1, 2, 3, 4, or 5) amino acid substitutions.

[218] In some embodiments, the anti-PD-L1 antibody moiety comprises: i) a V_H comprising an HC-CDR1 comprising the amino acid sequence of SEQ ID NO: 41, an HC-CDR2 comprising the amino acid sequence of SEQ ID NO: 42, and an HC-CDR3 comprising the amino acid sequence of a SEQ ID NO: 43; or a variant thereof comprising up to about 5 (such as about any of 1, 2, 3, 4, or 5) amino acid substitutions in the HC-CDR sequences; and ii) a V_L comprising an LC-CDR1 comprising the amino acid sequence of SEQ ID NO: 44, an LC-CDR2 comprising the amino acid sequence of SEQ ID NO: 45, and an LC-CDR3 comprising the amino acid sequence of SEQ ID NO: 46; or a variant thereof comprising up to about 5 (such as about any of 1, 2, 3, 4, or 5) amino acid substitutions in the LC-CDR sequences.

[219] In some embodiments, the anti-PD-L1 antibody moiety comprises: i) a V_H comprising an HC-CDR1 comprising the amino acid sequence of SEQ ID NO: 41, an HC-CDR2 comprising the amino acid sequence of SEQ ID NO: 42, and an HC-CDR3 comprising the amino acid sequence of a SEQ ID NO: 43; and ii) a V_L comprising an LC-CDR1 comprising the amino acid sequence of SEQ ID NO: 44, an LC-CDR2 comprising the amino acid sequence of SEQ ID NO: 45, and an LC-CDR3 comprising the amino acid sequence of SEQ ID NO: 46.

[220] In some embodiments, the anti-PD-L1 antibody moiety comprises: i) a V_H comprising the amino acid sequences of SEQ ID NO: 41, SEQ ID NO: 42, and SEQ ID NO: 43; and ii) a V_L comprising the amino acid sequences of SEQ ID NO: 44, SEQ ID NO: 45, and SEQ ID NO: 46.

[221] In some embodiments, the anti-PD-L1 antibody moiety comprises: i) a V_H comprising one, two or three CDRs of the VH comprising the amino acid sequence of SEQ ID NO: 1; and ii) a V_L comprising one, two or three CDRs of the VL comprising the amino acid sequence of SEQ ID NO: 3.

[222] In some embodiments, the anti-PD-L1 antibody moiety comprises: a) a V_H comprising the amino acid sequence of SEQ ID NO: 1, or a variant thereof having at least about 80% (such as at least about any one of 80%, 85%, 90%, 95%, 96%, 97%, 98%, or 99%) sequence identity to SEQ ID NO: 1; and b) a V_L comprising the amino acid sequence of SEQ ID NO: 2, or a variant thereof having at least about 80% (such as at least about

any one of 80%, 85%, 90%, 95%, 96%, 97%, 98%, or 99%) sequence identity to SEQ ID NO: 2. In some embodiment, the anti-PD-L1 antibody moiety comprises: a) a V_H comprising the amino acid sequence of SEQ ID NO: 1; and b) a V_L comprising the amino acid sequence of SEQ ID NO: 2.

[223] In some embodiments, the anti-PD-L1 antibody is a chimeric antibody. In some embodiments, the anti-PD-L1 antibody moiety comprises mouse variable regions and human constant regions. In some embodiments, the anti-PD-L1 antibody moiety comprises: a) a heavy chain comprising the amino acid sequence of SEQ ID NO: 5, or a variant thereof having at least about 80% (such as at least about any one of 80%, 85%, 90%, 95%, 96%, 97%, 98%, or 99%) sequence identity to SEQ ID NO: 5; and b) a light chain comprising the amino acid sequence of SEQ ID NO: 7, or a variant thereof having at least about 80% (such as at least about any one of 80%, 85%, 90%, 95%, 96%, 97%, 98%, or 99%) sequence identity to SEQ ID NO: 7. In some embodiment, the anti-PD-L1 antibody moiety comprises: a) a heavy chain comprising the amino acid sequence of SEQ ID NO: 5; and b) a light chain comprising the amino acid sequence of SEQ ID NO: 7.

[224] In some embodiments, the anti-PD-L1 antibody is a humanized antibody. In some embodiments, the anti-PD-L1 antibody moiety comprises: a) a V_H comprising the amino acid sequence of any one of SEQ ID NOs: 9, 11 and 13, or a variant thereof having at least about 80% (such as at least about any one of 80%, 85%, 90%, 95%, 96%, 97%, 98%, or 99%) sequence identity to any one of SEQ ID NOs: 9, 11 and 13; and b) a V_L comprising the amino acid sequence of any one of SEQ ID NOs: 15, 17 and 19, or a variant thereof having at least about 80% (such as at least about any one of 80%, 85%, 90%, 95%, 96%, 97%, 98%, or 99%) sequence identity to any one of SEQ ID NOs: 15, 17 and 19. In some embodiments, the anti-PD-L1 antibody moiety comprises: a) a V_H comprising the amino acid sequence of any one of SEQ ID NOs: 9, 11 and 13; and b) a V_L comprising the amino acid sequence of any one of SEQ ID NOs: 15, 17 and 19.

[225] In some embodiments, the anti-PD-L1 antibody moiety comprises: a) a V_H comprising the amino acid sequence of SEQ ID NO: 9, or a variant thereof having at least about 80% (such as at least about any one of 80%, 85%, 90%, 95%, 96%, 97%, 98%, or 99%) sequence identity to SEQ ID NO: 9; and b) a V_L comprising the amino acid sequence of SEQ ID NO: 15, or a variant thereof having at least about 80% (such as at least about any one of 80%, 85%, 90%, 95%, 96%, 97%, 98%, or 99%) sequence identity to SEQ ID NO: 15. In some embodiments, the anti-PD-L1 antibody moiety comprises: a) a V_H comprising the amino acid sequence of SEQ ID NO: 9; and b) a V_L comprising the amino acid sequence of SEQ ID NO: 15.

[226] In some embodiments, the anti-PD-L1 antibody moiety comprises: a) a V_H comprising the amino acid sequence of SEQ ID NO: 11, or a variant thereof having at least about 80% (such as at least about any one of 80%, 85%, 90%, 95%, 96%, 97%, 98%, or 99%) sequence identity to SEQ ID NO: 11; and b) a V_L comprising the amino acid sequence of SEQ ID NO: 15, or a variant thereof having at least about 80% (such as at least about any one of 80%, 85%, 90%, 95%, 96%, 97%, 98%, or 99%) sequence identity to SEQ ID NO: 15. In

some embodiments, the anti-PD-L1 antibody moiety comprises: a) a V_H comprising the amino acid sequence of SEQ ID NO: 11; and b) a V_L comprising the amino acid sequence of SEQ ID NO: 15.

[227] In some embodiments, the anti-PD-L1 antibody moiety comprises: a) a V_H comprising the amino acid sequence of SEQ ID NO: 13, or a variant thereof having at least about 80% (such as at least about any one of 80%, 85%, 90%, 95%, 96%, 97%, 98%, or 99%) sequence identity to SEQ ID NO: 13; and b) a V_L comprising the amino acid sequence of SEQ ID NO: 15, or a variant thereof having at least about 80% (such as at least about any one of 80%, 85%, 90%, 95%, 96%, 97%, 98%, or 99%) sequence identity to SEQ ID NO: 15. In some embodiments, the anti-PD-L1 antibody moiety comprises: a) a V_H comprising the amino acid sequence of SEQ ID NO: 13; and b) a V_L comprising the amino acid sequence of SEQ ID NO: 15.

[228] In some embodiments, the anti-PD-L1 antibody moiety comprises: a) a V_H comprising the amino acid sequence of SEQ ID NO: 9, or a variant thereof having at least about 80% (such as at least about any one of 80%, 85%, 90%, 95%, 96%, 97%, 98%, or 99%) sequence identity to SEQ ID NO: 9; and b) a V_L comprising the amino acid sequence of SEQ ID NO: 17, or a variant thereof having at least about 80% (such as at least about any one of 80%, 85%, 90%, 95%, 96%, 97%, 98%, or 99%) sequence identity to SEQ ID NO: 17. In some embodiments, the anti-PD-L1 antibody moiety comprises: a) a V_H comprising the amino acid sequence of SEQ ID NO: 9; and b) a V_L comprising the amino acid sequence of SEQ ID NO: 17.

[229] In some embodiments, the anti-PD-L1 antibody moiety comprises: a) a V_H comprising the amino acid sequence of SEQ ID NO: 11, or a variant thereof having at least about 80% (such as at least about any one of 80%, 85%, 90%, 95%, 96%, 97%, 98%, or 99%) sequence identity to SEQ ID NO: 11; and b) a V_L comprising the amino acid sequence of SEQ ID NO: 17, or a variant thereof having at least about 80% (such as at least about any one of 80%, 85%, 90%, 95%, 96%, 97%, 98%, or 99%) sequence identity to SEQ ID NO: 17. In some embodiments, the anti-PD-L1 antibody moiety comprises: a) a V_H comprising the amino acid sequence of SEQ ID NO: 11; and b) a V_L comprising the amino acid sequence of SEQ ID NO: 17.

[230] In some embodiments, the anti-PD-L1 antibody moiety comprises: a) a V_H comprising the amino acid sequence of SEQ ID NO: 13, or a variant thereof having at least about 80% (such as at least about any one of 80%, 85%, 90%, 95%, 96%, 97%, 98%, or 99%) sequence identity to SEQ ID NO: 13; and b) a V_L comprising the amino acid sequence of SEQ ID NO: 17, or a variant thereof having at least about 80% (such as at least about any one of 80%, 85%, 90%, 95%, 96%, 97%, 98%, or 99%) sequence identity to SEQ ID NO: 17. In some embodiments, the anti-PD-L1 antibody moiety comprises: a) a V_H comprising the amino acid sequence of SEQ ID NO: 13; and b) a V_L comprising the amino acid sequence of SEQ ID NO: 17.

[231] In some embodiments, the anti-PD-L1 antibody moiety comprises: a) a V_H comprising the amino acid sequence of SEQ ID NO: 9, or a variant thereof having at least about 80% (such as at least about any one of 80%, 85%, 90%, 95%, 96%, 97%, 98%, or 99%) sequence identity to SEQ ID NO: 9; and b) a V_L comprising

the amino acid sequence of SEQ ID NO: 19, or a variant thereof having at least about 80% (such as at least about any one of 80%, 85%, 90%, 95%, 96%, 97%, 98%, or 99%) sequence identity to SEQ ID NO: 19. In some embodiments, the anti-PD-L1 antibody moiety comprises: a) a V_H comprising the amino acid sequence of SEQ ID NO: 9; and b) a V_L comprising the amino acid sequence of SEQ ID NO: 19.

[232] In some embodiments, the anti-PD-L1 antibody moiety comprises: a) a V_H comprising the amino acid sequence of SEQ ID NO: 11, or a variant thereof having at least about 80% (such as at least about any one of 80%, 85%, 90%, 95%, 96%, 97%, 98%, or 99%) sequence identity to SEQ ID NO: 11; and b) a V_L comprising the amino acid sequence of SEQ ID NO: 19, or a variant thereof having at least about 80% (such as at least about any one of 80%, 85%, 90%, 95%, 96%, 97%, 98%, or 99%) sequence identity to SEQ ID NO: 19. In some embodiments, the anti-PD-L1 antibody moiety comprises: a) a V_H comprising the amino acid sequence of SEQ ID NO: 11; and b) a V_L comprising the amino acid sequence of SEQ ID NO: 19.

[233] In some embodiments, the anti-PD-L1 antibody moiety comprises: a) a V_H comprising the amino acid sequence of SEQ ID NO: 13, or a variant thereof having at least about 80% (such as at least about any one of 80%, 85%, 90%, 95%, 96%, 97%, 98%, or 99%) sequence identity to SEQ ID NO: 13; and b) a V_L comprising the amino acid sequence of SEQ ID NO: 19, or a variant thereof having at least about 80% (such as at least about any one of 80%, 85%, 90%, 95%, 96%, 97%, 98%, or 99%) sequence identity to SEQ ID NO: 19. In some embodiments, the anti-PD-L1 antibody moiety comprises: a) a V_H comprising the amino acid sequence of SEQ ID NO: 13; and b) a V_L comprising the amino acid sequence of SEQ ID NO: 19.

[234] The heavy and light chain variable domains can be combined in various pair-wise combinations to generate a number of anti-PD-L1 antibody moieties. Exemplary sequences of anti-PD-L1 antibodies are provided in Tables 3 and 4. The exemplary CDR, VH and VL sequences as shown in Table 3 are delimited according to the INTERNATIONAL IMMUNOGENETICS INFORMATION SYSTEM® (IMGT). *See*, for example, Lefranc, MP *et al.*, *Nucleic Acids Res.*, 43:D413-422 (2015), the disclosure of which is incorporated herein by reference in its entirety. Those skilled in the art will recognize that many algorithms are known for prediction of CDR positions in antibody heavy chain and light chain variable regions, and antibody agents comprising CDRs from antibodies described herein, but based on prediction algorithms other than IMGT, are within the scope of this invention. Table 4 lists exemplary CDR sequences under various other numbering schemes and/or definitions.

Table 3. Exemplary anti-PD-L1 antibody sequences.

SEQ ID NO.		Description	Amino acid sequence (CDR sequences are underlined and bold)
AA	DNA		
1	2	VH	EVQLQQSGAELVKPGASVKLSCTAS**GFNIKDTY**MYWVKQRPEQGLECIGR**ID**

			PANDNTKYDPKFQGKATITADTSSNTAYVQLASLTSEDTAVYYC**ARAKNLLN YFDY**WGQGTTLTVSS
3	4	VL	DIQMTQSPSSLSASLGERVTLSCRAS**QEISGY**LSWLQQKPDGTIKRLIY**ATS** TLDSGVPKRFSGSRSGSDYSLTISSLESEDFADYYC**LQYAIYPLT**FGAGTKL ELKR
41		HC-CDR1	GFNIKDTY
42		HC-CDR2	IDPANDNT
43		HC-CDR3	ARAKNLLNYFDY
44		LC-CDR1	QEISGY
45		LC-CDR2	ATS
46		LC-CDR3	LQYAIYPLT
5	6	Chimeric heavy chain	EVQLQQSGAELVKPGASVKLSCTAS**GFNIKDTY**MYWVKQRPEQGLECIGR**ID PANDNT**KYDPKFQGKATITADTSSNTAYVQLASLTSEDTAVYYC**ARAKNLLN YFDY**WGQGTTLTVSSASTKGPSVFPLAPSSKSTSGGTAALGCLVKDYFPEPV TVSWNSGALTSGVHTFPAVLQSSGLYSLSSVVTVPSSSLGTQTYICNVNHKP SNTKVDKKVEPKSCDKTHTCPPCPAPELLGGPSVFLFPPKPKDTLMISRTPE VTCVVVDVSHEDPEVKFNWYVDGVEVHNAKTKPREEQYNSTYRVVSVLTVLH QDWLNGKEYKCKVSNKALPAPIEKTISKAKGQPREPQVYTLPPSRDELTKNQ VSLTCLVKGFYPSDIAVEWESNGQPENNYKTTPPVLDSDGSFFLYSKLTVDK SRWQQGNVFSCSVMHEALHNHYTQKSLSLSPG
7	8	Chimeric light chain	DIQMTQSPSSLSASLGERVTLSCRAS**QEISGY**LSWLQQKPDGTIKRLIY**ATS** TLDSGVPKRFSGSRSGSDYSLTISSLESEDFADYYC**LQYAIYPLT**FGAGTKL ELKRTVAAPSVFIFPPSDEQLKSGTASVVCLLNNFYPREAKVQWKVDNALQS GNSQESVTEQDSKDSTYSLSSTLTLSKADYEKHKVYACEVTHQGLSSPVTKS FNRGEC
9	10	Humanized VH 1	QVQLVQSGAEVKKPGASVKVSCKAS**GFNIKDTY**MYWVRQAPGQGLEWMGR**ID PANDNT**KYAQKFQGRVTITADTSTSTAYMELSSLRSEDTAVYYC**ARAKNLLN YFDY**WGQGTLVTVSS
11	12	Humanized VH 2	QVQLVQSGAEVKKPGASVKVSCKAS**GFNIKDTY**MYWVRQAPGQGLEWIGR**ID PANDNT**KYAPKFQGRVTITADTSTNTAYMELSSLRSEDTAVYYC**ARAKNLLN YFDY**WGQGTLVTVSS
13	14	Humanized VH3	EVQLVQSGAEVKKPGASVKVSCKAS**GFNIKDTY**MYWVRQAPGQGLEWMGR**ID PANDNT**KYAQKFQGRVTITADTSTNTAYMELSSLRSEDTAVYYC**ARAKNLLN**

			YFDYWGQGTLVTVSS
15	16	Humanized VL 1	DIQMTQSPSSLSASVGDRVTITCRAS**QEISGY**LSWYQQKPGKAPKRLIY**ATS** TLDSGVPSRFSGSGSGTDFTLTISSLQPEDFATYYC**LQYAIYPLT**FGQGTKL EIKR
17	18	Humanized VL 2	DIQMTQSPSSLSASVGDRVTITCRAS**QEISGY**LSWLQQKPGKAPKRLIY**ATS** TLQSGVPSRFSGSRSGTDYTLTISSLQPEDFATYYC**LQYAIYPLT**FGQGTKL EIKR
19	20	Humanized VL 3	DIQMTQSPSSLSASVGDRVTITCRAS**QEISGY**LSWYQQKPGKAPKRLIY**ATS** TLDSGVPSRFSGSRSGSDYTLTISSLQPEDFATYYC**LQYAIYPLT**FGQGTKL EIKR

Table 4. Exemplary anti-PD-L1 CDRs under various CDR definitions

SEQ ID NO	**Description**	**Sequences**
41	CDR-H1	GFNIKDTY
52	CDR-H1 (Kabat)	DTYMY
53	CDR-H1 (Chothia)	GFNIKDT
54	CDR-H1 (AbM)	GFNIKDTYMY
55	CDR-H1 (Contact)	KDTYMY
42	CDR-H2	IDPANDNT
56	CDR-H2 (Kabat)	RIDPANDNTKYAQKFQG
57	CDR-H2 (Chothia)	DPANDN

58	CDR-H2 (AbM)	RIDPANDNTK
59	CDR-H2 (Contact)	WMGRIDPANDNTK
43	CDR-H3	ARAKNLLNYFDY
60	CDR-H3 (Kabat/Abm /Chothia)	AKNLLNYFDY
61	CDR-H3 (Contact)	ARAKNLLNYFD
44	CDR-L1	QEISGY
62	CDR-L1 (Kabat/Abm /Chothia)	RASQEISGYLS
63	CDR-L1 (Contact)	SGYLSWL
45	CDR-L2	ATS
64	CDR-L2 (Kabat/Abm /Chothia)	ATSTLQS
65	CDR-L2 (Contact)	RLIYATSTLQ
46	CDR-L3 (Kabat/Abm /Chothia)	LQYAIYPLT
66	CDR-L3	LQYAIYPL

	(Contact)	

[235] In some embodiments, the anti-PD-L1 antibody moiety competes for binding to a target PD-L1 with a second anti-PD-L1 antibody moiety according to any one of the anti-PD-L1 antibody moieties described herein. In some embodiments, the anti-PD-L1 antibody moiety binds to the same, or substantially the same, epitope as the second anti-PD-L1 antibody moiety. In some embodiments, binding of the anti-PD-L1 antibody moiety to the target PD-L1 inhibits binding of the second anti-PD-L1 antibody moiety to PD-L1 by at least about 70% (such as by at least about any one of 75%, 80%, 85%, 90%, 95%, 98% or 99%), or *vice versa*. In some embodiments, the anti-PD-L1 antibody moiety and the second anti-PD-L1 antibody moiety cross-compete for binding to the target PD-L1, *i.e.*, each of the anti-PD-L1 antibody moieties competes with the other for binding to the target PD-L1.

Anti-PD-L1 scFv

[236] In some embodiments, the anti-PD-L1 antibody moiety comprises an scFv. In some embodiments, the anti-PD-L1 antibody moiety is an scFv. In some embodiments, the anti-PD-L1 scFv has the configuration of (from N-terminus to C-terminus): V_L-L-V_H, or V_H-L-V_L, wherein L is a peptide linker. In some embodiments, the anti-PD-L1 scFv is chimeric, human, partially humanized, fully humanized, or semi-synthetic.

[237] In some embodiments, the anti-PD-L1 scFv is engineered to have enhanced thermal stability. In some embodiments, the anti-PD-L1 scFv is engineered to have a melting temperature of about 55-70°C, such as about any one of 55-60, 60-65, or 65-70°C. In some embodiments, the anti-PD-L1 scFv comprises one or more (such as 1, 2, 3, or more) engineered disulfide bonds. In some embodiments, the anti-PD-L1 scFv comprises a first engineered cysteine residue at position 44 of V_H and a second engineered cysteine residue at position 100 of V_L, and/or a first engineered cysteine residue at position 105 of V_H and a second engineered cysteine residue at position 43 of V_L, wherein the first engineered cysteine residue and the second engineered cysteine residue form a disulfide bond, and wherein the amino acid positions are based on the Kabat numbering system. Other engineered disulfide bonds may be introduced into the anti-PD-L1 scFv by engineering a cysteine in the VH and a cysteine in the VL at suitable positions based on the structure and sequences of the scFv.

[238] In some embodiments, the anti-PD-L1 scFv comprises: i) a V_H comprising an HC-CDR1 comprising the amino acid sequence of SEQ ID NO: 41, an HC-CDR2 comprising the amino acid sequence of SEQ ID NO: 42, and an HC-CDR3 comprising the amino acid sequence of a SEQ ID NO: 43; or a variant thereof comprising up to about 5 (such as about any of 1, 2, 3, 4, or 5) amino acid substitutions in the HC-CDR sequences; and ii) a V_L comprising an LC-CDR1 comprising the amino acid sequence of SEQ ID NO: 44, an LC-CDR2 comprising the amino acid sequence of SEQ ID NO: 45, and an LC-CDR3 comprising the amino acid sequence of SEQ ID

NO: 46; or a variant thereof comprising up to about 5 (such as about any of 1, 2, 3, 4, or 5) amino acid substitutions in the LC-CDR sequences. In some embodiments, the anti-PD-L1 scFv is humanized. In some embodiments, the anti-PD-L1 scFv comprises from the N-terminus to the C-terminus: a V_H, an optional peptide linker, and a V_L. In some embodiments, the anti-PD-L1 scFv comprises from the N-terminus to the C-terminus: a V_L, an optional peptide linker, and a V_H. In some embodiments, the scFv comprises a peptide linker comprising the amino acid sequence of SEQ ID NO: 47 or 48. In some embodiments, the anti-PD-L1 scFv comprises one or more (such as 1, 2, 3, or more) engineered disulfide bonds. In some embodiments, the anti-PD-L1 scFv comprises a first engineered cysteine residue at position 44 of V_H and a second engineered cysteine residue at position 100 of V_L, and/or a first engineered cysteine residue at position 105 of V_H and a second engineered cysteine residue at position 43 of V_L, wherein the first engineered cysteine residue and the second engineered cysteine residue form a disulfide bond, and wherein the amino acid positions are based on the Kabat numbering system.

[239] In some embodiments, the anti-PD-L1 scFv comprises: a V_H comprising the amino acid sequence of any one of SEQ ID NOs: 1, 5, 9, 11, and 13, or a variant thereof having at least about 80% (such as at least about any one of 80%, 85%, 90%, 95%, 96%, 97%, 98%, or 99%) sequence identity to the amino acid sequence of any one of SEQ ID NOs: 1, 5, 9, 11, and 13; and a V_L comprising the amino acid sequence of any one of SEQ ID NOs: 3, 7, 15, 17 and 19, or a variant thereof having at least about 80% (such as at least about any one of 80%, 85%, 90%, 95%, 96%, 97%, 98%, or 99%) sequence identity to the amino acid sequence of any one of SEQ ID NOs: 3, 7, 15, 17 and 19. In some embodiments, the anti-PD-L1 scFv is humanized. In some embodiments, the anti-PD-L1 scFv comprises from the N-terminus to the C-terminus: a V_H, an optional peptide linker, and a V_L. In some embodiments, the anti-PD-L1 scFv comprises from the N-terminus to the C-terminus: a V_L, an optional peptide linker, and a V_H. In some embodiments, the scFv comprises a peptide linker comprising the amino acid sequence of SEQ ID NO: 47 or 48. In some embodiments, the anti-PD-L1 scFv comprises one or more (such as 1, 2, 3, or more) engineered disulfide bonds. In some embodiments, the anti-PD-L1 scFv comprises a first engineered cysteine residue at position 44 of V_H and a second engineered cysteine residue at position 100 of V_L, and/or a first engineered cysteine residue at position 105 of V_H and a second engineered cysteine residue at position 43 of V_L, wherein the first engineered cysteine residue and the second engineered cysteine residue form a disulfide bond, and wherein the amino acid positions are based on the Kabat numbering system.

[240] In some embodiments, the anti-PD-L1 scFv comprises the amino acid sequence of any one of SEQ ID NOs: 25, 27, 29, 31, 33, 35, 37 and 39, or a variant thereof having at least about 80% (such as at least about any one of 80%, 85%, 90%, 95%, 96%, 97%, 98%, or 99%) sequence identity to the amino acid sequence of any one of SEQ ID NOs: 25, 27, 29, 31, 33, 35, 37 and 39. In some embodiments, the anti-PD-L1 scFv comprises a

His tag. In some embodiments, the anti-PD-L1 scFv comprises a His tag fused to the C-terminus of the anti-PD-L1 scFv moiety. In some embodiments, the anti-PD-L1 scFv comprises GGGGSHHHHHH (SEQ ID NO: 51). Exemplary anti-PD-L1 scFvs are illustrated in FIG. 9. Exemplary anti-PD-L1 scFv sequences are shown in Table 5.

Table 5. Exemplary anti-PD-L1 scFv sequences.

SEQ ID NO.		Description	Amino acid Sequence (CDR sequences are underlined and bold)
AA	DNA		
25	26	anti-human PD-L1 scFv variant 1	QVQLVQSGAEVKKPGASVKVSCKAS**GFNIKDTY**MYWVRQAPGQGLEWMGR**ID** **PANDNT**KYAQKFQGRVTITADTSTSTAYMELSSLRSEDTAVYYC**ARAKNLLN** **YFDY**WGQGTLVTVSSGGGGSGGGGSGGGGSGGGGSDIQMTQSPSSLSASVGD RVTITCRAS**QEISGY**LSWLQQKPGKAPKRLIY**ATS**TLQSGVPSRFSGSRSGT DYTLTISSLQPEDFATYYC**LQYAIYPLT**FGQGTKLEIKR
27	28	anti-human PD-L1 scFv variant 2	QVQLVQSGAEVKKPGASVKVSCKAS**GFNIKDTY**MYWVRQAPGQGLEWMGR**ID** **PANDNT**KYAQKFQGRVTITADTSTSTAYMELSSLRSEDTAVYYC**ARAKNLLN** **YFDY**WGQGTLVTVSSGSTSGSGKPGSGEGSTKGDIQMTQSPSSLSASVGDRV TITCRAS**QEISGY**LSWLQQKPGKAPKRLIY**ATS**TLQSGVPSRFSGSRSGTDY TLTISSLQPEDFATYYC**LQYAIYPLT**FGQGTKLEIKR
29	30	anti-human PD-L1 scFv variant 3	QVQLVQSGAEVKKPGASVKVSCKAS**GFNIKDTY**MYWVRQAPGQCLEWMGR**ID** **PANDNT**KYAQKFQGRVTITADTSTSTAYMELSSLRSEDTAVYYC**ARAKNLLN** **YFDY**WGQGTLVTVSSGGGGSGGGGSGGGGSGGGGSDIQMTQSPSSLSASVGD RVTITCRAS**QEISGY**LSWLQQKPGKAPKRLIY**ATS**TLQSGVPSRFSGSRSGT DYTLTISSLQPEDFATYYC**LQYAIYPLT**FGCGTKLEIKR
31	32	anti-human PD-L1 scFv variant 4	QVQLVQSGAEVKKPGASVKVSCKAS**GFNIKDTY**MYWVRQAPGQGLEWMGR**ID** **PANDNT**KYAQKFQGRVTITADTSTSTAYMELSSLRSEDTAVYYC**ARAKNLLN** **YFDY**WGCGTLVTVSSGGGGSGGGGSGGGGSGGGGSDIQMTQSPSSLSASVGD RVTITCRAS**QEISGY**LSWLQQKPGKCPKRLIY**ATS**TLQSGVPSRFSGSRSGT DYTLTISSLQPEDFATYYC**LQYAIYPLT**FGQGTKLEIKR
33	34	anti-human PD-L1 scFv variant 5	DIQMTQSPSSLSASVGDRVTITCRAS**QEISGY**LSWLQQKPGKAPKRLIY**ATS** TLQSGVPSRFSGSRSGTDYTLTISSLQPEDFATYYC**LQYAIYPLT**FGQGTKL EIKRGGGGSGGGGSGGGGSGGGGSQVQLVQSGAEVKKPGASVKVSCKAS**GFN** **IKDTY**MYWVRQAPGQGLEWMGR**IDPANDNT**KYAQKFQGRVTITADTSTSTAY MELSSLRSEDTAVYYC**ARAKNLLNYFDY**WGQGTLVTVSS

35	36	anti-human PD-L1 scFv variant 6	DIQMTQSPSSLSASVGDRVTITCRAS**QEISGY**LSWLQQKPGKAPKRLIY**ATS** TLQSGVPSRFSGSRSGTDYTLTISSLQPEDFATYYC**LQYAIYPLT**FGQGTKL EIKRGSTSGSGKPGSGEGSTKGQVQLVQSGAEVKKPGASVKVSCKAS**GFNIK DTY**MYWVRQAPGQGLEWMGR**IDPANDNT**KYAQKFQGRVTITADTSTSTAYME LSSLRSEDTAVYYC**ARAKNLLNYFDY**WGQGTLVTVSS
37	38	anti-human PD-L1 scFv variant 7	DIQMTQSPSSLSASVGDRVTITCRAS**QEISGY**LSWLQQKPGKCPKRLIY**ATS** TLQSGVPSRFSGSRSGTDYTLTISSLQPEDFATYYC**LQYAIYPLT**FGQGTKL EIKRGGGGSGGGGSGGGGSGGGGSQVQLVQSGAEVKKPGASVKVSCKAS**GFN IKDTY**MYWVRQAPGQGLEWMGR**IDPANDNT**KYAQKFQGRVTITADTSTSTAY MELSSLRSEDTAVYYC**ARAKNLLNYFDY**WGCGTLVTVSS
39	40	anti-human PD-L1 scFv variant 8	DIQMTQSPSSLSASVGDRVTITCRAS**QEISGY**LSWLQQKPGKAPKRLIY**ATS** TLQSGVPSRFSGSRSGTDYTLTISSLQPEDFATYYC**LQYAIYPLT**FGCGTKL EIKRGGGGSGGGGSGGGGSGGGGSQVQLVQSGAEVKKPGASVKVSCKAS**GFN IKDTY**MYWVRQAPGQCLEWMGR**IDPANDNT**KYAQKFQGRVTITADTSTSTAY MELSSLRSEDTAVYYC**ARAKNLLNYFDY**WGQGTLVTVSS

Anti-PD-L1 scFv-Fc

[241] In some embodiments, the anti-PD-L1 antibody moiety is an anti-PD-L1 scFv according to any one of the anti-PD-L1 scFvs described herein fused to an Fc fragment. In some embodiments, the anti-PD-L1 antibody moiety is fused to an Fc fragment via a peptide linker. The anti-PD-L1 antibody moiety may comprise any of the Fc fragments described in the "Antibody moieties" section above. In some embodiments, the Fc fragment is a human IgG1 Fc fragment. In some embodiments, the Fc fragment comprises one or more mutations to increase clearance or decrease half-life. For example, the Fc fragment may have H310A and/or H435Q mutations, wherein the amino acid positions are based on the Kabat numbering system.

[242] In some embodiments, each chain of the Fc fragment is fused to the same entity. In some embodiments, the anti-PD-L1 scFv-Fc comprises two identical anti-PD-L1 scFvs described herein, each fused with one chain of the Fc fragment. In some embodiments, the anti-PD-L1 scFv-Fc is a homodimer. In some embodiments, the anti-PD-L1 scFv-Fc is a heterodimer.

[243] In some embodiments, the anti-PD-L1 scFv-Fc comprises the amino acid sequence of SEQ ID NO: 21 or 23, or a variant thereof having at least about 80% (such as at least about any one of 80%, 85%, 90%, 95%, 96%, 97%, 98%, or 99%) sequence identity to the amino acid sequence of SEQ ID NO: 21 or 23. Exemplary anti-PD-L1 scFv-Fc sequences are shown in Table 6.

Table 6. Exemplary anti-PD-L1 scFv-Fc sequences.

SEQ ID NO.		Description	Amino acid Sequence (CDR sequences are underlined and bold)
AA	DNA		
21	22	hPD-L1 scFv-hFc wt	QVQLVQSGAEVKKPGASVKVSCKAS**GFNIKDTY**MYWVRQAPGQGLEWMGR**IDP ANDNT**KYAQKFQGRVTITADTSTSTAYMELSSLRSEDTAVYYC**ARAKNLLNYF DY**WGQGTLVTVSSGSTSGSGKPGSGEGSTKGDIQMTQSPSSLSASVGDRVTIT CRAS**QEISGY**LSWLQQKPGKAPKRLIY**ATS**TLQSGVPSRFSGSRSGTDYTLTI SSLQPEDFATYYC**LQYAIYPLT**FGQGTKLEIKRDKTHTCPPCPAPELLGGPSV FLFPPKPKDTLMISRTPEVTCVVVDVSHEDPEVKFNWYVDGVEVHNAKTKPRE EQYNSTYRVVSVLTVLHQDWLNGKEYKCKVSNKALPAPIEKTISKAKGQPREP QVYTLPPSRDELTKNQVSLTCLVKGFYPSDIAVEWESNGQPENNYKTTPPVLD SDGSFFLYSKLTVDKSRWQQGNVFSCSVMHEALHNHYTQKSLSLSPG
23	24	hPD-L1 scFv-hFc Mt	QVQLVQSGAEVKKPGASVKVSCKAS**GFNIKDTY**MYWVRQAPGQGLEWMGR**IDP ANDNT**KYAQKFQGRVTITADTSTSTAYMELSSLRSEDTAVYYC**ARAKNLLNYF DY**WGQGTLVTVSSGSTSGSGKPGSGEGSTKGDIQMTQSPSSLSASVGDRVTIT CRAS**QEISGY**LSWLQQKPGKAPKRLIY**ATS**TLQSGVPSRFSGSRSGTDYTLTI SSLQPEDFATYYC**LQYAIYPLT**FGQGTKLEIKRDKTHTCPPCPAPELLGGPSV FLFPPKPKDTLMISRTPEVTCVVVDVSHEDPEVKFNWYVDGVEVHNAKTKPRE EQYNSTYRVVSVLTVLAQDWLNGKEYKCKVSNKALPAPIEKTISKAKGQPREP QVYTLPPSRDELTKNQVSLTCLVKGFYPSDIAVEWESNGQPENNYKTTPPVLD SDGSFFLYSKLTVDKSRWQQGNVFSCSVMHEALHNQYTQKSLSLSPG

Anti-PD-L1 imaging agent

[244] Any one of the anti-PD-L1 antibody moieties described herein may be incorporated in an imaging agent for detection of PD-L1. The features described herein in this section regarding anti-PD-L1 antibody agents may be combined with the features described in the section "Imaging agents" above in any suitable combination.

[245] In some embodiments, there is provided an imaging agent comprising any one of the anti-PD-L1 antibody moieties described herein, wherein the antibody moiety is labeled with a radionuclide. In some embodiments, the radionuclide is selected from the group consisting of ^{62}Cu, ^{64}Cu, ^{67}Cu, ^{18}F, ^{67}Ga, ^{68}Ga, ^{111}In, ^{177}Lu, ^{86}Y, ^{90}Y, and ^{89}Zr. In some embodiments, the anti-PD-L1 antibody moiety is conjugated to a chelating compound that chelates the radionuclide. In some embodiments, the chelating compound is NOTA, DOTA or derivatives thereof.

[246] In some embodiments, there is provided an imaging agent comprising any one of the isolated anti-PD-L1 antibody agents described herein, wherein the anti-PD-L1 antibody moiety is labeled with a radionuclide. In some embodiments, the radionuclide is selected from the group consisting of ^{64}Cu, ^{18}F, ^{67}Ga, ^{68}Ga, ^{111}In, ^{177}Lu, ^{90}Y, ^{89}Zr, ^{61}Cu, ^{62}Cu, ^{67}Cu, ^{19}F, ^{66}Ga, ^{72}Ga, ^{44}Sc, ^{47}Sc, ^{86}Y, ^{88}Y and ^{45}Ti. In some embodiments, the anti-PD-L1 antibody moiety is conjugated to a chelating compound that chelates the radionuclide. In some embodiments, the chelating compound is NOTA, DOTA or derivatives thereof.

[247] In some embodiments, there is provided an imaging agent comprising an anti-PD-L1 antibody moiety labeled with a radionuclide, wherein the anti-PD-L1 antibody moiety comprises: a V_H comprising a HC-CDR1 comprising the amino acid sequence of SEQ ID NO: 41, a HC-CDR2 comprising the amino acid sequence of SEQ ID NO: 42, and a HC-CDR3 comprising the amino acid sequence of SEQ ID NO: 43, or a variant thereof comprising up to about 5 amino acid substitutions; and a V_L comprising a LC-CDR1 comprising the amino acid sequence of SEQ ID NO: 44, a LC-CDR2 comprising the amino acid sequence of SEQ ID NO: 45, and a LC-CDR3 comprising the amino acid sequence of SEQ ID NO: 46, or a variant thereof comprising up to about 5 amino acid substitutions. In some embodiments, the anti-PD-L1 antibody moiety comprises: a V_H comprising the amino acid sequence of any one of SEQ ID NOs: 1, 5, 9, 11, and 13, or a variant thereof having at least about 80% (such as at least about any one of 80%, 85%, 90%, 95%, 96%, 97%, 98%, or 99%) sequence identity to the amino acid sequence of any one of SEQ ID NOs: 1, 5, 9, 11, and 13; and a V_L comprising the amino acid sequence of any one of SEQ ID NOs: 3, 7, 15, 17 and 19, or a variant thereof having at least about 80% (such as at least about any one of 80%, 85%, 90%, 95%, 96%, 97%, 98%, or 99%) sequence identity to the amino acid sequence of any one of SEQ ID NOs: 3, 7, 15, 17 and 19. In some embodiments, the anti-PD-L1 antibody moiety is humanized. In some embodiments, the radionuclide is selected from the group consisting of ^{64}Cu, ^{18}F, ^{67}Ga, ^{68}Ga, ^{111}In, ^{177}Lu, ^{90}Y, ^{89}Zr, ^{61}Cu, ^{62}Cu, ^{67}Cu, ^{19}F, ^{66}Ga, ^{72}Ga, ^{44}Sc, ^{47}Sc, ^{86}Y, ^{88}Y and ^{45}Ti. In some embodiments, the radionuclide is ^{68}Ga. In some embodiments, the anti-PD-L1 antibody moiety is conjugated to a chelating compound that chelates the radionuclide. In some embodiments, the chelating compound is NOTA, DOTA or derivatives thereof.

[248] In some embodiments, there is provided an imaging agent comprising an anti-PD-L1 scFv labeled with a radionuclide, wherein the anti-PD-L1 scFv comprises: a V_H comprising a HC-CDR1 comprising the amino acid sequence of SEQ ID NO: 41, a HC-CDR2 comprising the amino acid sequence of SEQ ID NO: 42, and a HC-CDR3 comprising the amino acid sequence of SEQ ID NO: 43, or a variant thereof comprising up to about 5 amino acid substitutions; and a V_L comprising a LC-CDR1 comprising the amino acid sequence of SEQ ID NO: 44, a LC-CDR2 comprising the amino acid sequence of SEQ ID NO: 45, and a LC-CDR3 comprising the amino acid sequence of SEQ ID NO: 46, or a variant thereof comprising up to about 5 amino acid substitutions. In some embodiments, the anti-PD-L1 antibody moiety comprises: a V_H comprising the amino

acid sequence of any one of SEQ ID NOs: 1, 5, 9, 11, and 13, or a variant thereof having at least about 80% (such as at least about any one of 80%, 85%, 90%, 95%, 96%, 97%, 98%, or 99%) sequence identity to the amino acid sequence of any one of SEQ ID NOs: 1, 5, 9, 11, and 13; and a V_L comprising the amino acid sequence of any one of SEQ ID NOs: 3, 7, 15, 17 and 19, or a variant thereof having at least about 80% (such as at least about any one of 80%, 85%, 90%, 95%, 96%, 97%, 98%, or 99%) sequence identity to the amino acid sequence of any one of SEQ ID NOs: 3, 7, 15, 17 and 19. In some embodiments, the anti-PD-L1 antibody moiety is humanized. In some embodiments, the anti-PD-L1 scFv comprises a first engineered cysteine residue at position 44 of V_H and a second engineered cysteine residue at position 100 of V_L, or a first engineered cysteine residue at position 105 of V_H and a second engineered cysteine residue at position 43 of V_L, wherein the first engineered cysteine residue and the second engineered cysteine residue form a disulfide bond, and wherein the amino acid positions are based on the Kabat numbering system. In some embodiments, the Fc fragment is an IgG1 Fc fragment. In some embodiments, the Fc fragment has H310A and H435Q mutations, wherein the amino acid positions are based on the Kabat numbering system. In some embodiments, the anti-PD-L1 scFv comprises the amino acid sequence of any one of SEQ ID NOs: 25, 27, 29, 31, 33, 35, 37 and 39, or a variant thereof having at least about 80% (such as at least about any one of 80%, 85%, 90%, 95%, 96%, 97%, 98%, or 99%) sequence identity to the amino acid sequence of any one of SEQ ID NOs: 25, 27, 29, 31, 33, 35, 37 and 39. In some embodiments, the radionuclide is selected from the group consisting of ^{64}Cu, ^{18}F, ^{67}Ga, ^{68}Ga, ^{111}In, ^{177}Lu, ^{90}Y, ^{89}Zr, ^{61}Cu, ^{62}Cu, ^{67}Cu, ^{19}F, ^{66}Ga, ^{72}Ga, ^{44}Sc, ^{47}Sc, ^{86}Y, ^{88}Y and ^{45}Ti. In some embodiments, the radionuclide is ^{68}Ga. In some embodiments, the anti-PD-L1 antibody moiety is conjugated to a chelating compound that chelates the radionuclide. In some embodiments, the chelating compound is NOTA, DOTA or derivatives thereof.

[249] In some embodiments, there is provided an imaging agent comprising an anti-PD-L1 antibody moiety labeled with a radionuclide, wherein the anti-PD-L1 antibody moiety is an anti-PD-L1 scFv fused to an Fc fragment, wherein the anti-PD-L1 antibody moiety comprises: a V_H comprising a HC-CDR1 comprising the amino acid sequence of SEQ ID NO: 41, a HC-CDR2 comprising the amino acid sequence of SEQ ID NO: 42, and a HC-CDR3 comprising the amino acid sequence of SEQ ID NO: 43, or a variant thereof comprising up to about 5 amino acid substitutions; and a V_L comprising a LC-CDR1 comprising the amino acid sequence of SEQ ID NO: 44, a LC-CDR2 comprising the amino acid sequence of SEQ ID NO: 45, and a LC-CDR3 comprising the amino acid sequence of SEQ ID NO: 46, or a variant thereof comprising up to about 5 amino acid substitutions. In some embodiments, the anti-PD-L1 antibody moiety comprises: a V_H comprising the amino acid sequence of any one of SEQ ID NOs: 1, 5, 9, 11, and 13, or a variant thereof having at least about 80% (such as at least about any one of 80%, 85%, 90%, 95%, 96%, 97%, 98%, or 99%) sequence identity to the amino acid sequence of any one of SEQ ID NOs: 1, 5, 9, 11, and 13; and a V_L comprising the amino acid

sequence of any one of SEQ ID NOs: 3, 7, 15, 17 and 19, or a variant thereof having at least about 80% (such as at least about any one of 80%, 85%, 90%, 95%, 96%, 97%, 98%, or 99%) sequence identity to the amino acid sequence of any one of SEQ ID NOs: 3, 7, 15, 17 and 19. In some embodiments, the anti-PD-L1 antibody moiety is humanized. In some embodiments, the anti-PD-L1 scFv comprises a first engineered cysteine residue at position 44 of V_H and a second engineered cysteine residue at position 100 of V_L, or a first engineered cysteine residue at position 105 of V_H and a second engineered cysteine residue at position 43 of V_L, wherein the first engineered cysteine residue and the second engineered cysteine residue form a disulfide bond, and wherein the amino acid positions are based on the Kabat numbering system. In some embodiments, the Fc fragment is an IgG1 Fc fragment. In some embodiments, the Fc fragment has H310A and H435Q mutations, wherein the amino acid positions are based on the Kabat numbering system. In some embodiments, the anti-PD-L1 scFv comprises the amino acid sequence of any one of SEQ ID NOs: 25, 27, 29, 31, 33, 35, 37 and 39, or a variant thereof having at least about 80% (such as at least about any one of 80%, 85%, 90%, 95%, 96%, 97%, 98%, or 99%) sequence identity to the amino acid sequence of any one of SEQ ID NOs: 25, 27, 29, 31, 33, 35, 37 and 39. In some embodiments, the radionuclide is selected from the group consisting of ^{64}Cu, ^{18}F, ^{67}Ga, ^{68}Ga, ^{111}In, ^{177}Lu, ^{90}Y, ^{89}Zr, ^{61}Cu, ^{62}Cu, ^{67}Cu, ^{19}F, ^{66}Ga, ^{72}Ga, ^{44}Sc, ^{47}Sc, ^{86}Y, ^{88}Y and ^{45}Ti. In some embodiments, the radionuclide is ^{68}Ga. In some embodiments, the anti-PD-L1 antibody moiety is conjugated to a chelating compound that chelates the radionuclide. In some embodiments, the chelating compound is NOTA, DOTA or derivatives thereof.

[250] In some embodiments, there is provided an imaging agent comprising an anti-PD-L1 antibody moiety conjugated to NOTA that chelates a radionuclide (*e.g.*, ^{68}Ga), wherein the anti-PD-L1 antibody moiety comprises: a V_H comprising a HC-CDR1 comprising the amino acid sequence of SEQ ID NO: 41, a HC-CDR2 comprising the amino acid sequence of SEQ ID NO: 42, and a HC-CDR3 comprising the amino acid sequence of SEQ ID NO: 43, or a variant thereof comprising up to about 5 amino acid substitutions; and a V_L comprising a LC-CDR1 comprising the amino acid sequence of SEQ ID NO: 44, a LC-CDR2 comprising the amino acid sequence of SEQ ID NO: 45, and a LC-CDR3 comprising the amino acid sequence of SEQ ID NO: 46, or a variant thereof comprising up to about 5 amino acid substitutions. In some embodiments, the anti-PD-L1 antibody moiety comprises: a V_H comprising the amino acid sequence of any one of SEQ ID NOs: 1, 5, 9, 11, and 13, or a variant thereof having at least about 80% (such as at least about any one of 80%, 85%, 90%, 95%, 96%, 97%, 98%, or 99%) sequence identity to the amino acid sequence of any one of SEQ ID NOs: 1, 5, 9, 11, and 13; and a V_L comprising the amino acid sequence of any one of SEQ ID NOs: 3, 7, 15, 17 and 19, or a variant thereof having at least about 80% (such as at least about any one of 80%, 85%, 90%, 95%, 96%, 97%, 98%, or 99%) sequence identity to the amino acid sequence of any one of SEQ ID NOs: 3, 7, 15, 17 and 19. In some embodiments, the anti-PD-L1 antibody moiety is humanized. In some embodiments, the radionuclide is

selected from the group consisting of ^{64}Cu, ^{18}F, ^{67}Ga, ^{68}Ga, ^{111}In, ^{177}Lu, ^{90}Y, ^{89}Zr, ^{61}Cu, ^{62}Cu, ^{67}Cu, ^{19}F, ^{66}Ga, ^{72}Ga, ^{44}Sc, ^{47}Sc, ^{86}Y, ^{88}Y and ^{45}Ti.

[251] In some embodiments, there is provided an imaging agent comprising an anti-PD-L1 scFv conjugated to NOTA that chelates a radionuclide (*e.g.*, ^{68}Ga), wherein the anti-PD-L1 scFv comprises: a V_H comprising a HC-CDR1 comprising the amino acid sequence of SEQ ID NO: 41, a HC-CDR2 comprising the amino acid sequence of SEQ ID NO: 42, and a HC-CDR3 comprising the amino acid sequence of SEQ ID NO: 43, or a variant thereof comprising up to about 5 amino acid substitutions; and a V_L comprising a LC-CDR1 comprising the amino acid sequence of SEQ ID NO: 44, a LC-CDR2 comprising the amino acid sequence of SEQ ID NO: 45, and a LC-CDR3 comprising the amino acid sequence of SEQ ID NO: 46, or a variant thereof comprising up to about 5 amino acid substitutions. In some embodiments, the anti-PD-L1 antibody moiety comprises: a V_H comprising the amino acid sequence of any one of SEQ ID NOs: 1, 5, 9, 11, and 13, or a variant thereof having at least about 80% (such as at least about any one of 80%, 85%, 90%, 95%, 96%, 97%, 98%, or 99%) sequence identity to the amino acid sequence of any one of SEQ ID NOs: 1, 5, 9, 11, and 13; and a V_L comprising the amino acid sequence of any one of SEQ ID NOs: 3, 7, 15, 17 and 19, or a variant thereof having at least about 80% (such as at least about any one of 80%, 85%, 90%, 95%, 96%, 97%, 98%, or 99%) sequence identity to the amino acid sequence of any one of SEQ ID NOs: 3, 7, 15, 17 and 19. In some embodiments, the anti-PD-L1 antibody moiety is humanized. In some embodiments, the anti-PD-L1 scFv comprises a first engineered cysteine residue at position 44 of V_H and a second engineered cysteine residue at position 100 of V_L, or a first engineered cysteine residue at position 105 of V_H and a second engineered cysteine residue at position 43 of V_L, wherein the first engineered cysteine residue and the second engineered cysteine residue form a disulfide bond, and wherein the amino acid positions are based on the Kabat numbering system. In some embodiments, the Fc fragment is an IgG1 Fc fragment. In some embodiments, the Fc fragment has H310A and H435Q mutations, wherein the amino acid positions are based on the Kabat numbering system. In some embodiments, the anti-PD-L1 scFv comprises the amino acid sequence of any one of SEQ ID NOs: 25, 27, 29, 31, 33, 35, 37 and 39, or a variant thereof having at least about 80% (such as at least about any one of 80%, 85%, 90%, 95%, 96%, 97%, 98%, or 99%) sequence identity to the amino acid sequence of any one of SEQ ID NOs: 25, 27, 29, 31, 33, 35, 37 and 39. In some embodiments, the radionuclide is selected from the group consisting of ^{64}Cu, ^{18}F, ^{67}Ga, ^{68}Ga, ^{111}In, ^{177}Lu, ^{90}Y, ^{89}Zr, ^{61}Cu, ^{62}Cu, ^{67}Cu, ^{19}F, ^{66}Ga, ^{72}Ga, ^{44}Sc, ^{47}Sc, ^{86}Y, ^{88}Y and ^{45}Ti.

[252] In some embodiments, there is provided an imaging agent comprising an anti-PD-L1 antibody moiety conjugated to NOTA that chelates a radionuclide (*e.g.*, ^{68}Ga), wherein the anti-PD-L1 antibody moiety is an anti-PD-L1 scFv fused to an Fc fragment, and wherein the anti-PD-L1 antibody moiety comprises: a V_H comprising a HC-CDR1 comprising the amino acid sequence of SEQ ID NO: 41, a HC-CDR2 comprising the amino acid sequence of SEQ ID NO: 42, and a HC-CDR3 comprising the amino acid sequence of SEQ ID NO:

43, or a variant thereof comprising up to about 5 amino acid substitutions; and a V_L comprising a LC-CDR1 comprising the amino acid sequence of SEQ ID NO: 44, a LC-CDR2 comprising the amino acid sequence of SEQ ID NO: 45, and a LC-CDR3 comprising the amino acid sequence of SEQ ID NO: 46, or a variant thereof comprising up to about 5 amino acid substitutions. In some embodiments, the anti-PD-L1 antibody moiety comprises: a V_H comprising the amino acid sequence of any one of SEQ ID NOs: 1, 5, 9, 11, and 13, or a variant thereof having at least about 80% (such as at least about any one of 80%, 85%, 90%, 95%, 96%, 97%, 98%, or 99%) sequence identity to the amino acid sequence of any one of SEQ ID NOs: 1, 5, 9, 11, and 13; and a V_L comprising the amino acid sequence of any one of SEQ ID NOs: 3, 7, 15, 17 and 19, or a variant thereof having at least about 80% (such as at least about any one of 80%, 85%, 90%, 95%, 96%, 97%, 98%, or 99%) sequence identity to the amino acid sequence of any one of SEQ ID NOs: 3, 7, 15, 17 and 19. In some embodiments, the anti-PD-L1 antibody moiety is humanized. In some embodiments, the anti-PD-L1 scFv comprises a first engineered cysteine residue at position 44 of V_H and a second engineered cysteine residue at position 100 of V_L, or a first engineered cysteine residue at position 105 of V_H and a second engineered cysteine residue at position 43 of V_L, wherein the first engineered cysteine residue and the second engineered cysteine residue form a disulfide bond, and wherein the amino acid positions are based on the Kabat numbering system. In some embodiments, the Fc fragment is an IgG1 Fc fragment. In some embodiments, the Fc fragment has H310A and H435Q mutations, wherein the amino acid positions are based on the Kabat numbering system. In some embodiments, the anti-PD-L1 scFv comprises the amino acid sequence of any one of SEQ ID NOs: 25, 27, 29, 31, 33, 35, 37 and 39, or a variant thereof having at least about 80% (such as at least about any one of 80%, 85%, 90%, 95%, 96%, 97%, 98%, or 99%) sequence identity to the amino acid sequence of any one of SEQ ID NOs: 25, 27, 29, 31, 33, 35, 37 and 39. In some embodiments, the radionuclide is selected from the group consisting of ^{64}Cu, ^{18}F, ^{67}Ga, ^{68}Ga, ^{111}In, ^{177}Lu, ^{90}Y, ^{89}Zr, ^{61}Cu, ^{62}Cu, ^{67}Cu, ^{19}F, ^{66}Ga, ^{72}Ga, ^{44}Sc, ^{47}Sc, ^{86}Y, ^{88}Y and ^{45}Ti.

PD-1

<u>The PD-1/PD-L1 Pathway and anti-PD immunotherapy</u>

[253] The gene encoding programmed cell death 1 (PD-1) was first isolated from a murine T cell hybridoma and a hematopoietic progenitor cell line undergoing classic apoptosis in 1992 (Ishida Y, *et al. The EMBO journal* 1992; 11:3887-3895). Structurally, as a CD28 and CTLA4 homologue, PD-1 is a type I transmembrane protein and belongs to the Ig superfamily (Sharpe AH and Freeman GJ. *Nature Reviews Immunology* 2002; 2:116-126). The critical role of PD-1 in negatively modulating T cell responses and maintaining peripheral tolerance was shown by PD-1 gene ablation studies using different mouse models. PD-1–deficient mice in C57BL/6 background develop lupus-like autoimmune diseases due to enhanced proliferation of PD-1 deficient

T cells against allogeneic antigen (Nishimura H *et al. Immunity* 1999; 11:141-151). In BALB/c, but not in immune-deficient BALB/c RAG2−/− background, PD-1 knockout mice develop dilated cardiomyopathy and suffered sudden death from congestive heart failure (Nishimura H *et al. Science* 2001; 291:319-322). One of the major contributing causes was later identified to be the generation of high-titer autoantibodies against the heart-specific protein cardiac troponin I (Okazaki T, *et al. Nature medicine* 2003; 9:1477-1483). In the NOD (non-obese diabetic) background, PD-1 deficiency leads to early onset of type I diabetes due to the accelerated islet-specific T cell expansion and infiltration into the pancreas islets (Yantha J *et al. Diabetes* 2010; 59:2588-2596). Overall, the PD-1 molecule acts as an inhibitory receptor involved in peripheral tolerance (Nishimura H, Honjo T. *Trends in immunology* 2001; 22:265-268). Unlike CTLA-4, the immunoreceptor tyrosine-based switch motif (ITSM) in PD-1, but not the nearby ITIM domain located in the cytoplasmic tail of PD-1, recruits SHP-2 phosphatase and reverses activation-induced phosphorylation after TCR signaling (Ishida Y, *et al. The EMBO journal* 1992; 11:3887-3895).

[254] Several studies contributed to the discovery of the molecules that interact with PD-1. In 1999, the B7 homolog 1 (B7H1, also referred to as programmed death ligand-1 [PD-L1] in the later literature) was identified as a 290-amino-acid type I transmembrane glycoprotein belonging to the B7-CD28 family of the immunoglobulin superfamily that served as a negative regulator of human T cell response *in vitro* (Dong H *et al. Nature medicine* 1999; 5:1365-1369). One year later, it was shown that PD-L1 is a binding and functional counterpart of PD-1 (Freeman GJ *et al. The Journal of experimental medicine* 2000; 192:1027-1034). It was later demonstrated that PD-L1 deficient mice were prone to autoimmune conditions (Dong H *et al. Immunity* 2004; 20:327-336). Notably, although the mRNAs for PD-1 and PD-L1 are expressed with broad spectrum in many cell types, both are inducible molecules as their expression patterns are strictly controlled by posttranscriptional regulation. PD-1 protein is not detectable on resting T cells, but is found on the cell surface within 24 hours of T-cell activation (Keir ME *et al. Annual review of immunology* 2008; 26:677-704). Under normal physiological conditions, PD-L1 protein is only expressed in the peripheral tissues, such as the tonsil, placenta, and a small fraction of macrophage-like cells in the lung and liver (Dong H *et al. Nature medicine* 2002; 8:793-800; Petroff MG *et al. Placenta* 2002; 23 Suppl A:S95-101). Extrinsic induction of PD-L1 is largely mediated by proinflammatory cytokines, such as interferon γ (IFN-γ), which indicates that PD-1/PD-L1 interaction plays an important role in the control of inflammatory response in the peripheral tissues (Zou W, Chen L. *Nature reviews Immunology* 2008; 8:467-477).

[255] In addition to PD-L1, another PD-1 ligand called B7-DC (also known as PD-L2) was also independently identified by two laboratories (Tseng SY *et al. The Journal of experimental medicine* 2001; 193:839-846; Latchman Y, *et al. Nature immunology* 2001; 2:261-268). PD-L2 was found to be selectively expressed on dendritic cells (DCs) and also negatively regulate T cell response by binding to PD-1. Recently,

PD-L2 was also found to interact with repulsive guidance molecule family member b (RGMb), a molecule that is highly enriched in lung macrophages and may be required for induction of respiratory tolerance (Xiao Y, *et al. The Journal of experimental medicine* 2014; 211:943-959). Interestingly, PD-L1 was also found to have another receptor CD80 on activated T cells to deliver inhibitory signal, which may also contribute to the formation of T cell tolerance in vivo (Butte MJ *et al. Immunity* 2007; 27:111-122; Park JJ *et al. Blood* 2010; 116:1291-1298). Currently, at least five interacting molecules are known to be involved in the PD network. Thus, the original PD-1/PD-L1 pathway is renamed to the more suitable "PD pathway." The presence of two ligands (PD-L1 and PD-L2) for PD-1 and two inhibitory receptors (PD-1 and CD80) for PD-L1 suggests that neither PD-1 blockade nor PD-L1 blockade would completely disrupt the PD pathway. Complete abrogation of the PD inhibitory pathway may require a combination strategy targeting both molecules.

[256] The crucial function of the PD pathway in modulating the activity of T cells in the peripheral tissues in an inflammatory response to infection and in limiting autoimmunity appears to be hijacked by tumor cells and by viruses during chronic viral infections. PD-L1 is overexpressed on many freshly isolated human tumors from multiple tissue origins (Dong *et al. Nature Medicine* 2002; 8:793-800; Romano *et al. Journal for Immunotherapy of Cancer* 2015; 3:15; Hirano *et al. Cancer Research* 2005; 65:1089-1096). The expression of PD-L1 has been correlated with the progression and poor prognosis of certain types of human cancers (Wang *et al. European journal of surgical oncology: the journal of the European Society of Surgical Oncology and the British Association of Surgical Oncology* 2015; 41:450-456; Cierna *et al. Annals of oncology : official journal of the European Society for Medical Oncology / ESMO* 2016; 27:300-305; Gandini *et al. Critical reviews in oncology/hematology* 2016; 100:88-98; Thierauf *et al. Melanoma research* 2015; 25:503-509; Taube *et al. Clinical cancer research : an official journal of the American Association for Cancer Research* 2014; 20:5064-5074). During chronic viral infections, PD-L1 is persistently expressed on many tissues, while PD-1 is up-regulated on virus-specific CTLs (Yao *et al. Trends in molecular medicine* 2006; 12:244-246). Tumor- or virus-induced PD-L1 may utilize multiple mechanisms to facilitate the evasion of host immune surveillance, including T cell anergy, apoptosis, exhaustion, IL-10 production, DC suppression, as well as Treg induction and expansion (Zou *et al. Nature reviews Immunology* 2008; 8:467-477).

[257] The PD pathway mediated escape of tumor immunity could be viewed as an "adaptive resistance" model (Chen *et al. The Journal of clinical investigation* 2015; 125:3384-3391; Yao *et al. European journal of immunology* 2013; 43:576-579). Specifically, activated tumor-specific T cells reach the tumor sites and become tumor-infiltrating lymphocytes (TILs). Upon recognition of the cognate antigen, TILs produce IFN-γ, which induces PD-L1 expression in many cell types in the tumor microenvironment, including DCs, macrophages, neutrophils, and B lymphocytes. Upon binding to PD-1, PD-L1 delivers a suppressive signal to T cells and an anti-apoptotic signal to tumor cells, leading to T cell dysfunction and tumor survival (Taube et al. *Science*

translational medicine 2012; **4**:127ra137; Spranger et al. *Science translational medicine* 2013; **5**:200ra116). This model is not only supported by IHC-based observations that cell surface PD-L1 expression is detected only in cells that are adjacent to T cells, but also supported by studies showing a strong correlation between PD-L1 expression in tumor sites and the presence of TILs (Gandini et al. *Critical reviews in oncology/hematology* 2016; 100:88-98; Taube et al. *Science translational medicine* 2012; **4**:127ra137), as well as the demonstration of IFN-γ as a major PD-L1 inducer *in vivo* in mouse tumor models (Dong *et al. Nature medicine* 2002; 8:793-800). The PD pathway mediated adaptive resistance hypothesis supports the observation that the majority of the PD-L1$^+$ tumors can escape immune destruction even under strong anti-tumor immunity.

[258] Based on the "adaptive resistance" model, immunotherapies targeting the PD pathway are designed to block the interaction of PD-1 and PD-L1, thus preventing the resistance to anti-tumor immunity. Even though the discovery of PD-1 did not lead to its application in anti-tumor therapies until the abundant expression of PD-L1 was discovered in tumors, linking the PD pathway with cancer treatment (Dong *et al. Nature Medicine* 1999; 5:1365-1369), by now, the FDA has already approved two PD-1 monoclonal antibodies for treating human cancers. These are OPDIVO® (also known as nivolumab, MDX-1106, BMS-936558 and ONO-4538) developed by Bristol-Myers Squibb (68), and KEYTRUDA® (also known as pembrolizumab, lambrolizumab and MK-3475) developed by Merck (69). Multiple monoclonal antibodies targeting either PD-1 or PD-L1 are under intense evaluations in hundreds of clinical trials involving thousands of patients. Anti-PD therapy has generated significant clinical benefits including remarkable regression of tumors and substantial extension of survival rate. Since anti-PD therapy targets tumor-induced immune defects through immune-modulation in the tumor sites, it offers durable efficacy, tolerable toxicity and a broad spectrum of cancer indications[59] The clinical success of anti-PD immunotherapy further validates the effectiveness of PD pathway blockade as a unique category of cancer therapy that is distinct from personalized or tumor type-specific therapies. By targeting tumors that have exploited defined immune checkpoint pathway to escape immune surveillance, anti-PD immunotherapy has taken center stage in immunotherapies for human cancers, and especially solid tumors.

PD-L1 expression at tumor site as a biomarker to predict efficacy of anti-PD immunotherapy

[259] Multiple solid tumor types show positive correlation between response rate to anti-PD immunotherapy and PD-L1 expression level within the tumor, including melanoma, RCC, NSCLC, colorectal cancer, as well as several hematologic malignancies, such as classical Hodgkin's lymphoma. In the melanoma clinical trials, PD-L1 overexpression detected by IHC was in approximately 45%–75% of the samples. In the nivolumab study, 45% of patients were positive for PD-L1 expression based on a 5% cutoff using the 28-8 antibody. The response rate for PD-L1–positive patients was 44%, compared to 17% for PD-L1–negative patients. PD-L1–positive melanoma patients treated with nivolumab had an OS of 21.1 months and a PFS of 9.1 months, as compared to 12.5 months and 2 months in PD-L1 negative patients, respectively. Pembrolizumab (anti-PD-1) has also been

studied in advanced melanoma utilizing an IHC cutoff of 1%. PD-L1–positive patients (77%) had an ORR of 51%, while PD-L1–negative patients had an ORR of 6%. PD-L1–positive patients treated with pembrolizumab had a PFS of 12 months and a 1-year OS rate of 84%, while PD-L1–negative patients had a PFS of 3 months and a 1-year OS of 69%.

[260] A similar trend was seen in NSCLC, where PD-L1–positive patients seemed to preferentially benefit from PD-1/PD-L1–directed therapy. Nivolumab was studied in patients with refractory NSCLC, and PD-L1 IHC was performed using a DAKO IHC assay with a 5% cutoff. On the basis of these criteria, 60% of patients were classified as positive for PD-L1, and the response rate in PD-L1–positive patients was 67% compared with 0% in PD-L1–negative patients. Pembrolizumab has also been investigated in NSCLC, utilizing a unique 50% IHC cutoff for PD-L1 expression using an unreported assay. On the basis of this cutoff, 25% of tumors were positive for PD-L1 and, at 6 months, PD-L1–positive patients had a 67% immune-related ORR (irORR), a 67% PFS rate, and a 89% OS rate compared to PD-L1-negative patients who had a 0% irORR, a 11% PFS rate, and a 33% OS rate. MPDL3280A, an anti-PD-L1 antibody, has also been studied in NSCLC utilizing a proprietary IHC platform with 0–3+ grading (3+ for >10% cells, 2+ for >5% cells, 0–1 for <5% cells). NSCLC patients with a PD-L1 expression score of 3+ had an 83% response rate, compared with 46% in patients with a score of either 2+ or 3+. Patients with 1+/2+/3+ PD-L1 expression had a 31% ORR.

[261] PD-L1 IHC as a predictive biomarker has also been assessed in clinical trials involving multiple histologies. The nivolumab phase I study included patients with melanoma, RCC, NSCLC, metastatic colorectal cancer (mCRC), and metastatic castration-resistant prostate cancer (mCRPC). PD-L1 was detected by the 5H1 antibody utilizing a 5% threshold, and 60% of tumors were positive by this criterion. Patients with PD-L1–positive tumors had a 36% response rate, while patients with PD-L1 negative tumors had a 0% ORR. MPDL3280A has been studied in patients with melanoma, RCC, NSCLC, mCRC, and gastric cancer utilizing a proprietary PD-L1 IHC platform. PD-L1–positive patients had a 39% response rate, while PD-L1–negative patients had a 13% response rate.

[262] Data from these clinical trials appears to suggest that patients with higher levels of PD-L1 according to IHC appeared to have superior responses to PD-1/PD-L1–directed therapy. However, relatively less is known about the nature of responses or survival outcomes in PDL1–negative patients treated with anti-PD immunotherapy. Initial evaluations suggest that select PD-L1–negative patients with melanoma can still obtain durable responses to anti-PD-1/PD-L1 therapy, while response in PD-L1–negative NSCLC patients are rare. If this trend is reproduced in larger trials, it may represent a fundamental difference in the immunobiology between the two different tumor types, or it may represent a technical issue with IHC in different tissue types. In a phase I clinical trial for MPDL3280A in metastatic urothelial bladder cancer, PD-L1–positive patients had

a 52% ORR at 12 weeks, compared with 11% in PD-L1–negative patients. Therefore, the depth and duration of responses in PD-L1–negative patients remains to be seen.

V. Antibodies

[263] Also provided herein are antibodies that specifically recognize an immune checkpoint ligand, such as PD-L1 and PD-L1 like ligands. Such antibodies and antibody moieties derived therefrom can be incorporated into the methods and imaging agents described in the sections above. Suitable antibody moieties include, but are not limited to, scFv, Fab, and scFv fused to an Fc fragment (also referred herein as "scFv-Fc"). The antibody moieties (including the anti-PD-L1 antibody agents) described herein may have any one or more of the features described in the sections a)-h) below.

[264] In some embodiments, the antibody moiety comprises an scFv. In some embodiments, the antibody moiety is an scFv. In some embodiments, the scFv has the configuration of (from N-terminus to C-terminus): V_L-L-V_H, or V_H-L-V_L, wherein L is a peptide linker. In some embodiments, the scFv is chimeric, human, partially humanized, fully humanized, or semi-synthetic.

[265] In some embodiments, the scFv is engineered to have enhanced thermal stability. In some embodiments, the scFv is engineered to have a melting temperature of about 55-70°C, such as about any one of 55-60, 60-65, or 65-70°C. In some embodiments, the scFv comprises one or more (such as 1, 2, 3, or more) engineered disulfide bonds. In some embodiments, the scFv comprises a first engineered cysteine residue at position 44 of V_H and a second engineered cysteine residue at position 100 of V_L, and/or a first engineered cysteine residue at position 105 of V_H and a second engineered cysteine residue at position 43 of V_L, wherein the first engineered cysteine residue and the second engineered cysteine residue form a disulfide bond, and wherein the amino acid positions are based on the Kabat numbering system. Other engineered disulfide bonds may be introduced into the scFv by engineering a cysteine in the VH and a cysteine in the VL at suitable positions based on the structure and sequences of the scFv.

[266] In some embodiments, the antibody moiety comprises an Fc fragment. In some embodiments, the antibody moiety is an scFv fused to an Fc fragment. In some embodiments, the antibody moiety comprises a scFv fused to an Fc fragment via a peptide linker. In some embodiments, the Fc fragment is a human IgG1 Fc fragment. In some embodiments, the Fc fragment comprises one or more mutations to increase clearance or decrease half-life. For example, the Fc fragment may have H310A and/or H435Q mutations, wherein the amino acid positions are based on the Kabat numbering system.

[267] In some embodiments, the Fc fragment comprises an immunoglobulin IgG heavy chain constant region comprising a hinge region (starting at Cys226), an IgG CH2 domain and CH3 domain. The term "hinge region" or "hinge sequence" as used herein refers to the amino acid sequence located between the linker and the

CH2 domain. In some embodiments, the fusion protein comprises an Fc fragment comprising a hinge region. In some embodiments, the Fc fragment of the fusion protein starts at the hinge region and extends to the C-terminus of the IgG heavy chain. In some embodiments, the fusion protein comprises an Fc fragment that does not comprise the hinge region.

[268] In some embodiments, the antibody moiety comprises an Fc fragment selected from the group consisting of Fc fragments from IgG, IgA, IgD, IgE, IgM, and combinations and hybrids thereof. In some embodiments, the Fc fragment is derived from a human IgG. In some embodiments, the Fc fragment comprises the Fc region of human IgG1, IgG2, IgG3, IgG4, or a combination or hybrid IgG. In some embodiments, the Fc fragment is an IgG1 Fc fragment. In some embodiments, the Fc fragment comprises the CH2 and CH3 domains of IgG1. In some embodiments, the Fc fragment is an IgG4 Fc fragment. In some embodiments, the Fc fragment comprises the CH2 and CH3 domains of IgG4. IgG4 Fc is known to exhibit less effector activity than IgG1 Fc, and thus may be desirable for some applications. In some embodiments, the Fc fragment is derived from of a mouse immunoglobulin.

[269] In some embodiments, the IgG CH2 domain starts at Ala231. In some embodiments, the CH3 domain starts at Gly341. It is understood that the C-terminus Lys residue of human IgG can be optionally absent. It is also understood that conservative amino acid substitutions of the Fc region without affecting the desired structure and/or stability of Fc is contemplated within the scope of the invention.

[270] In some embodiments, each chain of the Fc fragment is fused to the same antibody moiety. In some embodiments, the scFv-Fc comprises two identical scFvs described herein, each fused with one chain of the Fc fragment. In some embodiments, the scFv-Fc is a homodimer.

[0101] In some embodiments, the scFv-Fc comprises two different scFvs, each fused with one chain of the Fc fragment. In some embodiments, the scFv-Fc is a heterodimer. Heterodimerization of non-identical polypeptides in the scFv-Fc can be facilitated by methods known in the art, including without limitation, heterodimerization by the knob-into-hole technology. The structure and assembly method of the knob-into-hole technology can be found in, e.g., US5,821,333, US7,642,228, US 201 1/0287009 and PCT/US2012/059810, hereby incorporated by reference in their entireties. This technology was developed by introducing a "knob" (or a protuberance) by replacing a small amino acid residue with a large one in the CH3 domain of one Fc and introducing a "hole" (or a cavity) in the CH3 domain of the other Fc by replacing one or more large amino acid residues with smaller ones. In some embodiments, one chain of the Fc fragment in the fusion protein comprises a knob, and the second chain of the Fc fragment comprises a hole.

[271] The preferred residues for the formation of a knob are generally naturally occurring amino acid residues and are preferably selected from arginine (R), phenylalanine (F), tyrosine (Y) and tryptophan (W). Most preferred are tryptophan and tyrosine. In one embodiment, the original residue for the formation of the

knob has a small side chain volume, such as alanine, asparagine, aspartic acid, glycine, serine, threonine or valine. Exemplary amino acid substitutions in the CH3 domain for forming the knob include without limitation the T366W, T366Y or F405W substitution.

[272] The preferred residues for the formation of a hole are usually naturally occurring amino acid residues and are preferably selected from alanine (A), serine (S), threonine (T) and valine (V). In one embodiment, the original residue for the formation of the hole has a large side chain volume, such as tyrosine, arginine, phenylalanine or tryptophan. Exemplary amino acid substitutions in the CH3 domain for generating the hole include without limitation the T366S, L368A, F405A, Y407A, Y407T and Y407V substitutions. In certain embodiments, the knob comprises T366W substitution, and the hole comprises the T366S/L368A/Y 407V substitutions. It is understood that other modifications to the Fc region known in the art that facilitate heterodimerization are also contemplated and encompassed by the instant application.

[273] Other scFv-Fc variants (including variants of isolated anti-PD-L1 scFv-Fc, *e.g.*, a full-length anti-PD-L1 antibody variants) comprising any of the variants described herein (*e.g.*, Fc variants, effector function variants, glycosylation variants, cysteine engineered variants), or combinations thereof, are contemplated.

a) Antibody affinity

[274] Binding specificity of the antibody moieties can be determined experimentally by methods known in the art. Such methods comprise, but are not limited to Western blots, ELISA-, RIA-, ECL-, IRMA-, EIA-, BIACORE^{TM} -tests and peptide scans.

[275] In some embodiments, the K_D of the binding between the antibody moiety and the immune checkpoint ligand (*e.g.*, PD-L1 or a PD-L1 like ligand) is about 10^{-7} M to about 10^{-12} M, about 10^{-7} M to about 10^{-8} M, about 10^{-8} M to about 10^{-9} M, about 10^{-9} M to about 10^{-10} M, about 10^{-10} M to about 10^{-11} M, about 10^{-11} M to about 10^{-12} M, about 10^{-7} M to about 10^{-12} M, about 10^{-8} M to about 10^{-12} M, about 10^{-9} M to about 10^{-12} M, about 10^{-10} M to about 10^{-12} M, about 10^{-7} M to about 10^{-11} M, about 10^{-8} M to about 10^{-11} M, about 10^{-9} M to about 10^{-11} M, about 10^{-7} M to about 10^{-10} M, about 10^{-8} M to about 10^{-10} M, or about 10^{-7} M to about 10^{-9} M. In some embodiments, the K_D of the binding between the antibody moiety and the immune checkpoint ligand (*e.g.*, PD-L1 or a PD-L1 like ligand) is stronger than about any one of 10^{-7} M, 10^{-8} M, 10^{-9} M, 10^{-10} M, 10^{-11} M, or 10^{-12} M. In some embodiments, the immune checkpoint ligand is human immune checkpoint ligand (*e.g.*, human PD-L1 or a PD-L1 like ligand). In some embodiments, the immune checkpoint ligand is cynomolgus monkey immune checkpoint ligand (*e.g.*, cynomolgus monkey PD-L1 or a PD-L1 like ligand). In some embodiments, the antibody moiety specifically recognizes an epitope in the extracellular domain of the immune checkpoint ligand, such as amino acids 19-238 of SEQ ID NO: 4.

[276] In some embodiments, the K_{on} of the binding between the antibody moiety and the immune checkpoint ligand (*e.g.*, PD-L1 or a PD-L1 like ligand) is about 10^3 $M^{-1}s^{-1}$ to about 10^8 $M^{-1}s^{-1}$, about 10^3 $M^{-1}s^{-1}$ to about 10^4

$M^{-1}s^{-1}$, about 10^4 $M^{-1}s^{-1}$ to about 10^5 $M^{-1}s^{-1}$, about 10^5 $M^{-1}s^{-1}$ to about 10^6 $M^{-1}s^{-1}$, about 10^6 $M^{-1}s^{-1}$ to about 10^7 $M^{-1}s^{-1}$, or about 10^7 $M^{-1}s^{-1}$ to about 10^8 $M^{-1}s^{-1}$. In some embodiments, the K_{on} of the binding between the antibody moiety and the immune checkpoint ligand (*e.g.*, PD-L1 or a PD-L1 like ligand) is about 10^3 $M^{-1}s^{-1}$ to about 10^5 $M^{-1}s^{-1}$, about 10^4 $M^{-1}s^{-1}$ to about 10^6 $M^{-1}s^{-1}$, about 10^5 $M^{-1}s^{-1}$ to about 10^7 $M^{-1}s^{-1}$, about 10^6 $M^{-1}s^{-1}$ to about 10^8 $M^{-1}s^{-1}$, about 10^4 $M^{-1}s^{-1}$ to about 10^7 $M^{-1}s^{-1}$, or about 10^5 $M^{-1}s^{-1}$ to about 10^8 $M^{-1}s^{-1}$. In some embodiments, the K_{on} of the binding between the antibody moiety and the immune checkpoint ligand (*e.g.*, PD-L1 or a PD-L1 like ligand) is no more than about any one of 10^3 $M^{-1}s^{-1}$, 10^4 $M^{-1}s^{-1}$, 10^5 $M^{-1}s^{-1}$, 10^6 $M^{-1}s^{-1}$, 10^7 $M^{-1}s^{-1}$ or 10^8 $M^{-1}s^{-1}$.

[277] In some embodiments, the K_{off} of the binding between the antibody moiety and the immune checkpoint ligand (*e.g.*, PD-L1 or a PD-L1 like ligand) is about 1 s^{-1} to about 10^{-6} s^{-1}, about 1 s^{-1} to about 10^{-2} s^{-1}, about 10^{-2} s^{-1} to about 10^{-3} s^{-1}, about 10^{-3} s^{-1} to about 10^{-4} s^{-1}, about 10^{-4} s^{-1} to about 10^{-5} s^{-1}, about 10^{-5} s^{-1} to about 10^{-6} s^{-1}, about 1 s^{-1} to about 10^{-5} s^{-1}, about 10^{-2} s^{-1} to about 10^{-6} s^{-1}, about 10^{-3} s^{-1} to about 10^{-6} s^{-1}, about 10^{-4} s^{-1} to about 10^{-6} s^{-1}, about 10^{-2} s^{-1} to about 10^{-5} s^{-1}, or about 10^{-3} s^{-1} to about 10^{-5} s^{-1}. In some embodiments, the K_{off} of the binding between the antibody moiety and the immune checkpoint ligand (*e.g.*, PD-L1 or a PD-L1 like ligand) is at least about any one of 1 s^{-1}, 10^{-2} s^{-1}, 10^{-3} s^{-1}, 10^{-4} s^{-1}, 10^{-5} s^{-1} or 10^{-6} s^{-1}.

b) Chimeric or humanized antibodies

[278] In some embodiments, the antibody moiety is a chimeric antibody. Certain chimeric antibodies are described, *e.g.*, in U.S. Patent No. 4,816,567; and Morrison et al., *Proc. Natl. Acad. Sci. USA*, 81:6851-6855 (1984)). In some embodiments, a chimeric antibody comprises a non-human variable region (*e.g.*, a variable region derived from mouse) and a human constant region. In some embodiments, a chimeric antibody is a "class switched" antibody in which the class or subclass has been changed from that of the parent antibody. Chimeric antibodies include antigen-binding fragments thereof.

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

[280] Humanized antibodies and methods of making them are reviewed, *e.g.*, in Almagro and Fransson, *Front. Biosci.* 13:1619-1633 (2008), and are further described, e.g., in Riechmann et al., *Nature* 332:323-329 (1988); Queen et al., *Proc. Nat'l Acad. Sci. USA* 86:10029-10033 (1989); US Patent Nos. 5, 821,337, 7,527,791,

6,982,321, and 7,087,409; Kashmiri *et al.*, *Methods* 36:25-34 (2005) (describing SDR (a-CDR) grafting); Padlan, *Mol. Immunol.* 28:489-498 (1991) (describing "resurfacing"); Dall'Acqua et al., *Methods* 36:43-60 (2005) (describing "FR shuffling"); and Osbourn et al., *Methods* 36:61-68 (2005) and Klimka et al., *Br. J. Cancer*, 83:252-260 (2000) (describing the "guided selection" approach to FR shuffling).

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

c) Human antibodies

[282] In some embodiments, the antibody moiety is a human antibody (known as human domain antibody, or human DAb). Human antibodies can be produced using various techniques known in the art. Human antibodies are described generally in van Dijk and van de Winkel, *Curr. Opin. Pharmacol.* 5: 368-74 (2001), Lonberg, *Curr. Opin. Immunol.* 20:450-459 (2008), and Chen, *Mol. Immunol.* 47(4):912-21 (2010). Transgenic mice or rats capable of producing fully human single-domain antibodies (or DAb) are known in the art. See, *e.g.*, US20090307787A1, U.S. Pat. No. 8,754,287, US20150289489A1, US20100122358A1, and WO2004049794.

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

[284] Human antibodies (e.g., human DAbs) can also be made by hybridoma-based methods. Human myeloma and mouse-human heteromyeloma cell lines for the production of human monoclonal antibodies have

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

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

d) Library-derived antibodies

[286] The antibody moieties may be isolated by screening combinatorial libraries for antibodies with the desired activity or activities. For example, a variety of methods are known in the art for generating phage display libraries and screening such libraries for antibodies possessing the desired binding characteristics. Such methods are reviewed, *e.g.*, in Hoogenboom et al. in *Methods in Molecular Biology* 178:1-37 (O'Brien et al., ed., Human Press, Totowa, NJ, 2001) and further described, *e.g.*, in the McCafferty et al., *Nature* 348:552-554; Clackson et al., *Nature* 352: 624-628 (1991); Marks et al., *J. Mol. Biol.* 222: 581-597 (1992); Marks and Bradbury, in *Methods in Molecular Biology* 248:161-175 (Lo, ed., Human Press, Totowa, NJ, 2003); Sidhu et al., *J. Mol. Biol.* 338(2): 299-310 (2004); Lee et al., *J. Mol. Biol.* 340(5): 1073-1093 (2004); Fellouse, *Proc. Natl. Acad. Sci. USA* 101(34): 12467-12472 (2004); and Lee et al., *J. Immunol. Methods* 284(1-2): 119-132(2004). Methods for constructing single-domain antibody libraries have been described, for example, see U.S. Pat. NO. 7371849.

[287] In certain phage display methods, repertoires of V_H and V_L genes are separately cloned by polymerase chain reaction (PCR) and recombined randomly in phage libraries, which can then be screened for antigen-binding phage as described in Winter et al., *Ann. Rev. Immunol.*, 12: 433-455 (1994). Phage typically displays antibody fragments, either as scFv fragments or as Fab fragments. Libraries from immunized sources provide high-affinity antibodies to the immunogen without the requirement of constructing hybridomas. Alternatively, the naive repertoire can be cloned (*e.g.*, from human) to provide a single source of antibodies to a wide range of non-self and also self-antigens without any immunization as described by Griffiths et al., *EMBO J,* 12: 725-734 (1993). Finally, naive libraries can also be made synthetically by cloning unrearranged V-gene segments from

stem cells, and using PCR primers containing random sequence to encode the highly variable CDR3 regions and to accomplish rearrangement *in vitro*, as described by Hoogenboom and Winter, *J. Mol. Biol.*, 227: 381-388 (1992). Patent publications describing human antibody phage libraries include, for example: US Patent No. 5,750,373, and US Patent Publication Nos. 2005/0079574, 2005/0119455, 2005/0266000, 2007/0117126, 2007/0160598, 2007/0237764, 2007/0292936, and 2009/0002360.

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

e) Substitution, insertion, deletion and variants

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

Table 2. Amino acid substitutions

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

[290] Amino acids may be grouped according to common side-chain properties: (1) hydrophobic: Norleucine, Met, Ala, Val, Leu, Ile; (2) neutral hydrophilic: Cys, Ser, Thr, Asn, Gln; (3) acidic: Asp, Glu; (4) basic: His, Lys, Arg; (5) residues that influence chain orientation: Gly, Pro; and (6) aromatic: Trp, Tyr, Phe.

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

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

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

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

[295] A useful method for identification of residues or regions of an antibody that may be targeted for mutagenesis is called "alanine scanning mutagenesis" as described by Cunningham and Wells (1989) *Science*, 244:1081-1085. In this method, a residue or group of target residues (*e.g.*, charged residues such as Arg, Asp, His, Lys, and Glu) are identified and replaced by a neutral or negatively charged amino acid (*e.g.*, alanine or polyalanine) to determine whether the interaction of the antibody with antigen is affected. Further substitutions may be introduced at the amino acid locations demonstrating functional sensitivity to the initial substitutions. Alternatively, or additionally, a crystal structure of an antigen-antibody complex to identify contact points between the antibody and antigen. Such contact residues and neighboring residues may be targeted or eliminated as candidates for substitution. Variants may be screened to determine whether they contain the desired properties.

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

f) Glycosylation variants

[297] In some embodiments, the antibody moiety is altered to increase or decrease the extent to which the construct is glycosylated. Addition or deletion of glycosylation sites to an antibody may be conveniently accomplished by altering the amino acid sequence such that one or more glycosylation sites is created or removed.

[298] Where the antibody moiety comprises an Fc region (*e.g.*, scFv-Fc), the carbohydrate attached thereto may be altered. Native antibodies produced by mammalian cells typically comprise a branched, biantennary oligosaccharide that is generally attached by an N-linkage to Asn297 of the C_H2 domain of the Fc region. *See, e.g.*, Wright et al. *TIBTECH* 15:26-32 (1997). The oligosaccharide may include various carbohydrates, *e.g.*, mannose, N-acetyl glucosamine (GlcNAc), galactose, and sialic acid, as well as a fucose attached to a GlcNAc in the "stem" of the biantennary oligosaccharide structure. In some embodiments, modifications of the oligosaccharide in the antibody moiety may be made in order to create antibody variants with certain improved properties.

[299] In some embodiments, the antibody moiety has a carbohydrate structure that lacks fucose attached (directly or indirectly) to an Fc region. For example, the amount of fucose in such antibody may be from 1% to 80%, from 1% to 65%, from 5% to 65% or from 20% to 40%. The amount of fucose is determined by calculating the average amount of fucose within the sugar chain at Asn297, relative to the sum of all

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

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

g) Fc region variants

[301] In some embodiments, one or more amino acid modifications may be introduced into the Fc region of the antibody moiety (*e.g.*, scFv-Fc), thereby generating an Fc region variant. The Fc region variant may comprise a human Fc region sequence (*e.g.*, a human IgG1, IgG2, IgG3 or IgG4 Fc region) comprising an amino acid modification (*e.g.* a substitution) at one or more amino acid positions.

[302] In some embodiments, the Fc fragment possesses some but not all effector functions, which make it a desirable candidate for applications in which the half-life of the antibody moiety *in vivo* is important yet certain effector functions (such as complement and ADCC) are unnecessary or deleterious. *In vitro* and/or *in vivo* cytotoxicity assays can be conducted to confirm the reduction/depletion of CDC and/or ADCC activities. For

example, Fc receptor (FcR) binding assays can be conducted to ensure that the antibody lacks FcγR binding (hence likely lacking ADCC activity), but retains FcRn binding ability. The primary cells for mediating ADCC, NK cells, express FcγRIII only, whereas monocytes express FcγRI, FcγRII and FcγRIII. FcR expression on hematopoietic cells is summarized in Table 2 on page 464 of Ravetch and Kinet, *Annu. Rev. Immunol.* 9:457-492 (1991). Non-limiting examples of *in vitro* assays to assess ADCC activity of a molecule of interest is described in U.S. Patent No. 5,500,362 (*see, e.g.* Hellstrom, I. et al. *Proc. Nat'l Acad. Sci. USA* 83:7059-7063 (1986)) and Hellstrom, I et al., *Proc. Nat'l Acad. Sci. USA* 82:1499-1502 (1985); 5,821,337 (*see* Bruggemann, M. et al., *J. Exp. Med.* 166:1351-1361 (1987)). Alternatively, non-radioactive assays methods may be employed (*see*, for example, ACTI™ non-radioactive cytotoxicity assay for flow cytometry (CellTechnology, Inc. Mountain View, CA; and CytoTox 96® non-radioactive cytotoxicity assay (Promega, Madison, WI). Useful effector cells for such assays include peripheral blood mononuclear cells (PBMC) and Natural Killer (NK) cells. Alternatively, or additionally, ADCC activity of the molecule of interest may be assessed *in vivo*, *e.g.*, in an animal model such as that disclosed in Clynes et al. *Proc. Nat'l Acad. Sci. USA* 95:652-656 (1998). C1q binding assays may also be carried out to confirm that the antibody is unable to bind C1q and hence lacks CDC activity. *See, e.g.*, C1q and C3c binding ELISA in WO 2006/029879 and WO 2005/100402. To assess complement activation, a CDC assay may be performed (*see*, for example, Gazzano-Santoro *et al.*, *J. Immunol. Methods* 202:163 (1996); Cragg, M.S. et al., *Blood* 101:1045-1052 (2003); and Cragg, M.S. and M.J. Glennie, *Blood* 103:2738-2743 (2004)). FcRn binding and *in vivo* clearance/half-life determinations can also be performed using methods known in the art (*see, e.g.*, Petkova, S.B. et al., *Int'l. Immunol.* 18(12):1759-1769 (2006)).

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

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

[305] In some embodiments, the antibody moiety comprises an Fc region with one or more amino acid substitutions which improve ADCC, *e.g.*, substitutions at positions 298, 333, and/or 334 of the Fc region (EU numbering of residues).

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

[307] In some embodiments, the antibody moiety (*e.g.*, scFv-Fc) variant comprising a variant Fc region comprising one or more amino acid substitutions which alters half-life and/or changes binding to the neonatal

Fc receptor (FcRn). Antibodies with increased half-lives and improved binding to the neonatal Fc receptor (FcRn), which is responsible for the transfer of maternal IgGs to the fetus (Guyer et al., *J. Immunol.* 117:587 (1976) and Kim et al., *J. Immunol.* 24:249 (1994)), are described in US2005/0014934A1 (Hinton et al.). Those antibodies comprise an Fc region with one or more substitutions therein which alters binding of the Fc region to FcRn. Such Fc variants include those with substitutions at one or more of Fc region residues, *e.g.*, substitution of Fc region residue 434 (US Patent No. 7,371,826).

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

h) Cysteine engineered antibody variants

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

i) Antibody derivatives

[310] In some embodiments, the antibody moiety described herein may be further modified to comprise additional nonproteinaceous moieties that are known in the art and readily available. The moieties suitable for derivatization of the antibody include but are not limited to water soluble polymers. Non-limiting examples of water soluble polymers include, but are not limited to, polyethylene glycol (PEG), copolymers of ethylene glycol/propylene glycol, carboxymethylcellulose, dextran, polyvinyl alcohol, polyvinyl pyrrolidone, poly-1, 3-dioxolane, poly-1,3,6-trioxane, ethylene/maleic anhydride copolymer, polyaminoacids (either homopolymers or random copolymers), and dextran or poly(n-vinyl pyrrolidone)polyethylene glycol, propropylene glycol homopolymers, prolypropylene oxide/ethylene oxide co-polymers, polyoxyethylated polyols (*e.g.*, glycerol), polyvinyl alcohol, and mixtures thereof. Polyethylene glycol propionaldehyde may have advantages in manufacturing due to its stability in water. The polymer may be of any molecular weight, and may be branched or unbranched. The number of polymers attached to the antibody may vary, and if more than one polymer are attached, they can be the same or different molecules. In general, the number and/or type of polymers used for derivatization can be determined based on considerations including, but not limited to, the particular properties

or functions of the antibody to be improved, whether the antibody derivative will be used in diagnosis under defined conditions, *etc.*

[311] In some embodiments, the antibody moiety may be further modified to comprise one or more biologically active protein, polypeptides or fragments thereof. "Bioactive" or "biologically active", as used herein interchangeably, means showing biological activity in the body to carry out a specific function. For example, it may mean the combination with a particular biomolecule such as protein, DNA, *etc.*, and then promotion or inhibition of the activity of such biomolecule. In some embodiments, the bioactive protein or fragments thereof include proteins and polypeptides that are administered to patients as the active drug substance for prevention of or treatment of a disease or condition, as well as proteins and polypeptides that are used for diagnostic purposes, such as enzymes used in diagnostic tests or *in vitro* assays, as well as proteins and polypeptides that are administered to a patient to prevent a disease such as a vaccine.

VI. Methods of preparation

[312] In some embodiments, there is provided a method of preparing an imaging agent targeting an immune checkpoint ligand. The imaging agents described herein may be prepared by a number of processes as generally described below and more specifically in the Examples.

[313] In some embodiments, there is provided a method of preparing an imaging agent targeting an immune checkpoint ligand, comprising: (a) conjugating a chelating compound to an antibody moiety (*e.g.*, scFv) specifically binding the immune checkpoint ligand to provide an antibody moiety conjugate; and (b) contacting a radionuclide with the antibody moiety conjugate, thereby providing the imaging agent. In some embodiments, the immune checkpoint ligand is PD-L1. In some embodiments, the immune checkpoint ligand is a PD-L1 like ligand. In some embodiments, the chelating compound is NOTA, DOTA or derivatives thereof. In some embodiments, the radionuclide is selected from the group consisting of ^{64}Cu, ^{18}F, ^{67}Ga, ^{68}Ga, ^{111}In, ^{177}Lu, ^{90}Y, ^{89}Zr, ^{61}Cu, ^{62}Cu, ^{67}Cu, ^{19}F, ^{66}Ga, ^{72}Ga, ^{44}Sc, ^{47}Sc, ^{86}Y, ^{88}Y and ^{45}Ti. In some embodiments, the radionuclide is ^{68}Ga. In some embodiments, the chelating compound is conjugated to a lysine of the antibody moiety. In some embodiments, the method further comprises isolating the imaging agent from the chelating compound and/or the radionuclide.

[314] In some embodiments, there is provided a method of preparing an imaging agent targeting an immune checkpoint ligand, comprising: (a) conjugating a chelating compound to any one of the antibody moieties described herein to provide an antibody moiety conjugate, wherein the antibody moiety specifically binds the immune checkpoint ligand; and (b) contacting a radionuclide with the antibody moiety conjugate, thereby providing the imaging agent. In some embodiments, the immune checkpoint ligand is PD-L1. In some embodiments, the immune checkpoint ligand is a PD-L1 like ligand. In some embodiments, the chelating

compound is NOTA, DOTA or derivatives thereof. In some embodiments, the radionuclide is selected from the group consisting of ^{64}Cu, ^{18}F, ^{67}Ga, ^{68}Ga, ^{111}In, ^{177}Lu, ^{90}Y, ^{89}Zr, ^{61}Cu, ^{62}Cu, ^{67}Cu, ^{19}F, ^{66}Ga, ^{72}Ga, ^{44}Sc, ^{47}Sc, ^{86}Y, ^{88}Y and ^{45}Ti. In some embodiments, the radionuclide is ^{68}Ga. In some embodiments, the chelating compound is conjugated to a lysine of the antibody moiety. In some embodiments, the method further comprises isolating the imaging agent from the chelating compound and/or the radionuclide.

[315] In some embodiments, there is provided a method of preparing an imaging agent targeting an immune checkpoint ligand, comprising: (a) contacting a chelating compound with a radionuclide; (b) conjugating the chelating compound that chelates the radionuclide to any one of the antibody moieties described herein, wherein the antibody moiety specifically binds the immune checkpoint ligand, thereby providing the imaging agent. In some embodiments, the immune checkpoint ligand is PD-L1. In some embodiments, the immune checkpoint ligand is a PD-L1 like ligand. In some embodiments, the chelating compound is NOTA, DOTA or derivatives thereof. In some embodiments, the radionuclide is selected from the group consisting of ^{64}Cu, ^{18}F, ^{67}Ga, ^{68}Ga, ^{111}In, ^{177}Lu, ^{90}Y, ^{89}Zr, ^{61}Cu, ^{62}Cu, ^{67}Cu, ^{19}F, ^{66}Ga, ^{72}Ga, ^{44}Sc, ^{47}Sc, ^{86}Y, ^{88}Y and ^{45}Ti. In some embodiments, the radionuclide is ^{68}Ga. In some embodiments, the chelating compound is conjugated to a lysine of the antibody moiety. In some embodiments, the method further comprises isolating the imaging agent from the chelating compound and/or the radionuclide.

[316] In some embodiments, there is provided a method of preparing an imaging agent targeting an immune checkpoint ligand, comprising: (a) conjugating an scFv specifically binding the immune checkpoint ligand (*e.g.*, PD-L1 or a PD-L1 like ligand) to p-SCN-Bn-NOTA to provide an scFv conjugate; (b) contacting ^{68}Ga with the scFv conjugate, thereby providing the imaging agent. In some embodiments, the scFv conjugate is isolated by passing the mixture of the scFv and p-SCN-Bn-NOTA through a column (*e.g.*, NAP-5 column). In some embodiments, the imaging agent is isolated by passing the mixture of ^{68}Ga with the scFv conjugate through a column (*e.g.*, NAP-5 column).

Antibody Expression and Production

[317] The antibody moieties described herein can be prepared using any known methods in the art, including those described below and in the Examples.

Monoclonal antibodies

[318] Monoclonal antibodies are obtained from a population of substantially homogeneous antibodies, *i.e.*, the individual antibodies comprising the population are identical except for possible naturally occurring mutations and/or post-translational modifications (*e.g.*, isomerizations, amidations) that may be present in minor amounts. Thus, the modifier "monoclonal" indicates the character of the antibody as not being a mixture of discrete antibodies. For example, the monoclonal antibodies may be made using the hybridoma method first

described by Kohler *et al.*, *Nature,* 256:495 (1975), or may be made by recombinant DNA methods (U.S. Pat. No. 4,816,567). In the hybridoma method, a mouse or other appropriate host animal, such as a hamster or a llama, is immunized as hereinabove described to elicit lymphocytes that produce or are capable of producing antibodies that will specifically bind the protein used for immunization. Alternatively, lymphocytes may be immunized *in vitro*. Lymphocytes then are fused with myeloma cells using a suitable fusing agent, such as polyethylene glycol, to form a hybridoma cell (Goding, *Monoclonal Antibodies: Principles and Practice*, pp. 59-103 (Academic Press, 1986). Also see Example 1 for immunization in Camels.

[319] The immunizing agent will typically include the antigenic protein or a fusion variant thereof. Generally either peripheral blood lymphocytes ("PBLs") 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.

[320] 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 thus prepared are seeded and grown in a suitable culture medium that preferably contains one or more substances that inhibit the growth or survival of the unfused, parental myeloma cells. For example, if the parental myeloma 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 are substances that prevent the growth of HGPRT-deficient cells.

[321] Preferred immortalized myeloma cells are those that fuse efficiently, support stable high-level production of antibody by the selected antibody-producing cells, and are sensitive to a medium such as HAT medium. Among these, preferred are murine myeloma lines, such as those derived from MOPC-21 and MPC-11 mouse tumors available from the Salk Institute Cell Distribution Center, San Diego, Calif. USA, and SP-2 cells (and derivatives thereof, *e.g.*, X63-Ag8-653) available from the American Type Culture Collection, Manassas, Va. USA. Human myeloma and mouse-human heteromyeloma cell lines also have been described for the production of human monoclonal antibodies (Kozbor, *J. Immunol.*, 133:3001 (1984); Brodeur *et al.*, *Monoclonal Antibody Production Techniques and Applications*, pp. 51-63 (Marcel Dekker, Inc., New York, 1987)).

[322] Culture medium in which hybridoma cells are growing is assayed for production of monoclonal antibodies directed against the antigen. Preferably, the binding specificity of monoclonal antibodies produced by hybridoma cells is determined by immunoprecipitation or by an in vitro binding assay, such as radioimmunoassay (RIA) or enzyme-linked immunosorbent assay (ELISA).

[323] The culture medium in which the hybridoma cells are cultured can be assayed for the presence of monoclonal antibodies directed against the desired antigen. Preferably, the binding affinity and specificity of the monoclonal antibody can be determined by immunoprecipitation or by an in vitro binding assay, such as radioimmunoassay (RIA) or enzyme-linked assay (ELISA). Such techniques and assays are known in the in art. For example, binding affinity may be determined by the Scatchard analysis of Munson *et al.*, *Anal. Biochem.*, 107:220 (1980).

[324] After hybridoma cells are identified that produce antibodies of the desired specificity, affinity, and/or activity, the clones may be subcloned by limiting dilution procedures and grown by standard methods (Goding, supra). Suitable culture media for this purpose include, for example, D-MEM or RPMI-1640 medium. In addition, the hybridoma cells may be grown in vivo as tumors in a mammal.

[325] The monoclonal antibodies secreted by the subclones are suitably separated from the culture medium, ascites fluid, or serum by conventional immunoglobulin purification procedures such as, for example, protein A-Sepharose, hydroxylapatite chromatography, gel electrophoresis, dialysis, or affinity chromatography.

[326] Monoclonal antibodies may also be made by recombinant DNA methods, such as those described in U.S. Pat. No. 4,816,567, and as described above. DNA encoding the monoclonal antibodies is readily isolated and sequenced using conventional procedures (*e.g.*, by using oligonucleotide probes that are capable of binding specifically to genes encoding the heavy and light chains of murine antibodies). The hybridoma cells serve as a preferred source of such DNA. Once isolated, the DNA may be placed into expression vectors, which are then transfected into host cells such as *E. coli* cells, simian COS cells, Chinese hamster ovary (CHO) cells, or myeloma cells that do not otherwise produce immunoglobulin protein, in order to synthesize monoclonal antibodies in such recombinant host cells. Review articles on recombinant expression in bacteria of DNA encoding the antibody include Skerra *et al.*, *Curr. Opinion in Immunol.*, 5:256-262 (1993) and Plückthun, *Immunol. Revs.* 130:151-188 (1992).

[327] In a further embodiment, antibodies can be isolated from antibody phage libraries generated using the techniques described in McCafferty *et al.*, *Nature,* 348:552-554 (1990). Clackson *et al.*, *Nature,* 352:624-628 (1991) and Marks *et al.*, *J. Mol. Biol.,* 222:581-597 (1991) describe the isolation of murine and human antibodies, respectively, using phage libraries. Subsequent publications describe the production of high affinity (nM range) human antibodies by chain shuffling (Marks *et al.*, *Bio/Technology,* 10:779-783 (1992)), as well as combinatorial infection and in vivo recombination as a strategy for constructing very large phage libraries (Waterhouse *et al.*, *Nucl. Acids Res.,* 21:2265-2266 (1993)). Thus, these techniques are viable alternatives to traditional monoclonal antibody hybridoma techniques for isolation of monoclonal antibodies.

[328] The DNA also may be modified, for example, by substituting the coding sequence for human heavy- and light-chain constant domains in place of the homologous murine sequences (U.S. Pat. No. 4,816,567;

Morrison, *et al.*, *Proc. Natl Acad. Sci. USA,* 81:6851 (1984)), or by covalently joining to the immunoglobulin coding sequence all or part of the coding sequence for a non-immunoglobulin polypeptide. Typically such non-immunoglobulin polypeptides are substituted for the constant domains of an antibody, or they are substituted for the variable domains of one antigen-combining site of an antibody to create a chimeric bivalent antibody comprising one antigen-combining site having specificity for an antigen and another antigen-combining site having specificity for a different antigen.

[329] The monoclonal antibodies described herein may by monovalent, the preparation of which is well known in the art. For example, one method involves recombinant expression of immunoglobulin light chain and a modified heavy chain. The heavy chain is truncated generally at any point in the Fc region so as to prevent heavy chain crosslinking. Alternatively, the relevant cysteine residues may be substituted with another amino acid residue or are deleted so as to prevent crosslinking. *In vitro* methods are also suitable for preparing monovalent antibodies. Digestion of antibodies to produce fragments thereof, particularly Fab fragments, can be accomplished using routine techniques known in the art.

[330] Chimeric or hybrid antibodies also may be prepared *in vitro* using known methods in synthetic protein chemistry, including those involving crosslinking agents. For example, immunotoxins may 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.

[331] Also, *see*, Example 1 for monoclonal antibody production.

Nucleic Acid Molecules Encoding antibody moieties

[332] The present application further provides isolated nucleic acid molecules comprising polynucleotides that encode one or more chains of the antibody moieties (*e.g.*, anti-PD-L1 antibody moieties) described herein. In some embodiments, a nucleic acid molecule comprises a polynucleotide that encodes a heavy chain or a light chain of an antibody moiety (*e.g.*, anti-PD-L1 antibody moiety). In some embodiments, a nucleic acid molecule comprises both a polynucleotide that encodes a heavy chain and a polynucleotide that encodes a light chain, of an antibody moiety (*e.g.*, anti-PD-L1 antibody moiety). In some embodiments, a first nucleic acid molecule comprises a first polynucleotide that encodes a heavy chain and a second nucleic acid molecule comprises a second polynucleotide that encodes a light chain. In some embodiments, a nucleic acid molecule encoding an scFv (*e.g.*, anti-PD-L1 scFv) is provided.

[333] In some such embodiments, the heavy chain and the light chain are expressed from one nucleic acid molecule, or from two separate nucleic acid molecules, as two separate polypeptides. In some embodiments, such as when an antibody is an scFv, a single polynucleotide encodes a single polypeptide comprising both a heavy chain and a light chain linked together.

[334] In some embodiments, a polynucleotide encoding a heavy chain or light chain of an antibody moiety (*e.g.*, anti-PD-L1 antibody moiety) comprises a nucleotide sequence that encodes a leader sequence, which, when translated, is located at the N terminus of the heavy chain or light chain. As discussed above, the leader sequence may be the native heavy or light chain leader sequence, or may be another heterologous leader sequence.

[335] Nucleic acid molecules may be constructed using recombinant DNA techniques conventional in the art. In some embodiments, a nucleic acid molecule is an expression vector that is suitable for expression in a selected host cell.

Vectors

[336] Vectors comprising polynucleotides that encode the heavy chains and/or light chains of any one of the antibody moieties described herein (*e.g.*, anti-PD-L1 antibody moieties) are provided. Vectors comprising polynucleotides that encode any of the scFvs described herein (*e.g.*, anti-PD-L1 scFv) are also provided. Such vectors include, but are not limited to, DNA vectors, phage vectors, viral vectors, retroviral vectors, *etc.* In some embodiments, a vector comprises a first polynucleotide sequence encoding a heavy chain and a second polynucleotide sequence encoding a light chain. In some embodiments, the heavy chain and light chain are expressed from the vector as two separate polypeptides. In some embodiments, the heavy chain and light chain are expressed as part of a single polypeptide, such as, for example, when the antibody is an scFv.

[337] In some embodiments, a first vector comprises a polynucleotide that encodes a heavy chain and a second vector comprises a polynucleotide that encodes a light chain. In some embodiments, the first vector and second vector are transfected into host cells in similar amounts (such as similar molar amounts or similar mass amounts). In some embodiments, a mole- or mass-ratio of between 5:1 and 1:5 of the first vector and the second vector is transfected into host cells. In some embodiments, a mass ratio of between 1:1 and 1:5 for the vector encoding the heavy chain and the vector encoding the light chain is used. In some embodiments, a mass ratio of 1:2 for the vector encoding the heavy chain and the vector encoding the light chain is used.

[338] In some embodiments, a vector is selected that is optimized for expression of polypeptides in CHO or CHO-derived cells, or in NSO cells. Exemplary such vectors are described, *e.g.*, in Running Deer *et al.*, *Biotechnol. Prog.* 20:880-889 (2004).

Host Cells

[339] In some embodiments, the antibody moieties described herein (*e.g.*, anti-PD-L1 antibody moieties) may be expressed in prokaryotic cells, such as bacterial cells; or in eukaryotic cells, such as fungal cells (such as yeast), plant cells, insect cells, and mammalian cells. Such expression may be carried out, for example, according to procedures known in the art. Exemplary eukaryotic cells that may be used to express polypeptides

include, but are not limited to, COS cells, including COS 7 cells; 293 cells, including 293-6E cells; CHO cells, including CHO-S, DG44. Lec13 CHO cells, and FUT8 CHO cells; PER.C6® cells (Crucell); and NSO cells. In some embodiments, the antibody moieties described herein (*e.g.*, anti-PD-L1 antibody moieties) may be expressed in yeast. See, *e.g.*, U.S. Publication No. US 2006/0270045 A1. In some embodiments, a particular eukaryotic host cell is selected based on its ability to make desired post-translational modifications to the heavy chains and/or light chains of the antibody moiety. For example, in some embodiments, CHO cells produce polypeptides that have a higher level of sialylation than the same polypeptide produced in 293 cells.

[340] Introduction of one or more nucleic acids into a desired host cell may be accomplished by any method, including but not limited to, calcium phosphate transfection, DEAE-dextran mediated transfection, cationic lipid-mediated transfection, electroporation, transduction, infection, *etc.* Non-limiting exemplary methods are described, *e.g.*, in Sambrook *et al.*, Molecular Cloning, A Laboratory Manual, 3rd ed. Cold Spring Harbor Laboratory Press (2001). Nucleic acids may be transiently or stably transfected in the desired host cells, according to any suitable method.

[341] The invention also provides host cells comprising any of the polynucleotides or vectors described herein. In some embodiments, the invention provides a host cell comprising an anti-PD-L1 antibody. Any host cells capable of over-expressing heterologous DNAs can be used for the purpose of isolating the genes encoding the antibody, polypeptide or protein of interest. Non-limiting examples of mammalian host cells include but not limited to COS, HeLa, and CHO cells. See also PCT Publication No. WO 87/04462. Suitable non-mammalian host cells include prokaryotes (such as *E. coli* or *B. subtillis*) and yeast (such as *S. cerevisae*, *S. pombe*; or *K. lactis*).

[342] In some embodiments, the antibody moiety is produced in a cell-free system. Non-limiting exemplary cell-free systems are described, *e.g.*, in Sitaraman *et al.*, *Methods Mol. Biol.* 498: 229-44 (2009); Spirin, *Trends Biotechnol.* 22: 538-45 (2004); Endo *et al.*, *Biotechnol. Adv.* 21: 695-713 (2003).

Purification of antibody moieties

[343] The antibody moieties (*e.g.*, anti-PD-L1 antibody moieties) may be purified by any suitable method. Such methods include, but are not limited to, the use of affinity matrices or hydrophobic interaction chromatography. Suitable affinity ligands include the ROR1 ECD and ligands that bind antibody constant regions. For example, a Protein A, Protein G, Protein A/G, or an antibody affinity column may be used to bind the constant region and to purify an antibody moiety comprising an Fc fragment. Hydrophobic interactive chromatography, for example, a butyl or phenyl column, may also suitable for purifying some polypeptides such as antibodies. Ion exchange chromatography (*e.g.* anion exchange chromatography and/or cation exchange chromatography) may also suitable for purifying some polypeptides such as antibodies. Mixed-mode chromatography (*e.g.* reversed phase/anion exchange, reversed phase/cation exchange, hydrophilic

interaction/anion exchange, hydrophilic interaction/cation exchange, *etc.*) may also suitable for purifying some polypeptides such as antibodies. Many methods of purifying polypeptides are known in the art.

VII. Methods of Treatment

[344] For the prevention or treatment of disease, the anti-PD-L1 antibody agent as described herein can be administered to individuals (e.g., mammals such as humans) to treat or prevent a disease or condition.

[345] In some embodiments, there is provided a method of treating a disease or condition in an individual, comprising administering to the individual an effective amount of an anti-PD-L1 antibody agent, wherein the anti-PD-L1 antibody agent comprises an antibody moiety comprising a heavy chain variable region (V_H) and a light chain variable region (V_L), wherein: a) the V_H comprises a heavy chain complementarity determining region (HC-CDR) 1 comprising the amino acid sequence of SEQ ID NO: 41, an HC-CDR2 comprising the amino acid sequence of SEQ ID NO: 42, and an HC-CDR3 comprising the amino acid sequence of SEQ ID NO: 43, or a variant thereof comprising up to a total of about 5 amino acid substitutions in the HC-CDRs; and b) the V_L comprises a light chain complementarity determining region (LC-CDR) 1 comprising the amino acid sequence of SEQ ID NO: 44, an LC-CDR2 comprising the amino acid sequence of SEQ ID NO: 45, and an LC-CDR3 comprising the amino acid sequence of SEQ ID NO: 46, or a variant thereof comprising up to a total of about 5 amino acid substitutions in the LC-CDRs. In some embodiments, there is provided a method of treating a disease or condition in an individual, comprising administering to the individual an effective amount of an anti-PD-L1 antibody agent, wherein the anti-PD-L1 antibody agent comprises an antibody moiety comprising a heavy chain variable region (V_H) and a light chain variable region (V_L), wherein: a) the V_H comprises an HC-CDR1 comprising the amino acid sequence of SEQ ID NO: 41, an HC-CDR2 comprising the amino acid sequence of SEQ ID NO: 42, and an HC-CDR3 comprising the amino acid sequence of SEQ ID NO: 43; and b) the V_L comprises an LC-CDR1 comprising the amino acid sequence of SEQ ID NO: 44, an LC-CDR2 comprising the amino acid sequence of SEQ ID NO: 45, and an LC-CDR3 comprising the amino acid sequence of SEQ ID NO: 46. In some embodiments, there is provided a method of treating a disease or condition in an individual, comprising administering to the individual an effective amount of an anti-PD-L1 antibody agent, wherein the anti-PD-L1 antibody agent comprises an antibody moiety comprising a heavy chain variable region (V_H) and a light chain variable region (V_L), wherein: a) the V_H comprises an amino acid sequence having at least about 80% (such as at least about 80%, 85%, 90%, 92%, 95%, 96%, 97%, 98%, or 99%) sequence identity to the amino acid sequence of any one of SEQ ID NOs: 1, 5, 9, 11, and 13; and/or b) the V_L comprises an amino acid sequence having at least about 80% (such as at least about 80%, 85%, 90%, 92%, 95%, 96%, 97%, 98%, or 99%) sequence identity to the amino acid sequence of any one of SEQ ID NOs: 3, 7, 15, 17 and 19. In some embodiments, the disease or condition is a cancer. In some embodiments, the cancer is selected from the group consisting of melanoma, renal cell carcinoma, colorectal cancer, urothelial carcinoma, Hodgkin's lymphoma,

small cell lung cancer, non-small cell lung cancer, head and neck tumors, stomach cancer, B cell lymphoma, Merkel cell carcinoma, liver cancer, and cervical cancer. In some embodiments, the individual is a human. In some embodiments, the antibody moiety is chimeric or humanized. In some embodiments, the antibody moiety is selected from the group consisting of a single-chain Fv (scFv), a Fab, a Fab', a F(ab')2, an Fv fragment, a disulfide stabilized Fv fragment (dsFv), a (dsFv)2, a V_HH, a Fv-Fc fusion, an scFv-Fc fusion, an scFv-Fv fusion, a diabody, a tribody, and a tetrabody. In some embodiments, the antibody moiety is a single-chain antibody. In some embodiments, the antibody moiety comprises an Fc fragment. In some embodiments, the antibody moiety is a full-length antibody. In some embodiments, the antibody moiety has an isotype selected from the group consisting of an IgG, an IgM, an IgA, an IgD, and an IgE. In some embodiments, the Fc fragment is an Fc fragment of IgG. In some embodiments, the Fc fragment is an Fc fragment of IgG1 or IgG4. In some embodiments, the Fc fragment comprises H310A and H435Q mutations, wherein the amino acid positions are based on the Kabat numbering system. In some embodiments, the effective amount of the anti-PD-L1 antibody agent is about 0.005 μg/kg to about 5g/kg of total body weight of the individual. In some embodiments, the antibody agent is administered intravenously, intraperitoneally, intramuscularly, subcutaneously, or orally.

[346] In some embodiments, there is provided a method of treating a disease or condition in an individual, comprising administering to the individual an effective amount of an anti-PD-L1 antibody agent, wherein the anti-PD-L1 antibody agent comprises an antibody moiety comprising a heavy chain variable region (V_H) and a light chain variable region (V_L), wherein: (a) a V_H comprising the amino acid sequence of SEQ ID NO: 9, and a V_L comprising the amino acid sequence of SEQ ID NO: 15; (b) a V_H comprising the amino acid sequence of SEQ ID NO: 9, and a V_L comprising the amino acid sequence of SEQ ID NO: 17; (c) a V_H comprising the amino acid sequence of SEQ ID NO: 9, and a V_L comprising the amino acid sequence of SEQ ID NO: 19; (d) a V_H comprising the amino acid sequence of SEQ ID NO: 11, and a V_L comprising the amino acid sequence of SEQ ID NO: 15; (e) a V_H comprising the amino acid sequence of SEQ ID NO: 11, and a V_L comprising the amino acid sequence of SEQ ID NO: 17; (f) a V_H comprising the amino acid sequence of SEQ ID NO: 11, and a V_L comprising the amino acid sequence of SEQ ID NO: 19; (g) a V_H comprising the amino acid sequence of SEQ ID NO: 13, and a V_L comprising the amino acid sequence of SEQ ID NO: 15; (h) a V_H comprising the amino acid sequence of SEQ ID NO: 13, and a V_L comprising the amino acid sequence of SEQ ID NO: 17; or (i) a V_H comprising the amino acid sequence of SEQ ID NO: 13, and a V_L comprising the amino acid sequence of SEQ ID NO: 19.

[347] In some embodiments, there is provided a method of treating a disease or condition in an individual, comprising administering to the individual an effective amount of an anti-PD-L1 antibody agent, wherein the anti-PD-L1 antibody agent comprises an antibody moiety, wherein the antibody moiety comprises an amino

acid sequence having at least about 80% (such as at least about 80%, 85%, 90%, 92%, 95%, 96%, 97%, 98%, or 99%) sequence identity to the amino acid sequence of SEQ ID NO: 21 or 23.

[348] In some embodiments, there is provided a method of treating a disease or condition in an individual, comprising administering to the individual an effective amount of an anti-PD-L1 antibody agent, wherein the anti-PD-L1 antibody agent comprises an antibody moiety comprising: a) a HC-CDR1, a HC-CDR2, and a HC-CDR3, respectively comprising the amino acid sequences of a CDR1, a CDR2, and a CDR3 within a heavy chain variable region (V_H) having the sequence set forth in any of SEQ ID NOs: 1, 5, 9, 11, and 13; and b) a LC-CDR1, a LC-CDR2, and a LC-CDR3, respectively comprising the amino acid sequences of a CDR1, a CDR2, and a CDR3 within a light chain variable region (V_L) having the sequence set forth in any of SEQ ID NOs: 3, 7, 15, 17 and 19.

[349] In some embodiments, there is provided a method of treating melanoma in an individual, comprising administering to the individual an effective amount of an anti-PD-L1 antibody agent, wherein the anti-PD-L1 antibody agent comprises an antibody moiety comprising a heavy chain variable region (V_H) and a light chain variable region (V_L), wherein: a) the V_H comprises an HC-CDR1 comprising the amino acid sequence of SEQ ID NO: 41, an HC-CDR2 comprising the amino acid sequence of SEQ ID NO: 42, and an HC-CDR3 comprising the amino acid sequence of SEQ ID NO: 43; and b) the V_L comprises an LC-CDR1 comprising the amino acid sequence of SEQ ID NO: 44, an LC-CDR2 comprising the amino acid sequence of SEQ ID NO: 45, and an LC-CDR3 comprising the amino acid sequence of SEQ ID NO: 46. In some embodiments, there is provided a method of treating melanoma in an individual, comprising administering to the individual an effective amount of an anti-PD-L1 antibody agent, wherein the anti-PD-L1 antibody agent comprises an antibody moiety comprising: a) a HC-CDR1, a HC-CDR2, and a HC-CDR3, respectively comprising the amino acid sequences of a CDR1, a CDR2, and a CDR3 within a heavy chain variable region (V_H) having the sequence set forth in any of SEQ ID NOs: 1, 5, 9, 11, and 13; and b) a LC-CDR1, a LC-CDR2, and a LC-CDR3, respectively comprising the amino acid sequences of a CDR1, a CDR2, and a CDR3 within a light chain variable region (V_L) having the sequence set forth in any of SEQ ID NOs: 3, 7, 15, 17 and 19.

[350] In some embodiments, there is provided a method of treating renal cell carcinoma in an individual, comprising administering to the individual an effective amount of an anti-PD-L1 antibody agent, wherein the anti-PD-L1 antibody agent comprises an antibody moiety comprising a heavy chain variable region (V_H) and a light chain variable region (V_L), wherein: a) the V_H comprises an HC-CDR1 comprising the amino acid sequence of SEQ ID NO: 41, an HC-CDR2 comprising the amino acid sequence of SEQ ID NO: 42, and an HC-CDR3 comprising the amino acid sequence of SEQ ID NO: 43; and b) the V_L comprises an LC-CDR1 comprising the amino acid sequence of SEQ ID NO: 44, an LC-CDR2 comprising the amino acid sequence of SEQ ID NO: 45, and an LC-CDR3 comprising the amino acid sequence of SEQ ID NO: 46. In some

embodiments, there is provided a method of treating renal cell carcinoma in an individual, comprising administering to the individual an effective amount of an anti-PD-L1 antibody agent, wherein the anti-PD-L1 antibody agent comprises an antibody moiety comprising: a) a HC-CDR1, a HC-CDR2, and a HC-CDR3, respectively comprising the amino acid sequences of a CDR1, a CDR2, and a CDR3 within a heavy chain variable region (V_H) having the sequence set forth in any of SEQ ID NOs: 1, 5, 9, 11, and 13; and b) a LC-CDR1, a LC-CDR2, and a LC-CDR3, respectively comprising the amino acid sequences of a CDR1, a CDR2, and a CDR3 within a light chain variable region (V_L) having the sequence set forth in any of SEQ ID NOs: 3, 7, 15, 17 and 19.

[351] In some embodiments, there is provided a method of treating colorectal cancer in an individual, comprising administering to the individual an effective amount of an anti-PD-L1 antibody agent, wherein the anti-PD-L1 antibody agent comprises an antibody moiety comprising a heavy chain variable region (V_H) and a light chain variable region (V_L), wherein: a) the V_H comprises an HC-CDR1 comprising the amino acid sequence of SEQ ID NO: 41, an HC-CDR2 comprising the amino acid sequence of SEQ ID NO: 42, and an HC-CDR3 comprising the amino acid sequence of SEQ ID NO: 43; and b) the V_L comprises an LC-CDR1 comprising the amino acid sequence of SEQ ID NO: 44, an LC-CDR2 comprising the amino acid sequence of SEQ ID NO: 45, and an LC-CDR3 comprising the amino acid sequence of SEQ ID NO: 46. In some embodiments, there is provided a method of treating colorectal cancer in an individual, comprising administering to the individual an effective amount of an anti-PD-L1 antibody agent, wherein the anti-PD-L1 antibody agent comprises an antibody moiety comprising: a) a HC-CDR1, a HC-CDR2, and a HC-CDR3, respectively comprising the amino acid sequences of a CDR1, a CDR2, and a CDR3 within a heavy chain variable region (V_H) having the sequence set forth in any of SEQ ID NOs: 1, 5, 9, 11, and 13; and b) a LC-CDR1, a LC-CDR2, and a LC-CDR3, respectively comprising the amino acid sequences of a CDR1, a CDR2, and a CDR3 within a light chain variable region (V_L) having the sequence set forth in any of SEQ ID NOs: 3, 7, 15, 17 and 19.

[352] In some embodiments, there is provided a method of treating urothelial carcinoma in an individual, comprising administering to the individual an effective amount of an anti-PD-L1 antibody agent, wherein the anti-PD-L1 antibody agent comprises an antibody moiety comprising a heavy chain variable region (V_H) and a light chain variable region (V_L), wherein: a) the V_H comprises an HC-CDR1 comprising the amino acid sequence of SEQ ID NO: 41, an HC-CDR2 comprising the amino acid sequence of SEQ ID NO: 42, and an HC-CDR3 comprising the amino acid sequence of SEQ ID NO: 43; and b) the V_L comprises an LC-CDR1 comprising the amino acid sequence of SEQ ID NO: 44, an LC-CDR2 comprising the amino acid sequence of SEQ ID NO: 45, and an LC-CDR3 comprising the amino acid sequence of SEQ ID NO: 46. In some embodiments, there is provided a method of treating urothelial carcinoma in an individual, comprising

administering to the individual an effective amount of an anti-PD-L1 antibody agent, wherein the anti-PD-L1 antibody agent comprises an antibody moiety comprising: a) a HC-CDR1, a HC-CDR2, and a HC-CDR3, respectively comprising the amino acid sequences of a CDR1, a CDR2, and a CDR3 within a heavy chain variable region (V_H) having the sequence set forth in any of SEQ ID NOs: 1, 5, 9, 11, and 13; and b) a LC-CDR1, a LC-CDR2, and a LC-CDR3, respectively comprising the amino acid sequences of a CDR1, a CDR2, and a CDR3 within a light chain variable region (V_L) having the sequence set forth in any of SEQ ID NOs: 3, 7, 15, 17 and 19.

[353] In some embodiments, there is provided a method of treating Hodgkin's lymphoma in an individual, comprising administering to the individual an effective amount of an anti-PD-L1 antibody agent, wherein the anti-PD-L1 antibody agent comprises an antibody moiety comprising a heavy chain variable region (V_H) and a light chain variable region (V_L), wherein: a) the V_H comprises an HC-CDR1 comprising the amino acid sequence of SEQ ID NO: 41, an HC-CDR2 comprising the amino acid sequence of SEQ ID NO: 42, and an HC-CDR3 comprising the amino acid sequence of SEQ ID NO: 43; and b) the V_L comprises an LC-CDR1 comprising the amino acid sequence of SEQ ID NO: 44, an LC-CDR2 comprising the amino acid sequence of SEQ ID NO: 45, and an LC-CDR3 comprising the amino acid sequence of SEQ ID NO: 46. In some embodiments, there is provided a method of treating Hodgkin's lymphoma in an individual, comprising administering to the individual an effective amount of an anti-PD-L1 antibody agent, wherein the anti-PD-L1 antibody agent comprises an antibody moiety comprising: a) a HC-CDR1, a HC-CDR2, and a HC-CDR3, respectively comprising the amino acid sequences of a CDR1, a CDR2, and a CDR3 within a heavy chain variable region (V_H) having the sequence set forth in any of SEQ ID NOs: 1, 5, 9, 11, and 13; and b) a LC-CDR1, a LC-CDR2, and a LC-CDR3, respectively comprising the amino acid sequences of a CDR1, a CDR2, and a CDR3 within a light chain variable region (V_L) having the sequence set forth in any of SEQ ID NOs: 3, 7, 15, 17 and 19.

[354] In some embodiments, there is provided a method of treating small cell lung cancer in an individual, comprising administering to the individual an effective amount of an anti-PD-L1 antibody agent, wherein the anti-PD-L1 antibody agent comprises an antibody moiety comprising a heavy chain variable region (V_H) and a light chain variable region (V_L), wherein: a) the V_H comprises an HC-CDR1 comprising the amino acid sequence of SEQ ID NO: 41, an HC-CDR2 comprising the amino acid sequence of SEQ ID NO: 42, and an HC-CDR3 comprising the amino acid sequence of SEQ ID NO: 43; and b) the V_L comprises an LC-CDR1 comprising the amino acid sequence of SEQ ID NO: 44, an LC-CDR2 comprising the amino acid sequence of SEQ ID NO: 45, and an LC-CDR3 comprising the amino acid sequence of SEQ ID NO: 46. In some embodiments, there is provided a method of treating small cell lung cancer in an individual, comprising administering to the individual an effective amount of an anti-PD-L1 antibody agent, wherein the anti-PD-L1

antibody agent comprises an antibody moiety comprising: a) a HC-CDR1, a HC-CDR2, and a HC-CDR3, respectively comprising the amino acid sequences of a CDR1, a CDR2, and a CDR3 within a heavy chain variable region (V_H) having the sequence set forth in any of SEQ ID NOs: 1, 5, 9, 11, and 13; and b) a LC-CDR1, a LC-CDR2, and a LC-CDR3, respectively comprising the amino acid sequences of a CDR1, a CDR2, and a CDR3 within a light chain variable region (V_L) having the sequence set forth in any of SEQ ID NOs: 3, 7, 15, 17 and 19.

[355] In some embodiments, there is provided a method of treating non-small cell lung cancer in an individual, comprising administering to the individual an effective amount of an anti-PD-L1 antibody agent, wherein the anti-PD-L1 antibody agent comprises an antibody moiety comprising a heavy chain variable region (V_H) and a light chain variable region (V_L), wherein: a) the V_H comprises an HC-CDR1 comprising the amino acid sequence of SEQ ID NO: 41, an HC-CDR2 comprising the amino acid sequence of SEQ ID NO: 42, and an HC-CDR3 comprising the amino acid sequence of SEQ ID NO: 43; and b) the V_L comprises an LC-CDR1 comprising the amino acid sequence of SEQ ID NO: 44, an LC-CDR2 comprising the amino acid sequence of SEQ ID NO: 45, and an LC-CDR3 comprising the amino acid sequence of SEQ ID NO: 46. In some embodiments, there is provided a method of treating non-small cell lung cancer in an individual, comprising administering to the individual an effective amount of an anti-PD-L1 antibody agent, wherein the anti-PD-L1 antibody agent comprises an antibody moiety comprising: a) a HC-CDR1, a HC-CDR2, and a HC-CDR3, respectively comprising the amino acid sequences of a CDR1, a CDR2, and a CDR3 within a heavy chain variable region (V_H) having the sequence set forth in any of SEQ ID NOs: 1, 5, 9, 11, and 13; and b) a LC-CDR1, a LC-CDR2, and a LC-CDR3, respectively comprising the amino acid sequences of a CDR1, a CDR2, and a CDR3 within a light chain variable region (V_L) having the sequence set forth in any of SEQ ID NOs: 3, 7, 15, 17 and 19.

[356] In some embodiments, there is provided a method of treating head and neck tumors in an individual, comprising administering to the individual an effective amount of an anti-PD-L1 antibody agent, wherein the anti-PD-L1 antibody agent comprises an antibody moiety comprising a heavy chain variable region (V_H) and a light chain variable region (V_L), wherein: a) the V_H comprises an HC-CDR1 comprising the amino acid sequence of SEQ ID NO: 41, an HC-CDR2 comprising the amino acid sequence of SEQ ID NO: 42, and an HC-CDR3 comprising the amino acid sequence of SEQ ID NO: 43; and b) the V_L comprises an LC-CDR1 comprising the amino acid sequence of SEQ ID NO: 44, an LC-CDR2 comprising the amino acid sequence of SEQ ID NO: 45, and an LC-CDR3 comprising the amino acid sequence of SEQ ID NO: 46. In some embodiments, there is provided a method of treating head and neck tumors in an individual, comprising administering to the individual an effective amount of an anti-PD-L1 antibody agent, wherein the anti-PD-L1 antibody agent comprises an antibody moiety comprising: a) a HC-CDR1, a HC-CDR2, and a HC-CDR3,

respectively comprising the amino acid sequences of a CDR1, a CDR2, and a CDR3 within a heavy chain variable region (V_H) having the sequence set forth in any of SEQ ID NOs: 1, 5, 9, 11, and 13; and b) a LC-CDR1, a LC-CDR2, and a LC-CDR3, respectively comprising the amino acid sequences of a CDR1, a CDR2, and a CDR3 within a light chain variable region (V_L) having the sequence set forth in any of SEQ ID NOs: 3, 7, 15, 17 and 19.

[357] In some embodiments, there is provided a method of treating stomach cancer in an individual, comprising administering to the individual an effective amount of an anti-PD-L1 antibody agent, wherein the anti-PD-L1 antibody agent comprises an antibody moiety comprising a heavy chain variable region (V_H) and a light chain variable region (V_L), wherein: a) the V_H comprises an HC-CDR1 comprising the amino acid sequence of SEQ ID NO: 41, an HC-CDR2 comprising the amino acid sequence of SEQ ID NO: 42, and an HC-CDR3 comprising the amino acid sequence of SEQ ID NO: 43; and b) the V_L comprises an LC-CDR1 comprising the amino acid sequence of SEQ ID NO: 44, an LC-CDR2 comprising the amino acid sequence of SEQ ID NO: 45, and an LC-CDR3 comprising the amino acid sequence of SEQ ID NO: 46. In some embodiments, there is provided a method of treating stomach cancer in an individual, comprising administering to the individual an effective amount of an anti-PD-L1 antibody agent, wherein the anti-PD-L1 antibody agent comprises an antibody moiety comprising: a) a HC-CDR1, a HC-CDR2, and a HC-CDR3, respectively comprising the amino acid sequences of a CDR1, a CDR2, and a CDR3 within a heavy chain variable region (V_H) having the sequence set forth in any of SEQ ID NOs: 1, 5, 9, 11, and 13; and b) a LC-CDR1, a LC-CDR2, and a LC-CDR3, respectively comprising the amino acid sequences of a CDR1, a CDR2, and a CDR3 within a light chain variable region (V_L) having the sequence set forth in any of SEQ ID NOs: 3, 7, 15, 17 and 19.

[358] In some embodiments, there is provided a method of treating B cell lymphoma in an individual, comprising administering to the individual an effective amount of an anti-PD-L1 antibody agent, wherein the anti-PD-L1 antibody agent comprises an antibody moiety comprising a heavy chain variable region (V_H) and a light chain variable region (V_L), wherein: a) the V_H comprises an HC-CDR1 comprising the amino acid sequence of SEQ ID NO: 41, an HC-CDR2 comprising the amino acid sequence of SEQ ID NO: 42, and an HC-CDR3 comprising the amino acid sequence of SEQ ID NO: 43; and b) the V_L comprises an LC-CDR1 comprising the amino acid sequence of SEQ ID NO: 44, an LC-CDR2 comprising the amino acid sequence of SEQ ID NO: 45, and an LC-CDR3 comprising the amino acid sequence of SEQ ID NO: 46. In some embodiments, there is provided a method of treating B cell lymphoma in an individual, comprising administering to the individual an effective amount of an anti-PD-L1 antibody agent, wherein the anti-PD-L1 antibody agent comprises an antibody moiety comprising: a) a HC-CDR1, a HC-CDR2, and a HC-CDR3, respectively comprising the amino acid sequences of a CDR1, a CDR2, and a CDR3 within a heavy chain variable region (V_H) having the sequence set forth in any of SEQ ID NOs: 1, 5, 9, 11, and 13; and b) a LC-

CDR1, a LC-CDR2, and a LC-CDR3, respectively comprising the amino acid sequences of a CDR1, a CDR2, and a CDR3 within a light chain variable region (V_L) having the sequence set forth in any of SEQ ID NOs: 3, 7, 15, 17 and 19.

[359] In some embodiments, there is provided a method of treating liver cancer in an individual, comprising administering to the individual an effective amount of an anti-PD-L1 antibody agent, wherein the anti-PD-L1 antibody agent comprises an antibody moiety comprising a heavy chain variable region (V_H) and a light chain variable region (V_L), wherein: a) the V_H comprises an HC-CDR1 comprising the amino acid sequence of SEQ ID NO: 41, an HC-CDR2 comprising the amino acid sequence of SEQ ID NO: 42, and an HC-CDR3 comprising the amino acid sequence of SEQ ID NO: 43; and b) the V_L comprises an LC-CDR1 comprising the amino acid sequence of SEQ ID NO: 44, an LC-CDR2 comprising the amino acid sequence of SEQ ID NO: 45, and an LC-CDR3 comprising the amino acid sequence of SEQ ID NO: 46. In some embodiments, there is provided a method of treating liver cancer in an individual, comprising administering to the individual an effective amount of an anti-PD-L1 antibody agent, wherein the anti-PD-L1 antibody agent comprises an antibody moiety comprising: a) a HC-CDR1, a HC-CDR2, and a HC-CDR3, respectively comprising the amino acid sequences of a CDR1, a CDR2, and a CDR3 within a heavy chain variable region (V_H) having the sequence set forth in any of SEQ ID NOs: 1, 5, 9, 11, and 13; and b) a LC-CDR1, a LC-CDR2, and a LC-CDR3, respectively comprising the amino acid sequences of a CDR1, a CDR2, and a CDR3 within a light chain variable region (V_L) having the sequence set forth in any of SEQ ID NOs: 3, 7, 15, 17 and 19.

[360] In some embodiments, there is provided a method of treating Merkel cell carcinoma in an individual, comprising administering to the individual an effective amount of an anti-PD-L1 antibody agent, wherein the anti-PD-L1 antibody agent comprises an antibody moiety comprising a heavy chain variable region (V_H) and a light chain variable region (V_L), wherein: a) the V_H comprises an HC-CDR1 comprising the amino acid sequence of SEQ ID NO: 41, an HC-CDR2 comprising the amino acid sequence of SEQ ID NO: 42, and an HC-CDR3 comprising the amino acid sequence of SEQ ID NO: 43; and b) the V_L comprises an LC-CDR1 comprising the amino acid sequence of SEQ ID NO: 44, an LC-CDR2 comprising the amino acid sequence of SEQ ID NO: 45, and an LC-CDR3 comprising the amino acid sequence of SEQ ID NO: 46. In some embodiments, there is provided a method of treating Merkel cell carcinoma in an individual, comprising administering to the individual an effective amount of an anti-PD-L1 antibody agent, wherein the anti-PD-L1 antibody agent comprises an antibody moiety comprising: a) a HC-CDR1, a HC-CDR2, and a HC-CDR3, respectively comprising the amino acid sequences of a CDR1, a CDR2, and a CDR3 within a heavy chain variable region (V_H) having the sequence set forth in any of SEQ ID NOs: 1, 5, 9, 11, and 13; and b) a LC-CDR1, a LC-CDR2, and a LC-CDR3, respectively comprising the amino acid sequences of a CDR1, a CDR2,

and a CDR3 within a light chain variable region (V_L) having the sequence set forth in any of SEQ ID NOs: 3, 7, 15, 17 and 19.

[361] In some embodiments, there is provided a method of treating cervical cancer in an individual, comprising administering to the individual an effective amount of an anti-PD-L1 antibody agent, wherein the anti-PD-L1 antibody agent comprises an antibody moiety comprising a heavy chain variable region (V_H) and a light chain variable region (V_L), wherein: a) the V_H comprises an HC-CDR1 comprising the amino acid sequence of SEQ ID NO: 41, an HC-CDR2 comprising the amino acid sequence of SEQ ID NO: 42, and an HC-CDR3 comprising the amino acid sequence of SEQ ID NO: 43; and b) the V_L comprises an LC-CDR1 comprising the amino acid sequence of SEQ ID NO: 44, an LC-CDR2 comprising the amino acid sequence of SEQ ID NO: 45, and an LC-CDR3 comprising the amino acid sequence of SEQ ID NO: 46. In some embodiments, there is provided a method of treating cervical cancer in an individual, comprising administering to the individual an effective amount of an anti-PD-L1 antibody agent, wherein the anti-PD-L1 antibody agent comprises an antibody moiety comprising: a) a HC-CDR1, a HC-CDR2, and a HC-CDR3, respectively comprising the amino acid sequences of a CDR1, a CDR2, and a CDR3 within a heavy chain variable region (V_H) having the sequence set forth in any of SEQ ID NOs: 1, 5, 9, 11, and 13; and b) a LC-CDR1, a LC-CDR2, and a LC-CDR3, respectively comprising the amino acid sequences of a CDR1, a CDR2, and a CDR3 within a light chain variable region (V_L) having the sequence set forth in any of SEQ ID NOs: 3, 7, 15, 17 and 19.

[362] In some embodiments, the individual is a mammal (*e.g.*, human, non-human primate, rat, mouse, cow, horse, pig, sheep, goat, dog, cat, *etc.*). In some embodiments, the individual is a human. In some embodiments, the individual is a clinical patient, a clinical trial volunteer, an experimental animal, *etc.* In some embodiments, the individual is younger than about 60 years old (including for example younger than about any of 50, 40, 30, 25, 20, 15, or 10 years old). In some embodiments, the individual is older than about 60 years old (including for example older than about any of 70, 80, 90, or 100 years old). In some embodiments, the individual is diagnosed with or genetically prone to one or more of the diseases or disorders described herein (such as melanoma, renal cell carcinoma, colorectal cancer, urothelial carcinoma, Hodgkin's lymphoma, small cell lung cancer, non-small cell lung cancer, head and neck tumors, stomach cancer, B cell lymphoma, Merkel cell carcinoma, liver cancer, or cervical cancer). In some embodiments, the individual has one or more risk factors associated with one or more diseases or disorders described herein.

Dosing and Method of Administering the anti-PD-L1 antibody agent

[363] The dose of the anti-PD-L1 antibody agent (or the antibody moiety) used for treating a disease or disorder as described herein administered into the individual may vary with the particular antibody agent (or the antibody moiety), the mode of administration, and the type of disease or condition being treated. In some embodiments, the type of disease or condition is a cancer. In some embodiments, the effective amount of the

anti-PD-L1 antibody agent (or the antibody moiety) is an amount that is effective to result in an objective response (such as a partial response or a complete response). In some embodiments, the effective amount of the anti-PD-L1 antibody agent (or the antibody moiety) is an amount that is sufficient to result in a complete response in the individual. In some embodiments, the effective amount of the anti-PD-L1 antibody agent (or the antibody moiety) is an amount that is sufficient to result in a partial response in the individual. In some embodiments, the effective amount of the anti-PD-L1 antibody agent (or the antibody moiety) is an amount that is sufficient to produce an overall response rate of more than about any of 20%, 25%, 30%, 35%, 40%, 45%, 50%, 55%, 60%, 64%, 65%, 70%, 75%, 80%, 85%, or 90% among a population of individuals treated with the anti-PD-L1 antibody agent (or the antibody moiety). Responses of an individual to the treatment of the methods described herein can be determined, for example, based on RECIST levels.

[364] In some embodiments, the effective amount of the anti-PD-L1 antibody agent (or the antibody moiety) is an amount that is sufficient to prolong progress-free survival of the individual. In some embodiments, the effective amount of the anti-PD-L1 antibody agent (or the antibody moiety) is an amount that is sufficient to prolong overall survival of the individual. In some embodiments, the effective amount of the anti-PD-L1 antibody agent (or the antibody moiety) is an amount that is sufficient to produce clinical benefit of more than about any of 50%, 60%, 70%, or 77% among a population of individuals treated with the anti-PD-L1 antibody agent (or the antibody moiety).

[365] In some embodiments, the effective amount of the anti-PD-L1 antibody agent (or the antibody moiety) alone or in combination with a second, third, and/or fourth agent, is an amount sufficient to decrease the size of a tumor, decrease the number of cancer cells, or decrease the growth rate of a tumor by at least about any of 10%, 20%, 30%, 40%, 50%, 60%, 70%, 80%, 90%, 95% or 100% compared to the corresponding tumor size, number of cancer cells, or tumor growth rate in the same subject prior to treatment or compared to the corresponding activity in other subjects not receiving the treatment. Standard methods can be used to measure the magnitude of this effect, such as *in vitro* assays with purified enzyme, cell-based assays, animal models, or human testing.

[366] In some embodiments, the effective amount of the anti-PD-L1 antibody agent (or the antibody moiety) is an amount that is below the level that induces a toxicological effect (*i.e.*, an effect above a clinically acceptable level of toxicity) or is at a level where a potential side effect can be controlled or tolerated when the composition is administered to the individual.

[367] In some embodiments, the effective amount of the anti-PD-L1 antibody agent (or the antibody moiety) is an amount that is close to a maximum tolerated dose (MTD) of the composition following the same dosing regimen. In some embodiments, the effective amount of the anti-PD-L1 antibody agent (or the antibody moiety) is more than about any of 80%, 90%, 95%, or 98% of the MTD.

[368] In some embodiments, the effective amount of the anti-PD-L1 antibody agent (or the antibody moiety) is included in a range of about 0.001 μg to about 10 g, for example, about 0.001 μg to about 0.01 μg, about 0.01 μg to about 0.1 μg, about 0.1 μg to about 1 μg, about 1 μg to about 10 μg, about 10 μg to about 100 μg, about 100 μg to about 1 mg, about 1 mg to about 10 mg, about 10 mg to about 100 mg, about 100 mg to about 1 g, or about 1 g to about 10 g. In some embodiments, the effective amount of the anti-PD-L1 antibody agent (or the antibody moiety) is at least about 0.001 μg, 0.01 μg, 0.1 μg, 1 μg, 10 μg, 100 μg, 1 mg, 10 mg, 100 mg, 1 g, or 5 g. In some embodiments, the effective amount of the anti-PD-L1 antibody agent (or the antibody moiety) is no more than about 0.05 μg, 0.1 μg, 1 μg, 10 μg, 100 μg, 1 mg, 10 mg, 100 mg, 1 g, or 10 g.

[369] In some embodiments of any of the above aspects, the effective amount of an anti- PD-L1 antibody agent (or the antibody moiety) is in the range of about 0.005 μg/kg to about 5g/kg of total body weight, for example, about 0.005 μg/kg to about 0.05 μg/kg, about 0.05 μg/kg to about 0.5 μg/kg, about 0.5 μg/kg to about 5 μg/kg, about 5 μg/kg to about 50 μg/kg, about 50 μg/kg to about 500 μg/kg, about 500 μg/kg to about 5 mg/kg, about 5 mg/kg to about 50 mg/kg, about 50 mg/kg to about 500 mg/kg, or about 500 mg/kg to about 5g/kg. In some embodiments, the effective amount of an anti- PD-L1 antibody agent (or the antibody moiety) is at least about 0.005 μg/kg, 0.05 μg/kg, 0.5 μg/kg, 5 μg/kg, 50 μg/kg, 500 μg/kg, 5 mg/kg, 50 mg/kg, 500 mg/kg, or 2.5g/kg. In some embodiments, the effective amount of an anti- PD-L1 antibody agent (or the antibody moiety) is no more about 0.01 μg/kg, 0.05 μg/kg, 0.5 μg/kg, 5 μg/kg, 50 μg/kg, 500 μg/kg, 5 mg/kg, 50 mg/kg, 500 mg/kg, or 5 g/kg.

[370] The anti-PD-L1 antibody agent can be administered to an individual (such as human) via various routes, including, for example, intravenous, intra-arterial, intraperitoneal, intrapulmonary, oral, inhalation, intravesicular, intramuscular, intra-tracheal, subcutaneous, intraocular, intrathecal, transmucosal, and transdermal. In some embodiments, the anti-PD-L1 antibody agent is included in a pharmaceutical composition while administered into the individual. In some embodimetns, sustained continuous release formulation of the composition may be used. In some embodiments, the composition is administered intravenously. In some embodiments, the composition is administered intraperitoneally. In some embodiments, the composition is administered intravenously. In some embodiments, the composition is administered intraperitoneally. In some embodiments, the composition is administered intramuscularly. In some embodiments, the composition is administered subcutaneously. In some embodiments, the composition is administered intravenously. In some embodiments, the composition is administered orally.

Combination therapy

[371] This application also provides methods of administering an anti-PD-L1 antibody agent into an individual for treating a disease or condition (such as cancer), wherein the method further comprises

administering a second agent or therapy. In some embodiments, the second agent or therapy is a standard or commonly used agent or therapy for treating the disease or condition. In some embodiments, the second agent or therapy comprises a chemotherapeutic agent. In some embodiments, the second agent or therapy comprises a surgery. In some embodiments, the second agent or therapy comprises a radioation therapy. In some embodiments, the second agent or therapy comprises an immunotherapy. In some embodiments, the second agent or therapy comprises a hormonal therapy. In some embodiments, the second agent or therapy comprises an angiogenesis inhibitor. In some embodiments, the second agent or therapy comprises a tyrosine kinase inhibitor.

[372] In some embodiments, the anti-PD-L1 antibody agent is administered simultaneously with the second agent or therapy. In some embodiments, the anti-PD-L1 antibody agent is administered concurrently with the second agent or therapy. In some embodiments, the anti-PD-L1 antibody agent is administered sequentially with the second agent or therapy. In some embodiments, the anti-PD-L1 antibody agent is administered in the same unit dosage form as the second agent or therapy. In some embodiment, the anti-PD-L1 antibody agent is administered in a different unit dosage form from the second agent or therapy.

[373] In some embodiment, the second agent or therapy is selected from the group consisting of Brentuximab, BMS-986016, Urelumab, Mogamulizumab, Varlilumab, DS-8273a, Pomalidomide, elotuzumab, Epacadostat, BMS-986205, Indoximod, ABT-399, Motolimod, cetuximab, BMS-986012, Glembatumumab vedotin, BMS-986148, ALT-803, Cabiralizumab, ABBV-085, Bevacizumab, pemetrexed, erlotinib, crizotinib, BMS-986156, Lirilumab, Elotuzumab, Interferon-gamma, BMS-986179, BMS-986178, ABBV-368, Ramucirumab, Interleukin 2, Daratumumab, Oregovomab, NKTR-214, ABBV-927, JTX-2011, Andecaliximab, BMS-986207, Dinutuximab beta, Nimotuzumab, ABBV-428, X4P-001, Trastuzumab-DM1, Ipilimumab, Interferon alfa-2b, MK-4166, Indoximod, Rituximab, Necitumumab, Enoblituzumab, GSK3174998, Ublituximab, TGR-1202, MK-1248, PV-10, Mirvetuximab soravtansine, AFM13, Margetuximab, IMP321, APX005M, AMG820, sEphB4-HAS, MK-4280, Demcizumab, GSK3359609, Recombinant EphB4-HSA fusion protein, Resimmune, radiotherapy, AM0010, Interleukin-12, Interferon gamma-1b, MK-7684, IMM-101, entinostat, cobimetinib, vanucizumab, PEG- Interferon alfa-2b, obinutuzumab, Acetylsalicylic acid, MOXR0916, RO6874281, tazemetostat, ibrutinib, polatuzumab vedotin, lenalidomide, Bendamustine, CHOP, RG7888, Vanucizumab, RO7009789, Emactuzumab, Cergutuzumab amunaleukin, RO6958688, Daratumumab, daratumumab, CDX-1401, pertuzumab, Trastuzumab emtansine, doxorubicin, cyclophosphamide, docetaxel, RG6058, ALX148, Daratumumab, Efizonerimod, Mogamulizumab, Oleclumab, Monalizumab, MEDI0562, IMC-CS4, MEDI5083, Utomilumab, PF-04518600, PD 0360324, azacitidine, bendamustine, M9241, vemurafenib, PDR001, LY3321367, Durvalumab, Plozalizumab, vedolizumab, TAK-580, Rovalpituzumab tesirine, Brentuximab vedotin, Blinatumomab, MBG453, GWN323, Decitabine, Canakinumab, CJM112,

trametinib, EGF816, NIS793, REGN1979, REGN3767, Acalabrutinib, Lenvatinib, Vorinostat, Dinaciclib, dabrafenib, Axitinib, Ibrutinib, Abemaciclib, Eribulin, BL-8040, Dexamethasone, CMP-001, Afatinib, Amcasertib, ARRY-382, Azacitidine, romidepsin, B-701, BGB324, Binimetinib, Birinapant, Carfilzomib, GM-CSF, Defactinib, Encorafenib, Enobosarm, Exemestane, leuprolide, G100, GR-MD-02, Imatinib, IMO-2125, INCB054828, Itacitinib, INCB050465, letrozole, palbociclib, MK-1454, Napabucasin, Nintedanib, Niraparib, Olaparib, enzalutamide, prednisone, Pazopanib, PEGPH20, PLX3397, Preladenant, Ruxolitinib, Sargramostim, SCH 900353, SD-101, Vismodegib, X4P-001, XL888, Ziv-aflibercept, Ibrutinib, Dasatinib, Plinabulin, Veliparib, PT2385, EGF816, INC280, Ceritinib, Galunisertib, Temsirolimus, irinotecan, capecitabine, Amcasertib, IPI-549, Chidamide, CB-839, TAK-659, Sitravatinib, Glesatinib, sitravatinib, mocetinostat, Avadomide, RRx-001, Omaveloxolone, Valproate, CB-1158, Azacitidine, midostaurin, cytarabine, Ibrutinib, Mocetinostat, Osimertinib mesylate, Gefitinib, AZD6738, cediranib, MEDI9197, AZD5069, nab-paclitaxel, AZD4547, AZD1775, vistusertib, Galunisertib, LY2510924, AZD4635, Pexidartinib, Selumetinib, Trabectedin, Ensartinib, Alectinib, Rociletinib, CPI-444, Etoposide, carboplatin, trilaciclib, Rucaparib, Veliparib, lorlatinib, Sunitinib, Beigene-290, BGB-3111, Savolitinib, Apatinib, merestinib, Citarinostat, Emurafenib, Cabozantinib, pazopanib, WNT974, FGF401, PBF 509, LXH254, Regorafenib, Sorafenib, LCL101, everolimus, panobinostat, Capmatinib, BLZ945, Ad-CEA vaccine, Axalimogene filolisbac, Vigil, TPIV 200, PVX-410, Hiltonol, DC/AML Fusion Cell Vaccine, LTX-315, LV305, Intravesical BCG therapy, ADXS-PSA, p53MVA, pTVG-HP plasmid DNA vaccine, 6MHP, GVAX pancreatic, GVAX, DNX-2401, DPX-Survivac vaccine, Dendritic cell therapy, cryosurgery, Prevnar 13, mDC3/8, NY-ESO-1, gp100:280-288, CRS-207, ISA101, Viagenpumatucel-L, Dendritic Cell Vaccine, WT1 Vaccine, TG4010, CV-301, PD-L1/IDO peptide vaccine, DCVax-L, NEO-PV-01, CimaVax-EGF Vaccine, Attenuated measles virus, Prostvac, CMB305, Sipuleucel-T, ONCOS-102, Coxsackievirus A21, Coxsackievirus A22, Pelareorep, Ad-MAGEA3/MG1-MAGEA3, Talimogene laherparepvec, HSV-tk-expressing adenovirus, Pexa-Vec, Enadenotucirev, MCPyV TAg-specific autologous CD8+ T cells, IMCgp100, TIL therapy, iC9-GD2 T Cells, E7 TCR T cells, pIL-12, ISF35, NY-ESO-1 TCR PBMC, HPV Specific T Cells, NK immunotherapies, Axicabtagene ciloleucel, AZD9150, Poly ICLC, and Imprime PGG.

Disease or condition

[374] The anti-PD-L1 antibody agent described herein can be used for treating any disease or condition. In some embodiments, the disease or condition comprises an infection. In some embodiments, the disease or condition is an infection (such as a bacteria infection or virus infection). In some embodiments, the disease or condition is an autoimmune disorder. In some embodiments, the disease or condition is a cancer.

[375] In some embodiments, the anti-PD-L1 antibody agent is used in a method for treating a cancer. Cancers that may be treated using any of the methods described herein include tumors that are not vascularized,

or not yet substantially vascularized, as well as vascularized tumors. Types of cancers to be treated with the anti-PD-L1 antibody agent as described in this application include, but are not limited to, carcinoma, blastoma, sarcoma, benign and malignant tumors, and malignancies *e.g.*, sarcomas, carcinomas, and melanomas. Adult tumors/cancers and pediatric tumors/cancers are also included.

[376] In various embodiments, the cancer is early stage cancer, non-metastatic cancer, primary cancer, advanced cancer, locally advanced cancer, metastatic cancer, cancer in remission, recurrent cancer, cancer in an adjuvant setting, cancer in a neoadjuvant setting, or cancer substantially refractory to a therapy.

[377] Examples of cancers that may be treated by the methods of this applicaiton include, but are not limited to, anal cancer, astrocytoma (*e.g.*, cerebellar and cerebral), basal cell carcinoma, bladder cancer, bone cancer, (osteosarcoma and malignant fibrous histiocytoma), brain tumor (*e.g.*, glioma, brain stem glioma, cerebellar or cerebral astrocytoma (*e.g.*, astrocytoma, malignant glioma, medulloblastoma, and glioblastoma), breast cancer, central nervous system lymphoma, cervical cancer, colon cancer, colorectal cancer, endometrial cancer (*e.g.*, uterine cancer), esophageal cancer, eye cancer (*e.g.*, intraocular melanoma and retinoblastoma), gastric (stomach) cancer, gastrointestinal stromal tumor (GIST), head and neck cancer, hepatocellular (liver) cancer (*e.g.*, hepatic carcinoma and heptoma), leukemia, liver cancer, lung cancer (*e.g.*, small cell lung cancer, non-small cell lung cancer, adenocarcinoma of the lung, and squamous carcinoma of the lung), lymphoid neoplasm (*e.g.*, lymphoma), medulloblastoma, melanoma, mesothelioma, myelodysplastic syndromes, nasopharyngeal cancer, neuroblastoma, ovarian cancer, pancreatic cancer, parathyroid cancer, cancer of the peritoneal, pituitary tumor, lymphoma, rectal cancer, renal cancer, renal pelvis and ureter cancer (transitional cell cancer), rhabdomyosarcoma, skin cancer (*e.g.*, non-melanoma (*e.g.*, squamous cell carcinoma), melanoma, and Merkel cell carcinoma), small intestine cancer, squamous cell cancer, testicular cancer, thyroid cancer, tuberous sclerosis, and post-transplant lymphoproliferative disorder (PTLD),.

[378] In some embodiments, the cancer is selected from the group consisting of melanoma, renal cell carcinoma, colorectal cancer, urothelial carcinoma, Hodgkin's lymphoma, small cell lung cancer, non-small cell lung cancer, head and neck tumors, stomach cancer, B cell lymphoma, Merkel cell carcinoma, liver cancer, and cervical cancer.

VIII. Compositions, Kits and Articles of manufacture

[379] Also provided herein are compositions (such as formulations) comprising any one of the imaging agents or the isolated anti-PD-L1 antibody agents described herein, nucleic acid encoding the antibody moieties (*e.g.*, anti-PD-L1 antibody moieties), vector comprising the nucleic acid encoding the antibody moieties, or host cells comprising the nucleic acid or vector.

[380] Suitable formulations of the imaging agents or the isolated anti-PD-L1 antibody agents described herein can be obtained by mixing the imaging agents or the isolated anti-PD-L1 antibody agents having the

desired degree of purity with optional pharmaceutically acceptable carriers, excipients or stabilizers *(Remington's Pharmaceutical Sciences* 16th edition, Osol, A. Ed. (1980)), in the form of lyophilized formulations or aqueous solutions. Acceptable carriers, excipients, or stabilizers are nontoxic to recipients at the dosages and concentrations employed, and include buffers such as phosphate, citrate, and other organic acids; antioxidants including ascorbic acid and methionine; preservatives (such as octadecyldimethylbenzyl ammonium chloride; hexamethonium chloride; benzalkonium chloride, benzethonium chloride; phenol, butyl or benzyl alcohol; alkyl parabens such as methyl or propylparaben; catechol; resorcinol; cyclohexanol; 3-pentanol; and m-cresol); low molecular weight (less than about 10 residues) polypeptides; proteins, such as serum albumin, gelatin, or immunoglobulins; hydrophilic polymers such as olyvinylpyrrolidone; amino acids such as glycine, glutamine, asparagine, histidine, arginine, or lysine; monosaccharides, disaccharides, and other carbohydrates including glucose, mannose, or dextrins; chelating agents such as EDTA; sugars such as sucrose, mannitol, trehalose or sorbitol; salt-forming counter-ions such as sodium; metal complexes *(e.g.* Zn-protein complexes); and/or non-ionic surfactants such as TWEEN™, PLURONICS™ or polyethylene glycol (PEG). Lyophilized formulations adapted for subcutaneous administration are described in WO97/04801. Such lyophilized formulations may be reconstituted with a suitable diluent to a high protein concentration and the reconstituted formulation may be administered subcutaneously to the individual to be imaged, diagnosed, or treated herein.

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

[382] Also provided are kits comprising any one of the imaging agents, the isolated anti-PD-L1 antibody agents, and/or optionally the chelating compound and/or the radionuclide described herein. The kits may be useful for any of the methods of imaging, diagnosis and treatment described herein.

[383] In some embodiments, there is provided a kit comprising an antibody moiety specifically binding an immune checkpoint ligand (*e.g.*, PD-L1 or a PD-L1 like ligand), and a chelating compound (*e.g.*, NOTA, DOTA or derivatives thereof). In some embodiments, the kit further comprises a radionuclide (*e.g.*, ^{68}Ga). In some embodiments, the chelating compound chelates the radionuclide.

[384] In some embodiments, there is provided a kit comprising an imaging agent comprising an antibody moiety labeled with a radionuclide (*e.g.*, ^{68}Ga), wherein the antibody moiety specifically binds an immune checkpoint ligand (*e.g.*, PD-L1 or a PD-L1 like ligand). In some embodiments, the antibody moiety is conjugated to a chelating moiety (*e.g.*, NOTA, DOTA or derivatives thereof) that chelates the radionuclide. In some embodiments, the kit further comprises an antibody moiety not labeled with a radionuclide.

[385] In some embodiments, the kit further comprises a device capable of delivering the imaging agent or the isolated anti-PD-L1 antibody agent. One type of device, for applications such as parenteral delivery, is a

syringe that is used to inject the composition into the body of a subject. Inhalation devices may also be used for certain applications.

[386] In some embodiments, the kit further comprises a therapeutic agent for treating a disease or condition, *e.g.*, cancer, infectious disease, autoimmune disease, or metabolic disease. In some embodiments, the therapeutic agent is an inhibitor of the immune checkpoint ligand or receptor thereof. In some embodiments, the therapeutic agent is a radiolabeled molecule specifically binding the immune checkpoint ligand or receptor thereof.

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

[388] The present application thus also provides articles of manufacture. The article of manufacture can comprise a container and a label or package insert on or associated with the container. Suitable containers include vials (such as sealed vials), bottles, jars, flexible packaging, and the like. Generally, the container holds a composition, and may have a sterile access port (for example the container may be an intravenous solution bag or a vial having a stopper pierceable by a hypodermic injection needle). The label or package insert indicates that the composition is used for imaging, diagnosing, or treating a particular condition in an individual. The label or package insert will further comprise instructions for administering the composition to the individual and for imaging the individual. The label may indicate directions for reconstitution and/or use. The container holding the composition may be a multi-use vial, which allows for repeat administrations (*e.g.* from 2-6 administrations) of the reconstituted formulation. Package insert refers to instructions customarily included in commercial packages of diagnostic products that contain information about the indications, usage, dosage, administration, contraindications and/or warnings concerning the use of such diagnostic products. Additionally, the article of manufacture may further comprise a second container comprising a pharmaceutically-acceptable buffer, such as bacteriostatic water for injection (BWFI), phosphate-buffered saline, Ringer's solution and dextrose solution. It may further include other materials desirable from a commercial and user standpoint, including other buffers, diluents, filters, needles, and syringes.

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

[390] Those skilled in the art will recognize that several embodiments are possible within the scope and spirit of this invention. The invention will now be described in greater detail by reference to the following non-limiting examples. The following examples further illustrate the invention but, of course, should not be construed as in any way limiting its scope.

EXEMPLARY EMBODIMENTS

[391] Embodiment 1. Use of an effective amount of an anti-PD-L1 antibody agent in the preparation of a medicament for treating a disease or condition in an individual, wherein the antibody agent comprises an antibody moiety comprising a heavy chain variable region (V_H) and a light chain variable region (V_L), wherein: a) the V_H comprises a heavy chain complementarity determination region 1 (HC-CDR1) comprising the amino acid sequence of SEQ ID NO: 41, an HC-CDR2 comprising the amino acid sequence of SEQ ID NO: 42, and an HC-CDR3 comprising the amino acid sequence of SEQ ID NO: 43, or a variant thereof comprising up to a total of about 5 amino acid substitutions in the HC-CDRs; and b) the V_L comprises a light chain complementarity determination region 1 (LC-CDR1) comprising the amino acid sequence of SEQ ID NO: 44, an LC-CDR2 comprising the amino acid sequence of SEQ ID NO: 45, and an LC-CDR3 comprising the amino acid sequence of SEQ ID NO: 46, or a variant thereof comprising up to a total of about 5 amino acid substitutions in the LC-CDRs.

[392] Embodiment 2. The use of embodiment 1, wherein: a) the V_H comprises an HC-CDR1 comprising the amino acid sequence of SEQ ID NO: 41, an HC-CDR2 comprising the amino acid sequence of SEQ ID NO: 42, and an HC-CDR3 comprising the amino acid sequence of SEQ ID NO: 43; and b) the V_L comprises an LC-CDR1 comprising the amino acid sequence of SEQ ID NO: 44, an LC-CDR2 comprising the amino acid sequence of SEQ ID NO: 45, and an LC-CDR3 comprising the amino acid sequence of SEQ ID NO: 46.

[393] Embodiment 3. The use of embodiment 1 or embodiment 2, wherein: a) the V_H comprises an amino acid sequence having at least about 80% sequence identity to the amino acid sequence of any one of SEQ ID NOs: 1, 5, 9, 11, and 13; and/or b) the V_L comprises an amino acid sequence having at least about 80% sequence identity to the amino acid sequence of any one of SEQ ID NOs: 3, 7, 15, 17 and 19.

[394] Embodiment 4. The use of embodiment 3, wherein the antibody moiety comprises: (a) a V_H comprising the amino acid sequence of SEQ ID NO: 9, and a V_L comprising the amino acid sequence of SEQ ID NO: 15; (b) a V_H comprising the amino acid sequence of SEQ ID NO: 9, and a V_L comprising the amino acid sequence of SEQ ID NO: 17; (c) a V_H comprising the amino acid sequence of SEQ ID NO: 9, and a V_L comprising the amino acid sequence of SEQ ID NO: 19; (d) a V_H comprising the amino acid sequence of SEQ ID NO: 11, and a V_L comprising the amino acid sequence of SEQ ID NO: 15; (e) a V_H comprising the amino acid sequence of SEQ ID NO: 11, and a V_L comprising the amino acid sequence of SEQ ID NO: 17; (f) a V_H comprising the amino acid sequence of SEQ ID NO: 11, and a V_L comprising the amino acid sequence of SEQ ID NO: 19; (g) a V_H comprising the amino acid sequence of SEQ ID NO: 13, and a V_L comprising the amino acid sequence of SEQ ID NO: 15; (h) a V_H comprising the amino acid sequence of SEQ ID NO: 13, and a V_L

comprising the amino acid sequence of SEQ ID NO: 17; or (i) a V_H comprising the amino acid sequence of SEQ ID NO: 13, and a V_L comprising the amino acid sequence of SEQ ID NO: 19.

[395] Embodiment 5. The use of embodiment 1, wherein the antibody moiety comprises an amino acid sequence having at least about 80% sequence identity to the amino acid sequence of SEQ ID NO: 21 or 23.

[396] Embodiment 6. Use of an effective amount of an anti-PD-L1 antibody agent in the preparation of a medicament for treating a disease or condition in an individual, wherein the antibody agent comprises an antibody moiety comprising: a) a HC-CDR1, a HC-CDR2, and a HC-CDR3, respectively comprising the amino acid sequences of a CDR1, a CDR2, and a CDR3 within a heavy chain variable region (V_H) having the sequence set forth in any of SEQ ID NOs: 1, 5, 9, 11, and 13; and b) a LC-CDR1, a LC-CDR2, and a LC-CDR3, respectively comprising the amino acid sequences of a CDR1, a CDR2, and a CDR3 within a light chain variable region (V_L) having the sequence set forth in any of SEQ ID NOs: 3, 7, 15, 17 and 19.

[397] Embodiment 7. The use of any one of embodiments 1-6, wherein the antibody moiety is chimeric or humanized.

[398] Embodiment 8. The use of any one of embodiments 1-4, 6 and 7, wherein the antibody moiety is selected from the group consisting of a single-chain Fv (scFv), a Fab, a Fab', a F(ab')2, an Fv fragment, a disulfide stabilized Fv fragment (dsFv), a $(dsFv)_2$, a V_HH, a Fv-Fc fusion, an scFv-Fc fusion, an scFv-Fv fusion, a diabody, a tribody, and a tetrabody.

[399] Embodiment 9. The use of any one of embodiments 1-4 and 6-8, wherein the antibody moiety is a single-chain antibody.

[400] Embodiment 10. The use of embodiment 9, wherein the antibody moiety is an scFv.

[401] Embodiment 11. The use of any one of embodiments 1-4 and 6-8, wherein the antibody moiety comprises an Fc fragment.

[402] Embodiment 12. The use of embodiment 11, wherein the antibody moiety is a full-length antibody.

[403] Embodiment 13. The use of embodiment 12, wherein the antibody moiety has an isotype selected from the group consisting of an IgG, an IgM, an IgA, an IgD, and an IgE.

[404] Embodiment 14. The use of any one of embodiments 11-13, wherein the Fc fragment is an Fc fragment of IgG.

[405] Embodiment 15. The use of embodiments 14, wherein the Fc fragment is an Fc fragment of IgG1 or IgG4.

[406] Embodiment 16. The use of any one of embodiments 11-15, wherein the Fc fragment comprises H310A and H435Q mutations, wherein the amino acid positions are based on the Kabat numbering system.

[407] Embodiment 17. The use of any one of embodiments 1-16, wherein the individual is a human.

[408] Embodiment 18. The use of any one of embodiments 1-17, wherein the disease or condition is a cancer.

[409] Embodiment 19. The use of embodiment 18, wherein the cancer is selected from the group consisting of melanoma, renal cell carcinoma, colorectal cancer, urothelial carcinoma, Hodgkin's lymphoma, small cell lung cancer, non-small cell lung cancer, head and neck tumors, stomach cancer, B cell lymphoma, Merkel cell carcinoma, liver cancer, and cervical cancer.

[410] Embodiment 20. The use of any one of embodiments 1-19, wherein the antibody agent is suitable for intravenous, intraperitoneal, intramuscular, subcutaneous, or oral administration.

[411] Embodiment 21. The use of any one of embodiments 1-20, wherein the medicament is used in combination with an effective amount of a second agent.

[412] Embodiment 22. The use of embodiment 21, wherein the second agent is a chemotherapeutic agent.

[413] Embodiment 23. A method of treating a disease or condition in an individual, comprising administering to the individual an effective amount of an anti-PD-L1 antibody agent, wherein the anti-PD-L1 antibody agent comprises an antibody moiety comprising a heavy chain variable region (V_H) and a light chain variable region (V_L), wherein: a) the V_H comprises a heavy chain complementarity determining region (HC-CDR) 1 comprising the amino acid sequence of SEQ ID NO: 41, an HC-CDR2 comprising the amino acid sequence of SEQ ID NO: 42, and an HC-CDR3 comprising the amino acid sequence of SEQ ID NO: 43, or a variant thereof comprising up to a total of about 5 amino acid substitutions in the HC-CDRs; and b) the V_L comprises a light chain complementarity determining region (LC-CDR) 1 comprising the amino acid sequence of SEQ ID NO: 44, an LC-CDR2 comprising the amino acid sequence of SEQ ID NO: 45, and an LC-CDR3 comprising the amino acid sequence of SEQ ID NO: 46, or a variant thereof comprising up to a total of about 5 amino acid substitutions in the LC-CDRs.

[414] Embodiment 24. The method of embodiment 23, wherein the effective amount of the anti-PD-L1 antibody agent is about 0.005 μg/kg to about 5g/kg of total body weight of the individual.

[415] Embodiment 25. A pharmaceutical composition comprising an anti-PD-L1 antibody agent and a pharmaceutical acceptable carrier, wherein the antibody agent comprises an antibody moiety comprising a heavy chain variable region (V_H) and a light chain variable region (V_L), wherein: a) the V_H comprises an HC-CDR1 comprising the amino acid sequence of SEQ ID NO: 41, an HC-CDR2 comprising the amino acid

sequence of SEQ ID NO: 42, and an HC-CDR3 comprising the amino acid sequence of SEQ ID NO: 43, or a variant thereof comprising up to a total of about 5 amino acid substitutions in the HC-CDRs; and b) the V_L comprises an LC-CDR1 comprising the amino acid sequence of SEQ ID NO: 44, an LC-CDR2 comprising the amino acid sequence of SEQ ID NO: 45, and an LC-CDR3 comprising the amino acid sequence of SEQ ID NO: 46, or a variant thereof comprising up to a total of about 5 amino acid substitutions in the LC-CDRs.

[416] Embodiment 26. The pharmaceutical composition of embodiment 25, wherein the pharmaceutical composition is lyophilized.

[417] Embodiment 27. The pharmaceutical composition of embodiment 25, wherein the pharmaceutical composition is a solution.

[418] Embodiment 28. The pharmaceutical composition of any one of embodiments 25-27, comprising about 0.001 μg to about 10 g of the antibody moiety.

[419] Embodiment 29. A kit for treating a disease or condition in an individual, comprising the pharmaceutical composition of any one of embodiments 25-28 and an instruction.

EXAMPLES

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

Example 1: Preparation and characterization of monoclonal antibodies against human PD-L1

Immunization

[421] 8-10 weeks old female PD-L1 deficient mice (H Dong et al. Immunity. 2004 Mar;20(3):327-36) were immunized subcutaneously (s.c.) at multiple sites with 200 μl of emulsion comprising 100 μg of hPD-L1mIg fusion protein and complete Freund's adjuvant (CFA) (Sigma-Aldrich). Each animal received two or three boosts with emulsion comprising the same concentration of hPD-L1mIg fusion protein formulated in incomplete Freund's adjuvant (IFA) (Sigma-Aldrich). Blood samples were collected from the animals two weeks after each immunization for serum titer testing. Upon achieving a sufficient titer, the animals received a booster injection with 60 μg of the PD-L1mIg fusion protein in PBS through intraperitoneal injection (i.p.). The animals were sacrificed and their spleens were harvested aseptically 5 days after the booster injection.

[422] Whole spleen was dissociated into single-cell suspensions. Red blood cells were lysed using the ACK buffer. The spleen cells were then mixed with SP2/0-Ag14 myeloma cells (from ATCC) at a 1:1 ratio in 50 ml conical centrifuge tubes. After centrifugation, the supernatant was discarded and cell fusion was induced with

50% polyethylene glycol (Roche). The fused cells were cultured for 8-10 days in the HAT selection medium. The contents in the supernatant were analyzed for their ability to bind to hPD-1-expressing cells using ELISA, and the positive clones were further confirmed using flow cytometry analysis. Subcloning of the positive hybridoma was performed using the limiting dilution technique for 5 times to achieve a pure monoclonal culture.

Characterization of the anti-hPD-L1 monoclonal antibody

Binding specificity of the anti-hPD-L1 monoclonal antibody

[423] The binding specificity of the anti-hPD-L1 mAb was determined using hPD-L1 transfected CHO cells (CHO/hPD-L1 cells) by flow cytometry (FACSVerse, BD Biosciences). Specifically, CHO/hPD-L1 cells were incubated with increasing amounts of the anti-hPD-L1 mAb 5B7 (0.06ng, 0.125ng, 0.25ng, 0.5ng, 1ng, 2ng, 4ng, and 100ng) on ice for 30 minutes. The cells were then washed and further incubated with anti-mIgG-APC (eBioscience) prior to flow cytometry analysis. As shown in FIGs. 1A and 1B, the anti-hPD-L1 mAb 5B7 bound to hPD-L1 with high specificity in a dose-dependent manner.

[424] The isotype of the monoclonal antibody was determined to be IgG1 κ using the Mouse Immunoglobulin Isotyping Kit (BD Biosciences).

Species cross-reactivity

[425] CHO cells transfected with mouse PD-L1 (CHO/mPD-L1) were used to assess the species cross-reactivity of the anti-hPD-L1 mAb with mouse PD-L1. The cells were incubated with the anti-hPD-L1 mAb prior to flow cytometry analysis. FIG. 2A shows the binding specificity of the anti-hPD-L1 mAb to human PD-L1. As shown in FIG. 2B, the anti-hPD-L1 mAb also binds to mouse PD-L1.

Blockade of ligand binding

[426] To assess the blockade effect of the anti-hPD-L1 mAb on binding between PD-L1 and PD-1, the following blockade experiment was conducted: 100 ng of hPD-L1hIg fusion protein were pre-incubated with indicated doses of the mAb (400, 300, 200, 100, 50 ng/10ul) or control Ig for 30 min at 4℃ before being used to bind CHO/hPD-1 cells. The cells were then washed and further stained with goat anti-hIgG-APC. The blockade effect of the mAb was assessed with flow cytometry. Using a similar method, the ability of the PD-L1 mAb to block the binding between PD-L1 and B7-1 (CD80) was also assessed.

[427] FIG. 3A shows that anti-hPD-L1mAb can block the binding of hPD-L1 to hPD-1 in a dose-dependent manner. FIG. 3B shows that the mAb can also block the binding of hPD-L1 to hB7-1 in a similar manner. As can be seen, the mAb can block the binding of PD-L1 to its binding partners.

Sequencing of anti-hPD-L1 antibody-producing hybridoma cells

[428] To sequence antibody-producing hybridoma cells, 1×10^7 hybridoma cells were harvested and washed with PBS. Messenger RNAs were extracted from hybridomas using RNeasy Mini Kit (Qiagen). RACE-Ready first-Strand cDNAs were synthesized using SMARTer RACE cDNA Amplification Kit (Clontech). Following reverse transcription, 5' RACE PCR reactions were performed with ready cDNA as template and with 5' universal primer (UPM) provided by the kit and 3'gene specific primers (GSP1) designed using the mouse IgG1 heavy chain variable region and light chain variable region sequences. RACE products were determined by gel electrophoresis analysis. PCR products were then cloned into a T vector using Zero Blunt TOPO PCR Cloning Kit (Invitrogen). After transformation, the plasmids were verified by sequencing analysis. Sequences of the heavy chain variable region and light chain variable region were analyzed using VBASE2 (worldwide wweb.vbase2.org), and listed in Table 3.

Example 2: Humanization and characterization of anti-hPD-L1 antibodies

Humanization of anti-hPD-L1 antibodies

[429] Humanization was performed based on the heavy chain variable region (VH) and light chain variable region (VL) sequences from anti-hPD-L1 hybridoma cells. As an initial step, a mouse-human chimeric mAb comprising the parental mouse VH and VL sequences, the human IgG constant region and the human κ chain was generated. Upon characterization of the chimeric antibody, three VH and three VL humanized sequences were designed and used to generate nine humanized antibodies. The VH and VL sequences of the chimeric and humanized anti-hPD-L1 antibodies are listed in Table 2.

Characterization of humanized anti-hPD-L1 antibodies

Blocking activities of humanized anti-hPD-L1 antibodies

[430] To examine the blocking activities of the humanized antibodies (combinations of three VHs and three VLs, nine in total) as compared to the parental chimeric antibody, blockade of ligand binding experiments similar to those of Example 1 were conduced, but hPD-L1mIg fusion protein was used instead for humanized and chimeric antibodies. FIG. 4 shows that the binding of PD-L1 to CHO/hPD-1 cells was inhibited in a dose-dependent manner by all humanized antibodies. As can be seen, all combinations have blocking activity close to that of parental chimeric antibody. At lower dosages, some humanized antibodies have even higher blocking activity than the parental chimeric antibody.

Example 3: Preparation and characterization of anti-hPD-L1 scFv-hFc antibodies

Sequence design and synthesis of scFv-hFc antibodies

[431] The heavy chain variable region (VH) and light chain variable region (VL) of the humanized anti-hPD-L1 antibody variant 2 was used to generate the scFv-hFc antibody. Specifically, the heavy chain and light chain were connected by a linker, which was followed by a hinge sequence (GACAAGACCCACACCTGCCCTCCCTGCCCC, SEQ ID NO: 50) and a human immunoglobulin IgG1 Fc portion (CH2-CH3 region). Additionally, H310A (*i.e.*, CAC to GCC) and H435Q (*i.e.*, CAC to CAG) mutations were introduced into the CH2 and CH3 regions for rapid clearance of the antibody *in vivo* (FIG. 5). The sequences of the scFv-hFc antibodies with the wild type CH2-CH3 regions (scFv-hFc Wt) and with the mutant CH2-CH3 regions (scFv-hFc Mt) are shown in Table 6.

[432] The DNA sequences of scFv-hFc Wt and scFv-hFc Mt were cloned into pcDNA3.3 vectors respectively and used to transiently transfected ExPi 293 cells. The proteins from the cell culture supernatant were purified with protein G sepharose column (GE healthcare) for functional analysis.

Characterization of scFv-hFc antibodies

[433] The anti-hPD-L1 scFv-hFc Wt and scFv-hFc Mt antibodies were identified by SDS Page Gel Electroporation. As shown in FIG. 6, the scFv-hFc antibodies were identified in both the reduced and non-reduced conditions on the SDS-PAGE gel. The binding affinities of the scFv-hFc antibodies to hPD-L1, as compared to that of the parental antibody were examined by FACS and the results are shown in FIG. 7. As shown in the histograms, both scFv-hFc Wt and scFv-hFc Mt demonstrated similar binding affinities as the parental antibody. The binding affinities and kinetics of the scFv-hFc antibodies (scFv-hFc Wt and scFv-hFc Mt) to hPD-L1, as compared to that of the parental antibody (anti-PD-L1 IgG1), were further analyzed using the Fortebio Octet system. Table 7 shows the binding affinities and kinetics parameters of anti-PD-L1 IgG1, anti-PD-L1 IgG1-C52W (*i.e.*, an anti-PD-L1 antibody having an IgG1 Fc region with a C52W mutation), scFv-hFc Mt and scFv-hFc Wt.

Table 7: Binding kinetics parameters of anti-hPD-L1 scFv-hFc and parental control antibodies

Sample	K_D (M)	K_{on} (1/Ms)	K_{off} (1/s)	R^2
anti-PD-L1 IgG1	1.54E-11	1.77E+5	2.72E-5	0.9834
anti-PD-L1 IgG1 C52W	3.40E-10	1.33E+5	4.53E-4	0.9732
anti-PD-L1 scFv-hFc Wt	3.48E-10	6.98E+6	2.43E-4	0.97
anti-PD-L1 scFv-hFc Mt	2.32E-10	1.66E+6	3.85E-4	0.9812

[434] Pharmacokinetics studies were performed by injection of scFv-hFc Wt and scFv-hFc Mt antibodies *in vivo*, followed by measuring of the serum titers of the scFv-hFc antibodies and hIgG on Day 1, 2, 3, 4 and 6

after injection. As shown in FIGs. 8A and 8B, scFv-hFc Wt showed higher serum titer of the antibody and of hIgG as compared to scFv-hFc Mt.

Example 4: Generation and characterization of anti-PD-L1 scFv

Generation and small-scale expression of humanized anti-PD-L1 scFv

[435] Schematic diagrams of the construct designs for anti-hPD-L1 scFvs, the parental humanized IgG1 positive control antibody and a negative control scFv are shown in FIG. 9. Specifically, fragments containing VH and VL antigen binding domains from the humanized anti-hPD-L1 antibody and a peptide linker in between were artificially synthesized, which included both orientations (i.e., VH-linker-VL and VL-linker-VH). The $(Gly4-Ser)_4$ (SEQ ID NO: 47) peptide and GSTSGSGKPGSGEGSTKG (SEQ ID NO: 48) peptide linker were used in the construction of all scFvs. In addition, sc-dsFvs were also constructed by introducing single mutations at VH44/VL100 or VH105/VL43 (according to the Kabat numbering system). The fragments containing VH and VL also included restriction endonuclease HindIII and EcoRI recognition sites at the 5' and 3' ends, respectively, as well as a His tag sequence at the C-terminal. The fragments containing VH and VL were fused with either human IgG1 heavy chain CH1-CH2-CH3 or IgG1 light chain CL, at the 5' end by overlapping PCR. The fused heavy chain and light chain were then cloned into the corresponding HindIII and EcoRI recognition sites of the pCDNA3.1(+) expression vector. A total of eight scFvs were designed and synthesized. The scFvs comprise the sequences shown in Table 5. In addition, each scFv comprises a His tag fused to the C-terminus via a short peptide linker (*i.e.*, SEQ ID NO: 51). Constructs for the parental humanized IgG1 positive control antibody and a negative control scFv (irr-control scFv) were also synthesized.

[436] The constructs were transiently transfected into expiCHO cells. A one-time feed was added 18 hours later and the supernatant was harvested after culturing for 5 consecutive days. The proteins from the supernatant were purified by protein L sepharose column (GE healthcare) and superdex-75 increase column (GE healthcare) for functional analysis. The parental humanized IgG1 positive control antibody and the negative control scFv were similarly transfected and purified. The titers of the scFvs were measured by Fortebio Octet. The concentrations of the final purified scFvs were determined by Nanodrop. The sc-dsFv with the highest yield of 50mg/L was chosen for isotope labelling, and is referred to as scFv (PD-L1). FIG. 10 shows the SDS-PAGE results of scFv (PD-L1), the parental humanized IgG1 positive control antibody, and the negative control scFv, under both reduced and non-reduced conditions.

Characterization of scFv (PD-L1)

PD-L1 binding activity of scFv (PD-L1) as measured by FACS

[437] CHO/PD-L1 stable cells were harvested and washed twice with FACS buffer (1XPBS with 1% FBS). Cells were then stained with the parental humanized IgG1 positive control antibody (anti-PD-L1 IgG1), or scFv

(PD-L1) at various dilutions for 30 minutes on ice. Cells were then washed twice with FACS buffer and further stained with either 60ng of PE-conjugated anti-human Fc antibody (anti-HuFc-PE, BD), or with 60ng of PE-conjugated anti-His tag antibody (anti-Histag-PE, eBioscience), for 30 minutes. Cells were washed twice with FACS buffer and suspended in 300mL of FACS buffer. As shown by the histograms in FIG. 11, the binding activity of both the parental humanized IgG1 positive control antibody (anti-PD-L1 IgG1) and scFv (PD-L1) demonstrated a concentration-dependent pattern.

PD-L1 binding affinity of scFv (PD-L1) as measured by ForteBio Octet RED96

[438] All samples were prepared in PBS buffer (pH=7.4). The biotin-labeled hPD-L1-mouse Fc fusion protein was loaded onto SA sensors at a pre-determined loading threshold. scFv (PD-L1) was applied to the sensors at a concentration gradient of 3.125nM-50nM. Background subtraction was used to correct for sensor drifting. The data was fit to a 1:1 binding model using ForteBio's data analysis software in order to obtain the association (K_{on}) and dissociation (K_{off}) rates. The K_D values were calculated based on K_{off}/K_{on}. As shown in Table 8, the binding affinity of scFv (PD-L1) was similar to that of the parental humanized IgG1 positive control antibody.

Table 8. Binding affinity analysis of scFv (PD-L1) and the parental humanized IgG1 positive control antibody

Sample	K_D(M)	K_{off}(1/s)	K_{on}(1/Ms)	R^2
Anti-Human PD-L1 IgG1	6.92E-09	2.25E-04	3.26E+04	0.9993
scFv (PD-L1)	7.11E-09	7.01E-04	9.85E+04	0.995

Temperature of hydrophobic exposure of scFv (PD-L1) as measured by Differential Scanning Fluorimetry (DSF)

[439] scFv (PD-L1), the parental humanized IgG1 positive control antibody, and the negative control scFv (irr-control scFv) were expressed and purified as described above. Two buffer conditions (1×PBS at pH 7.4 and Na-Citrate buffer at pH5.5) were used and were achieved via buffer exchange of a concentrated protein stock using Hitrap G25 columns (GE Healthcare). Following buffer change, the protein concentration of the eluate was determined on a Nanodrop (Thermofisher). The final concentrations of scFv (PD-L1) and irr-control scFv were 1mg/ml, and the concentration of the parental humanized IgG1 positive control antibody was 0.5mg/ml. Immediately before use, the SYPRO Orange stock solution (5000×) was diluted with the corresponding buffer to a concentration of 25×. The diluted dye was then added to the protein samples to achieve a final working concentration of 5×SYPRO Orange. The CFX96 Real-Time PCR system (Bio-Rad Laboratories) was used to measure SYPRO Orange signals. The excitation/emission filter settings were determined according to the "FRET" channel compatible with the SYPRO Orange fluorescence signal. The samples were exposed to a temperature gradient from 25°C to 95°C at a heating rate of 1°C/min with 0.5°C increments. The -d(RFU)/dT curves representing the change in SYPRO Orange signals over the temperature gradient are shown in FIG. 12.

The first trough of the -d(RFU)/dT curve was defined as the temperature of hydrophobic exposure (Tm1 or Th), and was calculated by the CFX MANAGER™ software using the mathematical second derivative method. As shown in FIG. 12, scFv (PD-L1) exhibited a Tm1 of 61°C, the parental humanized IgG1 positive control antibody exhibited a Tm1 of 62°C, while the negative control scFv exhibited a Tm1 of 54°C. scFv (PD-L1) exhibited similar thermo-stability as the parental antibody in both the PBS buffer and the Na-Citrate buffer.

Stability of scFv (PD-L1) at low temperature and acidic pH conditions

[440] A 40°C accelerated stability test was performed on scFv (PD-L1) and the parental humanized IgG1 positive control antibody. Briefly, the scFv (PD-L1) and control antibodies were placed in either a 4°C or a 40°C stability test chamber (Memmert ICH110L) at a concentration of 1mg/mL. Every two weeks, samples were removed from the chambers and their binding affinity to PD-L1 was measured using FACS. As shown in FIGs. 13A-13D, the binding affinity of scFv (PD-L1) dramatically reduced over time under the high temperature (40°C) and high pH (pH 7.4) condition. scFv (PD-L1) exhibited relatively stable characteristics at the low temperature (4°C) and acidic pH (pH 5.5) condition.

Example 5: Radio-labeling of anti-hPD-L1 scFv and characterization of radio-labeled anti-hPD-L1 scFv

Preparation of ^{68}Ga-NOTA-anti-PD-L1 antibody moieties

[441] The experiments described below used an anti-hPD-L1 scFv as an example to prepare an imaging agent for detection of human PD-L1. Imaging agents having other anti-hPD-L1 antibody moieties were prepared using similar experimental procedure by replacing the anti-hPD-L1 scFv below with another anti-hPD-L1 antibody moiety (*e.g.*, scFv, scFv-Fc, or full-length antibody).

Coupling of anti-hPD-L1 scFv and NOTA

[442] scFv antibodies were formulated at a concentration of 2.318mg/mL (buffered in 20mM citric acid/sodium citrate/ sodium chloride, +0.8% (m/v), pH 5.5). 400μL of 0.05M $NaHCO_3$- Na_2CO_3 (pH 8.7) was added to the scFv antibody solution, followed by centrifugation at 16000 rpm for 20 minutes. The supernatant was removed to achieve a final volume of 100μL. The above steps were repeated one more time and the final 100μL solution was transferred to a 1.5mL centrifuge tube. 2.64μL of p-SCN-Bn-NOTA was added to the centrifuge tube at a final concentration of 927μM, followed by incubation for one hour at 37°C.

[443] The NAP-5 column was pre-balanced with PBS before the scFv/NOTA solution was added to the column. The column was then washed with 0.4mL of PBS and eluted with 0.5mL of PBS. The eluate was aliquoted into 0.05mL portions and stored at -20°C.

Labeling NOTA-anti-PD-L1 scFv with ^{68}Ga

[444] ^{68}Ga was washed with 0.05N HCl to a final concentration of 21mCi/mL. 46.5μL of 1.25M sodium acetate was then added to arrive at a final pH of 4. 50μL of NOTA-anti-PD-L1 scFv (0.05mg/ml) was added to

350uL ^{68}Ga, followed by incubation for 10 min at 25°C. The labeling rate of ^{68}Ga-NOTA-anti-PD-L1 scFv was determined using the thin layer chromatography paper. When 0.1M citrate was used as the expansion agent the labeling rate was determined to be 17%. When PBS was used as the expansion agent, the labeling rate was determined to be 100%.

Purification of ^{68}Ga-NOTA-anti-PD-L1 ScFv

[445] Purification of ^{68}Ga -NOTA-PD-L1 scFv was performed by first balancing the NAP-5 column with PBS, followed by adding 400μL of ^{68}Ga-NOTA-anti-PD-L1 scFv. The column was then washed with 400μL of PBS, followed by elution with another 400μL of PBS.

Quality control of ^{68}Ga-NOTA-anti-PD-L1 scFv

[446] The radiochemical purity of the purified ^{68}Ga-NOTA-anti-PD-L1 scFv was measured using instant thin layer chromatography-Silica-Gel (ITLC-SG) with 0.1M sodium citrate as the developing agent, with the origin point being the labeled product, and the front edge of the developing agent being dissociated ^{68}Ga. As shown in FIGs. 14 and 15, the yield of ^{68}Ga-NOTA-anti-PD-L1 scFv was 17%, and the purity was 93.5%.

^{68}Ga-NOTA-anti-PD-L1 scFv specific binding to human PD-L1 in vitro

[447] ^{68}Ga-NOTA-anti-PD-L1 scFv cell binding assay. MC38 and MC38-PD-L1 cells were each plated in 12 wells of a 24-well plate at a density of 2.5×10^5 cells/well. Cells were cultured for overnight at 37°C. On the next day, ^{68}Ga-NOTA-anti-PD-L1 scFv was added to the wells at final concentrations of 0.20, 0.40, 0.80, 1.60, 3.20 and 6.40nM (each concentration had two duplicates), and the cells were incubated at 37°C for 1 hour. After three washes with ice-cold PBS, the cells were lysed with 0.1M NaOH, and radioactivity was measured using a gamma counting instrument. The K_D value of ^{68}Ga-NOTA-anti-PD-L1 scFv was calculated using the GraphPad Prism software. As shown in FIG. 16, ^{68}Ga-NOTA-anti-PD-L1 scFv exhibited much higher binding affinity for MC38-PD-L1 (also referred to as "MC38-B7H1") cells as compared to MC38 cells with the K_D value for MC38-PD-L1 binding being 1.53±0.44nM.

[448] ^{68}Ga-NOTA-anti-PD-L1 scFv Competitive Binding Assay. MC38 cells were plated in 8 wells and MC38-PD-L1 cells were plated in 16 wells of a 24-well plate, at a density of 2.5×10^5 cells/well. On the next day, anti-PD-L1 IgG1 was added at final concentrations of 20, 40, 80, and 160nM. Anti-PD-L1 IgG1 and ^{68}Ga-NOTA-anti-PD-L1 scFv were added in duplicates at final concentrations of 0.20, 0.40, 0.80, 1.60, 3.20 and 6.40nM. The cells were incubated at 37°C for 1 hour. After three washes with ice cold PBS, the cells were lysed with 0.1M NaOH and radioactivity was measured using a gamma counting instrument.

[449] As shown in FIG. 17, anti-PD-L1 IgG1 blocked the binding of ^{68}Ga-NOTA-anti-PD-L1 scFv to MC38-PD-L1cells. This result demonstrated that binding of ^{68}Ga-NOTA-anti-PD-L1 scFv to human PD-L1 was specific.

In vivo live imaging of ^{68}Ga-NOTA-anti-PD-L1 scFv

In vivo live imaging assay

[450] MC38 cells and MC38-PD-L1 cells were cultured and counted after trypsin digestion. 1×10^6 MC38-PD-L1 cells were injected into the right axilla of five 6-8 week old female mice, and 1 x 10^6 MC38 cells were injected into the left axilla of the same mice. Another five 6-8 week old female mice were injected only with 1×10^6 MC38-PD-L1 cells into the right axilla. The tumors reached a diameter of about 0.5cm after 6 days, at which point the animals received 200uCi ^{68}Ga-NOTA-anti-PD-L1 scFv via tail vein injection. Live imaging was carried out 0.5 hour, 1 hour and 2 hours post injection with an exposure time of 5 minutes.

[451] The maximum exposure images taken at 1 hour and 2 hours post injection are shown in FIG. 18. Tumors in the right axilla (injected with MC38-PD-L1 cells) showed stronger expression as compared to tumors in the left axilla (injected with MC38 cells). Tumors in the left axilla were more visible at 2 hours after injection, as compared to 1 hour after injection. Low uptake and exposure was observed in liver and heart tissues, while high uptake was observed in kidney.

[452] FIG. 19 shows in vivo imaging results of animals #1-#6 at 0.5 hours, 1 hour and 2 hours after injection of ^{68}Ga-NOTA-anti-PD-L1 scFv. Animals #1-#4 received MC38-PD-L1 cells in the right axilla only, while animals #5 and #6 also received MC38 cells in the left axilla in addition to MC38-PD-L1 cells in the right axilla. The results showed that radioactive signal was detectable by 0.5 hours, and the uptake by the kidney did not decrease significantly over time. Animal #1 showed clear imaging and may be related to the specific growth status of the tumor.

Binding competition in vivo imaging assay

[453] MC38 cells and MC38-PD-L1 cells were cultured and counted after trypsin digestion. 1×10^6 MC38-PD-L1 cells were injected into the right axilla of five 6-8 week old female mice (#2, #3, #4, #7 and #8). 1 x 10^6 MC38 cells were also injected into the left axilla of mice #7 and #8. The tumors reached a diameter of about 0.5cm after 6 days. Animals #3, #4, #7 and #8 received both the unlabeled antibody anti-PD-L1 IgG1 and 140uCi of ^{68}Ga-NOTA-anti-PD-L1 scFv via tail vein injections. The unlabeled antibody anti-PD-L1 IgG1was injected at a concentration that was 50 times of the concentration of ^{68}Ga-NOTA-anti-PD-L1 scFv. Animal #2 received 140uCi of ^{68}Ga-NOTA-anti-PD-L1 scFv only as control. Live imaging was carried out 0.5 hour, 1 hour and 2 hours post injection with an exposure time of 5 minutes. As shown in FIG. 20, animal #2 showed binding of ^{68}Ga-NOTA-anti-PD-L1 scFv to B7H1 (PD-L1), whereas unlabeled ab-220 antibody when injected at a concentration 50 times that of ^{68}Ga-NOTA-anti-PD-L1 scFv blocked the binding of ^{68}Ga-NOTA-anti-PD-L1 scFv to B7H1 (PD-L1).

Comparison between 68Ga-NOTA-anti-PD-L1 scFv and 68Ga-NOTA-anti-PD-L1 scFv-Fc

[454] MC38 cells and MC38-PD-L1 cells were cultured and counted after trypsin digestion. 1×10^6 MC38-PD-L1 cells were injected into the right axilla of five 6-8 week old female mice, and 1 x 10^6 MC38 cells were injected into the left axilla of the same mice. The tumors reached a diameter of about 0.5cm after 6 days, at which point the animals received 200μCi ^{68}Ga-NOTA-anti-PD-L1 scFv, unlabeled anti-PD-L1 scFv with 200μCi ^{68}Ga-NOTA-anti-PD-L1 scFv, or 200μCi ^{68}Ga-NOTA-anti-PD-L1 scFv-Fc via tail vein injection. Live imaging was carried out 0.5 hour, 1 hour and 2 hours post injection with an exposure time of 5 minutes. For mice injected with ^{68}Ga-NOTA-anti-PD-L1 scFv-Fc, images were taken with normal camera sensitivity setting, and a reduced camera sensitivity setting (1/4 of normal conditions).

[455] FIG. 21 shows a side-by-side comparison of imaging results at 30 minutes after injection of the imaging agents. From left to right of the figure shows imaging results of a mouse injected with ^{68}Ga-NOTA-anti-PD-L1 scFv, a mouse injected with unlabeled anti-PD-L1 scFv and ^{68}Ga-NOTA-anti-PD-L1 scFv, a mouse injected with ^{68}Ga-NOTA-anti-PD-L1 scFv-Fc(wt) at normal camera sensitivity setting, and the same mouse injected with ^{68}Ga-NOTA-anti-PD-L1 scFv-Fc(wt) at a reduced camera sensitivity setting.

[456] FIG. 22 shows a side-by-side comparison of imaging results at 60 minutes (top panel) and 120 minutes (bottom panel) after injection of the imaging agents. From left to right of the figure shows imaging results of a mouse injected with ^{68}Ga-NOTA-anti-PD-L1 scFv, a mouse injected with unlabeled anti-PD-L1 scFv and ^{68}Ga-NOTA-anti-PD-L1 scFv, a mouse injected with ^{68}Ga-NOTA-anti-PD-L1 scFv-Fc(wt) at normal camera sensitivity setting, and the same mouse injected with ^{68}Ga-NOTA-anti-PD-L1 scFv-Fc(wt) at a reduced camera sensitivity setting.

CLAIMS

What is claimed is:

1. Use of an effective amount of an anti-PD-L1 antibody agent in the preparation of a medicament for treating a disease or condition in an individual, wherein the antibody agent comprises an antibody moiety comprising a heavy chain variable region (V_H) and a light chain variable region (V_L), wherein:

a) the V_H comprises a heavy chain complementarity determination region 1 (HC-CDR1) comprising the amino acid sequence of SEQ ID NO: 41, an HC-CDR2 comprising the amino acid sequence of SEQ ID NO: 42, and an HC-CDR3 comprising the amino acid sequence of SEQ ID NO: 43, or a variant thereof comprising up to a total of about 5 amino acid substitutions in the HC-CDRs; and

b) the V_L comprises a light chain complementarity determination region 1 (LC-CDR1) comprising the amino acid sequence of SEQ ID NO: 44, an LC-CDR2 comprising the amino acid sequence of SEQ ID NO: 45, and an LC-CDR3 comprising the amino acid sequence of SEQ ID NO: 46, or a variant thereof comprising up to a total of about 5 amino acid substitutions in the LC-CDRs.

2. The use of claim 1, wherein:

a) the V_H comprises an HC-CDR1 comprising the amino acid sequence of SEQ ID NO: 41, an HC-CDR2 comprising the amino acid sequence of SEQ ID NO: 42, and an HC-CDR3 comprising the amino acid sequence of SEQ ID NO: 43; and

b) the V_L comprises an LC-CDR1 comprising the amino acid sequence of SEQ ID NO: 44, an LC-CDR2 comprising the amino acid sequence of SEQ ID NO: 45, and an LC-CDR3 comprising the amino acid sequence of SEQ ID NO: 46.

3. The use of claim 1 or claim 2, wherein:

a) the V_H comprises an amino acid sequence having at least about 80% sequence identity to the amino acid sequence of any one of SEQ ID NOs: 1, 5, 9, 11, and 13; and/or

b) the V_L comprises an amino acid sequence having at least about 80% sequence identity to the amino acid sequence of any one of SEQ ID NOs: 3, 7, 15, 17 and 19.

4. The use of claim 3, wherein the antibody moiety comprises:

(a) a V_H comprising the amino acid sequence of SEQ ID NO: 9, and a V_L comprising the amino acid sequence of SEQ ID NO: 15;

(b) a V_H comprising the amino acid sequence of SEQ ID NO: 9, and a V_L comprising the amino acid sequence of SEQ ID NO: 17;

(c) a V_H comprising the amino acid sequence of SEQ ID NO: 9, and a V_L comprising the amino acid sequence of SEQ ID NO: 19;

(d) a V_H comprising the amino acid sequence of SEQ ID NO: 11, and a V_L comprising the amino acid sequence of SEQ ID NO: 15;

(e) a V_H comprising the amino acid sequence of SEQ ID NO: 11, and a V_L comprising the amino acid sequence of SEQ ID NO: 17;

(f) a V_H comprising the amino acid sequence of SEQ ID NO: 11, and a V_L comprising the amino acid sequence of SEQ ID NO: 19;

(g) a V_H comprising the amino acid sequence of SEQ ID NO: 13, and a V_L comprising the amino acid sequence of SEQ ID NO: 15;

(h) a V_H comprising the amino acid sequence of SEQ ID NO: 13, and a V_L comprising the amino acid sequence of SEQ ID NO: 17; or

(i) a V_H comprising the amino acid sequence of SEQ ID NO: 13, and a V_L comprising the amino acid sequence of SEQ ID NO: 19.

5. The use of claim 1, wherein the antibody moiety comprises an amino acid sequence having at least about 80% sequence identity to the amino acid sequence of SEQ ID NO: 21 or 23.

6. Use of an effective amount of an anti-PD-L1 antibody agent in the preparation of a medicament for treating a disease or condition in an individual, wherein the antibody agent comprises an antibody moiety comprising:

a) a HC-CDR1, a HC-CDR2, and a HC-CDR3, respectively comprising the amino acid sequences of a CDR1, a CDR2, and a CDR3 within a heavy chain variable region (V_H) having the sequence set forth in any of SEQ ID NOs: 1, 5, 9, 11, and 13; and

b) a LC-CDR1, a LC-CDR2, and a LC-CDR3, respectively comprising the amino acid sequences of a CDR1, a CDR2, and a CDR3 within a light chain variable region (V_L) having the sequence set forth in any of SEQ ID NOs: 3, 7, 15, 17 and 19.

7. The use of any one of claims 1-6, wherein the antibody moiety is chimeric or humanized.

8. The use of any one of claims 1-4, 6 and 7, wherein the antibody moiety is selected from the group consisting of a single-chain Fv (scFv), a Fab, a Fab', a F(ab')2, an Fv fragment, a disulfide stabilized Fv fragment (dsFv), a $(dsFv)_2$, a V_HH, a Fv-Fc fusion, an scFv-Fc fusion, an scFv-Fv fusion, a diabody, a tribody, and a tetrabody.

9. The use of any one of claims 1-4 and 6-8, wherein the antibody moiety is a single-chain antibody.

10. The use of claim 9, wherein the antibody moiety is an scFv.

11. The use of any one of claims 1-4 and 6-8, wherein the antibody moiety comprises an Fc fragment.

12. The use of claim 11, wherein the antibody moiety is a full-length antibody.

13. The use of claim 12, wherein the antibody moiety has an isotype selected from the group consisting of an IgG, an IgM, an IgA, an IgD, and an IgE.

14. The use of any one of claims 11-13, wherein the Fc fragment is an Fc fragment of IgG.

15. The use of claims 14, wherein the Fc fragment is an Fc fragment of IgG1 or IgG4.

16. The use of any one of claims 11-15, wherein the Fc fragment comprises H310A and H435Q mutations, wherein the amino acid positions are based on the Kabat numbering system.

17. The use of any one of claims 1-16, wherein the individual is a human.

18. The use of any one of claims 1-17, wherein the disease or condition is a cancer.

19. The use of claim 18, wherein the cancer is selected from the group consisting of melanoma, renal cell carcinoma, colorectal cancer, urothelial carcinoma, Hodgkin's lymphoma, small cell lung cancer, non-small cell lung cancer, head and neck tumors, stomach cancer, B cell lymphoma, Merkel cell carcinoma, liver cancer, and cervical cancer.

20. The use of any one of claims 1-19, wherein the antibody agent is suitable for intravenous, intraperitoneal, intramuscular, subcutaneous, or oral administration.

21. The use of any one of claims 1-20, wherein the medicament is used in combination with an effective amount of a second agent.

22. The use of claim 21, wherein the second agent is a chemotherapeutic agent.

23. A method of treating a disease or condition in an individual, comprising administering to the individual an effective amount of an anti-PD-L1 antibody agent, wherein the anti-PD-L1 antibody agent comprises an antibody moiety comprising a heavy chain variable region (V_H) and a light chain variable region (V_L), wherein:

a) the V_H comprises a heavy chain complementarity determining region (HC-CDR) 1 comprising the amino acid sequence of SEQ ID NO: 41, an HC-CDR2 comprising the amino acid sequence of SEQ ID NO: 42, and an HC-CDR3 comprising the amino acid sequence of SEQ ID NO: 43, or a variant thereof comprising up to a total of about 5 amino acid substitutions in the HC-CDRs; and

b) the V_L comprises a light chain complementarity determining region (LC-CDR) 1 comprising the amino acid sequence of SEQ ID NO: 44, an LC-CDR2 comprising the amino acid sequence of SEQ ID NO: 45, and an LC-CDR3 comprising the amino acid sequence of SEQ ID NO: 46, or a variant thereof comprising up to a total of about 5 amino acid substitutions in the LC-CDRs.

24. The method of claim 23, wherein the effective amount of the anti-PD-L1 antibody agent is about 0.005 μg/kg to about 5g/kg of total body weight of the individual.

25. A pharmaceutical composition comprising an anti-PD-L1 antibody agent and a pharmaceutical acceptable carrier, wherein the antibody agent comprises an antibody moiety comprising a heavy chain variable region (V_H) and a light chain variable region (V_L), wherein:

a) the V_H comprises an HC-CDR1 comprising the amino acid sequence of SEQ ID NO: 41, an HC-CDR2 comprising the amino acid sequence of SEQ ID NO: 42, and an HC-CDR3 comprising the amino acid sequence of SEQ ID NO: 43, or a variant thereof comprising up to a total of about 5 amino acid substitutions in the HC-CDRs; and

b) the V_L comprises an LC-CDR1 comprising the amino acid sequence of SEQ ID NO: 44, an LC-CDR2 comprising the amino acid sequence of SEQ ID NO: 45, and an LC-CDR3 comprising the amino acid

sequence of SEQ ID NO: 46, or a variant thereof comprising up to a total of about 5 amino acid substitutions in the LC-CDRs.

26. The pharmaceutical composition of claim 25, wherein the pharmaceutical composition is lyophilized.

27. The pharmaceutical composition of claim 25, wherein the pharmaceutical composition is a solution.

28. The pharmaceutical composition of any one of claims 25-27, comprising about 0.001 μg to about 10 g of the antibody moiety.

29. A kit for treating a disease or condition in an individual, comprising the pharmaceutical composition of any one of claims 25-28 and an instruction.

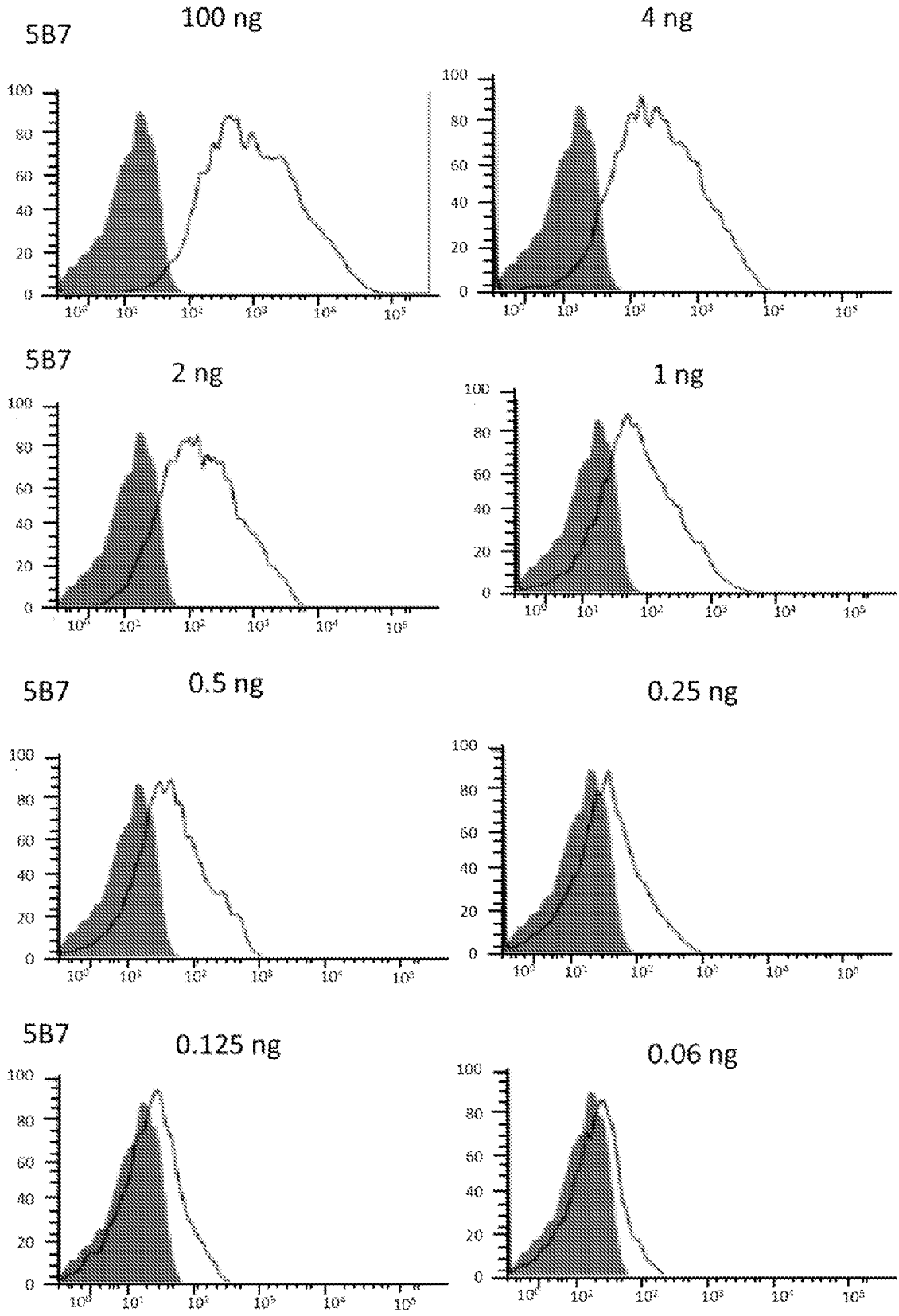


FIG. 1A

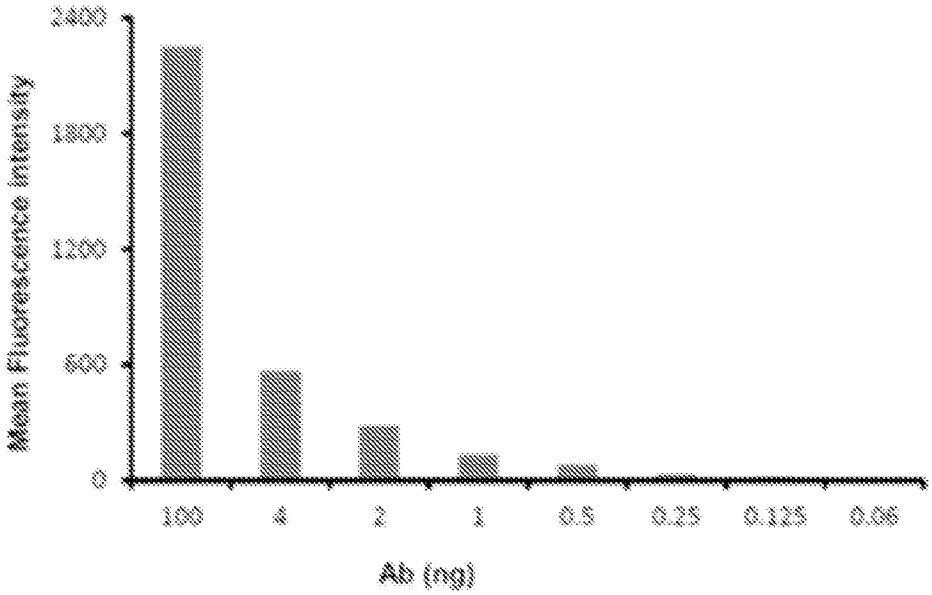

Mean Fluorescence Intensity
2400
1800
1200
600
0
100
4
2
1
0.5
0.25
0.125
0.06
Ab (ng)

FIG. 1B

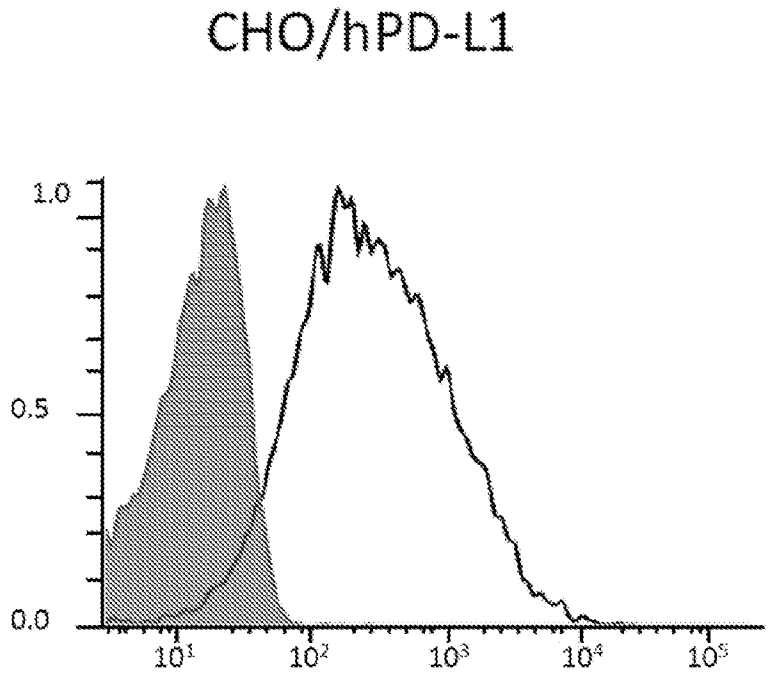

CHO/hPD-L1
1.0
0.5
0.0
10^1
10^2
10^3
10^4
10^5

FIG. 2A

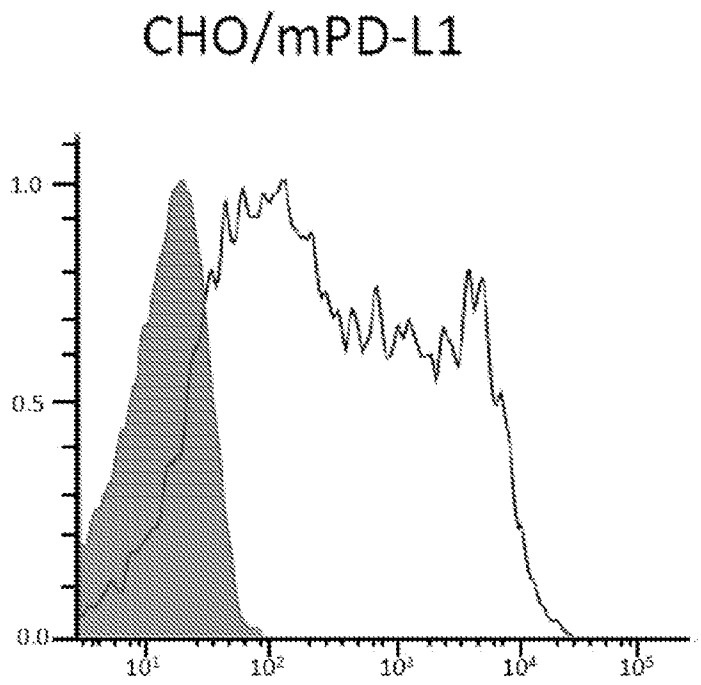


FIG. 2B

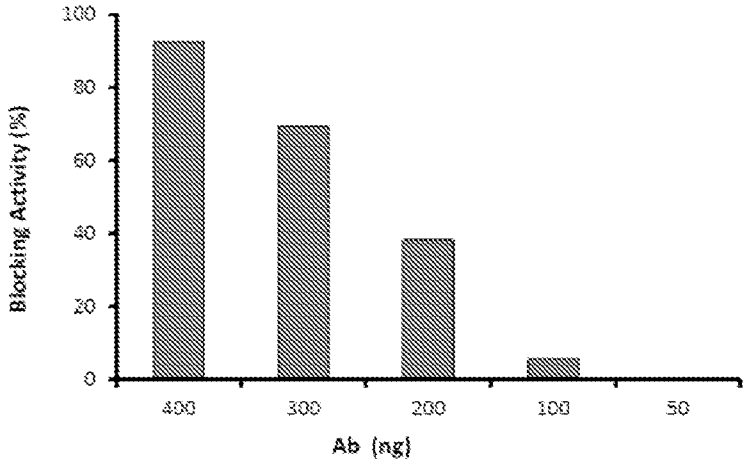

Blocking Activity (%)
100
80
60
40
20
0
400
300
200
100
50
Ab (ng)

FIG. 3A

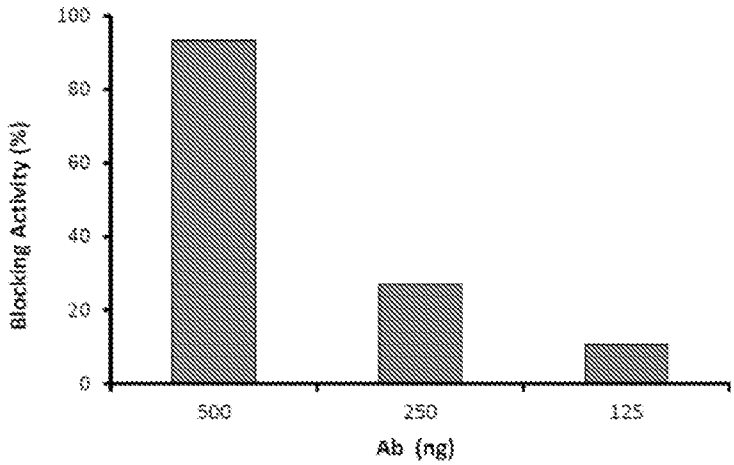

Blocking Activity (%)
100
80
60
40
20
0
500
250
125
Ab (ng)

FIG. 3B

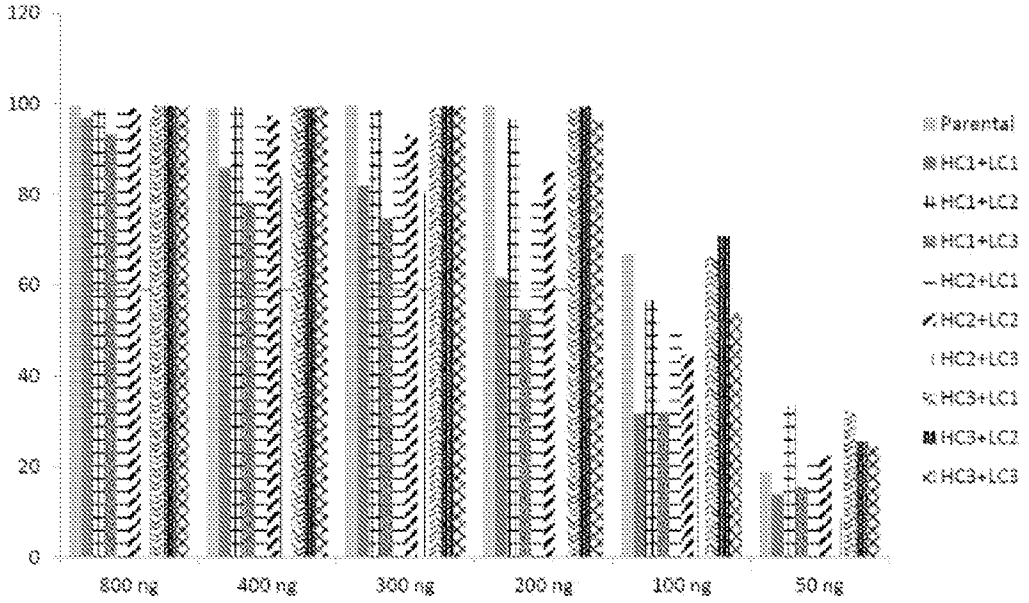

120
100
80
60
40
20
0
800 ng
400 ng
300 ng
200 ng
100 ng
50 ng
Parental
HC1+LC1
HC1+LC2
HC1+LC3
HC2+LC1
HC2+LC2
HC2+LC3
HC3+LC1
HC3+LC2
HC3+LC3

FIG. 4

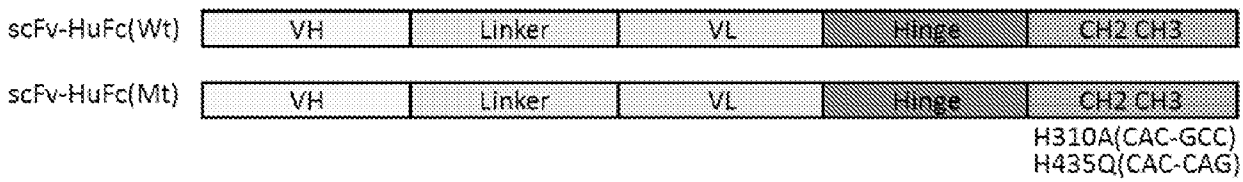

scFv-HuFc(Wt)
VH
Linker
VL
Hinge
CH2 CH3
scFv-HuFc(Mt)
VH
Linker
VL
Hinge
CH2 CH3
H310A(CAC-GCC)
H435Q(CAC-CAG)

FIG. 5

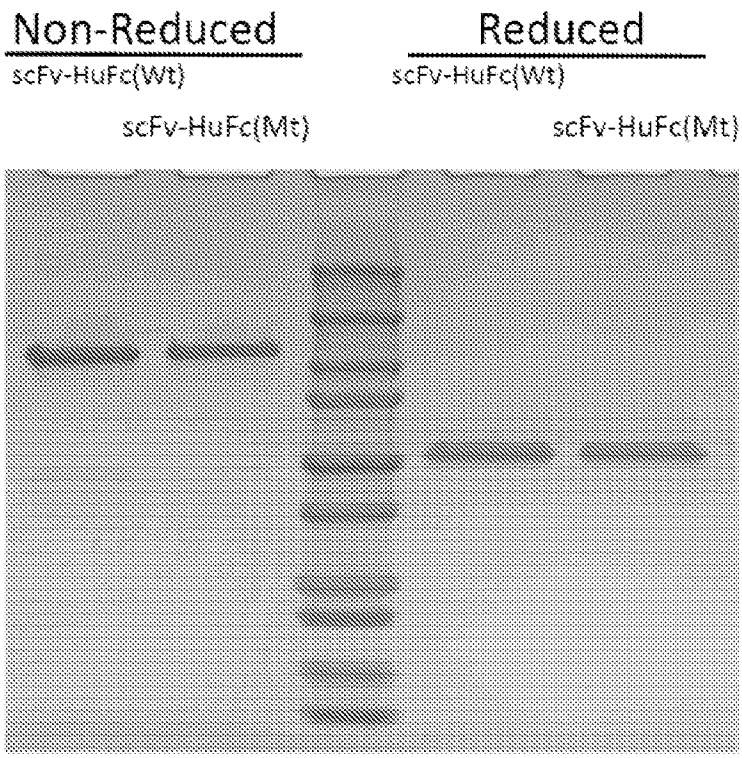

Non-Reduced
scFv-HuFc(Wt)
scFv-HuFc(Mt)
Reduced
scFv-HuFc(Wt)
scFv-HuFc(Mt)

FIG. 6

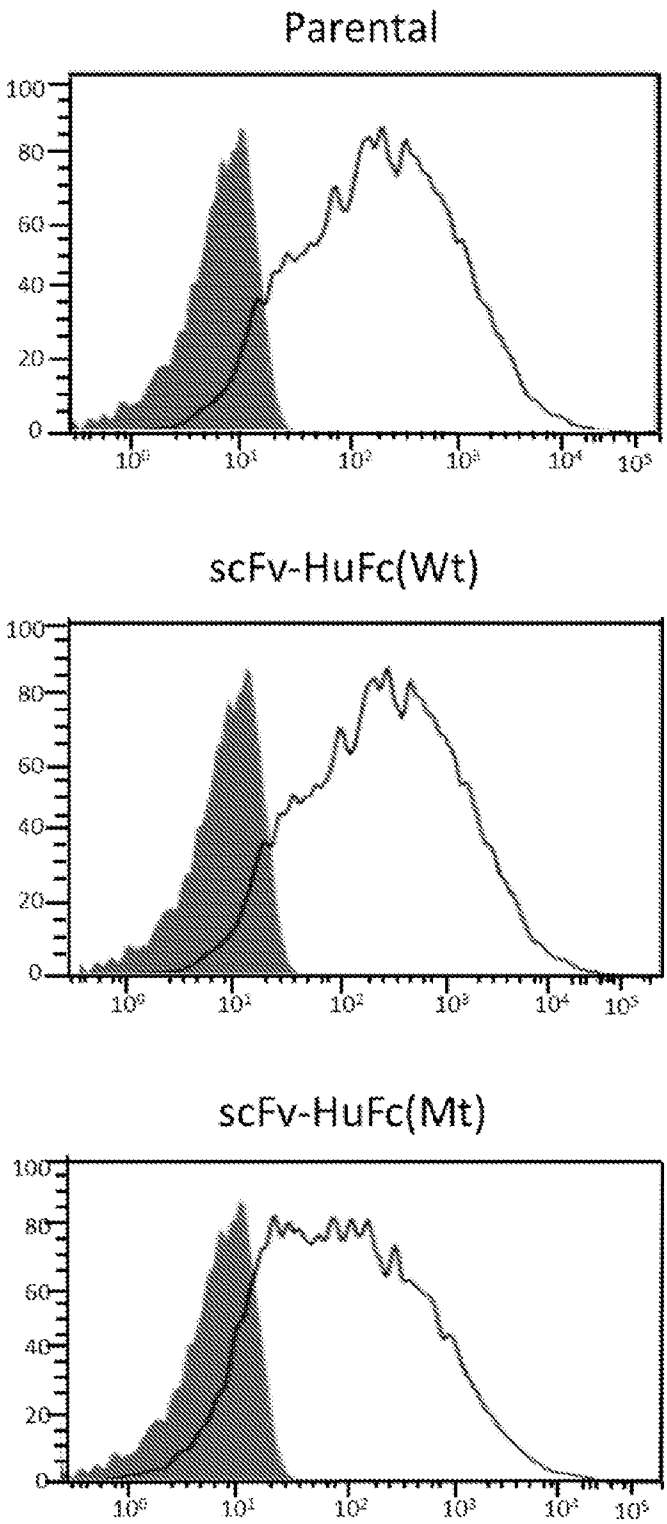


FIG. 7

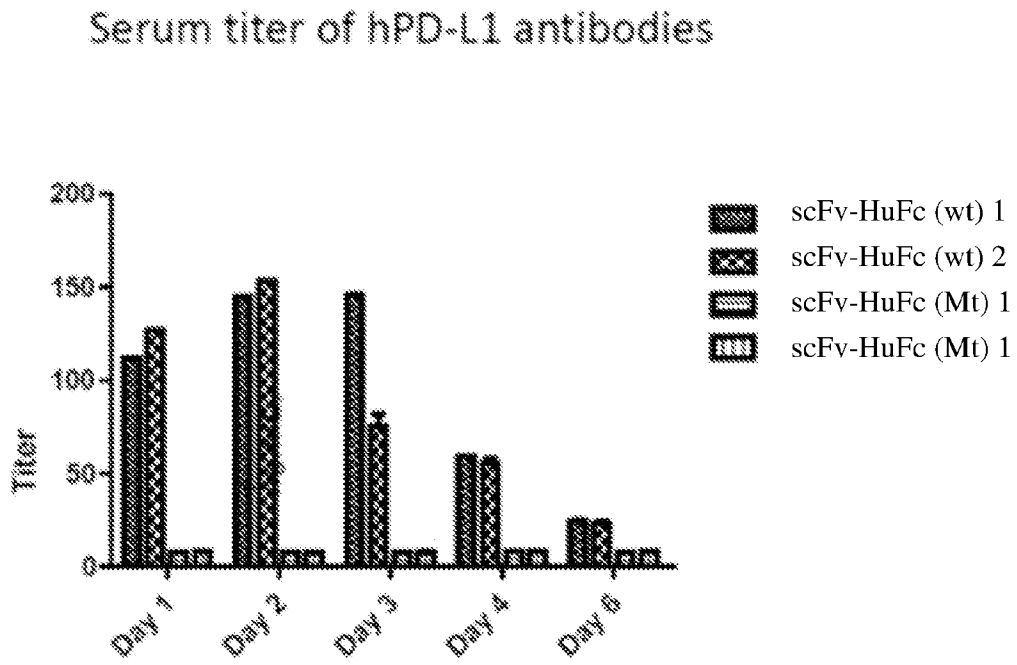

Serum titer of hPD-L1 antibodies
Titer
200
150
100
50
0
Day 1
Day 2
Day 3
Day 4
Day 6
scFv-HuFc (wt) 1
scFv-HuFc (wt) 2
scFv-HuFc (Mt) 1
scFv-HuFc (Mt) 1

FIG. 8A

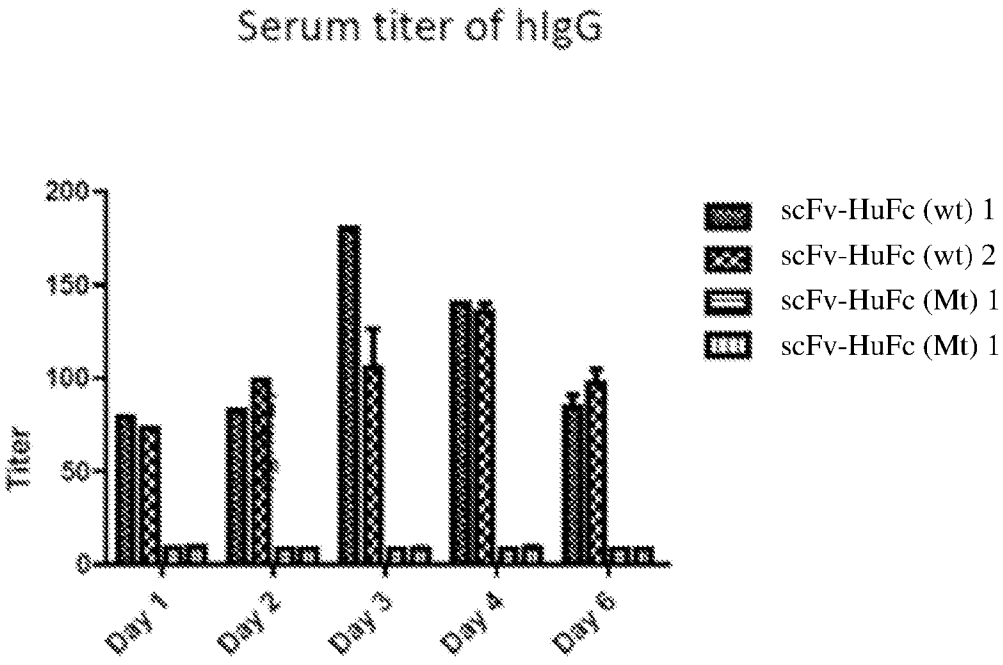

Serum titer of hIgG
Titer
200
150
100
50
0
Day 1
Day 2
Day 3
Day 4
Day 6
scFv-HuFc (wt) 1
scFv-HuFc (wt) 2
scFv-HuFc (Mt) 1
scFv-HuFc (Mt) 1

FIG. 8B

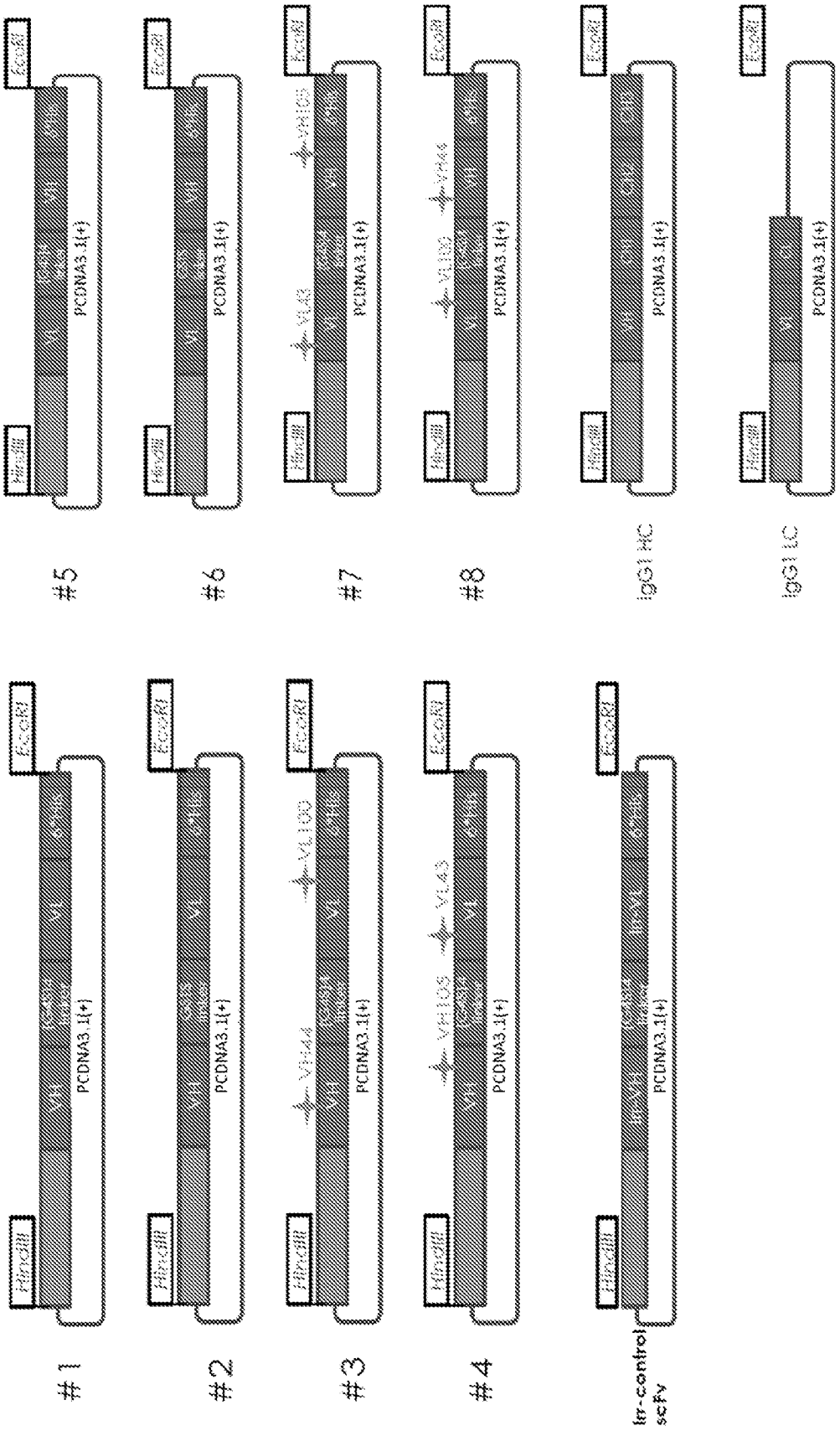
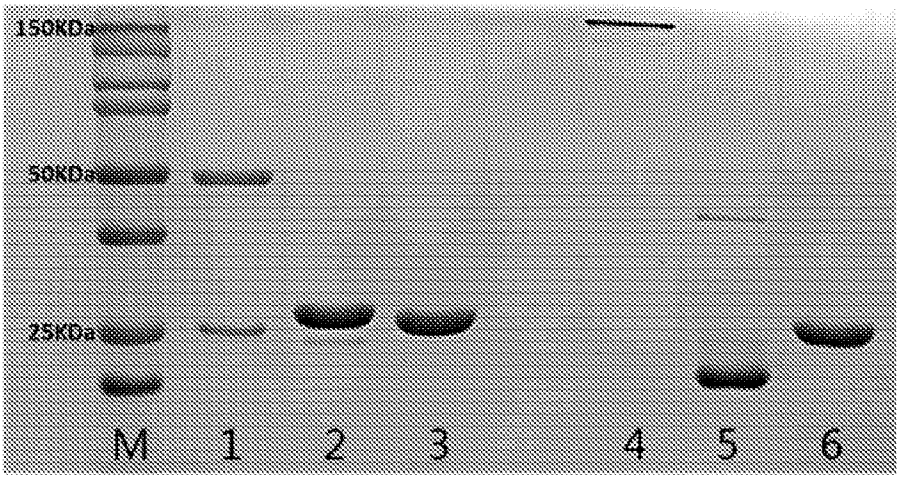


FIG. 9



Lane1. Reduced parental humanized IgG1 control
Lane2. Reduced scFv(PD-L1)
Lane3. Reduced scFv(irrelevant control)
Lane4. Non-reduced parental humanized IgG1 control
Lane5. Non-reduced scFv(PD-L1)
Lane6. Non-reduced scFv(irrelevant control)

FIG. 10

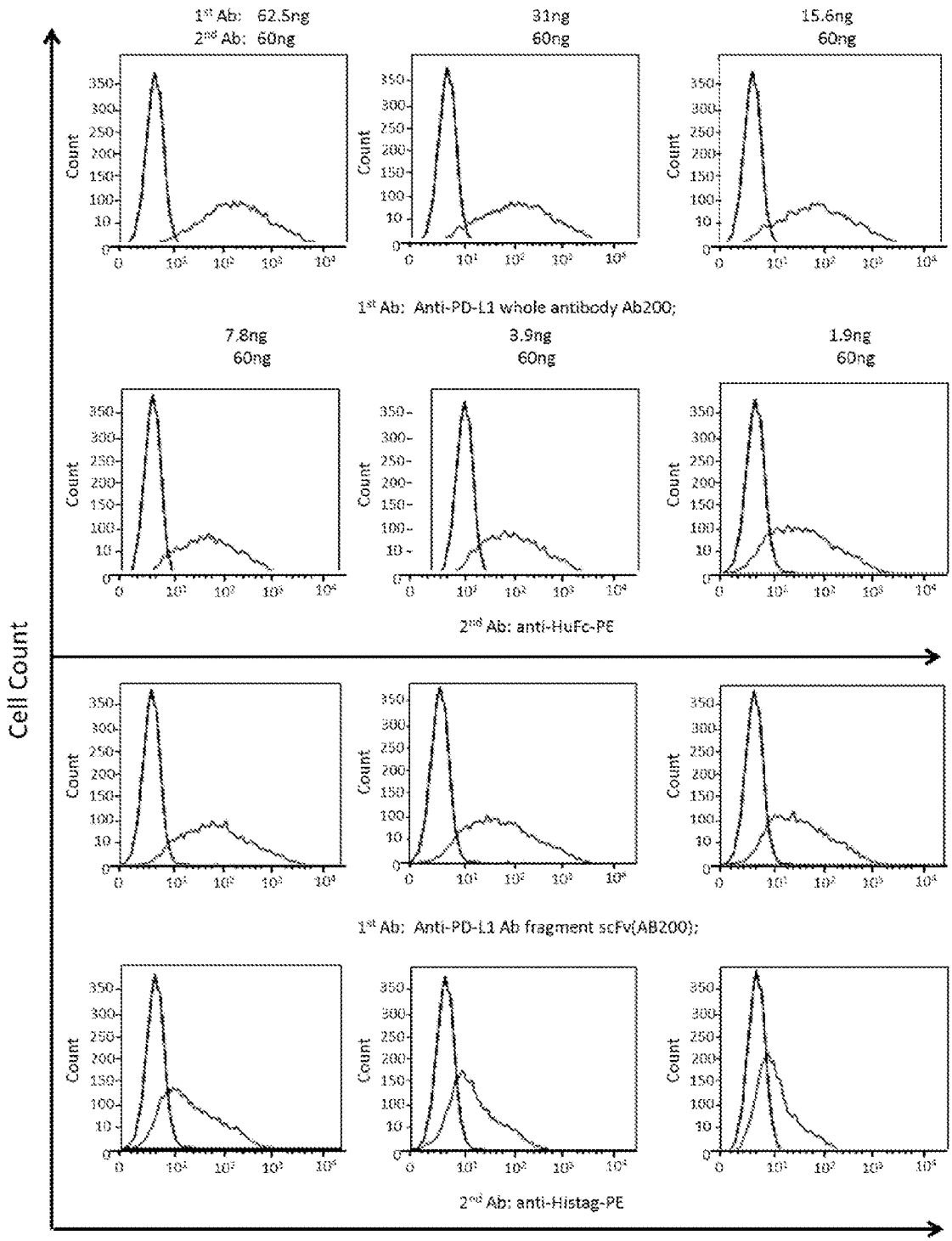
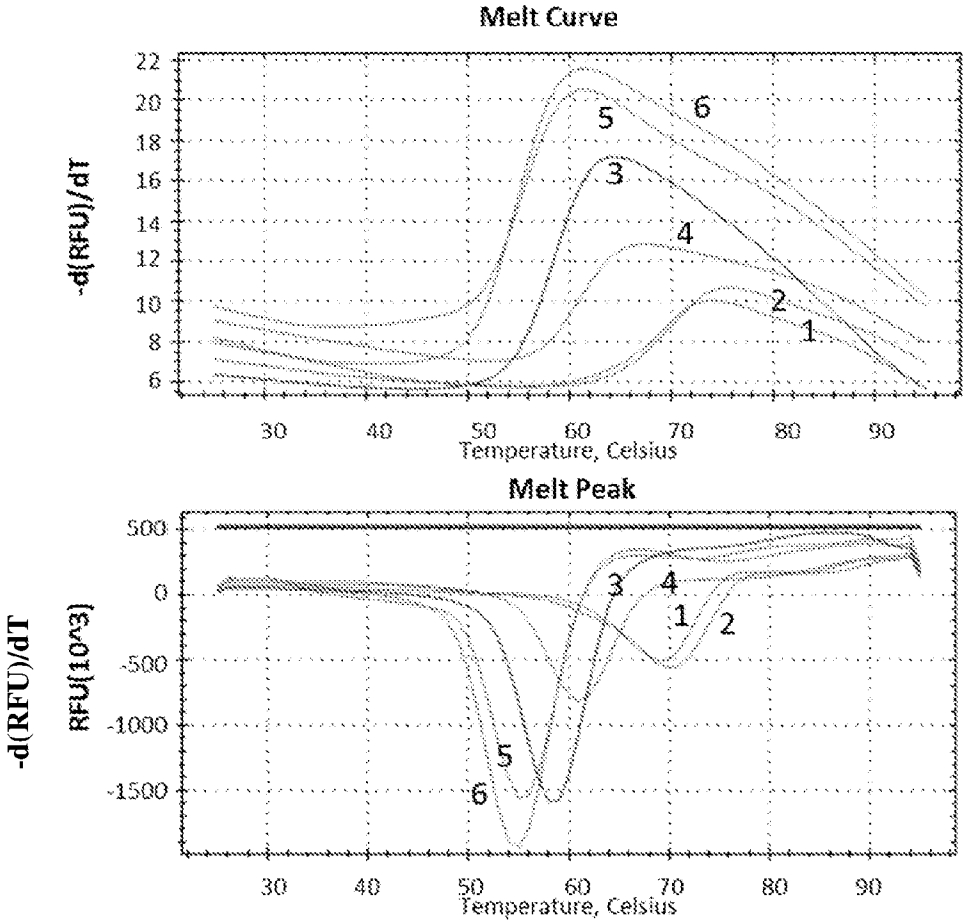

1st Ab: 62.5ng
2nd Ab: 60ng
31ng
60ng
15.6ng
60ng
1st Ab: Anti-PD-L1 whole antibody Ab200;
7.8ng
60ng
3.9ng
60ng
1.9ng
60ng
2nd Ab: anti-HuFc-PE
Cell Count
1st Ab: Anti-PD-L1 Ab fragment scFv(AB200);
2nd Ab: anti-Histag-PE
Count

FIG. 11



— 1: Parental anti-PD-L1 IgG; Buffer: Citric acid pH=5.5; 1.0mg/ml; Tm1=70°C

— 2: Parental anti-PD-L1 IgG; Buffer: PBS pH=7.4; 1.0mg/ml; Tm1=71°C

— 3: anti-PD-L1 scFv; Buffer: Citric acid pH=5.5; 1.0mg/ml; Tm1=59°C

— 4: anti-PD-L1 scFv; Buffer: PBS pH=7.4; 1.0mg/ml; Tm1=61°C

— 5: irrelevant scFv control; Buffer: Citric acid pH=5.5; 1.0mg/ml; Tm1=55°C

— 6: irrelevant scFv control; Buffer: PBS pH=7.4; 1.0mg/ml; Tm1=55°C

FIG. 12

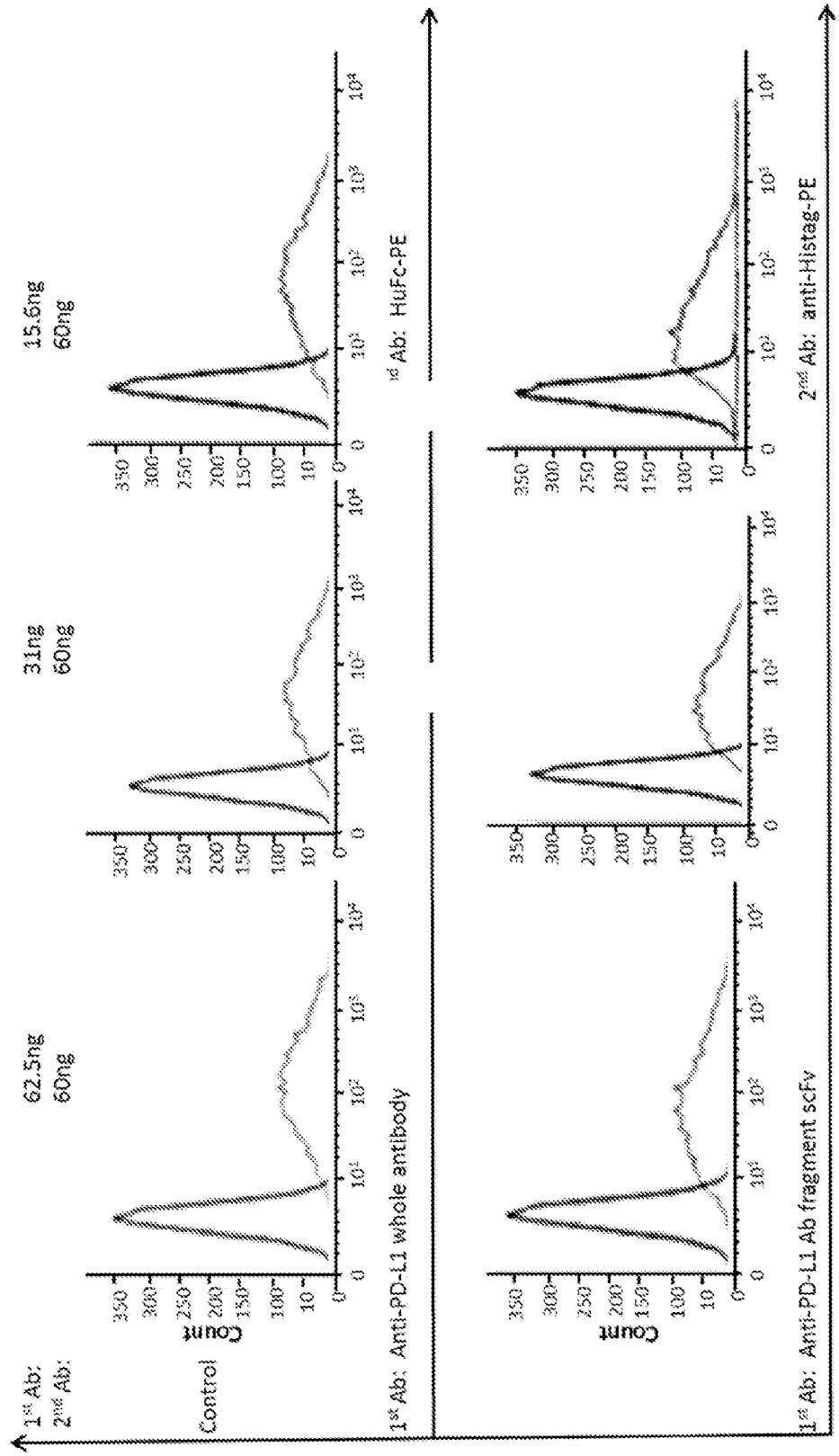

Week 0 in PBS, pH=7.4
1st Ab:
2nd Ab:
Control
62.5ng
60ng
31ng
60ng
15.6ng
60ng
Count
1st Ab: Anti-PD-L1 whole antibody
1st Ab: Anti-PD-L1 Ab fragment scFv
nd Ab: HuFc-PE
2nd Ab: anti-Histag-PE

FIG. 13A

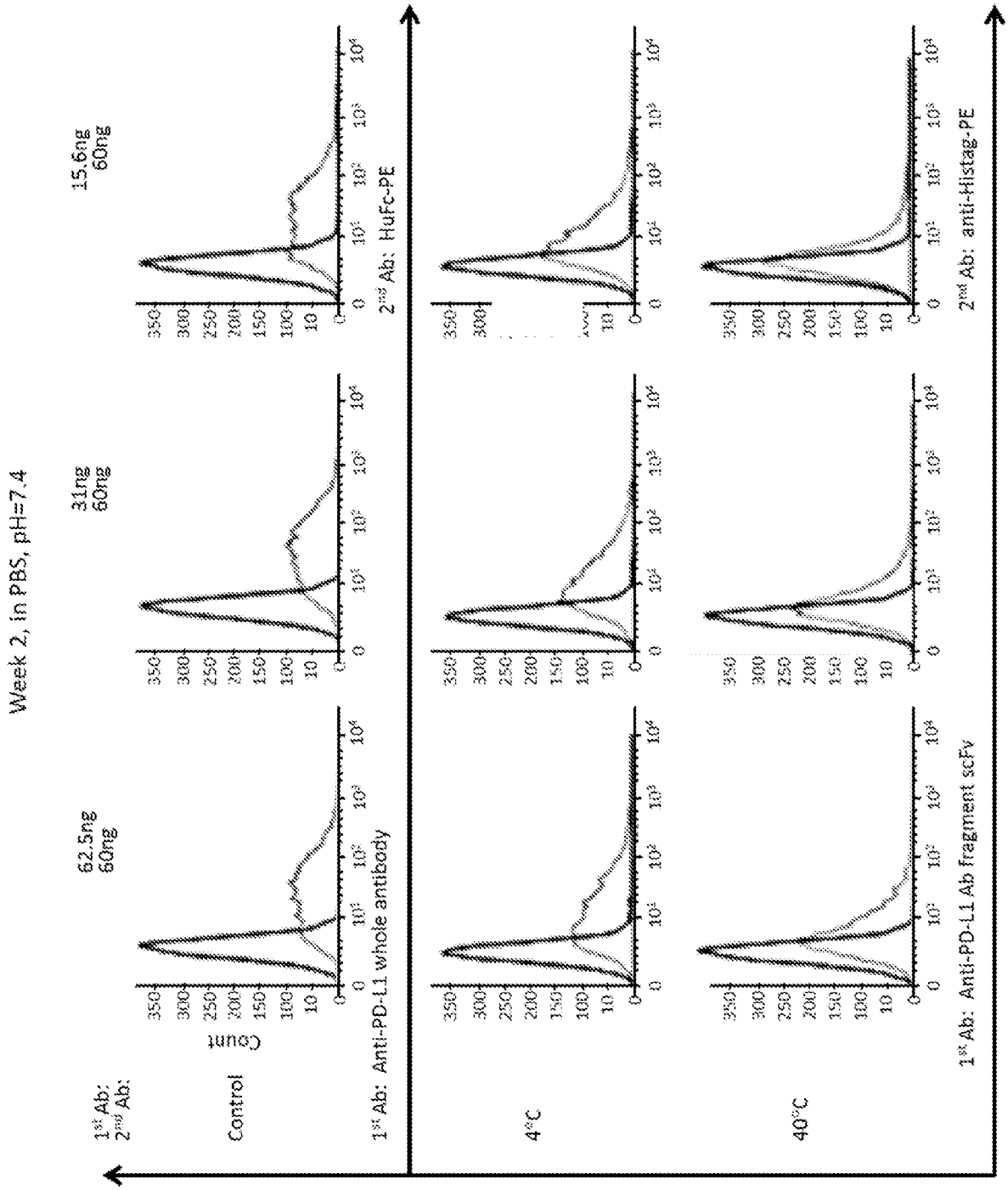

Week 2, in PBS, pH=7.4
62.5ng
60ng
31ng
60ng
15.6ng
60ng
1st Ab:
2nd Ab:
Control
4°C
40°C
Count
1st Ab: Anti-PD-L1 whole antibody
2nd Ab: HuFc-PE
1st Ab: Anti-PD-L1 Ab fragment scFv
2nd Ab: anti-Histag-PE

FIG. 13B

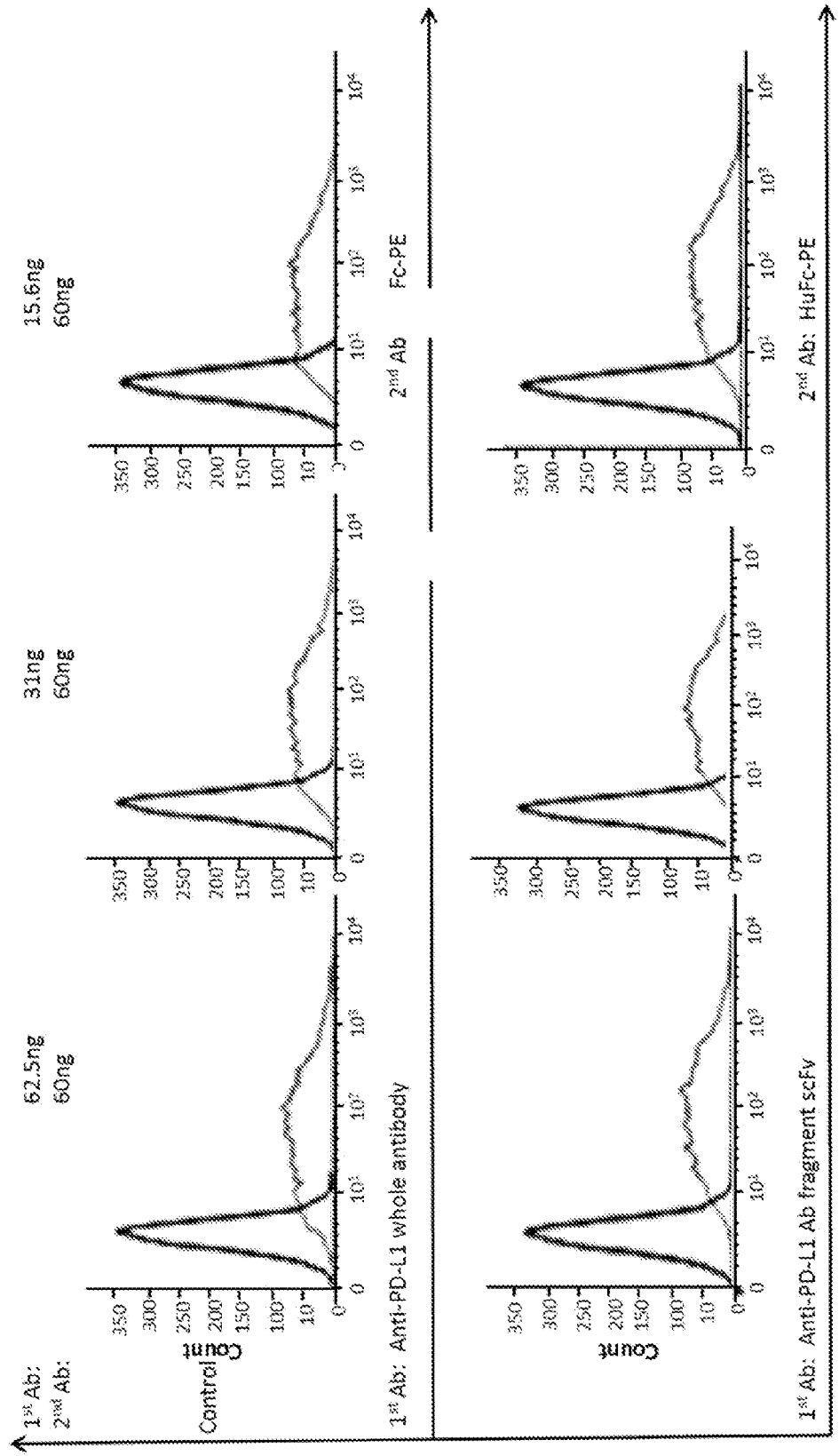

Week 0 in Na-citrate buffer, pH=5.5
1st Ab:
2nd Ab:
Control
62.5ng
60ng
31ng
60ng
15.6ng
60ng
Count
1st Ab: Anti-PD-L1 whole antibody
2nd Ab Fc-PE
1st Ab: Anti-PD-L1 Ab fragment scFv
2nd Ab: HuFc-PE

FIG. 13C

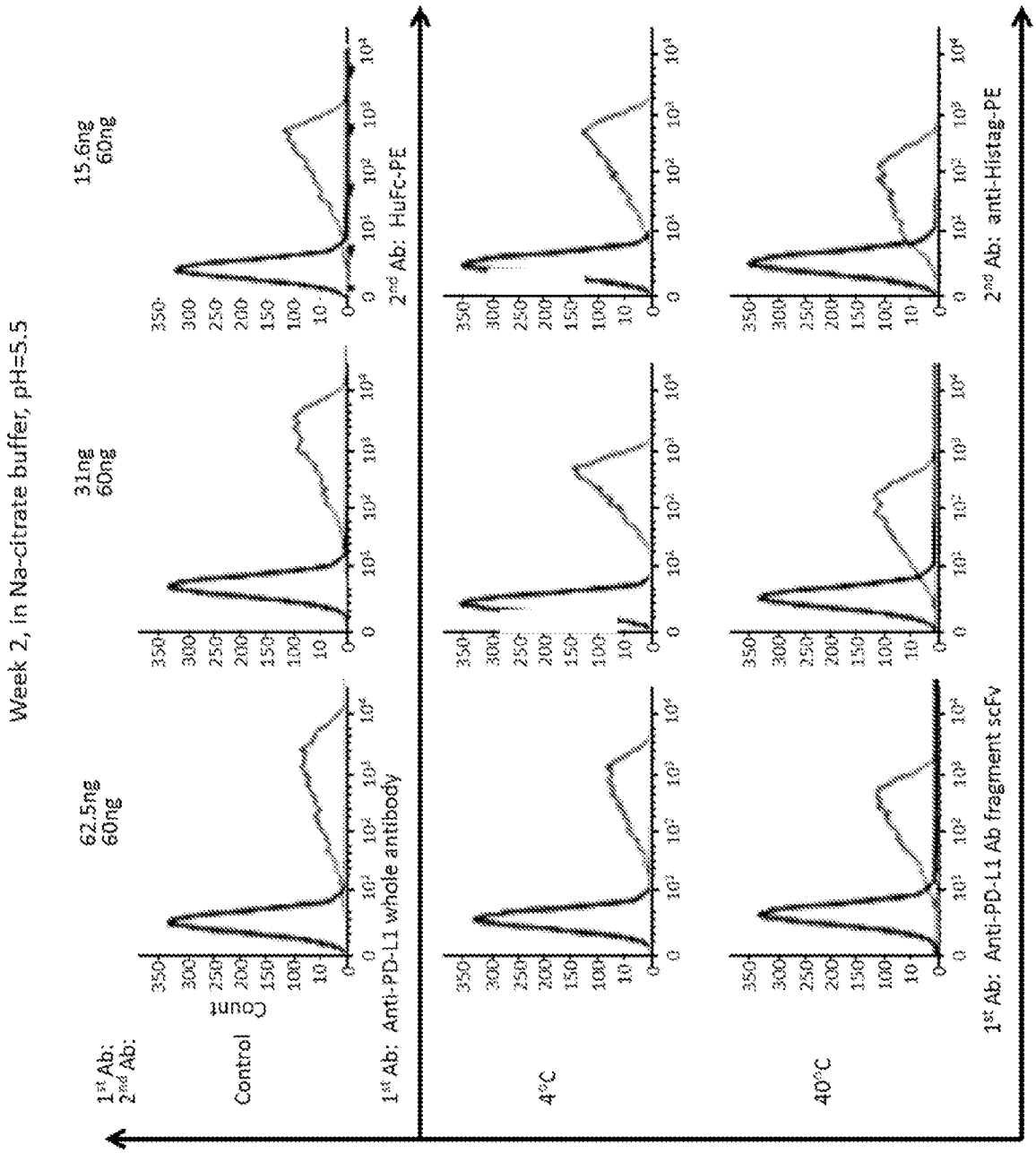

Week 2, in Na-citrate buffer, pH=5.5
62.5ng
60ng
31ng
60ng
15.6ng
60ng
1st Ab:
2nd Ab:
Control
4°C
40°C
Count
1st Ab: Anti-PD-L1 whole antibody
2nd Ab: HuFc-PE
1st Ab: Anti-PD-L1 Ab fragment scFv
2nd Ab: anti-Histag-PE

FIG. 13D

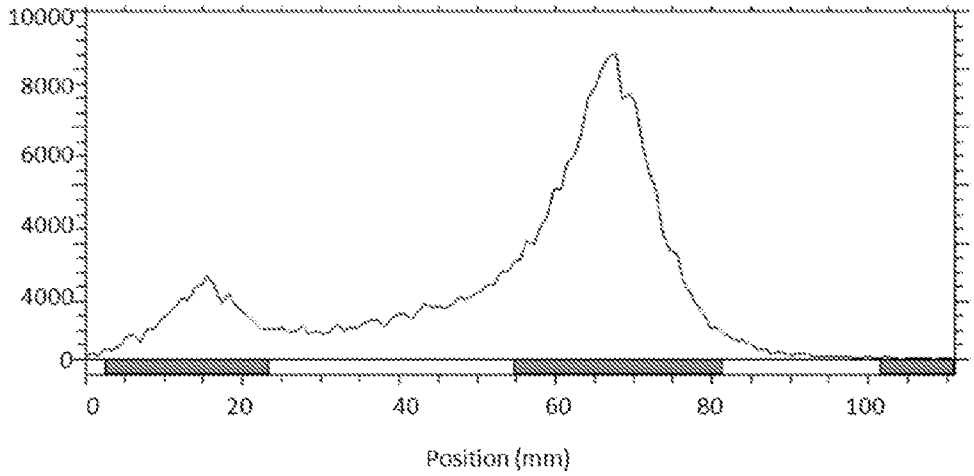

10000
8000
6000
4000
4000
0
0
20
40
60
80
100
Position (mm)

FIG. 14

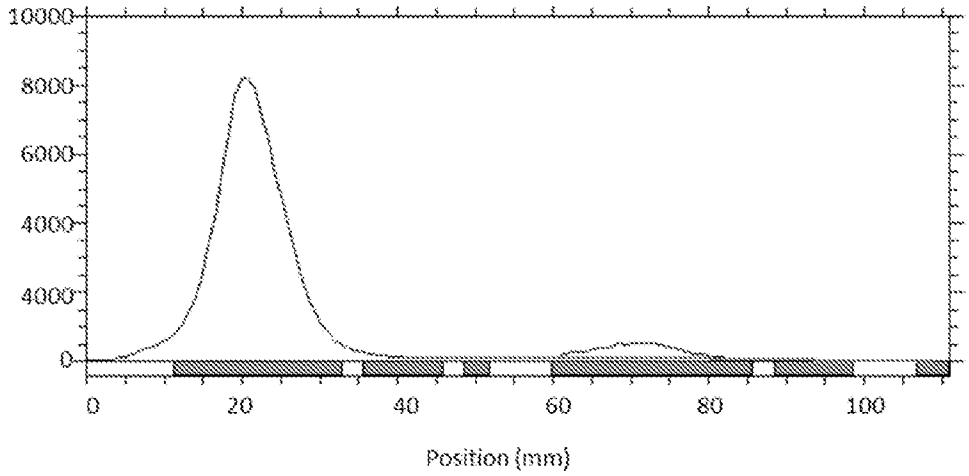

10000
8000
6000
4000
4000
0
0
20
40
60
80
100
Position (mm)

FIG. 15

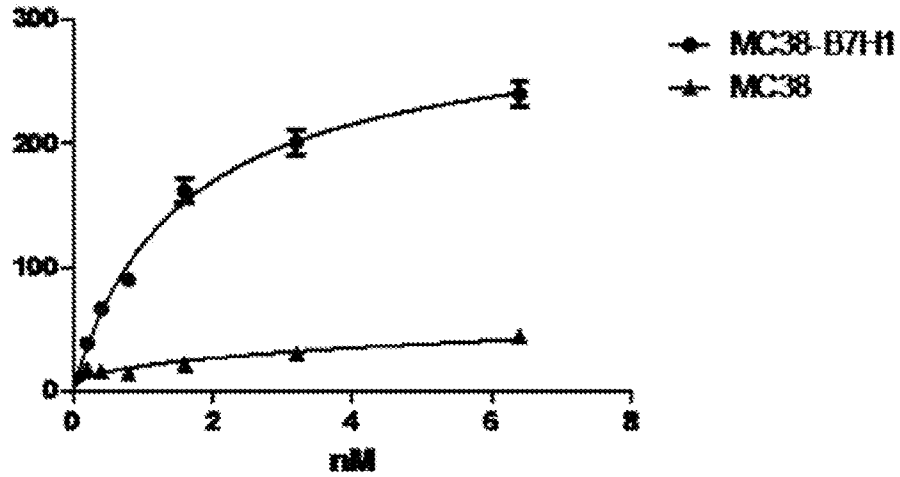

300
200
100
0
0
2
4
6
8
nM
MC38-B7H1
MC38

FIG. 16

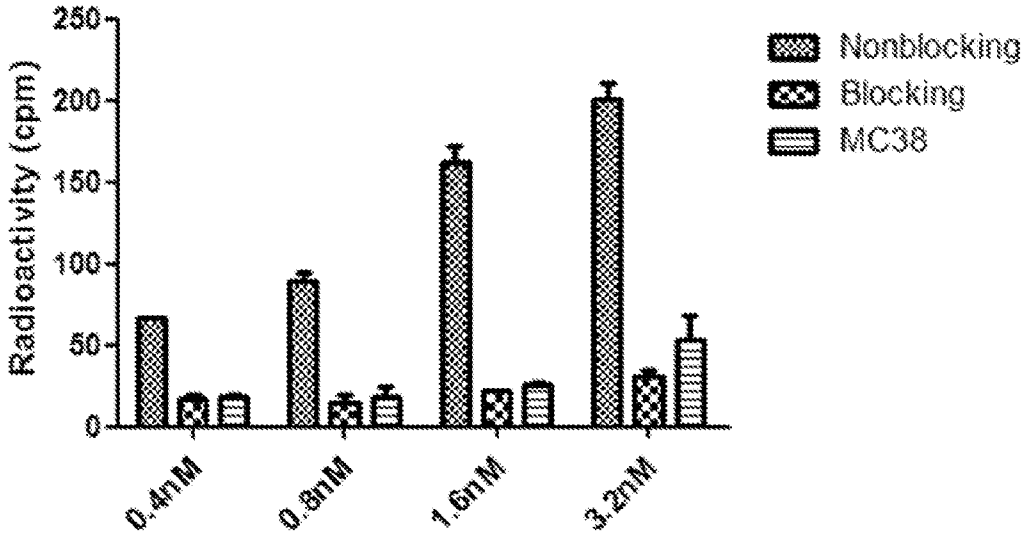

250
200
150
100
50
0
Radioactivity (cpm)
0.4nM
0.8nM
1.6nM
3.2nM
Nonblocking
Blocking
MC38

FIG. 17

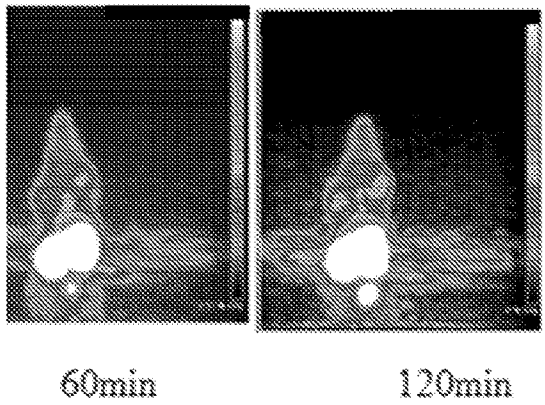


FIG. 18

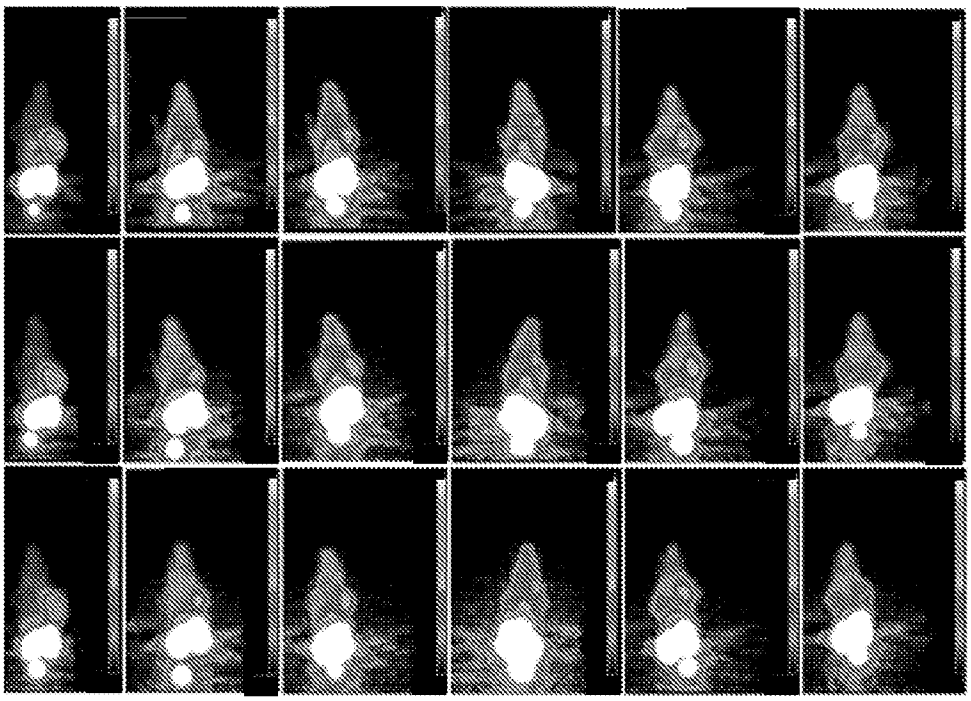


FIG. 19

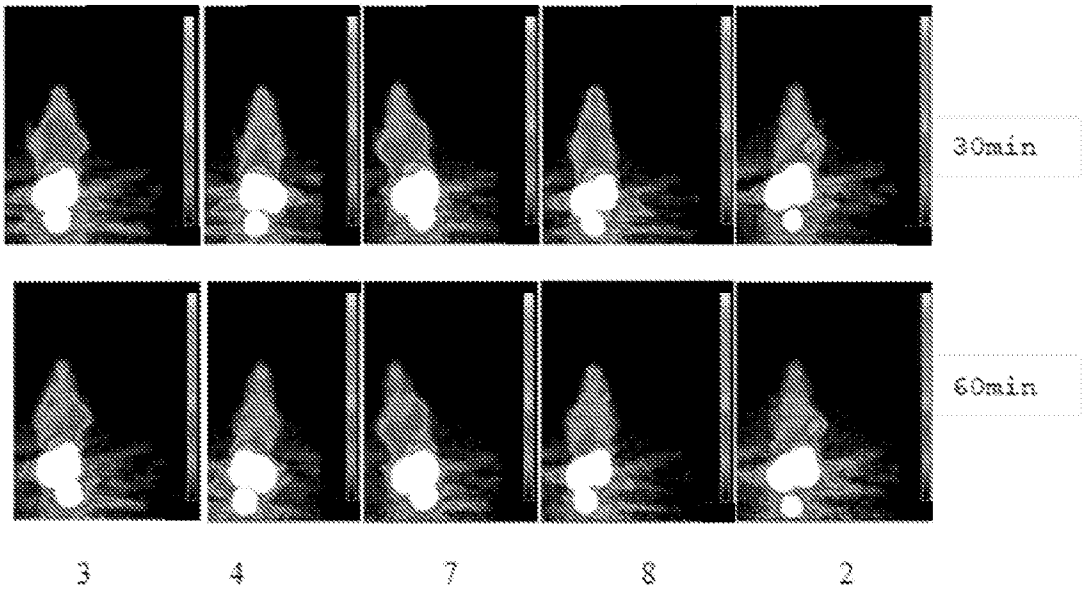


FIG. 20

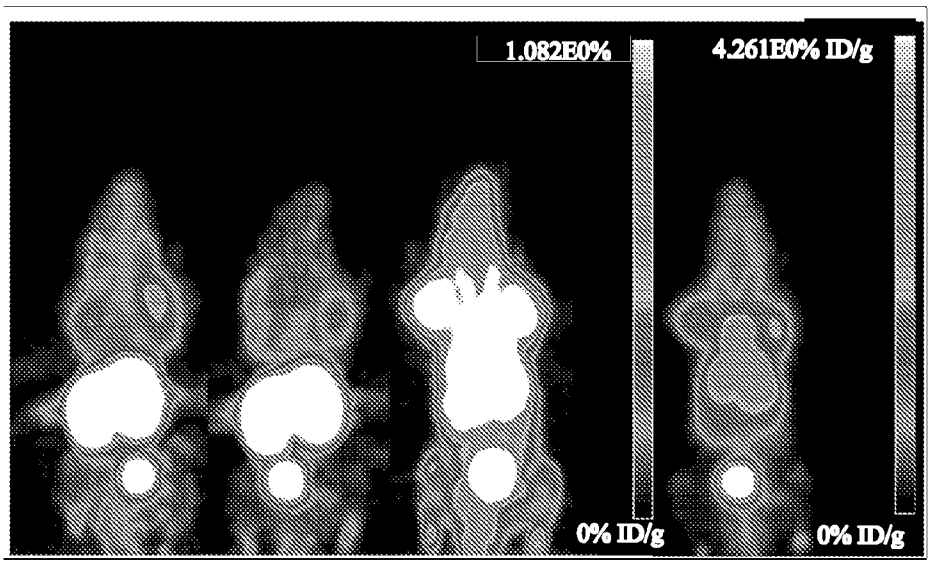


FIG. 21

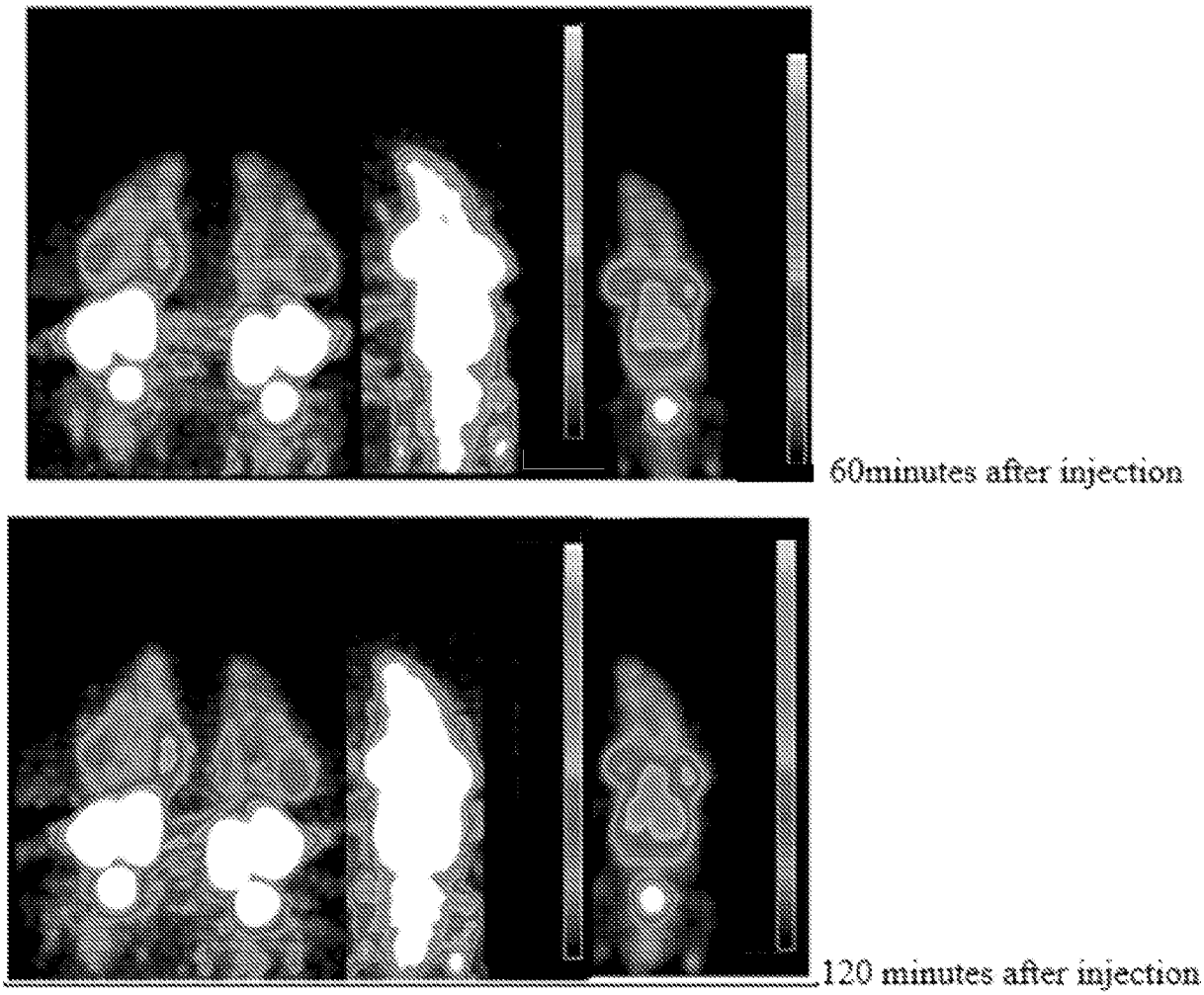


FIG. 22

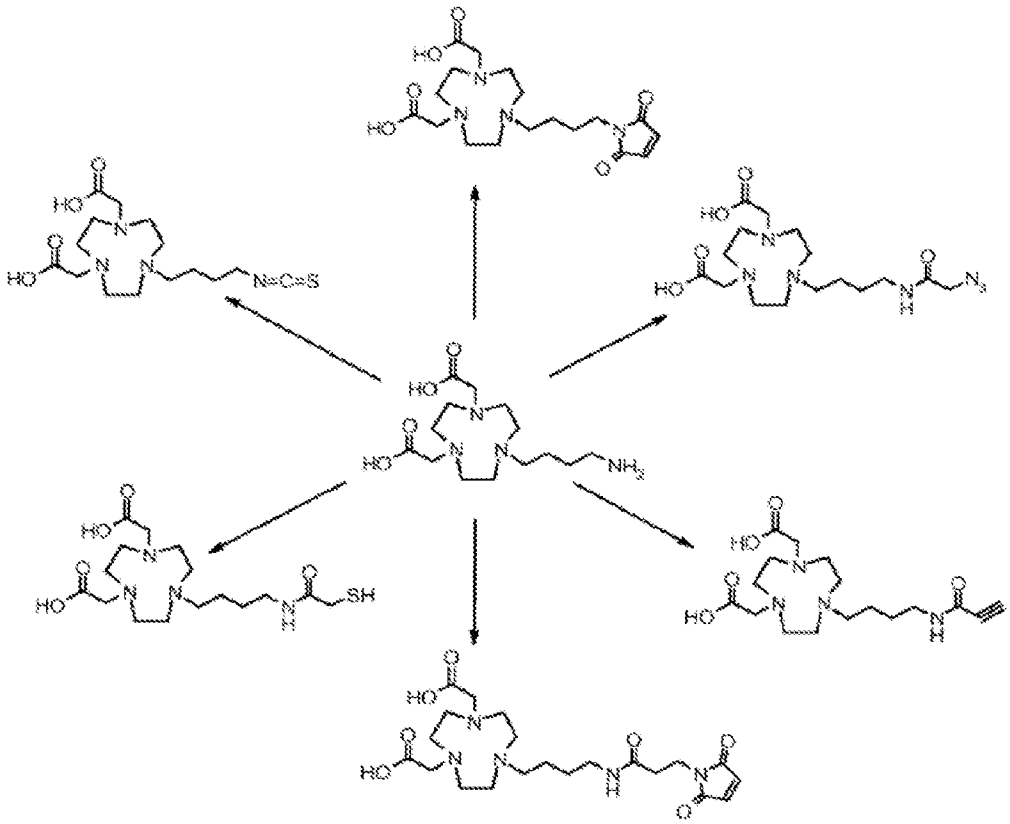


FIG. 23

INTERNATIONAL SEARCH REPORT

International application No.
PCT/CN2019/089606

A. CLASSIFICATION OF SUBJECT MATTER

C07K 16/28(2006.01)i; C12N 15/13(2006.01)i; A61K 39/395(2006.01)i; A61P 35/00(2006.01)i

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

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)

C07K; C12N; A61K; A61P

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

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

DWPI, SIPOABS, CPRSABS, CPEA, CNABS, JPABS, CNMED, KRABS, CNTXT, USTXT, WOTXT, EPTXT, JPTXT, KRTXT, CNKI, ISI Web of Science, Genbank: PD-L1, PDL1, PDL-1, anti-PD-L1, programmed cell death 1 ligand 1, CD274, B7 homolog 1, B7-H1, antibody, sequence search on SEQ ID NOs: 1,3,5,7,9,11,13,15,17,19,21,23,41-46

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
A	CN 107921131 A (LILLY CO ELI) 17 April 2018 (2018-04-17) see the whole document	1-29
A	WO 2017020858 A1 (WUXI BIOLOGICS SHANGHAI CO LTDET AL.) 09 February 2017 (2017-02-09) see the whole document	1-29

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

* Special categories of cited documents:

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

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

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

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

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

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

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

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

"&" document member of the same patent family

Date of the actual completion of the international search	Date of mailing of the international search report
30 August 2019	**10 September 2019**

Name and mailing address of the ISA/CN	Authorized officer
National Intellectual Property Administration, PRC **6, Xitucheng Rd., Jimen Bridge, Haidian District, Beijing 100088** **China**	**XING,Ying**
Facsimile No. **(86-10)62019451**	Telephone No. **62411044**

INTERNATIONAL SEARCH REPORT

International application No.
PCT/CN2019/089606

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

1. With regard to any nucleotide and/or amino acid sequence disclosed in the international application, the international search was carried out on the basis of a sequence listing:
   a. ☑ forming part of the international application as filed:
      ☑ in the form of an Annex C/ST.25 text file.
      ☐ on paper or in the form of an image file.
   b. ☐ furnished together with the international application under PCT Rule 13*ter*.1(a) for the purposes of international search only in the form of an Annex C/ST.25 text file.
   c. ☐ furnished subsequent to the international filing date for the purposes of international search only:
      ☐ in the form of an Annex C/ST.25 text file (Rule 13*ter*.1(a)).
      ☐ on paper or in the form of an image file (Rule 13*ter*.1(b) and Administrative Instructions, Section 713).

2. ☐ In addition, in the case that more than one version or copy of a sequence listing has been filed or furnished, the required statements that the information in the subsequent or additional copies is identical to that forming part of the application as filed or does not go beyond the application as filed, as appropriate, were furnished.

3. Additional comments:

INTERNATIONAL SEARCH REPORT

International application No.
PCT/CN2019/089606

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

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

1. ☑ Claims Nos.: **23,24**
because they relate to subject matter not required to be searched by this Authority, namely:

[1] The subject matter of claims 23 and 24 relates to a method for treatment of the human or animal body by therapy (PCT Rule 39.1(iv)). However, the search of claims 23 and 24 has been carried out on the basis of the reasonably anticipant subject i.e. use of an effective amount of the anti-PD-L1 antibody agent in preparation of a medicament for treating a disease or condition in an individual.

2. ☐ Claims Nos.:
because they relate to parts of the international application that do not comply with the prescribed requirements to such an extent that no meaningful international search can be carried out, specifically:

3. ☐ Claims Nos.:
because they are dependent claims and are not drafted in accordance with the second and third sentences of Rule 6.4(a).

INTERNATIONAL SEARCH REPORT
Information on patent family members

International application No.
PCT/CN2019/089606

Patent document cited in search report			Publication date (day/month/year)	Patent family member(s)			Publication date (day/month/year)
CN	107921131	A	17 April 2018	US	2019144544	A1	16 May 2019
				AR	105654	A1	25 October 2017
				CR	20180048	A	20 March 2018
				EP	3341020	A1	04 July 2018
				CA	2994425	A1	02 March 2017
				US	2017058033	A1	02 March 2017
				US	10214586	B2	26 February 2019
				BR	112018001506	A2	18 September 2018
				AU	2016313404	B2	04 April 2019
				KR	20180030899	A	26 March 2018
				PE	6042018	A1	09 April 2018
				TW	201718650	A	01 June 2017
				MX	2018002170	A	24 April 2018
				SV	2018005640	A	23 July 2018
				IL	256922	D0	29 March 2018
				JP	2018531219	A	25 October 2018
				CL	2018000454	A1	03 August 2018
				PH	12018500394	A1	29 August 2018
				DO	P2018000045	A	30 March 2018
				AU	2016313404	A1	08 February 2018
				JP	6518005	B2	22 May 2019
				WO	2017034916	A1	02 March 2017
				EA	201890278	A1	31 July 2018
WO	2017020858	A1	09 February 2017	EP	3332006	A4	09 January 2019
				KR	20180037222	A	11 April 2018
				BR	112018002428	A2	18 September 2018
				CA	2993272	A1	09 February 2017
				AU	2016301718	A1	18 January 2018
				EP	3332006	A1	13 June 2018
				JP	2018527949	A	27 September 2018
				IL	256803	D0	29 March 2018
				MX	2018001530	A	01 August 2018
				WO	2017020291	A1	09 February 2017