

CHECKPOINT INHIBITOR BISPECIFIC ANTIBODIES

The present invention relates to the field of medicine. More particularly, the present invention relates to bispecific antibodies that bind human programmed cell death 1 (PD-1) and human PD-1 ligand 1 (PD-L1), and may be useful for treating solid and hematological tumors alone and in combination with chemotherapy and other cancer therapeutics.

Immune checkpoint pathways are used in maintenance of self-tolerance and control of T cell activation, but cancer cells can use the pathways to suppress the anti-tumor response and prevent their destruction. The PD-1/PD-L1 pathway is one such immune checkpoint. Human PD-1 is found on T cells and human PD-L1 is aberrantly expressed by a variety of tumor types; binding of PD-L1 to PD-1 inhibits T cell proliferation and cytokine production. The PD-1/PD-L1 inhibitory axis has been subjugated by tumors as part of the natural selective process that shapes tumor evolution in the context of an anti-tumor immune response.

While therapeutics targeting the PD-1/PD-L1 pathway are clinically validated and have led to significant clinical advances for treatment of cancer, only a fraction of patients have benefited from such a treatment (see, for example, Sharma, P. and Allison, J.P., Immune checkpoint targeting in cancer therapy: toward combination strategies with curative potential. *Cell*. 2015; 161:2015-14). For example, only ~20% of the patients with non-small cell lung cancer (NSCLC) responded to PD-1 antibody treatment.

Although clinical trials involving co-administration of a PD-L1 antibody and a PD-1 antibody are currently underway (see, for example, EUROPEAN SOCIETY FOR MEDICAL ONCOLOGY (ESMO) Abstract #2130; Oct. 2016), these treatment regimens involve infusions of two separate antibody products at relatively high dosages for each antibody. Furthermore, it is not known yet if such combination therapies will provide improvements in efficacy without exacerbating the adverse event profile as compared to monotherapy.

WO2017/087547 specifically discloses anti-PD-L1 antibodies and generally discloses heterodimeric molecules (e.g., a bispecific agent) comprising a PD-L1-binding agent described therein and "a second immunotherapeutic agent". In some embodiments

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the second immunotherapeutic agent may include “an antibody that blocks immunosuppressive functions” such as an “anti-PD- antibody”. However, no specific PD-L1/PD-1 bispecific agents were disclosed in this publication. Thus, a bispecific antibody that binds PD-L1 and PD-1 with high affinity, effectively neutralizes PD-L1 and PD-1 activation by all PDx family ligands, and/or provides superior activity relative to known therapeutics targeting the PD-1/PD-L1 pathway, or even combinations thereof, is needed as a more effective pharmacological intervention for certain cancers.

5 and PD-1 activation by all PDx family ligands, and/or provides superior activity relative to known therapeutics targeting the PD-1/PD-L1 pathway, or even combinations thereof, is needed as a more effective pharmacological intervention for certain cancers. Particularly, desirable are such anti-PD-L1/PD-1 bispecific antibodies that i) may more effectively treat cancers characterized as having moderate or high PD-L1 or PD-1 expression levels and ii) demonstrate *in vivo* stability, physical and chemical stability including, but not limited to, thermal stability, solubility, low self-association, and pharmacokinetic characteristics which are acceptable for development and/or use in the treatment of cancer.

Accordingly, the present invention provides novel heterodimeric bispecific antibodies that can target PD-L1 and PD-1 simultaneously, via the pairing of two different heavy chains and two different light chains into a single IgG-like antibody. Furthermore, the present invention provides anti-human PD-L1 and anti-human PD-1 heterodimeric bispecific antibodies that possess one or more of the following features: block all three interactions of the PD axis (PD-L1 binding to PD-1, PD-L2 binding to PD-1 and PD-L1 binding to CD80), bridge PD-L1 and PD-1 over-expressing cells, increase T cell activation and tumor cell killing due to proximity of bound T cell and tumor cell, demonstrate significant antitumor activity at surprisingly low dosages in tumor cells with moderate to high PD ligand expression levels, and demonstrate unexpected physical and chemical stability including, but not limited to, *in vivo* stability, thermal stability, solubility, low self-association, and pharmacokinetic characteristics.

Accordingly, the present invention provides an antibody that binds human PD-L1 (SEQ ID NO: 1) and human PD-1 (SEQ ID NO: 2) comprising:

- a) a first heavy chain (HC1) comprising a heavy chain variable region (HCVR) comprising the amino acid sequence of SEQ ID NO: 3;
- 30 b) a first light chain (LC1) comprising a light chain variable region (LCVR) comprising the amino acid sequence of SEQ ID NO: 4;

- c) a second heavy chain (HC2) comprising a heavy chain variable region comprising the amino acid sequence of SEQ ID NO: 5; and
- d) a second light chain (LC2) comprising a light chain variable region comprising the amino acid sequence of SEQ ID NO: 8.

5 The present invention provides an antibody that binds human PD-L1 (SEQ ID NO: 1) and human PD-1 (SEQ ID NO: 2), comprising a HC1, a LC1, a HC2, and a LC2, wherein:

- a) said HC1 comprises a CDR1 having the amino acid sequence of SEQ ID NO: 16, a CDR2 having the amino acid sequence of SEQ ID NO: 17, and a CDR3 having the amino acid sequence of SEQ ID NO: 18 in the HCVR;
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- b) said LC1 comprises a CDR1 having the amino acid sequence of SEQ ID NO: 19, a CDR2 having the amino acid sequence of SEQ ID NO: 20, and a CDR3 having the amino acid sequence of SEQ ID NO: 21 in the LCVR;
- c) said HC2 comprises a CDR1 having the amino acid sequence of SEQ ID NO: 22, a CDR2 having the amino acid sequence of SEQ ID NO: 23 or SEQ ID NO: 24, and a CDR3 having the amino acid sequence of SEQ ID NO: 25 in the HCVR;
15
- d) said LC2 comprises a CDR1 having the amino acid sequence of SEQ ID NO: 26, a CDR2 having the amino acid sequence of SEQ ID NO: 27, and a CDR3 having the amino acid sequence of SEQ ID NO: 28 in the LCVR;
- 20 e) the CH1 domain of the HC1 comprises an amino acid substitution of S183K;
- f) the constant region of the LC1 is a human lambda variant comprising amino acid substitutions of S176E and Y178E;
- g) the CH1 domain of the HC2 comprises amino acid substitutions of L128E, K147T, and Q175E;
- 25 h) the constant region of the LC2 is a human kappa variant comprising amino acid substitutions of S131R, V133G, and S176R; and
- i) the CH3 domain of the HC1 comprises amino acid substitutions of T350V, L351Y, F405A, and Y407V and the CH3 domain of the HC2 comprises amino acid substitutions of T350V, T366L, K392L, and T394W; or the CH3 domain of the HC1
30 comprises amino acid substitutions of T350V, T366L, K392L, and T394W and the

CH3 domain of the HC2 comprises amino acid substitutions of T350V, L351Y, F405A, and Y407V; and,

- j) wherein the HC1 and HC2 are immune effector function null human IgG1 Fc variants, wherein the numbering is according to the EU index. Preferably, the HC1 and HC2
5 comprise amino acid substitutions of L234A, L235A, and D265S.

The present invention provides an antibody that binds human PD-L1 (SEQ ID NO: 1) and human PD-1 (SEQ ID NO: 2) comprising:

- a) a HC1 comprising, in order from the N-terminus to the C-terminus, the HCVR comprising the amino acid sequence of SEQ ID NO: 3, the amino acid sequence of
10 SEQ ID NO: 9 in the CH1 domain, the amino acid sequence of SEQ ID NO: 10 in the CH2 domain, and the amino acid sequence of SEQ ID NO: 11 or SEQ ID NO: 12 in the CH3 domain;
- b) a LC1 comprising, in order from the N-terminus to the C-terminus, the LCVR comprising the amino acid sequence of SEQ ID NO: 4 and the amino acid sequence
15 of SEQ ID NO: 14 in the constant region;
- c) a HC2 comprising, in order from the N-terminus to the C-terminus, the HCVR comprising the amino acid sequence of SEQ ID NO: 5, the amino acid sequence of
20 SEQ ID NO: 13 in the CH1 domain, the amino acid sequence of SEQ ID NO: 10 in the CH2 domain, and the amino acid sequence of SEQ ID NO: 12 or the amino acid sequence of SEQ ID NO: 11 in the CH3 domain; and
- d) the LC2 comprising, in order from N-terminus, the LCVR comprising the amino acid sequence of SEQ ID NO: 8 and the amino acid sequence of SEQ ID NO: 15 in the
constant region, provided that when the amino acid sequence of SEQ ID NO: 11 is present in the CH3 domain of said HC1, the amino acid sequence of SEQ ID NO: 12
25 is present in the CH3 domain of said HC2; or when the amino acid sequence of SEQ ID NO: 12 is present in the CH3 domain of said HC1, the amino acid sequence of SEQ ID NO: 11 is present in the CH3 domain of said HC2.

The present invention further provides an antibody comprising a HC1, LC1, HC2, and LC2 wherein:

- 30 a) said HC1 comprises, in order from the N-terminus to the C-terminus, a HCVR comprising the amino acid sequence of SEQ ID NO: 3, the amino acid sequence of

SEQ ID NO: 9 in the CH1 domain, the amino acid sequence of SEQ ID NO: 10 in the CH2 domain, and the amino acid sequence of SEQ ID NO: 11 in the CH3 domain;

- b) said LC1 comprising, in order from the N-terminus to the C-terminus, a LCVR comprising the amino acid sequence of SEQ ID NO: 4, and the amino acid sequence of SEQ ID NO: 14 in the constant region; and
- c) said HC2 comprising, in order from the N-terminus to the C-terminus, a HCVR comprising the amino acid sequence of SEQ ID NO: 6 the amino acid sequence of SEQ ID NO: 13 in the CH1 domain, the amino acid sequence of SEQ ID NO: 10 in the region of the CH2 domain, and the amino acid sequence of SEQ ID NO: 12 in the CH3 domain; and
- d) said LC2 comprising, in order from N-terminus, a LCVR comprising the amino acid sequence of SEQ ID NO: 8, and the amino acid sequence of SEQ ID NO: 15 in the constant region.

The present invention provides an antibody that binds human PD-L1 (SEQ ID NO: 1) and human PD-1 (SEQ ID NO: 2) comprising:

- a) a HC1 comprising the amino acid sequence of SEQ ID NO: 49;
- b) a LC1 comprising the amino acid sequence of SEQ ID NO: 30;
- c) a HC2 comprising the amino acid sequence of SEQ ID NO 31; and
- d) a LC2 comprising the amino acid sequence of SEQ ID NO: 34.

The present invention provides an antibody that binds human PD-L1 (SEQ ID NO: 1) and human PD-1 (SEQ ID NO: 2) comprising:

- a) a HC1 comprising the amino acid sequence of SEQ ID NO: 49;
- b) a LC1 comprising the amino acid sequence of SEQ ID NO: 30;
- c) a HC2 comprising the amino acid sequence of SEQ ID NO 33; and
- d) a LC2 comprising the amino acid sequence of SEQ ID NO: 34.

The present invention provides an antibody that binds human PD-L1 (SEQ ID NO: 1) and human PD-1 (SEQ ID NO: 2) comprising:

- a) a HC1 comprising the amino acid sequence of SEQ ID NO: 29;
- b) a LC1 comprising the amino acid sequence of SEQ ID NO: 30;
- c) a HC2 comprising the amino acid sequence of SEQ ID NO: 33; and
- d) a LC2 comprising the amino acid sequence of SEQ ID NO: 34.

The present invention provides a mammalian cell comprising a DNA molecule comprising a polynucleotide sequence encoding polypeptides having the amino acid sequences of SEQ ID NO: 49, SEQ ID NO: 30, SEQ ID NO: 31, and SEQ ID NO: 34, wherein the cell is capable of expressing an antibody of the present invention.

5 The present invention provides a mammalian cell comprising a DNA molecule comprising a polynucleotide sequence encoding polypeptides having the amino acid sequences of SEQ ID NO: 49, SEQ ID NO: 30, SEQ ID NO: 33, and SEQ ID NO: 34, wherein the cell is capable of expressing an antibody of the present invention.

10 The present invention provides a mammalian cell comprising a DNA molecule comprising a polynucleotide sequence encoding polypeptides having the amino acid sequences of SEQ ID NO: 29, SEQ ID NO: 30, SEQ ID NO: 33, and SEQ ID NO: 34, wherein the cell is capable of expressing an antibody of the present invention.

15 The present invention provides a process for producing an antibody of the present invention comprising cultivating a mammalian cell of the present invention under conditions such that the antibody is expressed, and recovering the expressed antibody.

 The present invention provides an antibody produced by a process of the present invention.

 The present invention provides a pharmaceutical composition, comprising an antibody of the present invention and an acceptable carrier, diluent, or excipient.

20 The present invention provides a method of treating cancer, comprising administering to a patient in need thereof, an effective amount of an antibody of the present invention. The present invention further provides a method of treating cancer wherein said method comprises administering to a patient in need thereof, an effective amount of an antibody of the present invention, wherein the cancer is Hodgkin's or non-Hodgkin's lymphomas, melanoma, renal cell cancer, kidney cancer, lung cancer, bladder cancer, gastric and esophageal cancer, colorectal cancer, liver cancer, hepatocellular cancer, cholangiocarcinoma, pancreatic cancer, breast cancer, triple-negative breast cancer, ovarian cancer, endometrial cancer, prostate cancer, small cell lung cancer (SCLC), non-small cell lung cancer (NSCLC), mesothelioma, squamous cancer of head
25 neck cancer (SCCHN), soft tissue sarcoma, or glioblastoma multiforme.
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The present invention provides a method of treating cancer, wherein the cancer is melanoma. The present invention further provides a method of treating cancer, wherein the cancer is lung cancer. The present invention further provides a method of treating cancer, wherein the lung cancer is NSCLC (squamous and non-squamous), small cell lung cancer, or mesothelioma. The present invention further provides a method of treating cancer, wherein the cancer is head and neck cancer. The present invention further provides a method of treating cancer, wherein the cancer is liver cancer. The present invention further provides a method of treating cancer, wherein the cancer is colorectal cancer. The present invention further provides a method of treating cancer, wherein the cancer is pancreatic cancer. The present invention further provides a method of treating cancer, wherein the cancer is gastric cancer. The present invention further provides a method of treating cancer, wherein the cancer is kidney cancer. The present invention further provides a method of treating cancer, wherein the cancer is bladder cancer. The present invention further provides a method of treating cancer, wherein the cancer is prostate cancer. The present invention further provides a method of treating cancer, wherein the cancer is breast cancer. The present invention further provides a method of treating cancer, wherein the cancer is ovarian cancer. The present invention further provides a method of treating cancer, wherein the cancer is endometrial cancer. The present invention further provides a method of treating cancer, wherein the cancer is esophageal cancer. The present invention further provides a method of treating cancer, wherein the cancer is soft tissue sarcoma. The present invention further provides a method of treating cancer, wherein the cancer is cholangiocarcinoma. The present invention further provides a method of treating cancer, wherein the cancer is hepatocellular carcinoma.

The present invention further provides methods comprising the administration of an effective amount of the antibody of the present invention in simultaneous, separate, or sequential combination with one or more anti-tumor agents selected from the group consisting of cisplatin, carboplatin, dacarbazine, liposomal doxorubicin, docetaxel, cyclophosphamide and doxorubicin, navelbine, eribulin, paclitaxel, paclitaxel protein-bound particles for injectable suspension, ixabepilone, capecitabine, FOLFOX (leucovorin, fluorouracil, and oxaliplatin), FOLFIRI (leucovorin, fluorouracil, and

irinotecan), gemcitabine, topotecan, liposomal irinotecan, pemetrexed, cetuximab, nivolumab, ipilimumab, pidilizumab, pembrolizumab, tremelimumab, urelumab, lirilumab, atezolizumab, epacadostat, and durvalumab.

5 The present invention further provides methods comprising the administration of an effective amount of the antibody of the present invention comprising simultaneous, separate, or sequential combination with ionizing radiation.

10 The present invention provides an antibody of the present invention, for use in therapy. The present invention provides an antibody of the present invention, for use in the treatment of cancer. The present invention provides an antibody of the present invention, for use in the treatment of cancer, wherein the cancer is Hodgkin's or non-Hodgkin's lymphomas, melanoma, renal cell cancer, kidney cancer, lung cancer, bladder cancer, gastric and esophageal cancer, colorectal cancer, liver cancer, hepatocellular cancer, cholangiocarcinoma, pancreatic cancer, breast cancer, triple-negative breast cancer, ovarian cancer, endometrial cancer, prostate cancer, small cell lung cancer
15 (SCLC), non-small cell lung cancer (NSCLC), mesothelioma, squamous cancer of head neck cancer (SCCHN), soft tissue sarcoma, or glioblastoma multiforme.

The present invention provides an antibody of the present invention, for use in the treatment of melanoma. The present invention provides an antibody of the present invention, for use in the treatment of lung cancer. The present invention further provides
20 an antibody of the present invention, wherein the lung cancer is NSCLC (squamous and non-squamous), small cell lung cancer, or mesothelioma. The present invention provides an antibody of the present invention, for use in the treatment of head and neck cancer. The present invention provides an antibody of the present invention, for use in the treatment of liver cancer. The present invention provides an antibody of the present invention, for use in the treatment of colorectal cancer. The present invention provides an
25 antibody of the present invention, for use in the treatment of pancreatic cancer. The present invention provides an antibody of the present invention, for use in the treatment of gastric cancer. The present invention provides an antibody of the present invention, for use in the treatment of kidney cancer. The present invention provides an antibody of the present invention, for use in the treatment of bladder cancer. The present invention
30 provides an antibody of the present invention, for use in the treatment of prostate cancer.

The present invention provides an antibody of the present invention, for use in the treatment of breast cancer. The present invention provides an antibody of the present invention, for use in the treatment of ovarian cancer. The present invention provides an antibody of the present invention, for use in the treatment of endometrial cancer. The present invention provides an antibody of the present invention, for use in the treatment of esophageal cancer. The present invention provides an antibody of the present invention, for use in the treatment of soft tissue sarcoma. The present invention provides an antibody of the present invention, for use in the treatment of cholangiocarcinoma. The present invention provides an antibody of the present invention, for use in the treatment of hepatocellular carcinoma.

The present invention provides the antibody of the present invention for use in simultaneous, separate, or sequential combination with one or more anti-tumor agents selected from the group consisting of cisplatin, carboplatin, dacarbazine, liposomal doxorubicin, docetaxel, cyclophosphamide and doxorubicin, navelbine, eribulin, paclitaxel, paclitaxel protein-bound particles for injectable suspension, ixabepilone, capecitabine, FOLFOX (leucovorin, fluorouracil, and oxaliplatin), FOLFIRI (leucovorin, fluorouracil, and irinotecan), gemcitabine, topotecan, liposomal irinotecan, pemetrexed, cetuximab, nivolumab, ipilimumab, pidilizumab, pembrolizumab, tremelimumab, urelumab, lirilumab, atezolizumab, epacadostat, and durvalumab, in the treatment of cancer.

The present invention provides the antibody of the present invention for use in simultaneous, separate, or sequential combination with ionizing radiation, in the treatment of cancer.

The present invention provides a pharmaceutical composition for use in treating cancer, comprising an effective amount of an antibody of the present invention. The present invention further provides a pharmaceutical composition for use in treating cancer, comprising an effective amount of an antibody of the present invention, wherein the cancer is Hodgkin's or non-Hodgkin's lymphomas, melanoma, renal cell cancer, kidney cancer, lung cancer, bladder cancer, gastric and esophageal cancer, colorectal cancer, liver cancer, hepatocellular cancer, cholangiocarcinoma, pancreatic cancer, breast cancer, triple-negative breast cancer, ovarian cancer, endometrial cancer, prostate cancer,

small cell lung cancer (SCLC), non-small cell lung cancer (NSCLC), mesothelioma, squamous cancer of head neck cancer (SCCHN), soft tissue sarcoma, or glioblastoma multiforme. The present invention further provides a pharmaceutical composition for use in treating cancer, comprising an effective amount of an antibody of the present
5 invention, wherein the lung cancer is NSCLC (squamous and non-squamous), small cell lung cancer, or mesothelioma.

The present invention provides a pharmaceutical composition for use in treating melanoma, comprising an effective amount of an antibody of the present invention. The present invention provides a pharmaceutical composition for use in treating lung cancer,
10 including, but not limited to, NSCLC (squamous and non-squamous), small cell lung cancer, or mesothelioma comprising an effective amount of an antibody of the present invention. The present invention provides a pharmaceutical composition for use in treating head and neck cancer, comprising an effective amount of an antibody of the present invention. The present invention provides a pharmaceutical composition for use
15 in treating liver cancer, comprising an effective amount of an antibody of the present invention. The present invention provides a pharmaceutical composition for use in treating colorectal cancer, comprising an effective amount of an antibody of the present invention. The present invention provides a pharmaceutical composition for use in treating pancreatic cancer, comprising an effective amount of an antibody of the present
20 invention. The present invention provides a pharmaceutical composition for use in treating gastric cancer, comprising an effective amount of an antibody of the present invention. The present invention provides a pharmaceutical composition for use in treating kidney cancer, comprising an effective amount of an antibody of the present invention. The present invention provides a pharmaceutical composition for use in
25 treating bladder cancer, comprising an effective amount of an antibody of the present invention. The present invention provides a pharmaceutical composition for use in treating prostate cancer, comprising an effective amount of an antibody of the present invention. The present invention provides a pharmaceutical composition for use in treating breast cancer, comprising an effective amount of an antibody of the present
30 invention. The present invention provides a pharmaceutical composition for use in treating ovarian cancer, comprising an effective amount of an antibody of the present

invention. The present invention provides a pharmaceutical composition for use in treating endometrial cancer, comprising an effective amount of an antibody of the present invention. The present invention provides a pharmaceutical composition for use in treating esophageal cancer, comprising an effective amount of an antibody of the present invention. The present invention provides a pharmaceutical composition for use in treating soft tissue sarcoma, comprising an effective amount of an antibody of the present invention. The present invention provides a pharmaceutical composition for use in treating cholangiocarcinoma, comprising an effective amount of an antibody of the present invention. The present invention provides a pharmaceutical composition for use in treating hepatocellular carcinoma, comprising an effective amount of an antibody of the present invention.

The present invention further provides a pharmaceutical composition for use in treating cancer, wherein said pharmaceutical composition is administered in simultaneous, separate, or sequential combination with one or more antitumor agents selected from the group consisting of cisplatin, carboplatin, dacarbazine, liposomal doxorubicin, docetaxel, cyclophosphamide and doxorubicin, navelbine, eribulin, paclitaxel, paclitaxel protein-bound particles for injectable suspension, ixabepilone, capecitabine, FOLFOX (leucovorin, fluorouracil, and oxaliplatin), FOLFIRI (leucovorin, fluorouracil, and irinotecan), gemcitabine, topotecan, liposomal irinotecan, pemetrexed, cetuximab, nivolumab, ipilimumab, pidilizumab, pembrolizumab, tremelimumab, urelumab, lirilumab, atezolizumab, epacadostat, and durvalumab.

The present invention further provides a pharmaceutical composition for use in treating cancer, wherein said pharmaceutical composition is administered in simultaneous, separate, or sequential combination with ionizing radiation.

The present invention provides the use of an antibody of the present invention for the manufacture of a medicament for the treatment of cancer. The present invention further provides the use of an antibody of the present invention for the manufacture of a medicament for the treatment of cancer, wherein the cancer is Hodgkin's or non-Hodgkin's lymphomas, melanoma, renal cell cancer, kidney cancer, lung cancer, bladder cancer, gastric and esophageal cancer, colorectal cancer, liver cancer, hepatocellular cancer, cholangiocarcinoma, pancreatic cancer, breast cancer, triple-negative breast

cancer, ovarian cancer, endometrial cancer, prostate cancer, small cell lung cancer (SCLC), non-small cell lung cancer (NSCLC), mesothelioma, squamous cancer of head neck cancer (SCCHN), soft tissue sarcoma, or glioblastoma multiforme. The present invention further provides the use of an antibody of the present invention for the manufacture of a medicament for the treatment of cancer, wherein the lung cancer is NSCLC (squamous and non-squamous), small cell lung cancer, or mesothelioma.

The present invention further provides the use of an antibody of the present invention in the manufacture of a medicament for the treatment of cancer wherein said medicament is to be administered simultaneously, separately, or sequentially with one or more antitumor agents selected from the group consisting of cisplatin, carboplatin, dacarbazine, liposomal doxorubicin, docetaxel, cyclophosphamide and doxorubicin, navelbine, eribulin, paclitaxel, paclitaxel protein-bound particles for injectable suspension, ixabepilone, capecitabine, FOLFOX (leucovorin, fluorouracil, and oxaliplatin), FOLFIRI (leucovorin, fluorouracil, and irinotecan), gemcitabine, topotecan, liposomal irinotecan, pemetrexed, cetuximab, nivolumab, ipilimumab, pidilizumab, pembrolizumab, tremelimumab, urelumab, lirilumab, atezolizumab, epacadostat, and durvalumab.

The present invention provides the use of an antibody of the present invention in the manufacture of a medicament for the treatment of cancer wherein said medicament is to be administered simultaneously, separately, or sequentially with ionizing radiation.

An antibody of the present invention is an engineered, non-naturally occurring polypeptide complex. A DNA molecule of the present invention is a non-naturally occurring DNA molecule that comprises a polynucleotide sequence encoding a polypeptide having the amino acid sequence of one of the polypeptides in an antibody of the present invention.

An antibody of the present invention is an IgG type antibody and has “heavy” chains and “light” chains that are cross-linked via intra- and inter-chain disulfide bonds. Each heavy chain is comprised of an N-terminal HCVR and a heavy chain constant region (“HCCR”). Each light chain is comprised of an N-terminal LCVR and a light chain constant region (“LCCR”). Light chains each form disulfide bonds with a heavy chain, and the two heavy chains form two disulfide bonds between each other.

The constant region of the heavy chains contains CH1, CH2, and CH3 domains. CH1 comes after the HCVR; the CH1 and HCVR form the heavy chain portion of a Fab. CH2 comes after the hinge region and before CH3. CH3 comes after CH2 and is at the carboxy-terminal end of the heavy chain.

5 The constant region of the light chains contains one domain, CL. CL comes after the LCVR; the CL and LCVR form the light chain portion of a Fab.

Antibodies of the present invention are heterodimeric in that each arm of the antibody exhibits selective monovalent binding to its cognate antigen due to two different heavy chains and two different light chains forming the antibody. In the present
10 invention one arm of the antibody binds human PD-L1 (SEQ ID NO: 1), and the other arm binds human PD-1 (SEQ ID NO: 2). The CH2 and/or CH3 domains of such polypeptide chains need not be identical in sequence, and advantageously are modified to foster complexing between the two polypeptide chains. For example, an amino acid substitution (preferably a substitution with an amino acid comprising a bulky side group
15 forming a "knob", e.g., tryptophan) can be introduced into the CH2 or CH3 domain such that steric interference will prevent interaction with a similarly mutated domain and will obligate the mutated domain to pair with a domain into which a complementary, or accommodating mutation has been engineered, i.e., "the hole" {e.g., a substitution with glycine). Such sets of mutations can be engineered into any pair of polypeptides
20 comprising CH2-CH3 Domains that form an Fc region. Methods of protein engineering to favor heterodimerization over homodimerization are well known in the art, in particular with respect to the engineering of immunoglobulin-like molecules (see e.g., WO 96/27011, WO 98/050431, EP 1870459, WO 2007/110205, WO 2007/147901, WO 2009/089004, WO 2010/129304, WO 2011/90754, WO 2011/143545, WO 2012/058768,
25 WO 2013/157954, WO 2013/096291, EP 1 870 459A1, as well as, Ridgway et al. (1996) " 'Knobs-Into-Holes' Engineering Of Antibody CH3 Domains For Heavy Chain Heterodimerization, " Protein Engr. 9:617-621, Atwell et al. (1997) "Stable Heterodimers From Remodeling The Domain Interface Of A Homodimer Using A Phage Display Library, " J. Mol. Biol. 270: 26-35, and Xie et al. (2005) "A New Format Of Bispecific
30 Antibody: Highly Efficient Heterodimerization, Expression And Tumor Cell Lysis, " J. Immunol. Methods 296:95-101). Typically, in the approaches known in the art, the CH3

domain of the first heavy chain and the CH3 domain of the second heavy chain are both engineered in a complementary manner so that the heavy chain comprising one engineered CH3 domain can no longer homodimerize with another heavy chain of the same structure (e.g. a CH3- engineered first heavy chain can no longer homodimerize with another CH3- engineered first heavy chain; and a CH3 -engineered second heavy chain can no longer homodimerize with another CH3 -engineered second heavy chain). Thereby the heavy chain comprising one engineered CH3 domain is forced to heterodimerize with another heavy chain comprising the CH3 domain, which is engineered in a complementary manner. For this embodiment of the invention, the CH3 domain of the first heavy chain and the CH3 domain of the second heavy chain are engineered in a complementary manner by amino acid substitutions, such that the first heavy chain and the second heavy chain are forced to heterodimerize, whereas the first heavy chain and the second heavy chain can no longer homodimerize (e.g. for steric reasons).

15 The different approaches for supporting heavy chain heterodimerization known in the art, are contemplated as different alternatives used in a bispecific antibody according to the invention. In some embodiments of the present invention, mutations are incorporated into the sequence of the heavy chains within the CH1 and CH3 region and into the sequence of the light chains within the light chain constant region. The CH1 and LC mutations are made to favor native pairing of the requisite light chain and heavy chain pairs and disfavor light chain mispairing. The CH3 mutations are made to favor heterodimeric pairing of the two distinct heavy chains and disfavor formation of homodimers.

20 Preferably, when mutations in the CH3 region of the anti-PD-L1 portion of the antibody includes positions 350, 351, 405, and 407 in EU numbering, mutations in the CH3 region of the anti-PD-1 portion of the antibody includes positions 350, 366, 392, and 394 in EU numbering; when mutations in the CH3 region of the anti-PD-L1 portion of the antibody includes positions 350, 366, 392, and 394 in EU numbering, mutations in the CH3 region of the anti-PD-1 portion of the antibody includes positions 350, 351, 356, 405, and 407 in EU numbering (as shown in the sequence alignment shown immediately below).

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Alignment of the amino acid sequences of wild-type human IgG1 and the constant regions of the heavy chain of antibodies v3.2 and v13884 (preferred modifications are underlined):

5	IgG1-wt	ASTKGPSVFPLAPSSKSTSGGTAALGCLVKDYFPEPVTVSWNSGALTSGV
	3.2PD-L1	ASTKGPSVFPLAPSSKSTSGGTAALGCLVKDYFPEPVTVSWNSGALTSGV
	844PD-1	ASTKGPSVFPEAPSSKSTSGGTAALGCLVTDYFPEPVTVSWNSGALTSGV
	3.2PD-1	ASTKGPSVFPEAPSSKSTSGGTAALGCLVTDYFPEPVTVSWNSGALTSGV
	844PD-L1	ASTKGPSVFPLAPSSKSTSGGTAALGCLVKDYFPEPVTVSWNSGALTSGV
10	IgG1-wt	HTFPAVLQSSGLYSLSSVVTVPSSSLGTQTYICNVNHKPSNTKVDKKVEP
	3.2PD-L1	HTFPAVLQSSGLYSLKSVVTVPSSSLGTQTYICNVNHKPSNTKVDKRVEP
	844PD-1	HTFPAVLESSGLYSLSSVVTVPSSSLGTQTYICNVNHKPSNTKVDKRVEP
	3.2PD-1	HTFPAVLESSGLYSLSSVVTVPSSSLGTQTYICNVNHKPSNTKVDKRVEP
	844PD-L1	HTFPAVLQSSGLYSLKSVVTVPSSSLGTQTYICNVNHKPSNTKVDKRVEP
15	IgG1-wt	KSCDKTHTCPPCPAPELLGGPSVFLFPPKPKDTLMISRTPEVTCVVDVSV
	3.2PD-L1	KSCDKTHTCPPCPAPEAAGGPSVFLFPPKPKDTLMISRTPEVTCVVSVSV
	844PD-1	KSCDKTHTCPPCPAPEAAGGPSVFLFPPKPKDTLMISRTPEVTCVVSVSV
	3.2PD-1	KSCDKTHTCPPCPAPEAAGGPSVFLFPPKPKDTLMISRTPEVTCVVSVSV
	844PD-L1	KSCDKTHTCPPCPAPEAAGGPSVFLFPPKPKDTLMISRTPEVTCVVSVSV
20	IgG1-wt	HEDPEVKFNWYVDGVEVHNAKTKPREEQYNSTYRVVSVLTVLHQDWLNGK
	3.2PD-L1	HEDPEVKFNWYVDGVEVHNAKTKPREEQYNSTYRVVSVLTVLHQDWLNGK
	844PD-1	HEDPEVKFNWYVDGVEVHNAKTKPREEQYNSTYRVVSVLTVLHQDWLNGK
	3.2PD-1	HEDPEVKFNWYVDGVEVHNAKTKPREEQYNSTYRVVSVLTVLHQDWLNGK
	844PD-L1	HEDPEVKFNWYVDGVEVHNAKTKPREEQYNSTYRVVSVLTVLHQDWLNGK
25	IgG1-wt	EYKCKVSNKALPAPIEKTISKAKGQPREPQVYTLPPSRDELTKNQVSLTLC
	3.2PD-L1	EYKCKVSNKALPAPIEKTISKAKGQPREPQVYVYPPSRDELTKNQVSLTLC
	844PD-1	EYKCKVSNKALPAPIEKTISKAKGQPREPQVYVYPPSRDELTKNQVSLTLC
	3.2PD-1	EYKCKVSNKALPAPIEKTISKAKGQPREPQVYVLPSPRDELTKNQVSLLC
	844PD-L1	EYKCKVSNKALPAPIEKTISKAKGQPREPQVYVLPSPRDELTKNQVSLLC
30	IgG1-wt	LVKGFYPSDIAVEWESNGQPENNYKTTPPVLDSDGSFFLYSKLTVDKSRW
	3.2PD-L1	LVKGFYPSDIAVEWESNGQPENNYKTTPPVLDSDGSFALVSKLTVDKSRW
	844PD-1	LVKGFYPSDIAVEWESNGQPENNYKTTPPVLDSDGSFALVSKLTVDKSRW
	3.2PD-1	LVKGFYPSDIAVEWESNGQPENNYLTWPPVLDSDGSFFLYSKLTVDKSRW
	844PD-L1	LVKGFYPSDIAVEWESNGQPENNYLTWPPVLDSDGSFFLYSKLTVDKSRW
35	IgG1-wt	LVKGFYPSDIAVEWESNGQPENNYKTTPPVLDSDGSFFLYSKLTVDKSRW
	3.2PD-L1	LVKGFYPSDIAVEWESNGQPENNYKTTPPVLDSDGSFALVSKLTVDKSRW
	844PD-1	LVKGFYPSDIAVEWESNGQPENNYKTTPPVLDSDGSFALVSKLTVDKSRW
	3.2PD-1	LVKGFYPSDIAVEWESNGQPENNYLTWPPVLDSDGSFFLYSKLTVDKSRW
	844PD-L1	LVKGFYPSDIAVEWESNGQPENNYLTWPPVLDSDGSFFLYSKLTVDKSRW
40	IgG1-wt	QQGNVFSCSVMHEALHNHYTQKSLSLSPGK (SEQ ID NO: 40)
	3.2PD-L1	QQGNVFSCSVMHEALHNHYTQKSLSLSPGK (SEQ ID NO: 41)
	844PD-1	QQGNVFSCSVMHEALHNHYTQKSLSLSPGK (SEQ ID NO: 42)
	3.2PD-1	QQGNVFSCSVMHEALHNHYTQKSLSLSPGK (SEQ ID NO: 43)
	844PD-L1	QQGNVFSCSVMHEALHNHYTQKSLSLSPGK (SEQ ID NO: 44)
45	IgG1-wt	QQGNVFSCSVMHEALHNHYTQKSLSLSPGK (SEQ ID NO: 40)
	3.2PD-L1	QQGNVFSCSVMHEALHNHYTQKSLSLSPGK (SEQ ID NO: 41)
	844PD-1	QQGNVFSCSVMHEALHNHYTQKSLSLSPGK (SEQ ID NO: 42)
	3.2PD-1	QQGNVFSCSVMHEALHNHYTQKSLSLSPGK (SEQ ID NO: 43)
	844PD-L1	QQGNVFSCSVMHEALHNHYTQKSLSLSPGK (SEQ ID NO: 44)

Preferably, as shown underlined in the alignment immediately above, mutations in the CH1 region of the anti-PD-L1 portion of the antibody preferably includes position

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183 in EU numbering while mutations in the CH1 region of the anti-PD-1 portion of the antibody preferably includes positions 128, 147, and 175 in EU numbering.

The CL region of the anti-PD-L1 portion of the antibody is preferably a human lambda subtype. More preferably, the CL region of the anti-PD-L1 portion of the antibody is a human lambda variant comprising amino acid substitutions of positions 176 and 178 in EU numbering (see alignment below)

Alignment of the amino acid sequence of wild-type human lambda with the constant region of the PD-L1 light chain of the v3.2, v3.0, and v13844 antibodies or PD-1 (preferred modifications are underlined).

10	Lambda-wt PD-L1	QPKANPTVTLFPPSSEELQANKATLVCLISDFYPGAVTVAWKADGSPVK QPKAAPSVTLFPPSSEELQANKATLVCLISDFYPGAVTVAWKADSSPVK
15	Lambda-wt PD-L1	AGVETTKPSKQSNKYAASSYLSLTPEQWKSHRSYSCQVTHEGSTVEKTV AGVETTTPSKQSNKYAAE <u>SE</u> LSLTPEQWKSHRSYSCQVTHEGSTVEKTV
20	Lambda PD-L1	APTECS (SEQ ID NO: 45) APAECS (SEQ ID NO: 46)

The CL region of the anti-PD-1 portion of the antibody is preferably a human kappa subtype. The CL region of the anti-PD-1 portion of the antibodies of the present invention is preferably a human kappa variant comprising amino acid substitutions at positions 131, 133, and 176 in EU numbering.

25

Alignment of the amino acid sequences of wild-type human kappa and the constant regions of the light chain of antibodies against PD-1 (preferred modifications are underlined).

30	Kappa-wt PD-1	RTVAAPSVFIFPPSDEQLKSGTASVVCLLNNFYPREAKVQWKVDNALQSG RTVAAPSVFIFPPSDEQLKSGTAR <u>VG</u> CLLNNFYPREAKVQWKVDNALQSG
35	Kappa-wt PD-1	NSQESVTEQDSKDYSLSTLTLSKADYEKHKVYACEVTHQGLSSPVTK NSQESVTEQDSKDYSLR <u>ST</u> LTLSKADYEKHKVYACEVTHQGLSSPVTK
	Kappa-wt PD-1	SFNRGEC (SEQ ID NO: 47) SFNRGEC (SEQ ID NO: 48)

In certain antibodies of the present invention, heavy chain heterodimeric pairing mutations yield a CH3 thermal stability greater than 78°C.

40

When expressed in certain biological systems, antibodies having Fc sequences are glycosylated in the Fc region. Typically, glycosylation occurs in the Fc region of the antibody at a highly conserved N-glycosylation site. N-glycans typically attach to asparagine. Antibodies may be glycosylated at other positions as well.

5 Preferably, antibodies of the present invention contain an Fc portion variant which is derived from human IgG1. IgG1 is well known to bind to Fc-gamma receptor family (FcγR) as well as C1q. Interaction with these receptors can induce antibody-dependent cell cytotoxicity (ADCC) and complement-dependent cytotoxicity (CDC). Therefore, certain amino acid substitutions are introduced into human IgG1 Fc region for antibodies
10 of the present invention to ablate immune effector functions. Mutations in the CH2 region of the antibody heavy chains may include positions 234, 235, and 265 in EU numbering to reduce or eliminate immune effector functions as shown in Figure 1.

 An isolated DNA encoding a HCVR region can be converted to a full-length heavy chain gene by operably linking the HCVR-encoding DNA to another DNA
15 molecule encoding a heavy chain constant region or a variant thereof. The sequences of human, as well as other mammalian, heavy chain constant region genes are known in the art. DNA fragments encompassing these regions can be obtained *e.g.*, by standard PCR amplification. Preferably, for antibodies of the present invention, the heavy chain constant regions of the heavy chains are variants of human IgG1.

20 An isolated DNA encoding a LCVR region may be converted to a full-length light chain gene by operably linking the LCVR-encoding DNA to another DNA molecule encoding a light chain constant region or a variant thereof. The sequences of human, as well as other mammalian, light chain constant region genes are known in the art. DNA fragments encompassing these regions can be obtained by standard PCR amplification.
25 The light chain constant region can be a kappa or lambda constant region. Preferably, for antibodies of the present invention, the light chain constant region of the anti-PD-L1 portion of the antibody is a variant of lambda light chain and the light chain constant region of the anti-PD-1 portion of the antibody is a variant of kappa light chain.

 The polynucleotides of the present invention may be expressed in a host cell after
30 the sequences have been operably linked to an expression control sequence. The expression vectors are typically replicable in the host organisms either as episomes or as

an integral part of the host chromosomal DNA. Commonly, expression vectors will contain selection markers, e.g., tetracycline, neomycin, glutamine synthetase, and dihydrofolate reductase, to permit detection of those cells transformed with the desired DNA sequences.

5 The antibody of the present invention may readily be produced in mammalian cells such as CHO, NS0, HEK293 or COS cells. The host cells are cultured using techniques well known in the art.

 The vectors containing the polynucleotide sequences of interest (e.g., the polynucleotides encoding the polypeptides of the antibody and expression control
10 sequences) can be transferred into the host cell by well-known methods, which vary depending on the type of cellular host.

 Various methods of protein purification may be employed and such methods are known in the art and described, for example, in Deutscher, *Methods in Enzymology* 182: 83-89 (1990) and Scopes, *Protein Purification: Principles and Practice*, 3rd Edition,
15 Springer, NY (1994).

 In another embodiment of the present invention, the antibody, or the nucleic acids encoding the same, is provided in isolated form. As used herein, the term "isolated" refers to a protein, peptide, or nucleic acid which is free or substantially free from any other macromolecular species found in a cellular environment. "Substantially free" as
20 used herein means the protein, peptide, or nucleic acid of interest comprises more than 80% (on a molar basis) of the macromolecular species present, preferably more than 90%, and more preferably more than 95%.

 The antibody of the present invention, or pharmaceutical compositions comprising the same, may be administered by parenteral routes (e.g., subcutaneous and intravenous).
25 An antibody of the present invention may be administered to a patient alone with pharmaceutically acceptable carriers, diluents, or excipients in single or multiple doses. Pharmaceutical compositions of the present invention can be prepared by methods well known in the art (e.g., *Remington: The Science and Practice of Pharmacy*, 22nd ed. (2012), A. Loyd et al., Pharmaceutical Press) and comprise an antibody, as disclosed
30 herein, and one or more pharmaceutically acceptable carriers, diluents, or excipients.

The term "treating" (or "treat" or "treatment") refers to slowing, interrupting, arresting, alleviating, stopping, reducing, or reversing the progression or severity of an existing symptom, disorder, condition, or disease.

"Binds" as used herein in reference to the affinity of an antibody for human PD-L1 (SEQ ID NO: 1), human PD-1 (SEQ ID NO: 2), or both is intended to mean, unless indicated otherwise, a K_D of less than about 1×10^{-6} M, preferably, less than about 1×10^{-9} M as determined by common methods known in the art, including by use of a surface plasmon resonance (SPR) biosensor at 37°C essentially as described herein.

"Effective amount" means the amount of an antibody of the present invention or pharmaceutical composition comprising an antibody of the present invention that will elicit the biological or medical response of or desired therapeutic effect on a tissue, system, animal, mammal or human that is being sought by the researcher, medical doctor, or other clinician. An effective amount of the antibody may vary according to factors such as the disease state, age, sex, and weight of the individual, and the ability of the antibody to elicit a desired response in the individual. An effective amount is also one in which any toxic or detrimental effect of the antibody is outweighed by the therapeutically beneficial effects.

This invention is further illustrated by the following non-limiting Examples.

Example 1: Antibody expression and purification

The polypeptides of the variable regions of the heavy chain and light chain, the complete heavy chain and light chain amino acid sequences of Antibodies v3.2, v3.0 and v13844, and the nucleotide sequences encoding the same, are listed below in the section entitled "Amino Acid and Nucleotide Sequences." In addition, the SEQ ID NOs of the amino acid sequences of the light chain, heavy chain, light chain variable region, and heavy chain variable region of Antibodies v3.2, v3.0 and v13844 are shown in Table 1(a) below. Furthermore, the SEQ ID NOs for the amino acid sequences of the CDRs of the PD-L1 and PD-1 binding variable regions of Antibodies v3.2, v3.0 and v13844 are shown in Table 1(b) and Table 1(c), respectively.

The antibodies of the present invention, including, but not limited to, Antibodies v3.2, v3.0 and v13844, can be made and purified essentially as follows. An appropriate

host cell, such as CHO, can be either transiently or stably transfected with an expression system for secreting antibodies using a quad vector (i.e., a single vector encoding for the expression of the two light chains and the two heavy chains), dual vectors (i.e., two vectors, which together encode the two different light chains and the two different heavy chains), or four single vectors (two of which encode a different light chain and two of which encode a different heavy chain). Media, into which the antibody has been secreted, may be purified using any of many commonly-used techniques. For example, the medium may be applied to a MabSelect column (GE Healthcare), or KappaSelect column (GE Healthcare) for Fab fragment, that has been equilibrated with a compatible buffer, such as phosphate buffered saline (pH 7.4). The column may be washed to remove nonspecific binding components. The bound antibody may be eluted, for example, by pH gradient (such as 20 mM Tris buffer pH 7 to 10 mM sodium citrate buffer pH 3.0, or phosphate buffered saline pH 7.4 to 100 mM glycine buffer pH 3.0). Antibody fractions may be detected, such as by SDS-PAGE, and then may be pooled. Soluble aggregate and multimers may be effectively removed by common techniques, including size exclusion, hydrophobic interaction, ion exchange, multimodal, or hydroxyapatite chromatography. The antibody may be concentrated and/or sterile filtered using common techniques. The product may be immediately frozen at -70°C or may be lyophilized.

Tables 1(a)-(c): SEQ ID Nos for Checkpoint Inhibitor Bispecific Antibody (BsAb) Sequences

1(a)

	Antibody v3.2	Antibody v3.0	Antibody v13844
HCVR – anti-PD-L1	3	3	3
HCVR – anti-PD-1	6	7	7
LCVR – anti-PD-L1	4	4	4
LCVR – anti-PD-1	8	8	8
Heavy chain 1 – anti-PD-L1	49	49	29
Heavy chain 2 – anti- PD-1	31	32	33
Light chain 1 – anti-PD-L1	30	30	30

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Light chain 2 – anti- PD-1	34	34	34
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1(b)

Anti-PD-L1	CDR1	CDR2	CDR3
HC	KASGGTFSSYAIS (SEQ ID NO: 16)	GIIPIFGTANYAQKFQG (SEQ ID NO: 17)	ARSPDYSPYYYYGMDV (SEQ ID NO: 18)
LC	SGSSSNIGSNTVN (SEQ ID NO: 19)	YGNSNRPS (SEQ ID NO: 20)	QSYDSSLGSGSV (SEQ ID NO: 21)

1(c)

Anti-PD1	CDR1	CDR2	CDR3
HC	KASGGTFSSYAIS (SEQ ID NO: 22)	LIIPSFDTAGYAQKFQG (SEQ ID NO: 23) or LIIPMFDTAGYAQKFQG (SEQ ID NO: 24)	ARAEHSSTGTFDY (SEQ ID NO: 25)
LC	RASQGISSWLA (SEQ ID NO: 26)	SAASSLQS (SEQ ID NO: 27)	QQANHLPFT (SEQ ID NO: 28)

5

Example 2: Binding to both human PD-L1 and PD-1 as measured by ELISA

The ability for antibodies of the present invention to bind both human PD-L1 and human PD-1 may be measured in a sandwich ELISA assay. For the PD-1 binding assay, a 96-well plate (Nunc) may be coated with human PD-1-Fc (R&D Systems, cat. #1086-PD) overnight at 4°C. Wells may be blocked for 2 hours with blocking buffer (PBS containing 5% nonfat dry milk). Wells may be washed three times with PBS containing 0.1% Tween-20. Anti-PD-1 antibody or control IgG (100 µl) may then be added and then the plate may be incubated at room temperature for 1 hour. After washing, the plate may be incubated with 100 µl of goat anti-human IgG F(ab')₂-HRP conjugate (Jackson Immuno Research, cat. #109-035-097) at room temperature for 1 hour. The plate may be washed and then may be incubated with 100 µl of 3,3', 5,5'-tetra-methylbenzidine. Absorbance at 450 nm may be read on a microplate reader. The half maximal effective concentration (EC₅₀) may be calculated using GraphPad prism 6 software.

10

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For the PD-L1 binding assay, a similar procedure may be applied, except that the 96-well plate (Nunc) may be coated with human PD-L1-Fc (R&D Systems, cat#156-B7) overnight at 4°C.

In experiments performed essentially as described above in this Example 2,
5 Antibody v3.2 binds to human PD-1 with an EC₅₀ of 0.0802 nM. In comparison, the binding affinity of the parent PD-1 antibody (as IgG4-PAA homodimer) is 0.1318 nM. Antibody v3.2 binds to human PD-L1 with an EC₅₀ of 0.4554 nM. In comparison, the binding affinity of the parent PD-L1 antibody (as IgG1-EN homodimer) is 0.4702 nM.

10 **Example 3: Bridging PD-1 expressing cells with PD-L1 expressing cells**

The ability for antibodies of the present invention to bridge PD-1 and PD-L1 expressing cells was determined by flow cytometry analysis using transfected CHO cells expressing either PD-1 or PD-L1. Briefly stated, CHO-PD1 and CHOK1-PDL1 over expressing cells may be differentially labeled with CFSE (carboxyfluorescein diacetate succinimidyl ester) (BD Horizon) or Cell Tracker Deep Red (CTDR/Thermo). CHO-PD1
15 and CHOK1-PDL1 cells are separately incubated for 2 hours with a test antibody, such as Antibody v3.2 (on ice in PBS + 1% BSA + 0.09% sodium azide). Unbound antibodies may be removed by washing (2X with 200 µl PBS + 1% BSA + 0.09% sodium azide). CHOK1-PDL1 cells are incubated 2 hours with 45 µg/ml of the parent PD-L1 antibody or
20 hIgG1 control on ice in PBS + 1% BSA + 0.09% sodium azide. CHO-PD1 cells are incubated 2 hours with 45 µg/ml of the parent of PD-1 antibody or huIgG4-PAA on ice in PBS + 1%BSA + 0.09% sodium azide. CHO-PD1/Antibody v3.2 cells are mixed with CHOK1-PDL1+ the parent PD-L1 antibody or hIgG1 at final concentration of 22.5ug/ml. CHOK1-PDL1/Antibody v3.2 cells may be mixed with CHO-PD1 + the parent of the PD-
25 1 antibody or huIgG4-PAA at a final concentration of 22.5 µg/ml. After an approximately 72 hour incubation (at 4°C) cells may be measured on Fortessa X20 (with HTS sampler) in channels suitable for CFSE and CTDR. Using flowJo® software (FlowJo, LLC, Ashland, OR), double positive events (CFSE+/ CTDR+) may be gated and % of total events may be calculated and reported (for 2 replicate wells). Fits and statistics may be
30 generated with Graphpad Prism using nonlinear regression (variable slope, 4 parameters).

In experiments performed essentially as described above in this Example 3, PD-1/PD-L1 bispecific antibodies mediate cell bridging which may be detected as double positive events by flow cytometry. Binding of Antibody v3.2 to CHO-PD1 or CHOK1-PDL1 cells (with subsequent removal of unbound) and then mixing with CHOK1-PDL1 or CHO-PD1 cells, respectively, caused a dose dependent increase in double positive events relative to background (up to 4-fold increase compared to buffer only). This increase in double positive events is blocked by the addition of excess competing PD-L1 and/or PD-1 mAbs at high concentration but not by matched non-specific IgG control, demonstrating specificity and dependence on target antigen expression.

Example 4: Blocking the interactions of PD-L1 with PD-L2, PD-L1 with CD80 and PD-L2 with PD-1

A PD-L1/PD-1 blocking assay can be performed, by mixing varying amounts of anti-PD-1 antibody or control IgG with a fixed amount of biotinylated PD-1-Fc fusion protein (100 ng/mL) and incubating at room temperature for about 1 hour. Afterwards, the mixture may be transferred to 96-well plates pre-coated with PD-L1-Fc (100 ng/well) or PD-L2-Fc (100 ng/well) and then incubated at room temperature for approximately another 1 hour. After washing, streptavidin HRP conjugate may be added, and the absorbance at 450 nm may be read. IC₅₀ represents the antibody concentration required for 50% inhibition of PD-1 binding to PD-L1 or binding to PD-L2.

Similarly, a PD-L1/B7-1 blocking assay may be performed by using plates coated with 1 µg/ml B7-1-Fc (R&D Systems, cat#140-B1-100). The antibody concentration required for 50% inhibition of PD-L1 binding to B7-1 (IC₅₀) is calculated using GraphPad prism 6 software.

In experiments performed essentially as described above in this Example 4, Antibody v3.2 appeared to block the interaction between PD-1 receptor and PD-L1 ligand at intermediate and higher concentration and seems to bridge the receptor and the ligand with the dual binding at lower and intermediate concentration, with stronger affinities than the natural ligand-receptor interaction. Furthermore, Antibody v3.2 appeared to block the interaction of PD-L1 with B7-1 with an IC₅₀ of 0.75 nM and the interaction of PD-L2 with PD-1 with an IC₅₀ of 2.27 nM.

Example 5: *In vitro* functional analysis of antibodies in Mixed leukocyte reaction (MLR)

CD14⁺ monocytes may be isolated from frozen human PBMC obtained from a healthy donor (AllCells cat. #PB005F) with MACS beads (Miltenyi, cat. #130-091-153). Immature dendritic cells (DC) may be generated by culturing these monocytes in 12 ml complete RPMI-1640 medium in the presence of 1000 IU/ml hGM-CSF and 500 IU/ml hIL-4 for 4 days. CD4⁺ T cells may be purified from fresh human PBMC of a different healthy donor (AllCells cat. #PB002) by negative selection (Miltenyi 130-096-533). Then, the two types of cells may be mixed in individual wells of a 96-well plate with 100 µl complete AIM-V medium containing 5 × 10⁴ CD4⁺ T cells and 4 × 10³ immature DC per well (E:T = 12.5:1). 100 µl complete AIM-V medium may be added containing human IgG1-EN, the parental anti-PD-1 antibody, the parental anti-PD-1 antibody, combinations of the parent antibodies, or Antibody v3.2 in 8 replicates (serially diluted by 3:1 from 32nM), respectively). After incubation for 72 hours at 37°C at 5% CO₂, supernatants may be harvested and then measured for human IFN-γ and IL-2 with ELISA kits (R&D cat# SIF50) and (R&D cat# S2050).

In experiments performed essentially as described above in this Example 5, addition of Antibody v3.2 to co-cultures of allogeneic CD4⁺ T cells and DC resulted in increased production of IFN-γ by responding CD4 T cell lymphocytes with an EC₅₀ of 0.005 nM, compared to 0.026 nM, 0.029 nM and 0.115 nM for the parental PD-L1 Ab, the parental PD-1 Ab and a combination of the two, respectively. Similarly, Antibody v3.2 also increased production of IL-2 in the co-culture with an EC₅₀ of 0.011 nM, compared to PD-L1 Ab, PD-1 Ab and PD-L1 Ab + PD-1 Ab combination (0.034 nM, 0.023 nM and 0.046 nM, respectively). The results indicate that Antibody v3.2 is superior in its ability to enhance T cell activation *in vitro* to the parental PD-L1 Ab alone, the parental PD-1 Ab alone or a combination thereof.

Example 6: Reinvigorating T cells

PD-L1 positive human T-activator CHOK1 cells (Promega part #CS187108) and PD-L1 negative human T-activator CHOK1 cells (Promega part #CS187110) may be

obtained from Promega. PD-L1/PD-L2 double positive human T-activator CHOK1 cells may be established by transfecting PD-L1 positive human T-activator CHOK1 cells with a vector encoding full-length PD-L2. These cells may be plated in a 96-well white opaque tissue culture plate at 40,000 cells per well in 100 μ L medium (10% FBS F-12, 0.2 mg/ml Hygromycin-B and 0.2 mg/ml G418) and incubated overnight at 37°C with 5% CO₂. Media may be removed from the assay plates on the following day and serially diluted test and control antibodies in assay buffer (2% FBS RPMI) may be added at 40 μ L per well. GloResponse NFAT-luc2/PD1 Jurkat cells (Promega part #CS187102) may be resuspended in assay buffer at a concentration of 1.25×10^6 /mL and added to the plate at 40 μ L per well. After 6 hours of induction, assay plates may be removed from the incubator and equilibrated at room temperature for 5 to 10 minutes. Bio-Glo™ Reagent (Promega G7941) may be prepared according to the manufacturer's instructions and added at 80 μ L per well. Then the plates may be incubated 5 to 10 minutes at room temperature. Luminescence may be measured in a plate reader and data may be analyzed using GraphPad Prism 7.

In a PD-1/NFAT Reporter Jurkat T cell assay performed essentially as described above in this Example 6, PD-L1 positive human T cell activator CHO cells were observed to suppress T cell activation. PD-1 Ab or PD-L1 Ab re-activated T cells by reversing PD-L1 – PD-1 mediated suppression. However, Antibody v3.2 appeared superior (EC50 0.12 nM) to either the parental PD-L1 Ab alone (1.92 nM), the parental PD-1 Ab alone (1.01 nM), or combination of the parental PD-1 Ab and the parental PD-L1 Ab (0.796 nM) in its ability to reinvigorate T cells. When PD-L1/PD-L2 double positive human T cell activator CHO cells were used in this system, activation of PD-1 positive reporter T cells was also suppressed. The parental PD-1 Ab but not the parental PD-L1 Ab was able to re-activated T cells. However, Antibody v3.2 is superior (EC50 0.181 nM) to either the parental PD-1 Ab alone (0.946 nM), or a combination of the parental PD-1 Ab and the parental PD-L1 Ab (1.251 nM) in its ability to reinvigorate T cells.

Example 7: *In vivo* efficacy assays

The efficacy of the antibodies of the present invention may be tested in xenograft models in immunodeficient mice reconstituted with human immune cells to assess the

ability to delay or destroy established tumors in the model through enhancement of T-cell response to allo-antigens. All animal in studies are approved by the Institutional Animal Care and Use Committee and performed in accordance with current regulations and standards of the United States Department of Agriculture and the National Institute of Health.

Part A: NCI-H292 human NSCLC xenograft model

NOD scid gamma (NSG) mice from Jackson Laboratories (7 weeks of age, female, in groups of 7-8 mice) are implanted into the flank subcutaneously with either 2 x 10⁶ NCI-H292 cells, or a mixture of 2 x 10⁶ NCI-H292 cells and 1 x 10⁶ human freshly isolated PBMCs in HBSS (0.2 ml total volume). Starting on Day 1, mice are treated with an intraperitoneal injection of either human IgG1-EN (control), the parental anti-PD-L1 antibody (0.25 mg/kg), or the parental anti-PD-1 antibody (0.25 mg/kg), or combination of the parental antibodies (0.25 mg/kg each), or Antibody v3.0, v3.2 or v13844 (0.5 mg/kg) once weekly for three doses. Animal well-being and behavior, including grooming and ambulation are monitored at least twice per week. Body weight and tumor volume are measured twice a week.

In experiments performed essentially as described above in Part A of Example 7, Antibodies v3.0, v3.2 and v13844 dosed at 10 mg/kg were well tolerated and safe as monitored by body weight and clinical observations. In mice implanted with mixture of NCI-H292 tumor cells and PBMC, treatment with anti-PD-L1 or anti-PD-1 antibody, or combination of anti-PD-L1 and anti-PD-1 antibodies at 10 mg/kg each all delayed tumor growth as compared to treatment with the control molecule, human IgG1-EN. In mice implanted with mixture of NCI-H292 tumor cells and PBMC, treatment with PD-1/PD-L1 bispecific Ab (v13844, v3.0 or v3.2) at 10 mg/kg qw were all more efficacious with a complete regression (CR) in 7/8, 5/8 and 5/8, respectively, than the combination therapy (parental PD-L1 antibody + parental PD-1 antibody) (CR in 2/8).

Part B: HCC827 human NSCLC xenograft model

NSG mice from Jackson Laboratories (7 weeks of age, female, in groups of 8 mice) are implanted into the flank subcutaneously with 10 x 10⁶ HCC827 cells in HBSS

(0.2 ml total volume). Bulk human T cells isolated from whole blood (New York Blood Center) are expanded using Human T-Activator CD3/CD28 Dynabeads® for 10 days and cryopreserved. T cells are thawed, washed, counted, and infused intravenously (3.5×10^6 T cells in 0.2 ml PBS per mouse) into HCC827 tumor-bearing mice on day 44 from

5 implantation. Starting the next day, mice are treated with an intraperitoneal injection of human IgG1-EN (control), parental anti-PD-L1 antibody, parental anti-PD-1 antibody, or the combination of the parental anti-PD-L1 antibody and the parental anti-PD-1 antibody, at 2 mg/kg each, or Antibody v3.2 at three dose levels (0.02, 0.2 or 2 mg/kg), once weekly for 4 weeks. Mice are given a second infusion of expanded T cells (2.5×10^6 T

10 cells in 0.2 ml PBS per mouse) on Day 56. Animal well-being and behavior, including grooming and ambulation are monitored at least twice per week. Body weight and tumor volume are measured twice a week. Tumor volumes are measured once per week using electronic calipers as described in the SOP entitled: IM-Tumor Growth Measurement. Tumor volume is calculated using a formula: Tumor Volume (mm^3) = $\pi/6$ * Length * Width².

15

In experiments performed essentially as described above in Part B of Example 7, Antibody v3.2 at all three dose levels (0.02, 0.2 or 2 mg/kg) shared similar anti-tumor response in the established HCC827 tumor model in the presence of expanded T cells. Moreover, Antibody v3.2 at 0.02 mg/kg delayed tumor growth more significantly than

20 anti-PD-L1 Antibody (2 mg/kg, $p=0.05$), anti-PD-1 Antibody (2 mg/kg, $p<0.001$) and the combination of both agents (2 mg/kg each, $p<0.001$) on Day 69 post tumor cell implantation (see, Table 2).

25 **Table 2: Tumor inhibition on Day 69 in HCC827 human NSCLC established tumor model**

Treatment Group	Tumor volume (in mm^3) on Day 69 (Mean $\pm$ SEM)	<i>p</i> value (vs. 0.02 mg/kg BsAb v3.2/T cells)
2 mg/kg Human IgG1-EN	1572.4 $\pm$ 87.8	<0.001
2 mg/kg Human IgG1-EN/T cells	1062.2 $\pm$ 81.7	<0.001
2 mg/kg Anti PD-L1 mAb/T cells	968.7 $\pm$ 58.7	0.005
2 mg/kg Anti PD-1 mAb/T cells	1275.0 $\pm$ 67.2	<0.001
2 mg/kg Anti PD-L1 mAb +	1021.0 $\pm$ 58.9	<0.001

2 mg/kg Anti PD-1 mAb/T cells		
0.02 mg/kg Antibody v3.2/T cells	761.8 ± 33.3	NA
0.2 mg/kg Antibody v3.2/T cells	759.8 ± 43.3	0.975
2 mg/kg Antibody v3.2/T cells	721.3 ± 49.2	0.516

SEM: standard estimation of mean

Amino Acid and Nucleotide Sequences

5 SEQ ID NO: 1 (human PD-L1)

MRIFAVFIFMTYWHLLNAFTVTVPKDLYVVEYGSNMTIECKFPVEKQLDLAALIVWEME
 DKNIIQFVHGEEEDLKVQHSSYRQRARLLKDQLSLGNAALQITDVKLQDAGVYRCMISYGG
 ADYKRITVKVNAPYNKINQRILVVDPTSEHELTCQAEGYPKAEVIWTSSDHQVLSGKTT
 TTNSKREEKLFNVTSTLRINTTTNEIFYCTFRRLDPEENHTAELVIPELPLAHPNERTH
 10 LVILGAILLCLGVALTFIFRLRKGRMMDVKKCGIQDTNSKKQSDTHLEET

SEQ ID NO: 2 (human PD-1)

MQIPQAPWPVVWAVLQLGWRPGWFLDSPDRPWNPPTFSPALLVVTEGDNATFTCSFSNTS
 ESFVLNWMSPSNQTDKLAAPFEDRSQPGQDCRFRTQLPNGRDFHMSVVRARRNDSTG
 15 YLCGAISLAPKAQIKESLRAELRVTERRAEVPTAHPSPSPRPAGQFQTLVVGVGGLLS
 LVLLVWVLAVICSRAARGTIGARRTGQPLKEDPSAVPVFSDYGEDLFQWREKTPEPPVP
 CVPEQTEYATIVFPSPGMGTSSPARRGSADGPRSAQPLRPEDGHCSWPL

SEQ ID NO: 3 (HCVR of PD-L1 Ab)

20 QVQLVQSGAEVKKPGSSVKVSKASGGTFSSYAISWVRQAPGQGLEWMGGIIPFGTANY
 AQKFQGRVTITADKSTSTAYMELSSLRSEDTAVYYCARSPDYSPYGYGMDVWGQGTITV
 VSS

SEQ ID NO: 4 (LCVR of PD-L1 Ab)

25 QSVLTQPPSASGTPGQRTISCSGSSSNIGSNTVNWYQQLPGTAPKLLIYGNSNRPSGVP
 DRFSGSKSGTSASLAISGLQSEDEADYYCQSYDSSLGSGSVFGGGIKLTVLG

SEQ ID NO: 5 (HCVR of PD-1 Ab-Xaa)

30 QVQLVQSGAEVKKPGSSVKVSKASGGTFSSYAISWVRQAPGQGLEWMGLIIPXaaFDTA
 GYAQKFQGRVAITVDESTSTAYMELSSLRSEDTAVYYCARAEHSSTGTFDYWGQGTITV
 SS

Xaa: M or S.

SEQ ID NO: 6 (HCVR of PD-1 Xaa-S)

35 QVQLVQSGAE VKKPGSSVKV SKASGGTFS SYAISWVRQA PGQGLEWMGL
 IIPSFDTAGY AQKFQGRVAI TVDESTSTAY MELSSLRSED TAVYYCARAE
 HSSTGTFDYW GQGTITVTVSS

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- SEQ ID NO: 7 (HCVR of PD-1 Xaa-M)
QVQLVQSGAE VKKPGSSVKV SCKASGGTFS SYAISWVRQA PGQGLEWMGL
IIPMFDTAGY AQKFQGRVAI TVDESTSTAY MELSSLRSED TAVYYCARAE
HSSTGTFDYW GQGTLLVTVSS
- 5
- SEQ ID NO: 8 (LCVR of PD-1 Ab)
DIQMTQSPSSVSASVGDRVTITCRASQGISSWLAWYQQKPGKAPKLLISAASSLQSGVPS
RFGSGSGTDFTLTISLQPEDFATYYCQQANHLPTFTGGGKVEIK
- 10
- SEQ ID NO: 9 (region from CH1 domain of PD-L1 Ab v3.2 and v13844 HC)
SLKSV
- SEQ ID NO: 10 (region from CH2 domain of PD-L1 Ab HC)
AAGGPSVFLFPPKPKDTLMISRTPEVTCVVS
- 15
- SEQ ID NO: 11 (region of CH3 domain of HC for PD-1 v13844 and PD-L1 v3.2)
VYPPSRDELTKNQVSLTCLVKGFYPSDIAVEWESNGQPENNYKTTPPVLDSDGSFALV
- SEQ ID NO: 12 (region of CH3 domain of HC for PD-1 v3.2 and PD-L1 v13844)
20 VLPPSRDELTKNQVSLTCLVKGFYPSDIAVEWESNGQPENNYLTW
- SEQ ID NO: 13 (region from CH1 domain of PD-1 Ab v3.2 and PD-1 v13844 HC)
EAPSSKSTSGGTAALGCLVTDYFPEPVTVSWNSGALTSGV HTFPAVLE
- 25
- SEQ ID NO: 14 (region from LC constant region of PD-L1 v3.2 and v13844 Abs)
AAESEL
- SEQ ID NO: 15 (region from LC constant region of PD-1 v3.2 and v13844 Ab)
RVGCLLNNFYPREAKVQWKVDNALQSGNSQESVTEQDSKDSTYSLR
- 30
- SEQ ID NO: 16 (HCDR1 of PD-L1 Ab)
KASGGTFSSYAIS
- SEQ ID NO: 17 (HCDR2 of PD-L1 Ab)
35 GIIFI FGTANYAQKFQG
- SEQ ID NO: 18 (HCDR3 of PD-L1 Ab)
ARSPDYSPYYYYGMDV
- 40
- SEQ ID NO: 19 (LCDR1 of PD-L1 Ab)
SGSSSNIGSNTVN

-30-

- SEQ ID NO: 20 (LCDR2 of PD-L1 Ab)
YGNNSNRPS
- 5 SEQ ID NO: 21 (LCDR3 of PD-L1 Ab)
QSYDSSLGSGSV
- SEQ ID NO: 22 (HCDR1 of PD-1 Ab)
KASGGTFSSYAIS
- 10 SEQ ID NO: 23 (HCDR2 of PD-1 Ab; v3.2)
LIIPSFDTAGYAQKFQG
- SEQ ID NO: 24 (HCDR2 of PD-1 Ab; v3.0)
15 LIIPMFDTAGYAQKFQG
- SEQ ID NO: 25 (HCDR3 of PD-1 Ab)
ARAEHSSTGTFDY
- 20 SEQ ID NO: 26 (LCDR1 of PD-1 Ab)
RASQGISSWLA
- SEQ ID NO: 27 (LCDR2 of PD-1 Ab)
SAASSLQS
- 25 SEQ ID NO: 28 (LCDR3 of PD-1 Ab)
QQANHLPFT
- SEQ ID NO: 29 (HC of PD-L1 Ab v13844)
30 QVQLVQSGAEVKKPGSSVKVSCASGGTFSSYAISWVRQAPGGGLEWMGGIIPIFGTANY
AQKFQGRVTITADKSTSTAYMELSSLRSEDTAVYYCARSPDYSPYYYYGMDVWGQGTTVT
VSSASTKGPSVFPLAPSSKSTSGGTAALGCLVKDYFPEPVTVSWNSGALTSGVHTFPAVL
QSSGLYSLKSVVTVPSSSLGTQTYICNVNHKPSNTKVDKRVEPKSCDKTHTCPPCPAPEA
AGGPSVFLFPPKPKDTLMISRTPEVTCVVVSVSHEDPEVKFNWYVDGVEVHNAKTKPREE
35 QYNSTYRVVSVLTVLHQDWLNGKEYKCKVSNKALPAPIEKTISKAKGQPREPQVYVLPSS
RDELTKNQVSLCLVKGFYPSDIAVEWESNGQPENNYLTWPPVLDSDGSFFLYSKLTVDK
SRWQQGNVFSCSVMHEALHNHYTQKSLSLSPGK
- SEQ ID NO: 30 (LC of PD-L1 Ab v3.2, v3.0 and v13844)
40 QSVLTQPPSASGTPGQRVTISCSGSSSNIGSNTVNWYQQLPGTAPKLLIYGNSNRPSGVP
DRFSGSKSGTSASLAISGLQSEDEADYYCQSYDSSLGSGSVFGGGIKLTVLGQPKAAPSVT
LFPPSSEELQANKATLVCLISDFYPGAVTVAWKADSSPVKAGVETTTTPSKQSNNKYAAES
ELSLTPEQWKSHRSYSCQVTHEGSTVEKTVAPAECS
- 45 SEQ ID NO: 31 (HC of PD-1 Ab v3.2)

-31-

QVQLVQSGAE VKKPGSSVKV SCKASGGTFS SYAISWVRQA PGQGLEWMGL
 IIPSFDTAGY AQKFQGRVAI TVDESTSTAY MELSSLRSED TAVYYCARAE
 HSSTGTFDYW GQGTLLTVSS ASTKGPSVFP EAPSSKSTSG GTAALGCLVT
 DYFPEPVTVS WNSGALTSGV HTFPAVLESS GLYSLSSVVT VPSSSLGTQT
 5 YICNVNHKPS NTKVDKRVEP KSCDKTHTCP PCPAPEAAGG PSVFLFPPKP
 KDTLMISRTP EVTCVVVSVS HEDPEVKFNW YVDGVEVHNA KTKPREEQYN
 STYRVVSVLT VLHQDWLNGK EYKCKVSNKA LPAPIEKTIS KAKGQPREPQ
 VYVLPISRDE LTKNQVSLC LVKGFYPSDI AVEWESNGQP ENNYLTWPPV
 LDSDGSFFLY SKLTVDKSRW QQGNVFSCSV MHEALHNHYT QKSLSLSPGK

10

SEQ ID NO: 32 (HC of PD-1 v3.0)

QVQLVQSGAE VKKPGSSVKV SCKASGGTFS SYAISWVRQA PGQGLEWMGL
 IIPMFDTAGY AQKFQGRVAI TVDESTSTAY MELSSLRSED TAVYYCARAE
 15 HSSTGTFDYW GQGTLLTVSS ASTKGPSVFP EAPSSKSTSG GTAALGCLVT
 DYFPEPVTVS WNSGALTSGV HTFPAVLESS GLYSLSSVVT VPSSSLGTQT
 YICNVNHKPS NTKVDKRVEP KSCDKTHTCP PCPAPEAAGG PSVFLFPPKP
 KDTLMISRTP EVTCVVVSVS HEDPEVKFNW YVDGVEVHNA KTKPREEQYN
 STYRVVSVLT VLHQDWLNGK EYKCKVSNKA LPAPIEKTIS KAKGQPREPQ
 20 VYVLPISRDE LTKNQVSLC LVKGFYPSDI AVEWESNGQP ENNYLTWPPV
 LDSDGSFFLY SKLTVDKSRW QQGNVFSCSV MHEALHNHYT QKSLSLSPGK

SEQ ID NO: 33 (HC of PD-1 Ab v13844)

QVQLVQSGAE VKKPGSSVKV SCKASGGTFS SYAISWVRQA PGQGLEWMGL
 25 IIPMFDTAGY AQKFQGRVAI TVDESTSTAY MELSSLRSED TAVYYCARAE
 HSSTGTFDYW GQGTLLTVSS ASTKGPSVFP EAPSSKSTSG GTAALGCLVT
 DYFPEPVTVS WNSGALTSGV HTFPAVLESS GLYSLSSVVT VPSSSLGTQT
 YICNVNHKPS NTKVDKRVEP KSCDKTHTCP PCPAPEAAGG PSVFLFPPKP
 KDTLMISRTP EVTCVVVSVS HEDPEVKFNW YVDGVEVHNA KTKPREEQYN
 30 STYRVVSVLT VLHQDWLNGK EYKCKVSNKA LPAPIEKTIS KAKGQPREPQ
 VYVLPISRDE LTKNQVSLC LVKGFYPSDI AVEWESNGQP ENNYKTTPPV
 LDSDGSFALV SKLTVDKSRW QQGNVFSCSV MHEALHNHYT QKSLSLSPGK

SEQ ID NO: 34 (LC of PD-1 Ab v3.2, v3.0 and v13844)

35 DIQMTQSPSS VSASVGDRVT ITCRASQGIS SWLAWYQQKP GKAPKLLISA
 ASSLQSGVPS RFSGSGSGTD FTLTISSLQP EDFATYYCQQ ANHLPFTFGG
 GTKVEIKRTV AAPSVFIFPP SDEQLKSGTA RVGCLLNIFY PREAKVQWKV
 DNALQSGNSQ ESVTEQDSKD STYSLRSTLT LSKADYEKHK VYACEVTHQG
 LSSPVTKSFN RGEC

40

SEQ ID NO: 35 DNA Sequence of PD-L1 LC

CAGTCCGTCC TGACTCAGCC ACCTTCCGCT AGCGGTACCC CCGGCCAGAG
 AGTGACAATC TCATGCTCCG GTTCCAGCTC TAACATTGGC TCTAACACTG
 TCAATTGGTA CCAGCAGCTG CCAGGAACCG CACCAAAGCT GCTGATCTAT
 45 GGAAACTCAA ATAGGCCTAG CGGGGTGCCA GACCGGTTTA GCGGATCTAA

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AAGTGGGACT TCAGCTTCCC TGGCAATTTT TGGACTGCAG AGTGAGGACG
AAGCTGATTA CTATTGCCAG TCCTACGATA GTTCACTGAG CGGTTCCTGT
TTCGGCGGAG GGATCAAGCT GACAGTCCTG GGCCAGCCCA AGGTGAGTTC
TAGAGGATCC ATCTGGGATA AGCATGCTGT TTTCTGTCTG TCCCTAACAT
5 GCCCTGTGAT TATCCGCAAA CAACACACCC AAGGGCAGAA CTTTGTACT
TAAACACCAT CCTGTTTGCT TCTTTCCTCA GGCCGCTCCT TCCGTGACTC
TGTTTCCCCC TTCCAGCGAG GAACTGCAGG CCAATAAGGC CACCCTGGTG
TGCTTGATTA GCGACTTCTA TCCTGGAGCT GTGACAGTCG CATGGAAGGC
CGATTCTAGT CCAGTGAAAG CAGGGGTCGA GACCACAAC CCCTCCAAGC
10 AGAGCAACAA CAAGTACGCA GCCGAGTCTG AACTGAGTCT GACCCAGAA
CAGTGGAAGT CCCACAGGAG TTATTCATGC CAGGTGACCC ATGAGGGCTC
CACAGTGGAG AAGACCGTGG CCCCTGCTGA GTGTAGC

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SEQ ID NO: 36 DNA Sequence of PD-1 LC

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15 GACATTTCAGATGACCCAGAGCCCAAGCAGCGTGAGCGCCAGCGTCGGGGA
CCGAGTGACCATCACATGCAGGGCCAGCCAGGGTATTTCTAGTTGGCTGG
CTTGGTACCAGCAGAAGCCAGGCAAAGCACCCAAGCTGCTGATCTCCGCC
GCTTCAAGCCTGCAGTCCGGAGTGCCCTCTCGATTCTCTGGTAGTGGCTC
AGGAACAGACTTTACTCTGACCATTTCTCTCTGCAGCCTGAGGATTTTCG
20 CTACTTACTATTGCCAGCAGGCAAACACCTGCCATTACCTTTGGCGGA
GGGACAAAAGTGGAGATCAAGAGAACCGTCGCGGCGCCAGTGTCTTCAT
TTTTCCCCCTAGCGACGAACAGCTGAAGTCTGGGACAGCCAGAGTGGGCT
GTCTGCTGAACAACTTCTACCCTAGAGAGGCTAAAGTGCAGTGGAAGGTC
GATAACGCACTGCAGTCCGGAAATTCTCAGGAGAGTGTGACTGAACAGGA
25 CTCAAAGATAGCACCTATTTCCCTGAGAAGCACACTGACTCTGAGCAAGG
CCGACTACGAGAAGCATAAAGTGTATGCTTGTGAAGTCACCCACCAGGGG
CTGAGTTCACCAGTCACAAAATCATTCAACAGAGGGGAGTGC

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SEQ ID NO: 37 DNA Sequence of PD-L1 HC

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30 CAGGTCCAGC TGGTGCAGAG CGGAGCCGAA GTGAAGAAAC CCGGTAGCAG
CGTCAAAGTG TCATGTAAAG CCTCAGGGGG AACATTCTCC AGCTACGCCA
TCTCCTGGGT GAGACAGGCT CCAGGACAGG GACTGGAGTG GATGGGAGGA
ATCATCCCTA TCTTCGGCAC CGCCAACCTAC GCTCAGAAGT TTCAGGGCCG
CGTGACCATC ACAGCCGACA AGAGCACCTC TACAGCTTAT ATGGAGCTGT
35 CTTCCCTGAG AAGCGAGGAT ACAGCCGTGT ACTATTGCGC TCGCTCCCCC
GACTACAGCC CTTACTATTA CTATGGCATG GACGTGTGGG GCCAGGGCAC
CACAGTGACC GTGAGCTCTG CTAGCACAAA GGGCCCATCC GTGTTCAC
TGGCTCCATC CAGCAAGTCC ACCAGCGGAG GAACAGCCGC TCTGGGCTGT
CTGGTGAAGG ACTATTTCCC AGAGCCAGTG ACCGTGTCCT GGAACAGCGG
40 CGCCCTGACC TCTGGAGTGC ACACATTTCC CGCTGTGCTG CAGTCTTCCG
GCCTGTACTC TCTGAAGTCC GTGGTGACCG TGCCTAGCTC TTCCCTGGGC
ACCCAGACAT ATATCTGCAA CGTGAATCAC AAGCCTTCCA ATACAAAGGT
GGACAAGAGG GTGGAGCCAA AGAGCTGTGA TAAGACCCAT ACATGCCCCC
CTTGTCCTGC TCCAGAGGCT GCTGGAGGAC CAAGCGTGTT CCTGTTTCCA
45 CCCAAGCCCA AGGACACCCT GATGATCTCT AGGACCCCTG AGGTGACATG
CGTGGTGGTG TCCGTGTCCC ACGAGGACCC AGAGGTGAAG TTAACTGGT

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-33-

ACGTGGATGG CGTGGAGGTG CATAATGCTA AGACCAAGCC TAGGGAGGAG
 CAGTACAACA GCACCTATCG GGTGGTGTCT GTGCTGACAG TGCTGCATCA
 GGATTGGCTG AACGGCAAGG AGTATAAGTG CAAGGTGTCT AATAAGGCCC
 TGCCCGCTCC TATCGAGAAG ACCATCTCCA AGGCCAAGGG CCAGCCTAGG
 5 GAGCCACAGG TGTACGTGCT GCCTCCAAGC CGGGACGAGC TGACAAAGAA
 CCAGGTGTCT CTGCTGTGCC TGGTGAAGGG CTTCTATCCA TCTGATATCG
 CTGTGGAGTG GGAGTCCAAT GGCCAGCCCG AGAACAATTA CCTGACCTGG
 CCCCCTGTGC TGGACAGCGA TGGCTCTTTC TTTCTGTATT CCAAGCTGAC
 AGTGGATAAG AGCCGGTGGC AGCAGGGCAA CGTGTTCTCC TGTCTGTGA
 10 TGCACGAGGC ACTGCACAAT CATTACACCC AGAAATCCCT GTCACTGAGC
 CCCGGCAAG

SEQ ID NO: 38 DNA Sequence of PD-1 HC (v3.2)

CAGGTCCAGCTGGTGCAGAGCGGGGCAGAGGTCAAGAAACCCGGTAGCTC
 15 CGTGAAGGTCAGCTGCAAGGCTTCCGGCGGAACCTTCTCTAGTTACGCCA
 TCAGCTGGGTGAGACAGGCTCCTGGCCAGGGACTGGAATGGATGGGCCTG
 ATCATTTCCATCTTTTGATACCGCTGGCTACGCACAGAAGTTTCAGGGACG
 GGTGGCAATTACAGTCGATGAGTCAACCAGCACAGCCTATATGGAGCTGT
 CAAGCCTGCGGTCCGAAGACACTGCCGTGTACTATTGCGCAAGGGCCGAA
 20 CACTCCTCTACTGGAACCTTCGATTACTGGGGGCAGGGTACCCTGGTGAC
 AGTCAGTTCAGCCAGCACTAAGGGACCCAGCGTGTTTCCAGAGGCCCCCT
 CTAGTAAATCCACTTCTGGAGGCACCGCTGCACTGGGCTGTCTGGTGACC
 GATTACTTTCCAGAGCCCGTCACAGTGAGCTGGAACCTCCGGGGCCCTGAC
 CAGCGGAGTCCATACATTTCCCTGCTGTGCTGGAGTCAAGCGGGCTGTACT
 25 CCCTGTCTCTGTGGTCACCGTGCCAAGTTCAAGCCTGGGAACTCAGACC
 TATATCTGCAACGTGAATCACAAGCCTTCAAATACAAAAGTTGACAAACG
 TGTGGAACCCAAGAGTTGTGATAAAACCCATACATGCCCCCTTGTCCGG
 CGCCAGAGGCTGCAGGAGGACCAAGCGTGTTCTGTTTCCACCCAAGCCT
 AAAGACACACTGATGATTTCCCGAACCCCCGAAGTCACATGCGTGGTTCGT
 30 GTCTGTGAGTCACGAGGACCCTGAAGTCAAGTCAAGTCAACTGGTACGTGGATG
 GCGTCGAGGTGCATAATGCCAAGACTAAACCTAGGGAGGAACAGTACAAC
 TCAACCTATCGCGTCGTGAGCGTCCTGACAGTGCTGCACCAGGATTGGCT
 GAACGGCAAAGAATATAAGTGCAAAGTGAGCAATAAGGCCCTGCCCCGCTC
 CTATCGAGAAAACCATTTCCAAGGCTAAAGGGCAGCCTCGCGAACCACAG
 35 GTCTACGTGCTGCCTCCATCCCGGGACGAGCTGACAAAGAACCAGGTCTC
 TCTGCTGTGCCTGGTGAAAGGCTTCTATCCATCAGATATTGCTGTGGAGT
 GGGAAAGCAATGGGCAGCCCGAGAACAATTACCTGACTTGGCCCCCTGTG
 CTGGACTCTGATGGGAGTTTCTTTCTGTATTCTAAGCTGACCGTGGATAA
 AAGTAGGTGGCAGCAGGGAAATGTCTTTAGTTGTTCAGTGATGCATGAAG
 40 CCCTGCATAACCACTACACCCAGAAAAGCCTGTCCCTGTCCCCCGGAAAA

SEQ ID NO: 39 DNA Sequence of PD-1 HC (v13844)

CAGGTCCAGCTGGTGCAGAGCGGGGCAGAGGTCAAGAAACCCGGTAGCTC
 45 CGTGAAGGTCAGCTGCAAGGCTTCCGGCGGAACCTTCTCTAGTTACGCCA
 TCAGCTGGGTGAGACAGGCTCCTGGCCAGGGACTGGAATGGATGGGCCTG
 ATCATTTCCAATGTTTCGACACCGCTGGCTACGCACAGAAGTTTCAGGGACG

GGTGGCAATTACAGTCGATGAGTCAACCAGCACAGCCTATATGGAGCTGT
 CAAGCCTGCGGTCCGAAGACACTGCCGTGTACTATTGCGCAAGGGCCGAA
 CACTCCTCTACTGGAACCTTCGATTACTGGGGGCAGGGTACCCTGGTGAC
 AGTCAGTTCAGCCAGCACTAAGGGACCCAGCGTGTTTCCAGAGGCCCCCT
 5 CTAGTAAATCCACTTCTGGAGGCACCGCTGCACTGGGCTGTCTGGTGACC
 GATTACTTCCCAGAGCCCCGTACAGTGAGCTGGAACCTCCGGGGCCCTGAC
 CAGCGGAGTCCATACATTTCCCTGCTGTGCTGGAGTCAAGCGGGCTGTACT
 CCCTGTCCTCTGTGGTCACCGTGCCAAGTTCAAGCCTGGGAACTCAGACC
 TATATCTGCAACGTGAATCACAAGCCTTCAAATACAAAAGTTGACAAACG
 10 TGTGGAACCCAAGAGTTGTGATAAAACCCATACATGCCCCCCTTGTCCGG
 CGCCAGAGGCTGCAGGAGGACCAAGCGTGTTCTGTTTCCACCCAAGCCT
 AAAGACACACTGATGATTTCCCGAACCCCCGAAGTCACATGCGTGGTCTG
 GTCTGTGAGTCACGAGGACCCTGAAGTCAAGTTCAACTGGTACGTGGATG
 GCGTCGAGGTGCATAATGCCAAGACTAAACCTAGGGAGGAACAGTACAAC
 15 TCAACCTATCGCGTCGTGAGCGTCCTGACAGTGCTGCACCAGGATTGGCT
 GAACGGCAAAGAATATAAGTGCAAAGTGAGCAATAAGGCCCTGCCCCGCTC
 CTATCGAGAAAACCATTTCCAAGGCTAAAGGGCAGCCTCGCGAACCACAG
 GTCTACGTGTATCCTCCAAGCCGGGACGAGCTGACAAAGAACCAGGTCTC
 CCTGACTTGTCTGGTGAAAGGGTTTTACCCTAGTGATATCGCTGTGGAGT
 20 GGAATCAAATGGACAGCCAGAGAACAATTATAAGACTACCCCCCCTGTG
 CTGGACAGTGATGGGTCAATTCGCACTGGTCTCCAAGCTGACAGTGGACAA
 ATCTCGGTGGCAGCAGGGAAATGTCTTTTCATGTAGCGTGATGCATGAAG
 CACTGCACAACCATTACACCCAGAAGTCACTGTCCTGTCACCAGGAAAA

25 **SEQ ID NO: 40 IgG1 CH wt**
 ASTKGPSVFPLAPSSKSTSGGTAALGCLVKDYFPEPVTVSWNSGALTSGV
 HTFPAVLQSSGLYSLSSVTVPSSSLGTQTYICNVNHKPSNTKVDKKVEP
 KSCDKHTHTCPPCPAPELLGGPSVFLFPPKPKDTLMISRTPEVTCVVDVS
 HEDPEVKFNWYVDGVEVHNAKTKPREEQYNSTYRVVSVLTVLHQDWLNGK
 30 EYKCKVSNKALPAPIEKTISKAKGQPREPQVYTLPPSRDELTKNQVSLTCL
 LVKGFYPDSIAVEWESNGQPENNYKTTTPVLDSDGSFFLYSKLTVDKSRW
 QQGNVFSCSVMHEALHNHYTQKSLSLSPGK

SEQ ID NO: 41 v3.2 PD-L1 CH
 35 ASTKGPSVFPLAPSSKSTSGGTAALGCLVKDYFPEPVTVSWNSGALTSGV
 HTFPAVLQSSGLYSLKSVTVPSSSLGTQTYICNVNHKPSNTKVDKRVPEP
 KSCDKHTHTCPPCPAPEAAGGPSVFLFPPKPKDTLMISRTPEVTCVVVSVS
 HEDPEVKFNWYVDGVEVHNAKTKPREEQYNSTYRVVSVLTVLHQDWLNGK
 EYKCKVSNKALPAPIEKTISKAKGQPREPQVYVYPPSRDELTKNQVSLTCL
 40 LVKGFYPDSIAVEWESNGQPENNYKTTTPVLDSDGSFALVSKLTVDKSRW
 QQGNVFSCSVMHEALHNHYTQKSLSLSPGK

SEQ ID NO: 42 v13844 PD-1 CH
 45 ASTKGPSVFPEAPSSKSTSGGTAALGCLVTDYFPEPVTVSWNSGALTSGV
 HTFPAVLESSGLYSLSSVTVPSSSLGTQTYICNVNHKPSNTKVDKRVPEP
 KSCDKHTHTCPPCPAPEAAGGPSVFLFPPKPKDTLMISRTPEVTCVVVSVS
 HEDPEVKFNWYVDGVEVHNAKTKPREEQYNSTYRVVSVLTVLHQDWLNGK
 EYKCKVSNKALPAPIEKTISKAKGQPREPQVYVYPPSRDELTKNQVSLTCL
 LVKGFYPDSIAVEWESNGQPENNYKTTTPVLDSDGSFALVSKLTVDKSRW

-35-

QQGNVFSCSVMHEALHNHYTQKSLSLSPGK

SEQ ID NO: 43 v3.2 PD-1 CH

5 ASTKGPSVFPEAPSSKSTSGGTAALGCLVTDYFPEPVTVSWNSGALTSGV
HTFPAVLESSGLYSLSSVTVPSSSLGTQTYICNVNHKPSNTKVDKRVEP
KSCDKTHTCPPCPAPEAAGGPSVFLFPPKPKDTLMISRTPEVTCVTVSVS
HEDPEVKFNWYVDGVEVHNAKTKPREEQYNSTYRVVSVLTVLHQDWLNGK
EYKCKVSNKALPAPIEKTISKAKGQPREPQVYVLPSSRDELTKNQVSLC
10 LVKGFYPSDIAVEWESNGQPENNYLTWPPVLDSDGSFFLYSKLTVDKSRW
QQGNVFSCSVMHEALHNHYTQKSLSLSPGK

SEQ ID NO: 44 v13844 PD-L1 CH

15 ASTKGPSVFPLAPSSKSTSGGTAALGCLVKDYFPEPVTVSWNSGALTSGV
HTFPAVLQSSGLYSLKSVTVPSSSLGTQTYICNVNHKPSNTKVDKRVEP
KSCDKTHTCPPCPAPEAAGGPSVFLFPPKPKDTLMISRTPEVTCVTVSVS
HEDPEVKFNWYVDGVEVHNAKTKPREEQYNSTYRVVSVLTVLHQDWLNGK
EYKCKVSNKALPAPIEKTISKAKGQPREPQVYVLPSSRDELTKNQVSLC
LVKGFYPSDIAVEWESNGQPENNYLTWPPVLDSDGSFFLYSKLTVDKSRW
20 QQGNVFSCSVMHEALHNHYTQKSLSLSPGK

SEQ ID NO: 45 CL Lambda-wildtype

QPKANPTVTLFPPSSEELQANKATLVCLISDFYPGAVTVAWKADGSPVKAGVETTKPSKQSNKY
AASSYLSTPEQWKSHRSYSCQVTHEGSTVEKTVAPTECS

25 SEQ ID NO: 46 CL PD-L1

QPKAAPSVTLFPPSSEELQANKATLVCLISDFYPGAVTVAWKADSSPVKAGVETTTPSKQSNKY
AAESELSTPEQWKSHRSYSCQVTHEGSTVEKTVAPAEC

SEQ ID NO: 47 CL Kappa-wildtype

30 RTVAAPSVFIFPPSDEQLKSGTASVCLLNNFYPREAKVQWKVDNALQSGNSQESVTEQDSKDST
YLSSTLTLSKADYEKHKVYACEVTHQGLSSPVTKSFNRGEC

SEQ ID NO: 48 CL Kappa-PD-1

35 RTVAAPSVFIFPPSDEQLKSGTARVCLLNNFYPREAKVQWKVDNALQSGNSQESVTEQDSKDST
YSLRSTLTLSKADYEKHKVYACEVTHQGLSSPVTKSFNRGEC

SEQ ID NO: 49 (HC of PD-L1 Ab v3.2)

40 QVQLVQSGAEVKKPGSSVKVSCKASGGTFSSYAISWVRQAPGQGLEWMGGIIPIFGTANY
AQKFQGRVTITADKSTSTAYMELSSLRSEDTAVYYCARSPDYSPYGYGMDVWGQGTTVT
VSSASTKGPSVFPLAPSSKSTSGGTAALGCLVKDYFPEPVTVSWNSGALTSGVHTFPAVL
QSSGLYSLKSVTVPSSSLGTQTYICNVNHKPSNTKVDKRVEPKSCDKTHTCPPCPAPEA
AGGPSVFLFPPKPKDTLMISRTPEVTCVTVSVSHEDPEVKFNWYVDGVEVHNAKTKPREE
QYNSTYRVVSVLTVLHQDWLNGKEYKCKVSNKALPAPIEKTISKAKGQPREPQVYVPPS
RDELTKNQVSLTCLVKGFYPSDIAVEWESNGQPENNYKTTTPVLDSDGSFALVSKLTVDK
45 SRWQQGNVFSCSVMHEALHNHYTQKSLSLSPGK

SEQ ID NO: 50 DNA Sequence of PD-L1 LC (codon variant 1)

5 CAGTCTGTGC TGACTCAGCC ACCTTCCGCC TCTGGAACCC CAGGACAGAG GGTCAACAATC
AGTTGCTCAG GGAGCTCCTC TAACATTGGA AGCAACACTG TGAATTGGTA CCAGCAGCTG
CCTGGGACCG CTCCAAAGCT GCTGATCTAT GGCAACTCCA ATCGACCATC TGGAGTGCCT
GACCGGTTCA GCGGCTCCAA ATCTGGCACC AGTGCTTCAC TGGCAATTAG TGGCCTGCAG
TCCGAGGACG AAGCCGATTA CTATTGCCAG AGCTACGATA GTTCACTGAG CGGCTCCGTG
TTCGGCGGGG GAATCAAGCT GACAGTCCTG GGACAGCCAA AAGCGGCGCC CAGCGTGACT
CTGTTTCCAC CCAGCTCCGA GGAAGTGCAG GCCAATAAGG CTACCCTGGT CTGTCTGATT
10 TCCGACTTCT ACCCCGGGGC TGTGACAGTC GCATGGAAGG CCGATTCTAG TCCTGTGAAA
GCAGGAGTCG AGACCACAAC TCCATCAAAG CAGAGCAACA ACAAGTACGC AGCCGAGAGC
GAGCTGTCTC TGACACCTGA ACAGTGGAAA AGCCACCGGT CTTATAGTTG TCAGGTGACT
CACGAGGGCT CAACAGTGGA AAAGACAGTC GCACCCGCAG AATGCTCA

SEQ ID NO: 51 DNA Sequence of PD-L1 HC (codon variant 1)

15 CAGGTCCAGC TGGTGCAGAG CGGAGCCGAA GTGAAGAAAC CAGGCAGCTC CGTCAAGGTG
TCATGCAAAG CCAGCGGCGG GACTTTCTCT AGTTACGCTA TCTCCTGGGT GAGACAGGCA
CCAGGACAGG GACTGGAGTG GATGGGAGGA ATCATTCCCTA TCTTCGGGAC AGCTAACTAC
GCACAGAAGT TTCAGGGAAG GGTGACTATT ACCGCCGACA AATCTACAAG TACTGCTTAT
ATGGAGCTGT CAAGCCTGAG GAGCGAAGAT ACCGCAGTGT ACTATTGCGC CCGTCCCCC
20 GACTACTCTC CTTACTATTA CTATGGCATG GACGTGTGGG GGCAGGGAAC CACAGTCACA
GTGTCCTCTG CCAGCACTAA GGGGCCTTCA GTGTTTCCAC TGGCACCCAG TTCAAAATCA
ACAAGCGGAG GAACTGCCGC TCTGGGATGT CTGGTGAAGG ACTATTTCCC AGAGCCAGTC
ACCGTGAGCT GGAAGTCCCG CGCACTGACT TCCGGAGTCC ACACCTTTCC AGCCGTGCTG
CAGAGCTCCG GACTGTACTC TCTGAAGAGT GTGGTCACAG TGCCTTCAAG CTCCCTGGGC
25 ACCCAGACAT ATATCTGCAA CGTGAATCAC AAGCCTAGTA ATACTAAGGT TGACAAAAGT
GTGGAACCAA AGAGCTGTGA TAAACTCAT ACCTGCCCCC CTTGTCCGGC GCCAGAGGCA
GCAGGAGGAC CAAGCGTGTT CCTGTTTCCA CCAAGCCCA AAGACACCCT GATGATTAGC
CGAACCCCTG AAGTCACATG CGTGGTCGTG TCCGTGTCTC ACGAGGACCC AGAAGTCAAG
TTCAACTGGT ACGTGGATGG CGTCGAGGTG CATAATGCCA AGACAAAACC CCGGGAGGAA
30 CAGTACAACA GCACCTATAG AGTCGTGTCC GTCCTGACAG TGCTGCACCA GGATTGGCTG
AACGGCAAGG AATATAAGTG CAAAGTGTCC AATAAGGCC TGCCCGCTCC TATCGAGAAA
ACCATTTCTA AGGCAAAAGG CCAGCCTCGC GAACACAGG TCTACGTGTA TCCTCCAAGC
CGGGACGAGC TGACAAAGAA CCAGGTCTCC CTGACTTGTC TGGTGAAAGG GTTTTACCCT
AGTGATATCG CTGTGGAGTG GGAATCAAAT GGACAGCCAG AGAACAATTA TAAGACTACC
35 CCCCTGTGC TGGACAGTGA TGGGTCATTG GCACTGGTCT CCAAGCTGAC AGTGGACAAA
TCTCGGTGGC AGCAGGAAA TGTCTTTTCA TGTAGCGTGA TGCATGAAGC ACTGCACAAC
CATTACACCC AGAAGTCACT GTCAGTGTCA CCAGGAAAA

WE CLAIM:

1. An antibody that binds human PD-L1 (SEQ ID NO: 1) and human PD-1 (SEQ ID NO: 2) comprising:
 - e) a first heavy chain (HC1) comprising a heavy chain variable region (HCVR) comprising the amino acid sequence of SEQ ID NO: 3;
 - f) a first light chain (LC1) comprising a light chain variable region (LCVR) comprising the amino acid sequence of SEQ ID NO: 4;
 - g) a second heavy chain (HC2) comprising a heavy chain variable region comprising the amino acid sequence of SEQ ID NO: 5; and
 - h) a second light chain (LC2) comprising a light chain variable region comprising the amino acid sequence of SEQ ID NO: 8.
2. The antibody of claim 1 comprising:
 - a) the HC1 comprising, in order from the N-terminus to the C-terminus, the HCVR comprising the amino acid sequence of SEQ ID NO: 3, the amino acid sequence of SEQ ID NO: 9 in the CH1 domain, the amino acid sequence of SEQ ID NO: 10 in the CH2 domain, and the amino acid sequence of SEQ ID NO: 11 or SEQ ID NO: 12 in the CH3 domain;
 - b) the LC1 comprising, in order from the N-terminus to the C-terminus, the LCVR comprising the amino acid sequence of SEQ ID NO: 4 and the amino acid sequence of SEQ ID NO: 14 in the constant region;
 - c) the HC2 comprising, in order from the N-terminus to the C-terminus, the HCVR comprising the amino acid sequence of SEQ ID NO: 5, the amino acid sequence of SEQ ID NO: 13 in the CH1 domain, the amino acid sequence of SEQ ID NO: 10 in the CH2 domain, and the amino acid sequence of SEQ ID NO: 12 or the amino acid sequence of SEQ ID NO: 11 in the CH3 domain; and
 - d) the LC2 comprising, in order from N-terminus, the LCVR comprising the amino acid sequence of SEQ ID NO: 8 and the amino acid sequence of SEQ ID NO: 15 in the constant region, provided that when the amino acid sequence of SEQ ID NO: 11 is present in the CH3 domain of said HC1, the amino acid sequence of SEQ ID NO: 12 is present in the CH3 domain of said HC2; or when the amino acid sequence of SEQ ID NO: 12 is present in the CH3 domain of said HC1, the

amino acid sequence of SEQ ID NO: 11 is present in the CH3 domain of said HC2.

3. The antibody of Claim 2, wherein said HC1 comprises the amino acid sequence of SEQ ID NO: 11 in the CH3 domain, and wherein said HC2 comprises the HCVR comprising the amino acid sequence of SEQ ID NO: 6 and said HC2 comprises the amino acid sequence of SEQ ID NO: 12 in the CH3 domain.
4. The antibody of Claim 3, wherein said HC1, LC1, HC2, and LC2 comprises the amino acid sequences of SEQ ID NO: 49, SEQ ID NO: 30, SEQ ID NO: 31, and SEQ ID NO: 34, respectively.
5. The antibody of Claim 3, wherein said HC1, LC1, HC2, and LC2 comprises the amino acid sequences of SEQ ID NO: 29, SEQ ID NO: 30, SEQ ID NO: 33, and SEQ ID NO: 34, respectively.
6. A mammalian cell comprising a DNA molecule comprising a polynucleotide sequence encoding polypeptides having the amino acid sequences of SEQ ID NO: 49, SEQ ID NO: 30, SEQ ID NO: 31, and SEQ ID NO: 34, wherein the cell is capable of expressing the antibody of Claim 4.
7. A mammalian cell comprising a DNA molecule comprising a polynucleotide sequence encoding polypeptides having the amino acid sequences of SEQ ID NO: 29, SEQ ID NO: 30, SEQ ID NO: 33, and SEQ ID NO: 34, wherein the cell is capable of expressing the antibody of Claim 5.
8. A process for producing an antibody comprising cultivating the mammalian cell of Claim 6 or 7 under conditions such that the antibody is expressed, and recovering the expressed antibody.
9. A pharmaceutical composition, comprising the antibody of any one of Claims 1-5 and an acceptable carrier, diluent, or excipient.
10. A method of treating cancer, comprising administering to a patient in need thereof, an effective amount of the antibody of any one of Claims 1-5.
11. The method of Claim 10, wherein the cancer is Hodgkin's or non-Hodgkin's lymphomas, melanoma, renal cell cancer, kidney cancer, lung cancer, bladder cancer, gastric and esophageal cancer, colorectal cancer, liver cancer, hepatocellular cancer, cholangiocarcinoma, pancreatic cancer, breast cancer,

triple-negative breast cancer, ovarian cancer, endometrial cancer, prostate cancer, small cell lung cancer (SCLC), non-small cell lung cancer (NSCLC), mesothelioma, squamous cancer of head neck cancer (SCCHN), soft tissue sarcoma, or glioblastoma multiforme.

- 5 12. The method of Claim 11, wherein the lung cancer is NSCLC, small cell lung cancer, or mesothelioma.
13. The method of any one of Claims 10-12, further comprising administering simultaneously, separately, or sequentially one or more antitumor agents selected from the group consisting of cisplatin, carboplatin, dacarbazine, liposomal
- 10 doxorubicin, docetaxel, cyclophosphamide and doxorubicin, navelbine, eribulin, paclitaxel, paclitaxel protein-bound particles for injectable suspension, ixabepilone, capecitabine, FOLFOX (leucovorin, fluorouracil, and oxaliplatin), FOLFIRI (leucovorin, fluorouracil, and irinotecan), gemcitabine, topotecan, liposomal irinotecan, pemetrexed, cetuximab, nivolumab, ipilimumab,
- 15 pidilizumab, pembrolizumab, tremelimumab, urelumab, lirilumab, atezolizumab, epacadostat, and durvalumab.
14. The antibody of any one of Claims 1-5 for use in therapy.
15. The antibody of any one of Claims 1-5 for use in the treatment of cancer.
16. The antibody for use of Claim 15, wherein the cancer is Hodgkin's or non-
- 20 Hodgkin's lymphomas, melanoma, renal cell cancer, kidney cancer, lung cancer, bladder cancer, gastric and esophageal cancer, colorectal cancer, liver cancer, hepatocellular cancer, cholangiocarcinoma, pancreatic cancer, breast cancer, triple-negative breast cancer, ovarian cancer, endometrial cancer, prostate cancer, small cell lung cancer (SCLC), non-small cell lung cancer (NSCLC),
- 25 mesothelioma, squamous cancer of head neck cancer (SCCHN), soft tissue sarcoma, or glioblastoma multiforme.
17. The antibody for use of Claim 16, wherein the cancer is non-small cell lung cancer, small cell lung cancer, or mesothelioma.
18. The antibody of any one of Claims 1-5 for use in simultaneous, separate, or
- 30 sequential combination with one or more antitumor agents selected from the group consisting of cisplatin, carboplatin, dacarbazine, liposomal doxorubicin,

- docetaxel, cyclophosphamide and doxorubicin, navelbine, eribulin, paclitaxel, paclitaxel protein-bound particles for injectable suspension, ixabepilone, capecitabine, FOLFOX (leucovorin, fluorouracil, and oxaliplatin), FOLFIRI (leucovorin, fluorouracil, and irinotecan), gemcitabine, topotecan, liposomal
5 irinotecan, pemetrexed, cetuximab, nivolumab, ipilimumab, pidilizumab, pembrolizumab, tremelimumab, urelumab, lirilumab, atezolizumab, epacadostat, and durvalumab, in the treatment of cancer.
19. A pharmaceutical composition for use in treating cancer, comprising an effective amount of the antibody of any one of Claims 1-5.
- 10 20. The composition for use of Claim 19, wherein the cancer is Hodgkin's or non-Hodgkin's lymphomas, melanoma, renal cell cancer, kidney cancer, lung cancer, bladder cancer, gastric and esophageal cancer, colorectal cancer, liver cancer, hepatocellular cancer, cholangiocarcinoma, pancreatic cancer, breast cancer, triple-negative breast cancer, ovarian cancer, endometrial cancer, prostate cancer,
15 small cell lung cancer (SCLC), non-small cell lung cancer (NSCLC), mesothelioma, squamous cancer of head neck cancer (SCCHN), soft tissue sarcoma, or glioblastoma multiforme.
21. The composition for use of Claim 20, wherein the cancer is NSCLC, small cell lung cancer, or mesothelioma.
- 20 22. The composition of any one of Claims 19-22, which is administered in simultaneous, separate, or sequential combination with one or more antitumor agents selected from the group consisting of cisplatin, carboplatin, dacarbazine, liposomal doxorubicin, docetaxel, cyclophosphamide and doxorubicin, navelbine, eribulin, paclitaxel, paclitaxel protein-bound particles for injectable suspension,
25 ixabepilone, capecitabine, FOLFOX (leucovorin, fluorouracil, and oxaliplatin), FOLFIRI (leucovorin, fluorouracil, and irinotecan), gemcitabine, topotecan, liposomal irinotecan, pemetrexed, cetuximab, nivolumab, ipilimumab, pidilizumab, pembrolizumab, tremelimumab, urelumab, lirilumab, atezolizumab, epacadostat, and durvalumab.