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(54) Title: PD-1 ANTIBODIES

(57) Abstract: The present invention provides antibodies that bind human programmed cell death 1 (PD-1). These antibodies may be useful for treating cancer alone and in combination with chemotherapy and other cancer therapeutics.

PD-1 Antibodies

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

Tumor cells escape detection and elimination by the immune system through multiple mechanisms. Immune checkpoint pathways are used in self-tolerance maintenance and activated T cell control, but cancer cells can use the pathways to prevent destruction. The PD-1 / human programmed cell death 1 ligand 1 (PD-L1) pathway is one such immune checkpoint. Human PD-1 is found on T cells, and the binding of PD-L1 and human programmed cell death 1 ligand 2 (PD-L2) to PD-1 inhibits T cell proliferation and cytokine production. Tumor cell production of PD-L1 and PD-L2 can therefore allow escape from T cell surveillance.

A fully human IgG4 (S228P) antibody against human PD-1, nivolumab, has been shown to inhibit the binding of PD-1 to PD-L1 and PD-L2, and has been tested in various clinical trials. (Wang et al., Cancer Immunol Res (2014) 2(9):846). A humanized IgG4 (S228P) antibody against PD-1, pembrolizumab (formerly lambrolizumab), has been shown to inhibit the binding of PD-1 to PD-L1 and PD-L2, and has been tested in various clinical trials. (WO2008156712 and Hamid et al., N Engl J Med (2013) 369:2).

There remains a need to provide alternative antibodies that bind and neutralize human PD-1 interaction with PD-L1 and PD-L2. In particular, there remains a need to provide antibodies that bind human PD-1 with high affinity but with different features than clinically approved PD-1 antibodies, such as binding to mouse and human PD-1. Further, there remains a need to provide antibodies that bind human PD-1 with affinity similar to clinically approved PD-1 but bind human PD-1 differently. Also, there remains a need to provide antibodies that more effectively block the human PD-1 interaction with PD-L1 and PD-L2 than certain prior art antibodies. Better blocking can translate into greater *in vivo* activity or lower required dosing amounts.

Certain antibodies of the present invention block the human PD-1 to PD-L1 and PD-L2 interactions in CHO cells more effectively than nivolumab and pembrolizumab.

Furthermore, certain antibodies of the present invention bind murine PD-1 on CHO cells whereas nivolumab and pembrolizumab binding to murine PD-1 is not detected.

Accordingly, in some embodiments the present invention provides an antibody that binds human PD-1 (SEQ ID NO: 1), comprising a light chain (LC) and a heavy chain (HC), wherein the light chain comprises light chain complementarity determining regions LCDR1, LCDR2, and LCDR3 consisting of the amino acid sequences RASQSVSSYLA (SEQ ID NO: 14), YDASKRAT (SEQ ID NO: 15), and DQRNNWPLT (SEQ ID NO: 16), respectively, and wherein the heavy chain comprises heavy chain complementarity determining regions HCDR1, HCDR2, and HCDR3, wherein HCDR1 consists of the amino acid sequence:

a. KASGYTFTSYYMH (SEQ ID NO: 2),
b. KASGYTFEGYYMH (SEQ ID NO: 3),
c. KASGYTFTAQYMH (SEQ ID NO: 4),
d. KASGYTFEKYYMH (SEQ ID NO: 5),
e. KASGYTFTSNYMH (SEQ ID NO: 6), or
f. KASGYTFSAYYMH (SEQ ID NO: 7);

wherein HCDR2 consists of the amino acid sequence:

a. IINPSGGSTSYAQKFQG (SEQ ID NO: 8),
b. IINPEGGETSYAQKFQG (SEQ ID NO: 9),
c. IINPSGGETGYAQKFQG (SEQ ID NO: 10),
d. IINPSEGSTGYAQKFQG (SEQ ID NO: 11), or
e. IINPDGGSTGYAQKFQG (SEQ ID NO: 12);

and wherein HCDR3 consists of the amino acid sequence AKEGVADGYGLVDV (SEQ ID NO: 13).

In some embodiments, the present invention provides an antibody that binds human PD-1 (SEQ ID NO: 1), wherein LCDR1, LCDR2, and LCDR3 consist of the amino acid sequences RASQSVSSYLA (SEQ ID NO: 14), YDASKRAT (SEQ ID NO: 15), and DQRNNWPLT (SEQ ID NO: 16), respectively, and wherein HCDR1, HCDR2, and HCDR3 consist of the amino acid sequences KASGYTFTSYYMH (SEQ ID NO: 2), IINPSGGSTSYAQKFQG (SEQ ID NO: 8), and AKEGVADGYGLVDV (SEQ ID NO: 13), respectively.

In some embodiments, the present invention provides an antibody that binds human PD-1 (SEQ ID NO: 1), wherein LCDR1, LCDR2, and LCDR3 consist of the amino acid sequences RASQSVSSYLA (SEQ ID NO: 14), YDASKRAT (SEQ ID NO: 15), and DQRNNWPLT (SEQ ID NO: 16), respectively, and wherein HCDR1, HCDR2, and HCDR3 consist of the amino acid sequences KASGYTFEGYYMH (SEQ ID NO: 3), IINPEGGETSYAQKFQG (SEQ ID NO: 9), and AKEGVADGYGLVDV (SEQ ID NO: 13), respectively.

In some embodiments, the present invention provides an antibody that binds human PD-1 (SEQ ID NO: 1), wherein LCDR1, LCDR2, and LCDR3 consist of the amino acid sequences RASQSVSSYLA (SEQ ID NO: 14), YDASKRAT (SEQ ID NO: 15), and DQRNNWPLT (SEQ ID NO: 16), respectively, and wherein HCDR1, HCDR2, and HCDR3 consist of the amino acid sequences KASGYTFTAQYMH (SEQ ID NO: 4), IINPSGGETGYAQKFQG (SEQ ID NO: 10), and AKEGVADGYGLVDV (SEQ ID NO: 13), respectively.

In some embodiments, the present invention provides an antibody that binds human PD-1 (SEQ ID NO: 1), wherein LCDR1, LCDR2, and LCDR3 consist of the amino acid sequences RASQSVSSYLA (SEQ ID NO: 14), YDASKRAT (SEQ ID NO: 15), and DQRNNWPLT (SEQ ID NO: 16), respectively, and wherein HCDR1, HCDR2, and HCDR3 consist of the amino acid sequences KASGYTFEKYYMH (SEQ ID NO: 5), IINPDGGSTGYAQKFQG (SEQ ID NO: 12), and AKEGVADGYGLVDV (SEQ ID NO: 13), respectively.

In some embodiments, the present invention provides an antibody that binds human PD-1 (SEQ ID NO: 1), wherein LCDR1, LCDR2, and LCDR3 consist of the amino acid sequences RASQSVSSYLA (SEQ ID NO: 14), YDASKRAT (SEQ ID NO: 15), and DQRNNWPLT (SEQ ID NO: 16), respectively, and wherein HCDR1, HCDR2, and HCDR3 consist of the amino acid sequences KASGYTFTSNYMH (SEQ ID NO: 6), IINPSEGSTGYAQKFQG (SEQ ID NO: 11), and AKEGVADGYGLVDV (SEQ ID NO: 13), respectively.

In some embodiments, the present invention provides an antibody that binds human PD-1 (SEQ ID NO: 1), wherein LCDR1, LCDR2, and LCDR3 consist of the amino acid sequences RASQSVSSYLA (SEQ ID NO: 14), YDASKRAT (SEQ ID NO:

15), and DQRNNWPLT (SEQ ID NO: 16), respectively, and wherein HCDR1, HCDR2, and HCDR3 consist of the amino acid sequences KASGYTFSAYYMH (SEQ ID NO: 7), IINPDGGSTGYAQKFQG (SEQ ID NO: 12), and AKEGVADGYGLVDV (SEQ ID NO: 13), respectively.

In some embodiments, the present invention provides an antibody, comprising a light chain (LC) and a heavy chain (HC), wherein the light chain comprises a light chain variable region (LCVR) and the heavy chain comprises a heavy chain variable region (HCVR), wherein the LCVR has the amino acid sequence given in SEQ ID NO: 23, and the HCVR has the amino acid sequence given in SEQ ID NO: 17, SEQ ID NO: 18, SEQ ID NO: 19, SEQ ID NO: 20, SEQ ID NO: 21, or SEQ ID NO: 22.

In a further embodiment, the present invention provides an antibody, wherein the LCVR has the amino acid sequence given in SEQ ID NO: 23, and the HCVR has the amino acid sequence given in SEQ ID NO: 17.

In a further embodiment, the present invention provides an antibody, wherein the LCVR has the amino acid sequence given in SEQ ID NO: 23, and the HCVR has the amino acid sequence given in SEQ ID NO: 18.

In a further embodiment, the present invention provides an antibody, wherein the LCVR has the amino acid sequence given in SEQ ID NO: 23, and the HCVR has the amino acid sequence given in SEQ ID NO: 19.

In a further embodiment, the present invention provides an antibody, wherein the LCVR has the amino acid sequence given in SEQ ID NO: 23, and the HCVR has the amino acid sequence given in SEQ ID NO: 20.

In a further embodiment, the present invention provides an antibody, wherein the LCVR has the amino acid sequence given in SEQ ID NO: 23, and the HCVR has the amino acid sequence given in SEQ ID NO: 21.

In a further embodiment, the present invention provides an antibody, wherein the LCVR has the amino acid sequence given in SEQ ID NO: 23, and the HCVR has the amino acid sequence given in SEQ ID NO: 22.

In some embodiments, the present invention provides an antibody, wherein the LC has the amino acid sequence given in SEQ ID NO: 30, and the HC has the amino acid

sequence given in SEQ ID NO: 24, SEQ ID NO: 25, SEQ ID NO: 26, SEQ ID NO: 27, SEQ ID NO: 28, or SEQ ID NO: 29.

In a further embodiment, the present invention provides an antibody, wherein the LC has the amino acid sequence given in SEQ ID NO: 30, and the HC has the amino acid sequence given in SEQ ID NO: 24.

In a further embodiment, the present invention provides an antibody, wherein the LC has the amino acid sequence given in SEQ ID NO: 30, and the HC has the amino acid sequence given in SEQ ID NO: 25.

In a further embodiment, the present invention provides an antibody, wherein the LC has the amino acid sequence given in SEQ ID NO: 30, and the HC has the amino acid sequence given in SEQ ID NO: 26.

In a further embodiment, the present invention provides an antibody, wherein the LC has the amino acid sequence given in SEQ ID NO: 30, and the HC has the amino acid sequence given in SEQ ID NO: 27.

In a further embodiment, the present invention provides an antibody, wherein the LC has the amino acid sequence given in SEQ ID NO: 30, and the HC has the amino acid sequence given in SEQ ID NO: 28.

In a further embodiment, the present invention provides an antibody, wherein the LC has the amino acid sequence given in SEQ ID NO: 30, and the HC has the amino acid sequence given in SEQ ID NO: 29.

In some embodiments, the present invention provides an antibody, comprising two light chains and two heavy chains, wherein each light chain has the amino acid sequence given in SEQ ID NO: 30, and each heavy chain has the amino acid sequence given in SEQ ID NO: 24, SEQ ID NO: 25, SEQ ID NO: 26, SEQ ID NO: 27, SEQ ID NO: 28, or SEQ ID NO: 29.

In a further embodiment, the present invention provides an antibody, wherein each light chain has the amino acid sequence given in SEQ ID NO: 30, and each heavy chain has the amino acid sequence given in SEQ ID NO: 24.

In a further embodiment, the present invention provides an antibody, wherein each light chain has the amino acid sequence given in SEQ ID NO: 30, and each heavy chain has the amino acid sequence given in SEQ ID NO: 25.

In a further embodiment, the present invention provides an antibody, wherein each light chain has the amino acid sequence given in SEQ ID NO: 30, and each heavy chain has the amino acid sequence given in SEQ ID NO: 26.

In a further embodiment, the present invention provides an antibody, wherein each light chain has the amino acid sequence given in SEQ ID NO: 30, and each heavy chain has the amino acid sequence given in SEQ ID NO: 27.

In a further embodiment, the present invention provides an antibody, wherein each light chain has the amino acid sequence given in SEQ ID NO: 30, and each heavy chain has the amino acid sequence given in SEQ ID NO: 28.

In a further embodiment, the present invention provides an antibody, wherein each light chain has the amino acid sequence given in SEQ ID NO: 30, and each heavy chain has the amino acid sequence given in SEQ ID NO: 29.

In an embodiment, the present invention provides an antibody, wherein one of the heavy chains forms an inter-chain disulfide bond with one of the light chains, and the other heavy chain forms an inter-chain disulfide bond with the other light chain, and one of the heavy chains forms two inter-chain disulfide bonds with the other heavy chain.

In an embodiment, the present invention provides an antibody, wherein the antibody is glycosylated.

In some embodiments, the present invention provides an antibody that binds both human PD-1 and mouse PD-1. In a further embodiment, the present invention provides an antibody that is an antagonistic antibody and that binds both human PD-1 and mouse PD-1. In a further embodiment, the present invention provides an antibody that binds both human PD-1 and mouse PD-1with a Kd for each less than 400 pM. In a further embodiment, the present invention provides an antibody that binds both human PD-1 and mouse PD-1with a Kd for each less than 200 pM.

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

In an embodiment, 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. In a further embodiment, 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, wherein the cancer is melanoma, lung cancer, head and neck cancer, colorectal cancer, pancreatic cancer, gastric cancer, kidney cancer, bladder cancer, prostate cancer, breast cancer, ovarian cancer, or hepatocellular carcinoma.

In a further embodiment, these methods comprise 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. Non-limiting examples of anti-tumor agents include ramucirumab, necitumumab, olaratumab, galunisertib, abemaciclib, 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), and cetuximab.

In a further embodiment, these methods comprise the administration of an effective amount of the compound of the present invention in simultaneous, separate, or sequential combination with one or more immuno-oncology agents. Non-limiting examples of immuno-oncology agents include nivolumab, ipilimumab, pidilizumab, pembrolizumab, tremelimumab, urelumab, lirilumab, atezolizumab, and durvalumab.

In an embodiment, the present invention provides an antibody of the present invention, for use in therapy. In an embodiment, the present invention provides an antibody of the present invention, for use in the treatment of cancer. In a further embodiment, the present invention provides an antibody of the present invention, for use in the treatment of cancer, wherein the cancer is melanoma, lung cancer, head and neck cancer, colorectal cancer, pancreatic cancer, gastric cancer, kidney cancer, bladder cancer, prostate cancer, breast cancer, ovarian cancer, or hepatocellular carcinoma. In a further embodiment, 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. In a further embodiment, 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 ramucirumab, necitumumab,

olaratumab, galunisertib, abemaciclib, 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), and cetuximab, in the treatment of cancer.

In a further embodiment, the present invention provides the antibody of the present invention for use in simultaneous, separate, or sequential combination with one or more immuno-oncology agents. In a further embodiment, the present invention provides the antibody of the present invention for use in simultaneous, separate, or sequential combination with one or more immuno-oncology agents selected from the group consisting of nivolumab, ipilimumab, pidilizumab, pembrolizumab, tremelimumab, urelumab, lirilumab, atezolizumab, and durvalumab, in the treatment of cancer.

In a further embodiment, the present invention provides the use of an antibody of the present invention for the manufacture of a medicament for the treatment of cancer. In a further embodiment, the present invention 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 melanoma, lung cancer, head and neck cancer, colorectal cancer, pancreatic cancer, gastric cancer, kidney cancer, bladder cancer, prostate cancer, breast cancer, ovarian cancer, or hepatocellular carcinoma.

In a further embodiment, 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 one or more anti-tumor agents. In a further embodiment, 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 one or more anti-tumor agents selected from the group consisting of ramucirumab, necitumumab, olaratumab, galunisertib, abemaciclib, 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), and cetuximab.

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 designed to have engineered CDRs and have some portions of the antibody (all or parts of the frameworks, hinge regions, and constant regions) to be of human origin that are identical with or substantially identical (substantially human) with frameworks and constant regions derived from human genomic sequences. Fully human frameworks, hinge regions, and constant regions are those human germline sequences as well as sequences with naturally-occurring somatic mutations and those with engineered mutations. An antibody of the present invention may comprise framework, hinge, or constant regions derived from a fully human framework, hinge, or constant region containing one or more amino acid substitutions, deletions, or additions therein. Further, an antibody of the present invention is preferably substantially non-immunogenic in humans.

The 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 a LCVR and a light chain constant region ("LCCR"). When expressed in certain biological systems, antibodies having native human 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.

Optionally, the antibody of the present invention contains an Fc portion which is derived from human IgG_4 Fc region because of a reduced ability to engage Fc receptor-mediated inflammatory mechanisms or to activate complement resulting in reduced effector function.

Certain antibodies of the present invention contain an IgG_4-Fc portion that has a serine to proline mutation at position 228. The S228P mutation is a hinge mutation that prevents half-antibody formation (phenomenon of dynamic exchange of half-molecules in IgG_4 antibodies).

The HCVR and LCVR regions can be further subdivided into regions of hyper-variability, termed complementarity determining regions ("CDRs"), interspersed with regions that are more conserved, termed framework regions ("FR"). Each HCVR and LCVR is composed of three CDRs and four FRs, arranged from amino-terminus to carboxy-terminus in the following order: FR1, CDR1, FR2, CDR2, FR3, CDR3, FR4. Herein, the three CDRs of the heavy chain are referred to as "HCDR1, HCDR2, and HCDR3" and the three CDRs of the light chain are referred to as "LCDR1, LCDR2 and LCDR3". The CDRs contain most of the residues which form specific interactions with the antigen. There are currently three systems of CDR assignments for antibodies that are used for sequence delineation. The Kabat CDR definition (Kabat *et al.*, "Sequences of Proteins of Immunological Interest," National Institutes of Health, Bethesda, Md. (1991)) is based upon antibody sequence variability. The Chothia CDR definition (Chothia *et al.*, "Canonical structures for the hypervariable regions of immunoglobulins", Journal of Molecular Biology, 196, 901-917 (1987); Al-Lazikani *et al.*, "Standard conformations for the canonical structures of immunoglobulins", Journal of Molecular Biology, 273, 927-948 (1997)) is based on three-dimensional structures of antibodies and topologies of the CDR loops. The Chothia CDR definitions are identical to the Kabat CDR definitions with the exception of HCDR1 and HCDR2. The North CDR definition (North *et al.*, "A New Clustering of Antibody CDR Loop Conformations", Journal of Molecular Biology, 406, 228-256 (2011)) is based on affinity propagation clustering with a large number of crystal structures.

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 molecule encoding heavy chain constant regions. 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.

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. 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. The light chain constant region can be a kappa or lambda constant region.

The polynucleotides of the present invention will be expressed in a host cell after 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, and dihydrofolate reductase, to permit detection of those cells transformed with the desired DNA sequences.

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 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, 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 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). 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*, 19th ed. (1995), A. Gennaro et al., Mack Publishing Co.) and comprise an antibody, as disclosed 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-1 is intended to mean, unless indicated otherwise, a K_D of less than about 1 x10-6 M, preferably, less than about 1 x 10-9 M as determined by common methods known in the art, including by use of MSD essentially as described herein.

For the purposes of the present disclosure, the term "high affinity" refers to a K_D of less than about 150 pM for human PD-1 as determined by MSD. The K_D values are established by binding kinetics as described in "Binding kinetics and affinity" in the Assays section.

"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 example.

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 Antibody A - Antibody F, 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 for the light chain, heavy chain, light chain variable region, and heavy chain variable region of Antibody A - Antibody F are shown in Table 1.

The antibodies of the present invention, including, but not limited to, Antibody A - Antibody F can be made and purified essentially as follows. An appropriate host cell, such as HEK 293 or CHO, can be either transiently or stably transfected with an expression system for secreting antibodies using an optimal predetermined HC:LC vector ratio or a single vector system encoding both HC and LC. Clarified media, into which the antibody has been secreted, may be purified using any of many commonly-used techniques. For example, the medium may be conveniently 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. Further purification is optional, depending on the intended use. The antibody may be concentrated and/or sterile filtered using common techniques. Soluble aggregate and multimers may be effectively removed by common techniques, including size exclusion, hydrophobic interaction, ion exchange, multimodal, or hydroxyapatite chromatography. The purity of the antibody after these chromatography steps is greater than 95%. The product may be immediately frozen at -70°C or may be lyophilized.

Table 1: SEQ ID NOs

	Antibody A	Antibody B	Antibody C	Antibody D	Antibody E	Antibody F
HCVR	17	18	19	20	21	22
LCVR	23	23	23	23	23	23
Heavy chain	24	25	26	27	28	29
Light chain	30	30	30	30	30	30

Assays

Binding kinetics and affinity

The kinetics and equilibrium dissociation constant (K_D) for human PD-1 is determined for antibodies of the present invention using MSD, and bio-layer interferometry (ForteBio) assay methods.

As used herein, nivolumab is a human IgG4 PD-1 antibody transiently expressed in 293 HEK cells that utilizes the heavy chain and light chain sequences from Proposed INN: List 107 (CAS #946414-94-4). As used herein, pembrolizumab is a human IgG4 PD-1 antibody transiently expressed in 293 HEK cells that utilizes the heavy chain and light chain sequences from Proposed INN: List 72.

MSD assay

Equilibrium affinity measurements are performed as previously described (Estep, P., et al., MAbs, 2013. **5**(2): p. 270-8). Solution equilibrium titrations (SET) are performed in PBS + 0.1% IgG-Free BSA (PBSF) where antigen (b-PD-1 monomer) is held constant at 10-100 pM and is incubated with 3-to 5-fold serial dilutions of Fab or mAbs starting at 5-100 nM (experimental condition is sample dependent). Antibodies diluted at 20 nM in PBS are coated onto standard bind MSD-ECL plates overnight at 4°C or at room temperature for 30 min. Plates are blocked with BSA for 30 min whilst shaking at 700 rpm. Plates are then washed 3x with wash buffer (PBSF + 0.05% Tween 20). SET samples are applied and incubated on the plates for 150s with shaking at 700

rpm followed by one wash. Antigen captured on a plate is detected with 250 ng/mL sulfotag-labeled streptavidin in PBSF by incubation on the plate for 3 min. The plates are washed three times with wash buffer and are then read on the MSD Sector Imager 2400 instrument using 1x Read Buffer T with surfactant. The percent free antigen is plotted as a function of titrated antibody in Prism and fit to a quadratic equation to extract the KD. To improve throughput, liquid handling robots are used throughout MSD-SET experiments, including for SET sample preparation.

In experiments performed essentially as described in this assay, Antibody C and Antibody E, in an IgG1 format and expressed in yeast, bind human PD-1 with a K_D of 120 pM and 91 pM, respectively. Pembrolizumab and nivolumab bind PD-1 with a K_D of 130 pM and 640 pM respectively. Avidity measurements for Antibody C and Antibody E result in a K_D of approximately 9 pM and 22 pM, respectively, to human PD-1. Pembrolizumab and nivolumab bind human PD-1 with a K_D of approximately 3 pM and 5 pM respectively. Antibody C and Antibody E, in an IgG1 format and expressed in yeast, bind murine PD-1 with a K_D of 1900 pM and 1100 pM, respectively. Avidity measurements for Antibody C and Antibody E result in a K_D of approximately 130 pM and 330 pM, respectively, to murine PD-1.

Table 1: Binding by MSD of antibodies of the invention in IgG1 format (P.F.: poor fit; N.D.: none detected)

Name	Monovalent KD (M) against human PD-1	Avid KD (M) against human PD-1	Monovalent KD (M) against murine PD-1	Avid KD (M) against murine PD-1
Antibody A	4.90E-09	4.90E-11	9.60E-09	P.F.
Antibody B	1.30E-10	1.40E-11	2.70E-09	3.80E-10
Antibody C	1.20E-10	9.30E-12	1.90E-09	1.30E-10
Antibody D	3.00E-10	3.50E-11	2.30E-09	P.F.
Antibody E	9.10E-11	2.20E-11	1.10E-09	3.30E-10
Antibody F	1.70E-10	1.80E-11	1.20E-09	4.40E-10
Pembrolizumab	1.30E-10	3.00E-12	N.D.	N.D.
Nivolumab	6.40E-10	5.10E-12	N.D.	N.D.

Bio-layer interferometry

ForteBio affinity measurements were performed generally as previously described (Estep, P., et al., *High throughput solution-based measurement of antibody-antigen affinity and epitope binning.* MAbs, 2013. **5**(2): p. 270-8.). Briefly, ForteBio affinity measurements were performed by loading IgGs online onto AHQ sensors. Sensors were equilibrated off-line in assay buffer for 30 min and then monitored on-line for 60 seconds for baseline establishment. Sensors with loaded IgGs were exposed to 100 nM antigen for 5 min, afterwards they were transferred to assay buffer for 5 min for off-rate measurement. Kinetics was analyzed using the 1:1 binding model.

Table 2: Binding by Bio-light interferometry of antibodies of the invention in IgG1 format (N.D.: none detected)

	Monovalent K_D (M) Fab in solution, human PD-1_Fc on sensor tip	Monovalent K_D (M) human PD-1_HIS in solution, IgG on sensor tip	Monovalent K_D (M) Fab in solution, cyno PD-1_Fc on sensor tip	Monovalent K_D (M) Fab in solution, murine PD-1_Fc on sensor tip
Antibody A	1.60E-08	4.30E-09	1.40E-08	3.30E-08
Antibody B	2.70E-09	1.40E-09	3.70E-09	1.70E-08
Antibody C	2.00E-09	1.00E-09	2.40E-09	1.00E-08
Antibody D	3.40E-09	1.90E-09	4.40E-09	7.80E-09
Antibody E	2.70E-09	1.20E-09	3.30E-09	7.60E-09
Antibody F	3.00E-09	1.60E-09	3.60E-09	6.10E-09
Pembrolizumab	2.00E-09	2.00E-09	4.70E-10	N.D.
Nivolumab	1.70E-09	4.10E-09	1.20E-09	N.D.

In experiments performed essentially as described in this assay, Antibody C and Antibody E, in an IgG1 format and expressed in yeast, bind human PD-1_Fc with a K_D similar to nivolumab and pembrolizumab when PD-1_Fc was on the sensor tip. When the antibody was on the sensor tip, Antibody C and Antibody E, in an IgG1 format and expressed in yeast, bind human PD-1_Fc with a K_D similarly to nivolumab and pembrolizumab. Antibody C and Antibody E, in an IgG1 format and expressed in yeast,

bind cyno PD-1_Fc with a similar K_D to nivolumab and pembrolizumab. Antibody C and Antibody E, in an IgG1 format and expressed in yeast, bind murine PD-1_Fc whereas no binding was detected with nivolumab and pembrolizumab.

Binding to human and murine PD-1 on CHO cells

The binding of an antibody of the present invention to human PD-1 may be measured in a flow cytometry assay.

CHO cells (0.2×10^6) are incubated with the experimental antibody from 100 nM titrated 14x by a factor of 2 to the lowest concentration of 6.1 pM for 30 min in PBS 1% BSA on ice. Cells are then washed 3x, and are incubated with a secondary antibody (PE-labelled, at final concentration of 5 μg/ml) in PBS 1% BSA for 30 min on ice (protected from light). Cells are washed 3x and analyzed via flow cytometry. Flow cytometry is performed on an Accuri C6 system (BD Biosciences) and MFIs are calculated on the C6 software. EC50s are calculated on Graphpad software.

In experiments performed essentially as described in this assay, Antibody A, Antibody C, and Antibody E, in an IgG1 format and expressed in yeast bind human PD-1 in a dose-dependent manner, with an EC50 value (n=1) of 10.22 nM, 4.115 nM and 4.587 nM, respectively, and pembrolizumab binds human PD-1 with an EC50 value (n=1) of 0.6921 nM and nivolumab with an EC50 value (n=1) of 0.8057 nM.

Antibody A, B, C, D, E, and F, in an IgG1 format and expressed in yeast bind to murine PD-1 in a dose dependent manner with an EC50 of 11.01 nM, 6.135 nM, 2.884 nM, 9.259 nM, and 5.044 nM, and 5.855 nM, respectively, whereas no binding was detected for nivolumab or pembrolizumab.

Blocking of human PD-1 to PD-L1 and PD-L2 in CHO cells.

The ability of an antibody of the present invention to block binding of human PD-1 to PD-L1 and PD-L2 may be measured by flow cytometry.

CHO cells 0.2×10^6 are incubated with the experimental antibody (100 nM) for 30 min in PBS 1% BSA on ice. Cells are then washed 3X, and are incubated with PD-L2 linked with NHS-FIuorescein (Promega) in PBS 1% BSA for 30 min on ice (protected from light). Cells are washed 3x and analyzed via flow cytometry. Flow cytometry is

performed on an Accuri C6 system (BD Biosciences) and MFIs are calculated on the C6 software. EC50s are calculated on Graphpad software.

In experiments performed essentially as described in this assay, Antibody C and Antibody E (IgG1 format expressed in yeast) blocked human PD-L2-FITC binding, resulting in an MFI of 30,123.4 and 38,682.1, respectively, as compared to control IgG which resulted in an MFI of 182,959.1. Pembrolizumab and nivolumab resulted in MFI's of 46,245.9 and 54,509.8 respectively.

Table 3: Blocking of human PD-1 on CHO cells

Test Sample	MFI (PD-L2-FITC)
Cells only	33,449.7
No IgG	199,716.0
IgG Control	182,959.1
Nivolumab	54,509.8
Pembrolizumab	46,245.9
Antibody A	90,866.5
Antibody C	30,123.4
Antibody E	38,682.1

Mixed Lymphocyte Reaction

The blocking of PD-1 signals by antibodies of the present invention may be evaluated by measuring the release of inhibitory signals during T cell activation.

2 x 10^6 PBMC are plated per well in a 6 well tissue culture plate or T25 tissue culture flask in complete T cell media. Cells are incubated for 2-3 hours, to allow for adherence of monocytes. If adherence is insufficient, serum free media is used. Unattached cells are removed by gently swirling the flask with fresh media 3X.

Immature myeloid DCs are generated by culturing monocytes (1 x 10^6 cells/ml) from PBMC in X-VIVO 15 media containing 1% AB serum, 10 mM HEPES, 50 μM β-Me, IL-4 (1000 U/ml) and GM-CSF (1000 U/ml), or 25-50 ng/ml of each. After 2 days fresh medium supplemented with IL-4 and GM-CSF is added. On Day 5, cells are either frozen or maturation is induced by adding a stimulation cocktail containing rTNFa (1000U/ml), IL-1b (5 ng/ml), IL-6 (10ng/ml) and 1 μM PGE_2 for 2 days at a cell density of 3 x 10^5 cells/ml.

T cell Isolation is performed as per manufacturer's instructions in the Untouched CD4+ T cell isolation kit (Invitrogen). A magnet fitted with a 1.5 ml tube rack is used to remove unwanted magnetic beads (QIAGEN). 100,000-200,000 isolated T cells are mixed with 10,000-20,000 allogeneic moDCs in a total volume of 200 µl in 96-round bottom tissue culture plates for 4-5 days at 37°C. T cells are stimulated using anti-CD3/CD28 DynaBeads at a ratio of 3:1 (cells:beads) as a positive control; beads are prepared as per the manufacturer's instructions. Test antibodies are added at the beginning of the MLR and incubated throughout the culture period. Detection of IL-2 and IFN-γ is carried out as per manufacturer's instructions (eBioscience). OD measurements are determined on a Multiskan FC system (Thermo).

In experiments performed essentially as described in this assay, Antibody C and Antibody E increase IL-2 and IFNg secretion by activated T cells in the mixed lymphocyte reaction with equivalent potency as compared with nivolumab and pembrolizumab.

Table 4: IL-2 secretion fold change vs. IgG control

IgG	Concentrations of IgG				
	100 nM	10 nM	1 nM	0.1 nM	0.01 nM
Pembrolizumab	2.03	2.49	2.04	1.47	1.06
Nivolumab	2.37	2.44	1.72	1.26	1.09
Antibody A	2.28	1.87	1.23	1.15	1.37
Antibody C	2.62	2.29	1.83	1.15	1.01
Antibody E	3.20	2.61	1.95	1.21	1.13

	Cytokine in supernatant	Concentrations of IgG				
		100 nM	10 nM	1 nM	0.1 nM	0.01 nM
IgG control	IL-2 (pg/ml)	619.16	521.57	500.33	508.82	515.02
	Stdev. (pg/ml)	18.53	4.13	25.45	18.92	18.33
Pembrolizumab	IL-2 (pg/ml)	1257.59	1299.83	1021.63	749.33	545.48
	Stdev. (pg/ml)	27.37	13.42	37.56	33.53	11.21
Nivolumab	IL-2 (pg/ml)	1469.85	1274.69	858.20	641.13	562.30

	Stdev. (pg/ml)	55.01	68.79	44.24	23.40	33.04
Antibody A	IL-2 (pg/ml)	1410.46	975.38	617.86	585.50	707.77
	Stdev. (pg/ml)	41.38	27.88	49.01	8.39	27.93
Antibody C	IL-2 (pg/ml)	1622.02	1194.17	913.62	587.10	518.69
	Stdev. (pg/ml)	49.88	46.17	30.18	42.17	12.79
Antibody E	IL-2 (pg/ml)	1983.95	1361.71	977.51	614.19	580.24
	Stdev. (pg/ml)	119.32	57.80	20.95	15.81	12.83

Table 5: IFNg secretion fold change vs. IgG control

IgG	Concentrations of IgG				
	100 nM	10 nM	1 nM	0.1 nM	0.01 nM
Pembrolizumab	1.78	1.77	1.76	1.99	1.03
Nivolumab	1.97	1.88	1.58	1.53	0.84
Antibody A	1.52	1.37	1.11	1.49	1.49
Antibody C	1.84	1.79	1.82	1.50	0.97
Antibody E	1.76	2.01	1.86	1.26	0.96

	Cytokine in supernatant	Concentrations of IgG				
		100 nM	10 nM	1 nM	0.1 nM	0.01 nM
IgG control	IFNγ (pg/ml)	14936.03	13497.03	11603.92	11007.43	14881.91
	Stdev. (pg/ml)	331.65	912.08	815.69	1400.66	453.67
Pembrolizumab	IFNγ (pg/ml)	26598.52	23903.29	20390.76	21894.58	15326.75
	Stdev. (pg/ml)	1143.84	409.35	1274.53	1038.25	280.19
Nivolumab	IFNγ (pg/ml)	29482.91	25333.89	18296.62	16820.33	12487.21
	Stdev. (pg/ml)	3935.40	3201.10	203.28	725.93	613.05
Antibody A	IFNγ (pg/ml)	22631.06	18442.75	12915.14	16368.19	22121.86
	Stdev.	712.64	3029.49	1683.58	580.07	160.46

	(pg/ml)					
Antibody C	IFNγ (pg/ml)	27443.11	24094.79	21136.75	16460.20	14382.09
	Stdev. (pg/ml)	1036.81	888.47	936.17	914.50	929.68
Antibody E	IFNγ (pg/ml)	26262.69	27124.92	21575.50	13825.35	14331.43
	Stdev. (pg/ml)	898.53	1884.76	508.65	513.84	1708.45

Tumor model

The antibodies of the present invention can be measured for *in vivo* immunomodulatory activity with the MC38 *in vivo* tumor mode. C57Bl/6 mice are inoculated subcutaneously with MC38 murine colon cancer cells 2 X 10^6 per mouse to establish the tumor-bearing model. Mice are selected 10 days after tumor inoculation with tumor volumes between 34.81 mm^3 ~ 148.24 mm^3 and then divided into five groups. These groups include a control group, RMP1-14 (Bio X Cell), Compound C-2.5, Compound C-5, and Compound C-10 (n=10). RMP1-14 group is given a dose of 10 mg/kg. Compound C-2.5, Compound C-5, and Compound C-10 groups are given 2.5 mg/kg, 5 mg/kg and 10 mg/kg of 11430 respectively (Compound C S228P IgG4 produced in HEK293 cells).

The control group receives an equal volume of saline, and all groups are dosed via intraperitoneal injection, dose frequency of 2 times weekly for 4 weeks. Weekly monitoring of animal body weight, and tumor volume (using formula $V=L\times W^2/2$) is conducted. After the experiment, tumors are photographed and weighed to calculate tumor weight and to calculate relative tumor inhibition.

In experiments performed essentially as described in this assay, the results show that administration of RMP1-14 and Compound C has no effect on animal weight. RMP1-14 and Compound C show significant anti-tumor effect after one week of administration. The three Compound C dose groups have higher anti-tumor (tumor volume reducing) effects compared to the RMP1-14 group. In groups Compound C-2.5 and Compound C-10, 1 and 2 mice, respectively, had complete tumor regression. After treatment, tumor weight is significantly reduced by the RMP1-14 and three Compound C

groups as compared to the control group. RMP1-14 group had a relative tumor inhibition value of 55.03%, with Compound C -2.5, Compound C -5, and Compound C -10 groups with relative tumor inhibition values of 69.70%, 76.53%, and 81.04%, respectively, showing a dose-dependent manner of Compound C on tumor weight. Thus, Compound C has significant anti-tumor efficacy in tumor-bearing mice, and has better effect than the positive control clone RMP1-14.

Amino Acid and Nucleotide Sequences

SEQ ID NO: 1 (human PD-1)
MQIPQAPWPVVWAVLQLGWRPGWFLDSPDRPWNPPTFSPALLVVTEGDNATFT
CSFSNTSESFVLNWYRMSPSNQTDKLAAFPEDRSQPGQDCRFRVTQLPNGRDFH
MSVVRARRNDSGTYLCGAISLAPKAQIKESLRAELRVTERRAEVPTAHPSPSPRPA
GQFQTLVVGVVGGLLGSLVLLVWVLAVICSRAARGTIGARRTGQPLKEDPSAVP
VFSVDYGELDFQWREKTPEPPVPCVPEQTEYATIVFPSGMGTSSPARRGSADGPR
SAQPLRPEDGHCSWPL

SEQ ID NO: 2 (HCDR1 of Antibody A)
KASGYTFTSYYMH

SEQ ID NO: 3 (HCDR1 of Antibody B)
KASGYTFEGYYMH

SEQ ID NO: 4 (HCDR1 of Antibody C)
KASGYTFTAQYMH

SEQ ID NO: 5 (HCDR1 of Antibody D)
KASGYTFEKYYMH

SEQ ID NO: 6 (HCDR1 of Antibody E)
KASGYTFTSNYMH

SEQ ID NO: 7 (HCDR1 of Antibody F)
KASGYTFSAYYMH

SEQ ID NO: 8 (HCDR2 of Antibody A)
IINPSGGSTSYAQKFQG

SEQ ID NO: 9 (HCDR2 of Antibody B)

IINPEGGETSYAQKFQG

SEQ ID NO: 10 (HCDR2 of Antibody C)

IINPSGGETGYAQKFQG

SEQ ID NO: 11 (HCDR2 of Antibody E)

IINPSEGSTGYAQKFQG

SEQ ID NO: 12 (HCDR2 of Antibody D and F)

IINPDGGSTGYAQKFQG

SEQ ID NO: 13 (HCDR3 of Antibody A-F)

AKEGVADGYGLVDV

SEQ ID NO: 14 (LCDR1 of Antibody A-F)

RASQSVSSYLA

SEQ ID NO: 15 (LCDR2 of Antibody A-F)

YDASKRAT

SEQ ID NO: 16 (LCDR3 of Antibody A-F)

DQRNNWPLT

SEQ ID NO: 17 (HCVR of Antibody A)

QVQLVQSGAEVKKPGASVKVSCKASGYTFTSYYMHWVRQAPGQGLEWMGIINP
SGGSTSYAQKFQGRVTMTRDTSTSTVYMELSSLRSEDTAVYYCAKEGVADGYG
LVDVWGQGTMVTISS

SEQ ID NO: 18 (HCVR of Antibody B)

QVQLVQSGAEVKKPGASVKVSCKASGYTFEGYYMHWVRQAPGQGLEWMGIIN
PEGGETSYAQKFQGRVTMTRDTSISTAYMELSRLRSDDTAVYYCAKEGVADGY
GLVDVWGQGTMVTVSS

SEQ ID NO: 19 (HCVR of Antibody C)

QVQLVQSGAEVKKPGASVKVSCKASGYTFTAQYMHWVRQAPGQGLEWMGIIN
PSGGETGYAQKFQGRVTMTRDTSTSTVYMELSSLRSEDTAVYYCAKEGVADGY
GLVDVWGQGTMVTVSS

SEQ ID NO: 20 (HCVR of Antibody D)

QVQLVQSGAEVKKPGASVKVSCKASGYTFEKYYMHWVRQAPGQGLEWMGIIN
PDGGSTGYAQKFQGRVTMTRDTSTSTVYMELSSLRSEDTAVYYCAKEGVADGY
GLVDVWGQGTMVTVSS

SEQ ID NO: 21 (HCVR of Antibody E)

QVQLVQSGAEVKKPGASVKVSCKASGYTFTSNYMHWVRQAPGQGLEWMGIINP
SEGSTGYAQKFQGRVTMTRDTSTSTVYMELSSLRSEDTAVYYCAKEGVADGYG
LVDVWGQGTMVTVSS

SEQ ID NO: 22 (HCVR of Antibody F)

QVQLVQSGAEVKKPGASVKVSCKASGYTFSAYYMHWVRQAPGQGLEWMGIINP
DGGSTGYAQKFQGRVTMTRDTSTSTVYMELSSLRSEDTAVYYCAKEGVADGYG
LVDVWGQGTMVTVSS

SEQ ID NO: 23 (LCVR of Antibody A-F)

EIVLTQSPATLSLSPGERATLSCRASQSVSSYLAWYQQKPGQAPRLLIYDASKRAT
GIPARFSGSGSGTDFTLTISSLEPEDFAVYYCDQRNNWPLTFGGGTKVEIK

SEQ ID NO: 24 (HC of Antibody A – S228P IgG4)

QVQLVQSGAEVKKPGASVKVSCKASGYTFTSYYMHWVRQAPGQGLEWMGIINP
SGGSTSYAQKFQGRVTMTRDTSTSTVYMELSSLRSEDTAVYYCAKEGVADGYG
LVDVWGQGTMVTISSASTKGPSVFPLAPCSRSTSESTAALGCLVKDYFPEPVTVS
WNSGALTSGVHTFPAVLQSSGLYSLSSVVTVPSSSLGTKTYTCNVDHKPSNTKVD
KRVESKYGPPCPPCPAPEFLGGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSQEDP
EVQFNWYVDGVEVHNAKTKPREEQFNSTYRVVSVLTVLHQDWLNGKEYKCKV
SNKGLPSSIEKTISKAKGQPREPQVYTLPPSQEEMTKNQVSLTCLVKGFYPSDIAV
EWESNGQPENNYKTTPPVLDSDGSFFLYSRLTVDKSRWQEGNVFSCSVMHEALH
NHYTQKSLSLSLGK

SEQ ID NO: 25 (HC of Antibody B – S228P IgG4)

QVQLVQSGAEVKKPGASVKVSCKASGYTFEGYYMHWVRQAPGQGLEWMGIIN
PEGGETSYAQKFQGRVTMTRDTSISTAYMELSRLRSDDTAVYYCAKEGVADGY
GLVDVWGQGTMVTVSSASTKGPSVFPLAPCSRSTSESTAALGCLVKDYFPEPVTV
SWNSGALTSGVHTFPAVLQSSGLYSLSSVVTVPSSSLGTKTYTCNVDHKPSNTKV

DKRVESKYGPPCPPCPAPEFLGGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSQED
PEVQFNWYVDGVEVHNAKTKPREEQFNSTYRVVSVLTVLHQDWLNGKEYKCK
VSNKGLPSSIEKTISKAKGQPREPQVYTLPPSQEEMTKNQVSLTCLVKGFYPSDIA
VEWESNGQPENNYKTTPPVLDSDGSFFLYSRLTVDKSRWQEGNVFSCSVMHEAL
HNHYTQKSLSLSLGK

SEQ ID NO: 26 (HC of Antibody C – S228P IgG4)

QVQLVQSGAEVKKPGASVKVSCKASGYTFTAQYMHWVRQAPGQGLEWMGIIN
PSGGETGYAQKFQGRVTMTRDTSTSTVYMELSSLRSEDTAVYYCAKEGVADGY
GLVDVWGQGTMVTVSSASTKGPSVFPLAPCSRSTSESTAALGCLVKDYFPEPVTV
SWNSGALTSGVHTFPAVLQSSGLYSLSSVVTVPSSSLGTKTYTCNVDHKPSNTKV
DKRVESKYGPPCPPCPAPEFLGGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSQED
PEVQFNWYVDGVEVHNAKTKPREEQFNSTYRVVSVLTVLHQDWLNGKEYKCK
VSNKGLPSSIEKTISKAKGQPREPQVYTLPPSQEEMTKNQVSLTCLVKGFYPSDIA
VEWESNGQPENNYKTTPPVLDSDGSFFLYSRLTVDKSRWQEGNVFSCSVMHEAL
HNHYTQKSLSLSLGK

SEQ ID NO: 27 (HC of Antibody D – S228P IgG4)

QVQLVQSGAEVKKPGASVKVSCKASGYTFEKYYMHWVRQAPGQGLEWMGIIN
PDGGSTGYAQKFQGRVTMTRDTSTSTVYMELSSLRSEDTAVYYCAKEGVADGY
GLVDVWGQGTMVTVSSASTKGPSVFPLAPCSRSTSESTAALGCLVKDYFPEPVTV
SWNSGALTSGVHTFPAVLQSSGLYSLSSVVTVPSSSLGTKTYTCNVDHKPSNTKV
DKRVESKYGPPCPPCPAPEFLGGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSQED
PEVQFNWYVDGVEVHNAKTKPREEQFNSTYRVVSVLTVLHQDWLNGKEYKCK
VSNKGLPSSIEKTISKAKGQPREPQVYTLPPSQEEMTKNQVSLTCLVKGFYPSDIA
VEWESNGQPENNYKTTPPVLDSDGSFFLYSRLTVDKSRWQEGNVFSCSVMHEAL
HNHYTQKSLSLSLGK

SEQ ID NO: 28 (HC of Antibody E – S228P IgG4)

QVQLVQSGAEVKKPGASVKVSCKASGYTFTSNYMHWVRQAPGQGLEWMGIINP
SEGSTGYAQKFQGRVTMTRDTSTSTVYMELSSLRSEDTAVYYCAKEGVADGYG
LVDVWGQGTMVTVSSASTKGPSVFPLAPCSRSTSESTAALGCLVKDYFPEPVTVS
WNSGALTSGVHTFPAVLQSSGLYSLSSVVTVPSSSLGTKTYTCNVDHKPSNTKVD
KRVESKYGPPCPPCPAPEFLGGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSQEDP
EVQFNWYVDGVEVHNAKTKPREEQFNSTYRVVSVLTVLHQDWLNGKEYKCKV
SNKGLPSSIEKTISKAKGQPREPQVYTLPPSQEEMTKNQVSLTCLVKGFYPSDIAV
EWESNGQPENNYKTTPPVLDSDGSFFLYSRLTVDKSRWQEGNVFSCSVMHEALH
NHYTQKSLSLSLGK

SEQ ID NO: 29 (HC of Antibody F – S228P IgG4)

QVQLVQSGAEVKKPGASVKVSCKASGYTFSAYYMHWVRQAPGQGLEWMGIINP
DGGSTGYAQKFQGRVTMTRDTSTSTVYMELSSLRSEDTAVYYCAKEGVADGYG
LVDVWGQGTMVTVSSASTKGPSVFPLAPCSRSTSESTAALGCLVKDYFPEPVTVS

WNSGALTSGVHTFPAVLQSSGLYSLSSVVTVPSSSLGTKTYTCNVDHKPSNTKVD
KRVESKYGPPCPPCPAPEFLGGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSQEDP
EVQFNWYVDGVEVHNAKTKPREEQFNSTYRVVSVLTVLHQDWLNGKEYKCKV
SNKGLPSSIEKTISKAKGQPREPQVYTLPPSQEEMTKNQVSLTCLVKGFYPSDIAV
EWESNGQPENNYKTTPPVLDSDGSFFLYSRLTVDKSRWQEGNVFSCSVMHEALH
NHYTQKSLSLSLGK

SEQ ID NO: 30 (LC of Antibody A-F)

EIVLTQSPATLSLSPGERATLSCRASQSVSSYLAWYQQKPGQAPRLLIYDASKRAT
GIPARFSGSGSGTDFTLTISSLEPEDFAVYYCDQRNNWPLTFGGGTKVEIKRTVAA
PSVFIFPPSDEQLKSGTASVVCLLNNFYPREAKVQWKVDNALQSGNSQESVTEQD
SKDSTYSLSSTLTLSKADYEKHKVYACEVTHQGLSSPVTKSFNRGEC

SEQ ID NO: 31 (DNA of HC of Antibody A S228P IgG4)

CAGGTGCAGCTGGTGCAGTCTGGGGCTGAGGTGAAGAAGCCTGGGGCCTCAG
TGAAGGTTTCCTGCAAGGCATCTGGATACACCTTCACCAGCTACTATATGCAC
TGGGTGCGACAGGCCCCTGGACAAGGGCTTGAGTGGATGGGAATAATCAACC
CTAGTGGTGGTAGCACAAGCTACGCACAGAAGTTCCAGGGCAGAGTCACCAT
GACCAGGGACACGTCCACGAGCACAGTCTACATGGAGCTGAGCAGCCTGAGA
TCTGAGGACACGGCGGTGTACTACTGCGCCAAAGAGGGAGTGGCCGACGGAT
ATGGATTGGTAGACGTATGGGGTCAGGGTACAATGGTCACCATCTCCTCAGC
CAGCACCAAGGGACCCTCCGTGTTCCCTCTGGCTCCTTGCAGCAGGTCCACCA
GCGAATCCACCGCTGCCCTGGGCTGTCTGGTGAAAGACTACTTTCCCGAGCCT
GTGACCGTGAGCTGGAACTCCGGCGCTCTGACCAGCGGCGTGCACACATTTC
CTGCCGTGCTGCAGAGCTCCGGCCTGTACTCCCTGTCCTCCGTGGTGACAGTC
CCCAGCAGCAGCCTGGGAACCAAGACCTACACCTGCAACGTCGACCACAAGC
CTTCCAACACCAAGGTGGACAAGAGGGTGGAGTCCAAATATGGCCCCCCCTG
CCCTCCTTGTCCCGCTCCTGAGTTCCTGGGCGGCCCTTCCGTGTTCCTGTTCCC
TCCCAAGCCCAAGGACACCCTGATGATCTCCCGGACCCCCGAGGTGACCTGT
GTGGTGGTGGACGTGTCCCAGGAGGACCCTGAGGTGCAATTCAACTGGTACG
TGGACGGCGTCGAGGTGCACAACGCCAAGACCAAGCCCCGGGAGGAGCAGT
TCAACAGCACCTACCGGGTCGTGTCCGTGCTGACCGTGCTGCACCAGGATTGG
CTCAACGGCAAGGAGTACAAGTGCAAAGTGTCCAATAAGGGCCTGCCCTCCT
CCATCGAGAAGACCATCTCCAAGGCCAAGGGACAACCCCGTGAGCCCCAGGT
GTACACCCTGCCTCCTTCCCAGGAGGAGATGACCAAGAATCAGGTGTCCCTC
ACCTGCCTGGTGAAGGGCTTCTACCCTTCCGACATCGCCGTGGAATGGGAGTC
CAACGGCCAGCCCGAGAACAACTACAAGACAACCCCCCCTGTCCTGGACAGC
GACGGCTCCTTCTTTCTGTACAGCAGGCTGACCGTGGACAAGAGCCGGTGGC
AGGAGGGCAACGTGTTTAGCTGTAGCGTCATGCACGAGGCCCTGCACAACCA
CTACACCCAGAAATCCCTGTCCCTGTCCCTGGGCAAGTGATGA

SEQ ID NO: 32 (DNA of HC of Antibody B S228P IgG4)

CAGGTGCAGCTGGTGCAGTCTGGGGCTGAGGTGAAGAAGCCTGGGGCCTCAG
TGAAGGTTTCCTGCAAGGCATCTGGATACACCTTCGAGGGTTACTATATGCAC
TGGGTGCGACAGGCCCCTGGACAAGGGCTTGAGTGGATGGGAATAATCAACC
CTGAGGGTGGTGAGACAAGCTACGCACAGAAGTTCCAGGGCAGAGTCACCAT
GACCAGGGACACGTCCATCAGCACAGCCTACATGGAGCTGAGCAGGCTGAGA
TCTGACGACACGGCGGTGTACTACTGCGCCAAAGAGGGAGTGGCCGACGGAT
ATGGATTGGTAGACGTATGGGGTCAGGGTACAATGGTCACCGTCTCCTCAGC
CAGCACCAAGGGACCCTCCGTGTTCCCTCTGGCTCCTTGCAGCAGGTCCACCA
GCGAATCCACCGCTGCCCTGGGCTGTCTGGTGAAAGACTACTTTCCCGAGCCT
GTGACCGTGAGCTGGAACTCCGGCGCTCTGACCAGCGGCGTGCACACATTTC
CTGCCGTGCTGCAGAGCTCCGGCCTGTACTCCCTGTCCTCCGTGGTGACAGTC
CCCAGCAGCAGCCTGGGAACCAAGACCTACACCTGCAACGTCGACCACAAGC
CTTCCAACACCAAGGTGGACAAGAGGGTGGAGTCCAAATATGGCCCCCCCTG
CCCTCCTTGTCCCGCTCCTGAGTTCCTGGGCGGCCCTTCCGTGTTCCTGTTCCC
TCCCAAGCCCAAGGACACCCTGATGATCTCCCGGACCCCCGAGGTGACCTGT
GTGGTGGTGGACGTGTCCCAGGAGGACCCTGAGGTGCAATTCAACTGGTACG
TGGACGGCGTCGAGGTGCACAACGCCAAGACCAAGCCCCGGGAGGAGCAGT
TCAACAGCACCTACCGGGTCGTGTCCGTGCTGACCGTGCTGCACCAGGATTGG
CTCAACGGCAAGGAGTACAAGTGCAAAGTGTCCAATAAGGGCCTGCCCTCCT
CCATCGAGAAGACCATCTCCAAGGCCAAGGGACAACCCCGTGAGCCCCAGGT
GTACACCCTGCCTCCTTCCCAGGAGGAGATGACCAAGAATCAGGTGTCCCTC
ACCTGCCTGGTGAAGGGCTTCTACCCTTCCGACATCGCCGTGGAATGGGAGTC
CAACGGCCAGCCCGAGAACAACTACAAGACAACCCCCCCTGTCCTGGACAGC
GACGGCTCCTTCTTTCTGTACAGCAGGCTGACCGTGGACAAGAGCCGGTGGC
AGGAGGGCAACGTGTTTAGCTGTAGCGTCATGCACGAGGCCCTGCACAACCA
CTACACCCAGAAATCCCTGTCCCTGTCCCTGGGCAAGTGATGA

SEQ ID NO: 33 (DNA of HC of Antibody C S228P IgG4)

CAGGTGCAGCTGGTGCAGTCTGGGGCTGAGGTGAAGAAGCCTGGGGCCTCAG
TGAAGGTTTCCTGCAAGGCATCTGGATACACCTTCACCGCTCAGTATATGCAC
TGGGTGCGACAGGCCCCTGGACAAGGGCTTGAGTGGATGGGAATAATCAACC
CTAGTGGTGGTGAGACAGGGTACGCACAGAAGTTCCAGGGCAGAGTCACCAT
GACCAGGGACACGTCCACGAGCACAGTCTACATGGAGCTGAGCAGCCTGAGA
TCTGAGGACACGGCGGTGTACTACTGCGCCAAAGAGGGAGTGGCCGACGGAT
ATGGATTGGTAGACGTATGGGGTCAGGGTACAATGGTCACCGTCTCCTCAGC
CAGCACCAAGGGACCCTCCGTGTTCCCTCTGGCTCCTTGCAGCAGGTCCACCA
GCGAATCCACCGCTGCCCTGGGCTGTCTGGTGAAAGACTACTTTCCCGAGCCT
GTGACCGTGAGCTGGAACTCCGGCGCTCTGACCAGCGGCGTGCACACATTTC
CTGCCGTGCTGCAGAGCTCCGGCCTGTACTCCCTGTCCTCCGTGGTGACAGTC
CCCAGCAGCAGCCTGGGAACCAAGACCTACACCTGCAACGTCGACCACAAGC
CTTCCAACACCAAGGTGGACAAGAGGGTGGAGTCCAAATATGGCCCCCCCTG
CCCTCCTTGTCCCGCTCCTGAGTTCCTGGGCGGCCCTTCCGTGTTCCTGTTCCC
TCCCAAGCCCAAGGACACCCTGATGATCTCCCGGACCCCCGAGGTGACCTGT
GTGGTGGTGGACGTGTCCCAGGAGGACCCTGAGGTGCAATTCAACTGGTACG
TGGACGGCGTCGAGGTGCACAACGCCAAGACCAAGCCCCGGGAGGAGCAGT

TCAACAGCACCTACCGGGTCGTGTCCGTGCTGACCGTGCTGCACCAGGATTGG
CTCAACGGCAAGGAGTACAAGTGCAAAGTGTCCAATAAGGGCCTGCCCTCCT
CCATCGAGAAGACCATCTCCAAGGCCAAGGGACAACCCCGTGAGCCCCAGGT
GTACACCCTGCCTCCTTCCCAGGAGGAGATGACCAAGAATCAGGTGTCCCTC
ACCTGCCTGGTGAAGGGCTTCTACCCTTCCGACATCGCCGTGGAATGGGAGTC
CAACGGCCAGCCCGAGAACAACTACAAGACAACCCCCCCTGTCCTGGACAGC
GACGGCTCCTTCTTTCTGTACAGCAGGCTGACCGTGGACAAGAGCCGGTGGC
AGGAGGGCAACGTGTTTAGCTGTAGCGTCATGCACGAGGCCCTGCACAACCA
CTACACCCAGAAATCCCTGTCCCTGTCCCTGGGCAAGTGATGA

SEQ ID NO: 34 (DNA of HC of Antibody D S228P IgG4)

CAGGTGCAGCTGGTGCAGTCTGGGGCTGAGGTGAAGAAGCCTGGGGCCTCAG
TGAAGGTTTCCTGCAAGGCATCTGGATACACCTTCGAGAAGTACTATATGCAC
TGGGTGCGACAGGCCCCTGGACAAGGGCTTGAGTGGATGGGAATAATCAACC
CTGATGGTGGTAGCACAGGGTACGCACAGAAGTTCCAGGGCAGAGTCACCAT
GACCAGGGACACGTCCACGAGCACAGTCTACATGGAGCTGAGCAGCCTGAGA
TCTGAGGACACGGCGGTGTACTACTGCGCCAAAGAGGGAGTGGCCGACGGAT
ATGGATTGGTAGACGTATGGGGTCAGGGTACAATGGTCACCGTCTCCTCAGC
CAGCACCAAGGGACCCTCCGTGTTCCCTCTGGCTCCTTGCAGCAGGTCCACCA
GCGAATCCACCGCTGCCCTGGGCTGTCTGGTGAAAGACTACTTTCCCGAGCCT
GTGACCGTGAGCTGGAACTCCGGCGCTCTGACCAGCGGCGTGCACACATTTC
CTGCCGTGCTGCAGAGCTCCGGCCTGTACTCCCTGTCCTCCGTGGTGACAGTC
CCCAGCAGCAGCCTGGGAACCAAGACCTACACCTGCAACGTCGACCACAAGC
CTTCCAACACCAAGGTGGACAAGAGGGTGGAGTCCAAATATGGCCCCCCCTG
CCCTCCTTGTCCCGCTCCTGAGTTCCTGGGCGGCCCTTCCGTGTTCCTGTTCCC
TCCCAAGCCCAAGGACACCCTGATGATCTCCCGGACCCCCGAGGTGACCTGT
GTGGTGGTGGACGTGTCCCAGGAGGACCCTGAGGTGCAATTCAACTGGTACG
TGGACGGCGTCGAGGTGCACAACGCCAAGACCAAGCCCCGGGAGGAGCAGT
TCAACAGCACCTACCGGGTCGTGTCCGTGCTGACCGTGCTGCACCAGGATTGG
CTCAACGGCAAGGAGTACAAGTGCAAAGTGTCCAATAAGGGCCTGCCCTCCT
CCATCGAGAAGACCATCTCCAAGGCCAAGGGACAACCCCGTGAGCCCCAGGT
GTACACCCTGCCTCCTTCCCAGGAGGAGATGACCAAGAATCAGGTGTCCCTC
ACCTGCCTGGTGAAGGGCTTCTACCCTTCCGACATCGCCGTGGAATGGGAGTC
CAACGGCCAGCCCGAGAACAACTACAAGACAACCCCCCCTGTCCTGGACAGC
GACGGCTCCTTCTTTCTGTACAGCAGGCTGACCGTGGACAAGAGCCGGTGGC
AGGAGGGCAACGTGTTTAGCTGTAGCGTCATGCACGAGGCCCTGCACAACCA
CTACACCCAGAAATCCCTGTCCCTGTCCCTGGGCAAGTGATGA

SEQ ID NO: 35 (DNA of HC of Antibody E S228P IgG4)

CAGGTGCAGCTGGTGCAGTCTGGGGCTGAGGTGAAGAAGCCTGGGGCCTCAG
TGAAGGTTTCCTGCAAGGCATCTGGATACACCTTCACCAGCAATTATATGCAC
TGGGTGCGACAGGCCCCTGGACAAGGGCTTGAGTGGATGGGAATAATCAACC
CTAGTGAGGGTAGCACAGGTTACGCACAGAAGTTCCAGGGCAGAGTCACCAT
GACCAGGGACACGTCCACGAGCACAGTCTACATGGAGCTGAGCAGCCTGAGA

TCTGAGGACACGGCGGTGTACTACTGCGCCAAAGAGGGAGTGGCCGACGGAT
ATGGATTGGTAGACGTATGGGGTCAGGGTACAATGGTCACCGTCTCCTCAGC
CAGCACCAAGGGACCCTCCGTGTTCCCTCTGGCTCCTTGCAGCAGGTCCACCA
GCGAATCCACCGCTGCCCTGGGCTGTCTGGTGAAAGACTACTTTCCCGAGCCT
GTGACCGTGAGCTGGAACTCCGGCGCTCTGACCAGCGGCGTGCACACATTTC
CTGCCGTGCTGCAGAGCTCCGGCCTGTACTCCCTGTCCTCCGTGGTGACAGTC
CCCAGCAGCAGCCTGGGAACCAAGACCTACACCTGCAACGTCGACCACAAGC
CTTCCAACACCAAGGTGGACAAGAGGGTGGAGTCCAAATATGGCCCCCCCTG
CCCTCCTTGTCCCGCTCCTGAGTTCCTGGGCGGCCCTTCCGTGTTCCTGTTCCC
TCCCAAGCCCAAGGACACCCTGATGATCTCCCGGACCCCCGAGGTGACCTGT
GTGGTGGTGGACGTGTCCCAGGAGGACCCTGAGGTGCAATTCAACTGGTACG
TGGACGGCGTCGAGGTGCACAACGCCAAGACCAAGCCCCGGGAGGAGCAGT
TCAACAGCACCTACCGGGTCGTGTCCGTGCTGACCGTGCTGCACCAGGATTGG
CTCAACGGCAAGGAGTACAAGTGCAAAGTGTCCAATAAGGGCCTGCCCTCCT
CCATCGAGAAGACCATCTCCAAGGCCAAGGGACAACCCCGTGAGCCCCAGGT
GTACACCCTGCCTCCTTCCCAGGAGGAGATGACCAAGAATCAGGTGTCCCTC
ACCTGCCTGGTGAAGGGCTTCTACCCTTCCGACATCGCCGTGGAATGGGAGTC
CAACGGCCAGCCCGAGAACAACTACAAGACAACCCCCCCTGTCCTGGACAGC
GACGGCTCCTTCTTTCTGTACAGCAGGCTGACCGTGGACAAGAGCCGGTGGC
AGGAGGGCAACGTGTTTAGCTGTAGCGTCATGCACGAGGCCCTGCACAACCA
CTACACCCAGAAATCCCTGTCCCTGTCCCTGGGCAAGTGATGA

SEQ ID NO: 36 (DNA of HC of Antibody F S228P IgG4)

CAGGTGCAGCTGGTGCAGTCTGGGGCTGAGGTGAAGAAGCCTGGGGCCTCAG
TGAAGGTTTCCTGCAAGGCATCTGGATACACCTTCAGTGCGTACTATATGCAC
TGGGTGCGACAGGCCCCTGGACAAGGGCTTGAGTGGATGGGAATAATCAACC
CTGATGGTGGTAGCACAGGGTACGCACAGAAGTTCCAGGGCAGAGTCACCAT
GACCAGGGACACGTCCACGAGCACAGTCTACATGGAGCTGAGCAGCCTGAGA
TCTGAGGACACGGCGGTGTACTACTGCGCCAAAGAGGGAGTGGCCGACGGAT
ATGGATTGGTAGACGTATGGGGTCAGGGTACAATGGTCACCGTCTCCTCAGC
CAGCACCAAGGGACCCTCCGTGTTCCCTCTGGCTCCTTGCAGCAGGTCCACCA
GCGAATCCACCGCTGCCCTGGGCTGTCTGGTGAAAGACTACTTTCCCGAGCCT
GTGACCGTGAGCTGGAACTCCGGCGCTCTGACCAGCGGCGTGCACACATTTC
CTGCCGTGCTGCAGAGCTCCGGCCTGTACTCCCTGTCCTCCGTGGTGACAGTC
CCCAGCAGCAGCCTGGGAACCAAGACCTACACCTGCAACGTCGACCACAAGC
CTTCCAACACCAAGGTGGACAAGAGGGTGGAGTCCAAATATGGCCCCCCCTG
CCCTCCTTGTCCCGCTCCTGAGTTCCTGGGCGGCCCTTCCGTGTTCCTGTTCCC
TCCCAAGCCCAAGGACACCCTGATGATCTCCCGGACCCCCGAGGTGACCTGT
GTGGTGGTGGACGTGTCCCAGGAGGACCCTGAGGTGCAATTCAACTGGTACG
TGGACGGCGTCGAGGTGCACAACGCCAAGACCAAGCCCCGGGAGGAGCAGT
TCAACAGCACCTACCGGGTCGTGTCCGTGCTGACCGTGCTGCACCAGGATTGG
CTCAACGGCAAGGAGTACAAGTGCAAAGTGTCCAATAAGGGCCTGCCCTCCT
CCATCGAGAAGACCATCTCCAAGGCCAAGGGACAACCCCGTGAGCCCCAGGT
GTACACCCTGCCTCCTTCCCAGGAGGAGATGACCAAGAATCAGGTGTCCCTC
ACCTGCCTGGTGAAGGGCTTCTACCCTTCCGACATCGCCGTGGAATGGGAGTC

CAACGGCCAGCCCGAGAACAACTACAAGACAACCCCCCCCTGTCCTGGACAGC
GACGGCTCCTTCTTTCTGTACAGCAGGCTGACCGTGGACAAGAGCCGGTGGC
AGGAGGGCAACGTGTTTAGCTGTAGCGTCATGCACGAGGCCCTGCACAACCA
CTACACCCAGAAATCCCTGTCCCTGTCCCTGGGCAAGTGATGA

SEQ ID NO: 37 (DNA of LC of Antibody C and E)

GAAATTGTGTTGACACAGTCTCCAGCCACCCTGTCTTTGTCTCCAGGGGAAAG
AGCCACCCTCTCCTGCAGGGCCAGTCAGAGTGTTAGCAGCTACTTAGCCTGGT
ACCAACAGAAACCTGGCCAGGCTCCCAGGCTCCTCATCTATGATGCATCCAA
AAGGGCCACTGGCATCCCAGCCAGGTTCAGTGGCAGTGGGTCTGGGACAGAC
TTCACTCTCACCATCAGCAGCCTAGAGCCTGAAGATTTTGCAGTTTATTACTG
TGACCAGAGAAACAATTGGCCTCTCACTTTTGGCGGAGGGACCAAGGTTGAG
ATCAAACGGACCGTGGCTGCCCCTAGCGTGTTCATCTTCCCTCCCTCCGATGA
GCAGCTCAAGTCCGGCACAGCCAGCGTGGTGTGCCTGCTGAATAACTTCTACC
CCCGGGAGGCCAAAGTGCAGTGGAAGGTGGACAACGCTCTGCAGTCCGGCAA
TTCCCAGGAGAGCGTCACCGAGCAGGACAGCAAGGACAGCACCTACTCCCTG
AGCTCCACCCTGACCCTGAGCAAGGCCGACTACGAGAAGCACAAGGTCTACG
CCTGCGAGGTCACCCATCAGGGCCTGTCCAGCCCCGTGACCAAGTCCTTCAAC
CGGGGCGAGTGCTGATGA

SEQ ID NO: 38 (mouse PD-1)

MWVRQVPWSFTWAVLQLSWQSGWLLEVPNGPWRSLTFYPAWLTVSEGANATF
TCSLSNWSEDLMLNWNRLSPSNQTEKQAAFCNGLSQPVQDARFQIIQLPNRHDF
HMNILDTRRNDSGIYLCGAISLHPKAKIEESPGAELVVTERILETSTRYPSPSPKPE
GRFQGMVIGIMSALVGIPVLLLLAWALAVFCSTSMSEARGAGSKDDTLKEEPSAA
PVPSVAYEELDFQGREKTPELPTACVHTEYATIVFTEGLGASAMGRRGSADGLQG
PRPPRHEDGHCSWPL

WE CLAIM:

1. An antibody that binds human PD-1 (SEQ ID NO: 1), comprising a light chain (LC) and a heavy chain (HC), wherein the light chain comprises light chain complementarity determining regions LCDR1, LCDR2, and LCDR3 consisting of the amino acid sequences RASQSVSSYLA (SEQ ID NO: 14), YDASKRAT (SEQ ID NO: 15), and DQRNNWPLT (SEQ ID NO: 16), respectively, and wherein the heavy chain comprises heavy chain complementarity determining regions HCDR1, HCDR2, and HCDR3, wherein HCDR1 consists of the amino acid sequence:
    a. KASGYTFTSYYMH (SEQ ID NO: 2),
    b. KASGYTFEGYYMH (SEQ ID NO: 3),
    c. KASGYTFTAQYMH (SEQ ID NO: 4),
    d. KASGYTFEKYYMH (SEQ ID NO: 5),
    e. KASGYTFTSNYMH (SEQ ID NO: 6), or
    f. KASGYTFSAYYMH (SEQ ID NO: 7);
wherein HCDR2 consists of the amino acid sequence:
    a. IINPSGGSTSYAQKFQG (SEQ ID NO: 8),
    b. IINPEGGETSYAQKFQG (SEQ ID NO: 9),
    c. IINPSGGETGYAQKFQG (SEQ ID NO: 10),
    d. IINPSEGSTGYAQKFQG (SEQ ID NO: 11), or
    e. IINPDGGSTGYAQKFQG (SEQ ID NO: 12);
and wherein HCDR3 consists of the amino acid sequence AKEGVADGYGLVDV (SEQ ID NO: 13).

2. The antibody of Claim 1, wherein LCDR1, LCDR2, and LCDR3 consist of the amino acid sequences RASQSVSSYLA (SEQ ID NO: 14), YDASKRAT (SEQ ID NO: 15), and DQRNNWPLT (SEQ ID NO: 16), respectively, and wherein HCDR1, HCDR2, and HCDR3 consist of the amino acid sequences KASGYTFTSYYMH (SEQ ID NO: 2),

IINPSGGSTSYAQKFQG (SEQ ID NO: 8), and AKEGVADGYGLVDV (SEQ ID NO: 13), respectively.

3. The antibody of Claim 1, wherein LCDR1, LCDR2, and LCDR3 consist of the amino acid sequences RASQSVSSYLA (SEQ ID NO: 14), YDASKRAT (SEQ ID NO: 15), and DQRNNWPLT (SEQ ID NO: 16), respectively, and wherein HCDR1, HCDR2, and HCDR3 consist of the amino acid sequences KASGYTFEGYYMH (SEQ ID NO: 3), IINPEGGETSYAQKFQG (SEQ ID NO: 9), and AKEGVADGYGLVDV (SEQ ID NO: 13), respectively.

4. The antibody of Claim 1, wherein LCDR1, LCDR2, and LCDR3 consist of the amino acid sequences RASQSVSSYLA (SEQ ID NO: 14), YDASKRAT (SEQ ID NO: 15), and DQRNNWPLT (SEQ ID NO: 16), respectively, and wherein HCDR1, HCDR2, and HCDR3 consist of the amino acid sequences KASGYTFTAQYMH (SEQ ID NO: 4), IINPSGGETGYAQKFQG (SEQ ID NO: 10), and AKEGVADGYGLVDV (SEQ ID NO: 13), respectively.

5. The antibody of Claim 1, wherein LCDR1, LCDR2, and LCDR3 consist of the amino acid sequences RASQSVSSYLA (SEQ ID NO: 14), YDASKRAT (SEQ ID NO: 15), and DQRNNWPLT (SEQ ID NO: 16), respectively, and wherein HCDR1, HCDR2, and HCDR3 consist of the amino acid sequences KASGYTFEKYYMH (SEQ ID NO: 5), IINPDGGSTGYAQKFQG (SEQ ID NO: 12), and AKEGVADGYGLVDV (SEQ ID NO: 13), respectively.

6. The antibody of Claim 1, wherein LCDR1, LCDR2, and LCDR3 consist of the amino acid sequences RASQSVSSYLA (SEQ ID NO: 14), YDASKRAT (SEQ ID NO: 15), and DQRNNWPLT (SEQ ID NO: 16), respectively, and wherein HCDR1, HCDR2, and HCDR3 consist of the amino acid sequences KASGYTFTSNYMH (SEQ ID NO: 6), IINPSEGSTGYAQKFQG (SEQ ID NO: 11), and AKEGVADGYGLVDV (SEQ ID NO: 13), respectively.

7. The antibody of Claim 1, wherein LCDR1, LCDR2, and LCDR3 consist of the amino acid sequences RASQSVSSYLA (SEQ ID NO: 14), YDASKRAT (SEQ ID NO: 15), and DQRNNWPLT (SEQ ID NO: 16), respectively, and wherein HCDR1, HCDR2, and HCDR3 consist of the amino acid sequences KASGYTFSAYYMH (SEQ ID NO: 7), IINPDGGSTGYAQKFQG (SEQ ID NO: 12), and AKEGVADGYGLVDV (SEQ ID NO: 13), respectively.
8. An antibody, comprising a light chain (LC) and a heavy chain (HC), wherein the light chain comprises a light chain variable region (LCVR) and the heavy chain comprises a heavy chain variable region (HCVR), wherein the LCVR has the amino acid sequence given in SEQ ID NO: 23, and the HCVR has the amino acid sequence given in SEQ ID NO: 17, SEQ ID NO: 18, SEQ ID NO: 19, SEQ ID NO: 20, SEQ ID NO: 21, or SEQ ID NO: 22.
9. The antibody of Claim 8, wherein the LCVR has the amino acid sequence given in SEQ ID NO: 23, and the HCVR has the amino acid sequence given in SEQ ID NO: 17.
10. The antibody of Claim 8, wherein the LCVR has the amino acid sequence given in SEQ ID NO: 23, and the HCVR has the amino acid sequence given in SEQ ID NO: 18.
11. The antibody of Claim 8, wherein the LCVR has the amino acid sequence given in SEQ ID NO: 23, and the HCVR has the amino acid sequence given in SEQ ID NO: 19.
12. The antibody of Claim 8, wherein the LCVR has the amino acid sequence given in SEQ ID NO: 23, and the HCVR has the amino acid sequence given in SEQ ID NO: 20.
13. The antibody of Claim 8, wherein the LCVR has the amino acid sequence given in SEQ ID NO: 23, and the HCVR has the amino acid sequence given in SEQ ID NO: 21.

14. The antibody of Claim 8, wherein the LCVR has the amino acid sequence given in SEQ ID NO: 23, and the HCVR has the amino acid sequence given in SEQ ID NO: 22.

15. The antibody of Claim 8, wherein the LC has the amino acid sequence given in SEQ ID NO: 30, and the HC has the amino acid sequence given in SEQ ID NO: 24, SEQ ID NO: 25, SEQ ID NO: 26, SEQ ID NO: 27, SEQ ID NO: 28, or SEQ ID NO: 29.

16. The antibody of Claim 15, wherein the LC has the amino acid sequence given in SEQ ID NO: 30, and the HC has the amino acid sequence given in SEQ ID NO: 24.

17. The antibody of Claim 15, wherein the LC has the amino acid sequence given in SEQ ID NO: 30, and the HC has the amino acid sequence given in SEQ ID NO: 25.

18. The antibody of Claim 15, wherein the LC has the amino acid sequence given in SEQ ID NO: 30, and the HC has the amino acid sequence given in SEQ ID NO: 26.

19. The antibody of Claim 15, wherein the LC has the amino acid sequence given in SEQ ID NO: 30, and the HC has the amino acid sequence given in SEQ ID NO: 27.

20. The antibody of Claim 15, wherein the LC has the amino acid sequence given in SEQ ID NO: 30, and the HC has the amino acid sequence given in SEQ ID NO: 28.

21. The antibody of Claim 15, wherein the LC has the amino acid sequence given in SEQ ID NO: 30, and the HC has the amino acid sequence given in SEQ ID NO: 29.

22. The antibody of Claim 15, comprising two light chains and two heavy chains, wherein each light chain has the amino acid sequence given in SEQ ID NO: 30, and each heavy chain has the amino acid sequence given in SEQ ID NO: 24, SEQ ID NO: 25, SEQ ID NO: 26, SEQ ID NO: 27, SEQ ID NO: 28, or SEQ ID NO: 29.

23. The antibody of Claim 22, wherein each light chain has the amino acid sequence given in SEQ ID NO: 30, and each heavy chain has the amino acid sequence given in SEQ ID NO: 24.
24. The antibody of Claim 22, wherein each light chain has the amino acid sequence given in SEQ ID NO: 30, and each heavy chain has the amino acid sequence given in SEQ ID NO: 25.
25. The antibody of Claim 22, wherein each light chain has the amino acid sequence given in SEQ ID NO: 30, and each heavy chain has the amino acid sequence given in SEQ ID NO: 26.
26. The antibody of Claim 22, wherein each light chain has the amino acid sequence given in SEQ ID NO: 30, and each heavy chain has the amino acid sequence given in SEQ ID NO: 27.
27. The antibody of Claim 22, wherein each light chain has the amino acid sequence given in SEQ ID NO: 30, and each heavy chain has the amino acid sequence given in SEQ ID NO: 28.
28. The antibody of Claim 22, wherein each light chain has the amino acid sequence given in SEQ ID NO: 30, and each heavy chain has the amino acid sequence given in SEQ ID NO: 29.
29. The antibody of any one of Claims 22-28, wherein one of the heavy chains forms an inter-chain disulfide bond with one of the light chains, and the other heavy chain forms an inter-chain disulfide bond with the other light chain, and one of the heavy chains forms two inter-chain disulfide bonds with the other heavy chain.
30. The antibody of any one of Claims 1-29, wherein the antibody is glycosylated.
31. A pharmaceutical composition, comprising the antibody of any one of Claims 1-30, and an acceptable carrier, diluent, or excipient.
32. A method of treating cancer, comprising administering to a patient in need thereof, an effective amount of the antibody of any one of Claims 1-30.
33. The method of Claim 32, wherein the cancer is melanoma, lung cancer, head and neck cancer, colorectal cancer, pancreatic cancer, gastric cancer,

kidney cancer, bladder cancer, prostate cancer, breast cancer, ovarian cancer, or hepatocellular carcinoma.

34. The method of Claim 32 or 33, further comprising administering simultaneously, separately, or sequentially one or more anti-tumor agents.
35. The antibody of any one of Claims 1-30, for use in therapy.
36. The antibody of any one of Claims 1-30, for use in the treatment of cancer.
37. The antibody for use of Claim 36, wherein the cancer is melanoma, lung cancer, head and neck cancer, colorectal cancer, pancreatic cancer, gastric cancer, kidney cancer, bladder cancer, prostate cancer, breast cancer, ovarian cancer, or hepatocellular carcinoma.
38. The antibody of any one of Claims 1-30 for use in simultaneous, separate, or sequential combination with one or more anti-tumor agents, in the treatment of cancer.
39. The combination for use of Claim 38, wherein the cancer is melanoma, lung cancer, head and neck cancer, colorectal cancer, pancreatic cancer, gastric cancer, kidney cancer, bladder cancer, prostate cancer, breast cancer, ovarian cancer, or hepatocellular carcinoma.

INTERNATIONAL SEARCH REPORT

International application No.
PCT/CN2016/073169

A. CLASSIFICATION OF SUBJECT MATTER

C07K 16/28(2006.01)i; C12N 5/10(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; CNKI; NCBI; ISI Web of knowledge; ELSEVIER and keywords: programmed cell death 1, PD-1, antibody, etc. GenBank; EMBL; Retrieving System for Biological Sequence of Chinese Patent and searched sequences: SEQ ID NOs: 2-29

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
A	CN 103242448 A (UNIV ZHENGZHOU) 14 August 2013 (2013-08-14) the abstract, claims 1-8	1-31, 35-39
A	CN 104250302 A (SHANGHAI JUNSHI BIOSCIENCES CO LTD) 31 December 2014 (2014-12-31) the abstract, claims 1-15	1-31, 35-39

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

* Special categories of cited documents:

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

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

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

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

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

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

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

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

"&" document member of the same patent family

Date of the actual completion of the international search	Date of mailing of the international search report
02 November 2016	**08 November 2016**

Name and mailing address of the ISA/CN	Authorized officer
STATE INTELLECTUAL PROPERTY OFFICE OF THE P.R.CHINA **6, Xitucheng Rd., Jimen Bridge, Haidian District, Beijing 100088** **China**	**YANG,Zhenyu**
Facsimile No. **(86-10)62019451**	Telephone No. **(86-10)62089435**

INTERNATIONAL SEARCH REPORT

International application No.
PCT/CN2016/073169

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 filed or furnished:

   a. (means)

   ☐ on paper

   ☑ in electronic form

   b. (time)

   ☐ in the international application as filed

   ☑ together with the international application in electronic form

   ☐ subsequently to this Authority for the purposes of search

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 in the application as filed or does not go beyond the application as filed, as appropriate, were furnished.

3. Additional comments:

   [1] The sequence listing contained in the description does not comply with the standard provided for in Annex C of the Administrative Instructions.

INTERNATIONAL SEARCH REPORT

International application No.
PCT/CN2016/073169

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

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

1. ☑ Claims Nos.: **32-34**
because they relate to subject matter not required to be searched by this Authority, namely:

[1] Rule 39.1(iv) PCT- Method for treatment of the human or animal body by surgery or therapy.

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/CN2016/073169

Patent document cited in search report	Publication date (day/month/year)	Patent family member(s)	Publication date (day/month/year)
CN 103242448 A	14 August 2013	CN 103242448 B	14 January 2015
CN 104250302 A	31 December 2014	EP 3026062 A1	01 June 2016
		PH 12015502819 A1	21 March 2016
		US 2016272708 A1	22 September 2016
		WO 2014206107 A1	31 December 2014
		JP 2016523265 A	08 August 2016