PD-1 Antibodies

Summary

In one aspect of the present disclosure, there is provided 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 RASQGISSWLA (SEQ ID NO: 9), SAASSLQS (SEQ ID NO: 10), and QQANHLPFT (SEQ ID NO: 11), respectively, and wherein the heavy chain comprises heavy chain complementarity determining regions HCDR1, HCDR2, and HCDR3,

wherein

(1) wherein LCDR1, LCDR2, and LCDR3 consist of the amino acid sequences RASQGISSWLA (SEQ ID NO: 9), SAASSLQS (SEQ ID NO: 10), and QQANHLPFT (SEQ ID NO: 11), respectively, and

wherein HCDR1, HCDR2, and HCDR3 consist of the amino acid sequences KASGGTFSSYAIS (SEQ ID NO: 2), LIIPMFGTAGYAQKFQG (SEQ ID NO: 4), and ARAEYSSTGTFDY (SEQ ID NO: 7), respectively; or

(2) wherein LCDR1, LCDR2, and LCDR3 consist of the amino acid sequences RASQGISSWLA (SEQ ID NO: 9), SAASSLQS (SEQ ID NO: 10), and QQANHLPFT (SEQ ID NO: 11), respectively, and wherein HCDR1, HCDR2, and HCDR3 consist of the amino acid sequences KASGGTFSSYAIS (SEQ ID NO: 2), LIIPMFDTAGYAQKFQG (SEQ ID NO: 5), and ARAEHSSTGTFDY (SEQ ID NO: 8), respectively; or

(3) wherein LCDR1, LCDR2, and LCDR3 consist of the amino acid sequences RASQGISSWLA (SEQ ID NO: 9), SAASSLQS (SEQ ID NO: 10), and QQANHLPFT (SEQ ID NO: 11), respectively, and wherein HCDR1, HCDR2, and HCDR3 consist of the amino acid sequences KASGGTLSSYAIS (SEQ ID NO: 3), LIIPMFGAAGYAQRFQG (SEQ ID NO: 6), and ARAEHSSTGTFDY (SEQ ID NO: 8), respectively.

In another aspect of the present disclosure, there is provided 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: 15, and the HCVR has the amino acid sequence given in SEQ ID NO: 12, SEQ ID NO: 13, or SEQ ID NO: 14.

In a further aspect of the present disclosure, there is provided a mammalian cell comprising a DNA molecule comprising a polynucleotide sequence encoding a polypeptide having an amino acid sequence of SEQ ID NO: 22 and a polynucleotide sequence encoding a polypeptide having an amino acid sequence of SEQ ID NO: 17 or SEQ ID NO: 20, wherein the cell is capable of expressing an antibody comprising a light chain having an amino acid sequence of SEQ ID NO: 22 and a heavy chain having an amino acid sequence of SEQ ID NO: 17 or SEQ ID NO: 20.

In yet another aspect of the present disclosure, there is provided a process for producing an antibody comprising a light chain having an amino acid sequence of SEQ ID NO: 22 and a heavy chain having an amino acid sequence of SEQ ID NO: 17 or SEQ ID NO: 20, comprising cultivating the mammalian cell as defined herein under conditions such that the antibody is expressed, and recovering the expressed antibody.

In another aspect of the present disclosure, there is provided an antibody produced by the process as defined herein.

In a further aspect of the present disclosure, there is provided a pharmaceutical composition comprising the antibody as defined herein, and an acceptable carrier, diluent, or excipient.

In yet another aspect of the present disclosure, there is provided a use of the antibody as defined herein, in the manufacture of a pharmaceutical composition for treating cancer in a patient in need thereof.

In another aspect of the present disclosure, there is provided a use of an antibody as defined herein, and one or more anti-tumor agents in the manufacture of a medicament for treating cancer, wherein the antibody is to be administered simultaneously, separately, or sequentially with said one or more anti-tumor agents.

In another aspect of the present disclosure, there is provided the antibody as defined herein, for use in therapy.

In a further aspect of the present disclosure, there is provided the antibody as defined herein, for use in the treatment of cancer.

In yet another aspect of the present disclosure, there is provided a combination of an antibody as defined herein and one or more anti-tumor agents, for simultaneous, separate, or sequential use in treating cancer.

Description

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 higher affinity than certain prior art antibodies. 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.

Higher affinity and better blocking, alone or together, can translate into greater *in vivo* activity or lower required dosing amounts.

Certain antibodies of the present invention bind human PD-1 with higher affinity than nivolumab and pembrolizumab. Furthermore, certain antibodies of the present invention mediate preferential enhanced alloreactivity compared to nivolumab and pembrolizumab in an *in vivo* model.

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 RASQGISSWLA (SEQ ID NO: 9), SAASSLQS (SEQ ID NO: 10), and QQANHLPFT (SEQ ID NO: 11), respectively, and wherein the heavy chain comprises heavy chain complementarity determining regions HCDR1, HCDR2, and HCDR3, wherein HCDR1 consists of the amino acid sequences KASGGTFSSYAIS (SEQ ID NO: 2) or KASGGTLSSYAIS (SEQ ID NO: 3), wherein HCDR2 consists of the amino acid sequences LIIPMFGTAGYAQKFQG (SEQ ID NO: 4), LIIPMFDTAGYAQKFQG (SEQ ID NO: 5) or LIIPMFGAAGYAQRFQG (SEQ ID NO: 6), and wherein HCDR3 consists of the amino acid sequences ARAEYSSTGTFDY (SEQ ID NO: 7) or ARAEHSSTGTFDY (SEQ ID NO: 8).

In some embodiments, the present invention provides an antibody, wherein LCDR1, LCDR2, and LCDR3 consist of the amino acid sequences RASQGISSWLA (SEQ ID NO: 9), SAASSLQS (SEQ ID NO: 10), and QQANHLPFT (SEQ ID NO: 11), respectively, and wherein HCDR1, HCDR2, and HCDR3 consist of the amino acid sequences KASGGTFSSYAIS (SEQ ID NO: 2), LIIPMFGTAGYAQKFQG (SEQ ID NO: 4), and ARAEYSSTGTFDY (SEQ ID NO: 7), respectively.

In some embodiments, the present invention provides an antibody, wherein LCDR1, LCDR2, and LCDR3 consist of the amino acid sequences RASQGISSWLA (SEQ ID NO: 9), SAASSLQS (SEQ ID NO: 10), and QQANHLPFT (SEQ ID NO: 11), respectively, and wherein HCDR1, HCDR2, and HCDR3 consist of the amino acid sequences KASGGTFSSYAIS (SEQ ID NO: 2), LIIPMFDTAGYAQKFQG (SEQ ID NO: 5), and ARAEHSSTGTFDY (SEQ ID NO: 8), respectively.

In some embodiments, the present invention provides an antibody, wherein LCDR1, LCDR2, and LCDR3 consist of the amino acid sequences RASQGISSWLA (SEQ ID NO: 9), SAASSLQS (SEQ ID NO: 10), and QQANHLPFT (SEQ ID NO: 11), respectively, and wherein HCDR1, HCDR2, and HCDR3 consist of the amino acid sequences KASGGTLSSYAIS (SEQ ID NO: 3), LIIPMFGAAGYAQRFQG (SEQ ID NO: 6), and ARAEHSSTGTFDY (SEQ ID NO: 8), 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: 15, and the HCVR has the amino acid sequence given in SEQ ID NO: 12, SEQ ID NO: 13, or SEQ ID NO: 14.

In further embodiments, the present invention provides an antibody, wherein the LCVR has the amino acid sequence given in SEQ ID NO: 15, and the HCVR has the amino acid sequence given in SEQ ID NO: 12. In further embodiments, the present invention provides an antibody, wherein the LCVR has the amino acid sequence given in SEQ ID NO: 15, and the HCVR has the amino acid sequence given in SEQ ID NO: 13. In further embodiments, the present invention provides an antibody, wherein the LCVR has the amino acid sequence given in SEQ ID NO: 15, and the HCVR has the amino acid sequence given in SEQ ID NO: 14.

In some embodiments, the present invention provides an antibody, wherein the LC has the amino acid sequence given in SEQ ID NO: 22, and the HC has the amino acid sequence given in SEQ ID NO: 16, SEQ ID NO: 17, SEQ ID NO: 18, SEQ ID NO: 19, SEQ ID NO: 20, or SEQ ID NO: 21.

In a further embodiment, the present invention provides an antibody, wherein the LC has the amino acid sequence given in SEQ ID NO: 22, and the HC has the amino acid sequence given in SEQ ID NO: 16. In a further embodiment, the present invention provides an antibody, wherein the LC has the amino acid sequence given in SEQ ID NO: 22, and the HC has the amino acid sequence given in SEQ ID NO: 17. In a further embodiment, the present invention provides an antibody, wherein the LC has the amino acid sequence given in SEQ ID NO: 22, and the HC has the amino acid sequence given in SEQ ID NO: 18. In a further embodiment, the present invention provides an antibody, wherein the LC has the amino acid sequence given in SEQ ID NO: 22, and the HC has the amino acid sequence given in SEQ ID NO: 19. In a further embodiment, the present invention provides an antibody, wherein the LC has the amino acid sequence given in SEQ ID NO: 22, and the HC has the amino acid sequence given in SEQ ID NO: 20. In a further embodiment, the present invention provides an antibody, wherein the LC has the

amino acid sequence given in SEQ ID NO: 22, and the HC has the amino acid sequence given in SEQ ID NO: 21.

In an embodiment, the present invention provides an antibody, wherein the LC has the amino acid sequence given in SEQ ID NO: 22, and the HC has the amino acid sequence given in SEQ ID NO: 23. In an embodiment, the present invention provides an antibody, wherein the LC has the amino acid sequence given in SEQ ID NO: 22, and the HC has the amino acid sequence given in SEQ ID NO: 24. In an embodiment, the present invention provides an antibody, wherein the LC has the amino acid sequence given in SEQ ID NO: 22, and the HC has the amino acid sequence given in SEQ ID NO: 25.

In an embodiment, 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: 22, and each heavy chain has the amino acid sequence given in SEQ ID NO: 16, SEQ ID NO: 17, SEQ ID NO: 18, SEQ ID NO: 19, SEQ ID NO: 20, or SEQ ID NO: 21.

In a further embodiment, the present invention provides an antibody, wherein each light chain has the amino acid sequence given in SEQ ID NO: 22, and each heavy chain has the amino acid sequence given in SEQ ID NO: 16. In a further embodiment, the present invention provides an antibody, wherein each light chain has the amino acid sequence given in SEQ ID NO: 22, and each heavy chain has the amino acid sequence given in SEQ ID NO: 17. In a further embodiment, the present invention provides an antibody, wherein each light chain has the amino acid sequence given in SEQ ID NO: 22, and each heavy chain has the amino acid sequence given in SEQ ID NO: 18. In a further embodiment, the present invention provides an antibody, wherein each light chain has the amino acid sequence given in SEQ ID NO: 22, and each heavy chain has the amino acid sequence given in SEQ ID NO: 19. In a further embodiment, the present invention provides an antibody, wherein each light chain has the amino acid sequence given in SEQ ID NO: 22, and each heavy chain has the amino acid sequence given in SEQ ID NO: 20. In a further embodiment, the present invention provides an antibody, wherein each light chain has the amino acid sequence given in SEQ ID NO: 22, and each heavy chain has the amino acid sequence given in SEQ ID NO: 21.

In an embodiment, 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: 22, and each heavy chain has the amino acid sequence given in SEQ ID NO: 23. In an embodiment, 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: 22, and each heavy chain has the amino acid sequence given in SEQ ID NO: 24. In an embodiment, 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: 22, and each heavy chain has the amino acid sequence given in SEQ ID NO: 25.

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 an embodiment, 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 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: 15, and the HCVR has the amino acid sequence given in SEQ ID NO: 12, SEQ ID NO: 13, or SEQ ID NO: 14.

In a further embodiment, the present invention provides an antibody that binds human PD-1 (SEQ ID NO: 1), wherein the LCVR has the amino acid sequence given in SEQ ID NO: 15, and the HCVR has the amino acid sequence given in SEQ ID NO: 12. In a further embodiment, the present invention provides an antibody that binds human PD-1 (SEQ ID NO: 1), wherein the LCVR has the amino acid sequence given in SEQ ID NO: 15, and the HCVR has the amino acid sequence given in SEQ ID NO: 13. In a further embodiment, the present invention provides an antibody that binds human PD-1 (SEQ ID NO: 1), wherein the LCVR has the amino acid sequence given in SEQ ID NO: 15, and the HCVR has the amino acid sequence given in SEQ ID NO: 14.

In an embodiment, the present invention provides an antibody that binds human PD-1 (SEQ ID NO: 1), wherein the LC has the amino acid sequence given in SEQ ID NO: 22, and the HC has the amino acid sequence given in SEQ ID NO: 16, SEQ ID NO: 17, SEQ ID NO: 18, SEQ ID NO: 19, SEQ ID NO: 20, or SEQ ID NO: 21.

In a further embodiment, the present invention provides an antibody that binds human PD-1 (SEQ ID NO: 1), wherein the LC has the amino acid sequence given in SEQ ID NO: 22, and the HC has the amino acid sequence given in SEQ ID NO: 16. In a further embodiment, the present invention provides an antibody that binds human PD-1 (SEQ ID NO: 1), wherein the LC has the amino acid sequence given in SEQ ID NO: 22, and the HC has the amino acid sequence given in SEQ ID NO: 17. In a further embodiment, the present invention provides an antibody that binds human PD-1 (SEQ ID NO: 1), wherein the LC has the amino acid sequence given in SEQ ID NO: 22, and the HC has the amino acid sequence given in SEQ ID NO: 18. In a further embodiment, the present invention provides an antibody that binds human PD-1 (SEQ ID NO: 1), wherein the LC has the amino acid sequence given in SEQ ID NO: 22, and the HC has the amino acid sequence given in SEQ ID NO: 19. In a further embodiment, the present invention provides an antibody that binds human PD-1 (SEQ ID NO: 1), wherein the LC has the amino acid sequence given in SEQ ID NO: 22, and the HC has the amino acid sequence given in SEQ ID NO: 20. In a further embodiment, the present invention provides an antibody that binds human PD-1 (SEQ ID NO: 1), wherein the LC has the amino acid sequence given in SEQ ID NO: 22, and the HC has the amino acid sequence given in SEQ ID NO: 21.

In an embodiment, the present invention provides an antibody that binds human PD-1 (SEQ ID NO: 1), wherein the LC has the amino acid sequence given in SEQ ID NO: 22, and the HC has the amino acid sequence given in SEQ ID NO: 23. In an embodiment, the present invention provides an antibody that binds human PD-1 (SEQ ID NO: 1), wherein the LC has the amino acid sequence given in SEQ ID NO: 22, and the HC has the amino acid sequence given in SEQ ID NO: 24. In an embodiment, the present invention provides an antibody that binds human PD-1 (SEQ ID NO: 1), wherein the LC has the amino acid sequence given in SEQ ID NO: 22, and the HC has the amino acid sequence given in SEQ ID NO: 25.

In an embodiment, the present invention provides an antibody that binds human PD-1 (SEQ ID NO: 1), comprising two light chains and two heavy chains, wherein each light chain has the amino acid sequence given in SEQ ID NO: 22, and each heavy chain has the amino acid sequence given in SEQ ID NO: 16, SEQ ID NO: 17, SEQ ID NO: 18, SEQ ID NO: 19, SEQ ID NO: 20, or SEQ ID NO: 21.

In a further embodiment, the present invention provides an antibody that binds human PD-1 (SEQ ID NO: 1), wherein each light chain has the amino acid sequence given in SEQ ID NO: 22, and each heavy chain has the amino acid sequence given in SEQ ID NO: 16. In a further embodiment, the present invention provides an antibody that binds human PD-1 (SEQ ID NO: 1), wherein each light chain has the amino acid sequence given in SEQ ID NO: 22, and each heavy chain has the amino acid sequence given in SEQ ID NO: 17. In a further embodiment, the present invention provides an antibody that binds human PD-1 (SEQ ID NO: 1), wherein each light chain has the amino acid sequence given in SEQ ID NO: 22, and each heavy chain has the amino acid sequence given in SEQ ID NO: 18. In a further embodiment, the present invention provides an antibody that binds human PD-1 (SEQ ID NO: 1), wherein each light chain has the amino acid sequence given in SEQ ID NO: 22, and each heavy chain has the amino acid sequence given in SEQ ID NO: 19. In a further embodiment, the present invention provides an antibody that binds human PD-1 (SEQ ID NO: 1), wherein each light chain has the amino acid sequence given in SEQ ID NO: 22, and each heavy chain has the amino acid sequence given in SEQ ID NO: 20. In a further embodiment, the present invention provides an antibody that binds human PD-1 (SEQ ID NO: 1), wherein each light chain has the amino acid sequence given in SEQ ID NO: 22, and each heavy chain has the amino acid sequence given in SEQ ID NO: 21.

In an embodiment, the present invention provides an antibody that binds human PD-1 (SEQ ID NO: 1), comprising two light chains and two heavy chains, wherein each light chain has the amino acid sequence given in SEQ ID NO: 22, and each heavy chain has the amino acid sequence given in SEQ ID NO: 23. In an embodiment, the present invention provides an antibody that binds human PD-1 (SEQ ID NO: 1), comprising two light chains and two heavy chains, wherein each light chain has the amino acid sequence given in SEQ ID NO: 22, and each heavy chain has the amino acid sequence given in

SEQ ID NO: 24. In an embodiment, the present invention provides an antibody that binds human PD-1 (SEQ ID NO: 1), comprising two light chains and two heavy chains, wherein each light chain has the amino acid sequence given in SEQ ID NO: 22, and each heavy chain has the amino acid sequence given in SEQ ID NO: 25.

In an embodiment, the present invention provides an antibody that binds human PD-1 (SEQ ID NO: 1), 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 that binds human PD-1 (SEQ ID NO: 1), wherein the antibody is glycosylated.

In an embodiment, the present invention provides a mammalian cell, comprising a DNA molecule comprising a polynucleotide sequence encoding a polypeptide having an amino acid sequence of SEQ ID NO: 22 and a polynucleotide sequence encoding a polypeptide having an amino acid sequence of SEQ ID NO: 16 or SEQ ID NO: 19, wherein the cell is capable of expressing an antibody comprising a light chain having an amino acid sequence of SEQ ID NO: 22 and a heavy chain having an amino acid sequence of SEQ ID NO: 16 or SEQ ID NO: 19.

In an embodiment, the present invention provides a mammalian cell, comprising a DNA molecule comprising a polynucleotide sequence encoding a polypeptide having an amino acid sequence of SEQ ID NO: 22 and a polynucleotide sequence encoding a polypeptide having an amino acid sequence of SEQ ID NO: 17 or SEQ ID NO: 20, wherein the cell is capable of expressing an antibody comprising a light chain having an amino acid sequence of SEQ ID NO: 22 and a heavy chain having an amino acid sequence of SEQ ID NO: 17 or SEQ ID NO: 20.

In an embodiment, the present invention provides a mammalian cell, comprising a DNA molecule comprising a polynucleotide sequence encoding a polypeptide having an amino acid sequence of SEQ ID NO: 22 and a polynucleotide sequence encoding a polypeptide having an amino acid sequence of SEQ ID NO: 18 or SEQ ID NO: 21, wherein the cell is capable of expressing an antibody comprising a light chain having an

amino acid sequence of SEQ ID NO: 22 and a heavy chain having an amino acid sequence of SEQ ID NO: 18 or SEQ ID NO: 21.

In an embodiment, the present invention provides a process for producing an antibody, comprising a light chain having an amino acid sequence of SEQ ID NO: 22 and a heavy chain having an amino acid sequence of SEQ ID NO: 16 or SEQ ID NO: 19, comprising cultivating a mammalian cell of the present invention under conditions such that the antibody is expressed, and recovering the expressed antibody.

In an embodiment, the present invention provides a process for producing an antibody, comprising a light chain having an amino acid sequence of SEQ ID NO: 22 and a heavy chain having an amino acid sequence of SEQ ID NO: 17 or SEQ ID NO: 20, comprising cultivating a mammalian cell of the present invention under conditions such that the antibody is expressed, and recovering the expressed antibody.

In an embodiment, the present invention provides a process for producing an antibody, comprising a light chain having an amino acid sequence of SEQ ID NO: 22 and a heavy chain having an amino acid sequence of SEQ ID NO: 18 or SEQ ID NO: 21, comprising cultivating a mammalian cell of the present invention under conditions such that the antibody is expressed, and recovering the expressed antibody.

In an embodiment, the present invention provides an antibody produced by a process of the present invention.

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. Further, certain antibodies of the present invention contain an IgG_4-PAA Fc portion. The IgG_4-PAA Fc portion has a serine to proline mutation at position 228, a phenylalanine to alanine mutation at position 234, and a leucine to alanine mutation at position 235. The S228P mutation is a hinge mutation

that prevents half-antibody formation (phenomenon of dynamic exchange of half-molecules in IgG_4 antibodies). The F234A and L235A mutations further reduce effector function of the already low human IgG_4 isotype. Further, certain antibodies of the present invention contain an IgG_4-PAA Fc portion with the C-terminal lysine removed (des-Lys) from the heavy chain.

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. For the purposes of the present invention, the North CDR definitions are used.

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 a surface plasmon resonance (SPR) biosensor at 37°C 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 or SPR. 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 I, 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 I are shown in Table 1.

The antibodies of the present invention, including, but not limited to, Antibody A - Antibody I 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 S228P IgG4	Antibody B PAA IgG4 des-Lys	Antibody C VK1-12 IgG4
HCVR	12	12	12
LCVR	15	15	15
Heavy chain	16	19	23
Light chain	22	22	22

	Antibody D S228P IgG4	Antibody E PAA IgG4 des-Lys	Antibody F VK1-12 IgG4
HCVR	13	13	13
LCVR	15	15	15
Heavy chain	17	20	24
Light chain	22	22	22

	Antibody G S228P IgG4	Antibody H PAA IgG4 des-Lys	Antibody I VK1-12 IgG4
HCVR	14	14	14
LCVR	15	15	15
Heavy chain	18	21	25
Light chain	22	22	22

Assays

***In vivo* activity - WINN assay**

The antibodies of the present invention can be measured for *in vivo* immunomodulatory activity with the Winn assay. In the Winn assay, human tumor cells and human immune cells (allogenic) are injected together into an immunodeficient mouse, and then followed by dosing with an immunomodulatory agent. Tumor volume is measured to determine the effect of the agent in the assay.

Enhancement of the immune response to allo-antigens by antibodies of the present invention may be tested in the NCI-H292 human NSCLC xenograft model. On day 0,

NSG mice from Jackson Laboratories (7 weeks of age, female, in groups of 8-10 mice) are implanted into the flank subcutaneously with either 2 x10^6 H292 cells, or a mixture of 2 x10^6 H292 cells and 1 x 10^6 human PBMCs in HBSS (0.2 ml total volume). Starting on Day 1, mice are treated with an i.p. injection of human IgG at 10 mg/kg, one time per week. 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 in this assay, Antibody A or Antibody D dosed at 10 mg/kg, qw, ip are well tolerated and safe as monitored by body weight and clinical observations. Tumor growth and T/C% results are shown in Table 2. Tumors in mice co-implanted with NCI-H292 and PBMCs and dosed with Antibody A or Antibody D at 10 mg/kg qw grew significantly slower and regressed over time. Under these conditions, Antibody A and Antibody D both mediate preferential enhanced alloreactivity compared to nivolumab and pembrolizumab.

Table 2: WINN assay

Days Post Tumor Inoculation		4 days	7 days	11 days	14 days	18 days
hIgG	Mean±SEM	22.5 ±7.3	106.1 ±14.5	322.2 ±27.3	365.5 ±36.7	838.8 ±134.7
hIgG + hPBMC	Mean±SEM	56.7 ±11.5	170.7 ±20.2	178.5 ±15.7	157.9 ±17.1	86.7 ±16.2
Nivolumab + hPBMC	Mean±SEM	61.0 ±7.5	135.5 ±11.2	215.8 ±18.7	180.0 ±31.8	93.5 ±14.3
	T/C%	**108%**	**79%**	**121%**	**114%**	**108%**
Pembrolizumab + hPBMC	Mean±SEM	63.3 ±5.4	145.0 ±19.4	197.2 ±22.1	144.9 ±14.0	107.9 ±15.9
	T/C%	**112%**	**85%**	**110%**	**92%**	**125%**
Antibody A + hPBMC	Mean±SEM	29.7 ±5.0	49.7 ±5.9	89.1 ±12.8	40.7 ±9.8	31.1 ±17.9

	T/C%	52%	29%	50%	26%	36%
Antibody D + hPBMC	Mean±SEM	31.9 ±2.6	69.2 ±6.6	99.8 ±13.3	52.6 ±12.3	21.9 ±7.9
	T/C%	**56%**	**41%**	**56%**	**33%**	**25%**

* T/C% is the ratio of tumor volume in control (hIgG + hPBMC) versus treated mice at a specified time.

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, surface plasmon resonance (Biacore), and bio-layer interferometry (ForteBio) assay methods.

As used herein, nivolumab is a human IgG4 PD-1 antibody transiently expressed by applicants 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 by applicants 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, Antibodies D and G in an IgG1 format and expressed in yeast, bind human PD-1 with a K_D of 12 pM and 14 pM respectively. Pembrolizumab and nivolumab, both in an IgG1 format, bind PD-1 with a K_D of 130 pM and 640 pM respectively. Avidity measurements for Antibodies D and G result in a K_D of approximately 0.9 pM and 1 pM respectively. Pembrolizumab and nivolumab bind human PD-1 with a K_D of approximately 3 pM and 5 pM respectively.

Table 3: Binding by MSD of antibodies of the invention in IgG1 format

Name	Monovalent KD (pM) against human PD-1	Avid KD (pM) against human PD-1
Antibody D in IgG1 format	12	0.9
Antibody G in IgG1 format	14	1
Pembrolizumab (IgG1)	130	3
Nivolumab (IgG1)	640	5

Bio-layer interferometry

ForteBio affinity measurements are 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 are performed by loading IgGs online onto AHQ sensors. Sensors are equilibrated off-line in assay buffer for 30 min and then monitored on-line for 60 seconds for baseline establishment. Sensors with loaded IgGs are exposed to 100 nM antigen for 5 min, afterwards they are transferred to assay buffer for 5 min for off-rate measurement. Kinetics are analyzed using the 1:1 binding model.

Table 4: Binding by Bio-layer interferometry of antibodies of the invention

	Monovalent K_D (pM) Fab in solution, hPD-1_Fc on sensor tip	Monovalent K_D (pM) hPD-1_HIS in solution, IgG on sensor tip	Monovalent K_D (pM) Fab in solution, cynoPD-1_Fc on sensor tip
Antibody D in IgG1 format	560	310	670
Antibody G in IgG1 format	490	440	590
Pembrolizumab in IgG1 format	2000	2000	470
Nivolumab in IgG1 format	1700	4100	1200

In experiments performed essentially as described in this assay, Antibodies D and G bind human PD-1_Fc with a K_D approximately threefold to fourfold lower than nivolumab and pembrolizumab when PD-1_Fc was on the sensor tip. When the antibody was on the sensor tip, Antibodies D and G bind human PD-1_Fc with a K_D approximately fourfold to sixfold lower than nivolumab and pembrolizumab. Antibodies D and G bind cynoPD-1_Fc with a similar K_D to nivolumab and pembrolizumab.

Surface Plasmon Resonance (SPR)

Immobilization of human PD-1-Fc (R&D Systems) as ligand on to sensor chip surface is performed at 25°C. Antibodies of the present invention are used as analyte, and injected over the human PD-1-Fc immobilized sensor chip surface. All sample analytes are run in 3-fold series dilutions from their starting concentration (90 nM), 8 total dilutions with one duplicate at a middle concentration and a zero. The analysis is performed at 37°C. The contact time for each sample is 180 sec at 30 µl/min. The dissociation time: 300 seconds for 5 lower concentrations and 1200 (Fab), or 2400 (T=0) or 3000 (4 weeks 4°C, 25°C, 40°C) seconds for 3 higher concentrations. The immobilized surface is regenerated for 6-8 seconds with 0.4% SDS at 30 µl/min, and then

stabilized for 5 seconds. Binding kinetics are analyzed using the Biacore T200 Evaluation software (Version 3.0). Data are referenced to a blank flow cell, and the data are fit to a 1:1 binding model.

In experiments performed essentially as described in this assay, Antibody D binds with a K_D to human PD-1 of 102 pM, nivolumab with a K_D of 246 pM, and pembrolizumab with a K_D of 181 pM. As shown in Table 6, Antibody D maintained binding activity at 4 weeks under elevated temperature conditions.

Table 5: Binding by SPR of Antibody D at extended times and temperatures

Binding to human PD-1-Fc	Kon (1/Ms)	Koff (1/s)	K_D (pM)
Antibody D	2.86E+05	2.98E-05	104
Antibody D, 4 weeks 4°C	3.77E+05	3.86E-05	103
Antibody D, 4 weeks 25°C	3.54E+05	3.60E-05	102
Antibody D, 4 weeks 40°C	3.58E+05	4.22E-05	118

Elisa Blocking of human PD-1 to PD-L1 and PD-L2.

For the receptor-ligand blocking assay, varying amounts (of anti-PD-1 antibody or control IgG are mixed with a fixed amount of biotinylated PD-1-Fc fusion protein (100 ng/mL) and incubated at room temperature for 1 hour. The mixture is 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 an additional 1 hour. Plates are washed and streptavidin HRP conjugate is added. Plates are read at an absorbance at 450 nm. IC50 represents the antibody concentration required for 50% inhibition of PD-1 binding to PD-L1 or binding to PD-L2.

In experiments performed essentially as described, Antibody D blocks the interaction of PD-1 with PD-L1 with an IC50 of 0.30 nM, and the interaction of PD-1 with PD-L2 with an IC50 of 0.34 nM.

Table 6: Elisa Blocking Assay of human PD-1

	Antibody D	Nivolumab	Pembrolizumab
Blocking PD-1/PD-L1 (IC50 nM)	0.30	0.25	0.24
Blocking PD-1/PD-L2 (IC50 nM)	0.34	0.26	0.27

Binding to human 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 antibody from 200 nM titrated 19x by a factor of 2 to the lowest concentration of 3.185 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 G binds PD-1 in a dose-dependent manner, with an EC50 value (n=1) of 1.756 nM and pembrolizumab binds PD-1 with an EC50 value (n=1) of 1.429 nM. Antibody D binds PD-1 in a dose-dependent manner, with an EC50 value (n=1) of 0.9784 nM, pembrolizumab with an EC50 value (n=1) of 0.9510 nM, and nivolumab with an EC50 value (n=1) of 0.9675 nM. Antibody D and Antibody G bind with a similar EC50 to human PD-1 as nivolumab and pembrolizumab under these conditions.

Blocking of human PD-1 to 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 mean fluorescence intensity (MFI) is calculated on the C6 software.

In experiments performed essentially as described in this assay, Antibody D and G in IgG1 format, expressed in yeast, blocked human PD-L2-FITC binding, resulting in an MFI of 24,697.7 and 31,390.5 respectively as compared to control IgG which resulted in an MFI of 182,959.1. Pembrolizumab and nivolumab resulted in less blocking of PD-L2 binding to PD-1 than Antibody D and G with MFI's of 46,245.9 and 54,509.8, respectively.

Table 7: 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 D in IgG1 format	24,697.7
Antibody G in IgG1 format	31,390.5

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. The levels of certain cytokines, such as IL-2, are expected to increase if T cell proliferation is promoted by treatment with antibodies of the present invention.

2×10^6 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 (1×10^6 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 3×10^5 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 D at each concentration increased IL-2 more than nivolumab and pembrolizumab. Antibody D and G resulted in a comparable increase of IFN-γ as nivolumab and pembrolizumab.

Table 8: IL-2 secretion fold change vs. IgG control

	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 D	3.08	2.63	2.45	1.64	1.25
Antibody G	2.45	2.62	3.12	1.91	1.36

Table 9: IFN-γ secretion fold change vs. IgG control

	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 D	1.72	1.99	2.26	1.94	1.32
Antibody G	2.07	2.04	2.48	1.91	1.17

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, B, C, D, E, and F)
KASGGTFSSYAIS

SEQ ID NO: 3 (HCDR1 of Antibody G, H, and I)
KASGGTLSSYAIS

SEQ ID NO: 4 (HCDR2 of Antibody A, B, and C)
LIIPMFGTAGYAQKFQG

SEQ ID NO: 5 (HCDR2 of Antibody D, E, and F)
LIIPMFDTAGYAQKFQG

SEQ ID NO: 6 (HCDR2 of Antibody G, H, and I)
LIIPMFGAAGYAQRFQG

SEQ ID NO: 7 (HCDR3 of Antibody A, B, and C)
ARAEYSSTGTFDY

SEQ ID NO: 8 (HCDR3 of Antibody D, E, F, G, H, and I)
ARAEHSSTGTFDY

SEQ ID NO: 9 (LCDR1 of Antibody A – Antibody I)
RASQGISSWLA

SEQ ID NO: 10 (LCDR2 of Antibody A – Antibody I)
SAASSLQS

SEQ ID NO: 11 (LCDR3 of Antibody A – Antibody I)
QQANHLPFT

SEQ ID NO: 12 (HCVR of Antibody A, B, and C)
QVQLVQSGAEVKKPGSSVKVSCKASGGTFSSYAISWVRQAPGQGLEWMGLIIPM
FGTAGYAQKFQGRVTITADESTSTAYMELSSLRSEDTAVYYCARAEYSSTGTFDY
WGQGTLVTVSS

SEQ ID NO: 13 (HCVR of Antibody D, E, and F)
QVQLVQSGAEVKKPGSSVKVSCKASGGTFSSYAISWVRQAPGQGLEWMGLIIPM
FDTAGYAQKFQGRVAITVDESTSTAYMELSSLRSEDTAVYYCARAEHSSTGTFD
YWGQGTLVTVSS

SEQ ID NO: 14 (HCVR of Antibody G, H, and I)
QVQLVQSGAEVKKPGSSVRVSCKASGGTLSSYAISWVRQAPGQGLEWMGLIIPM
FGAAGYAQRFQGRVTITADESASTAYMELSSLRSEDTAVYYCARAEHSSTGTFD
YWGQGTLVTVSS

SEQ ID NO: 15 (LCVR of Antibody A – Antibody I)
DIQMTQSPSSVSASVGDRVTITCRASQGISSWLAWYQQKPGKAPKLLISAASSLQS
GVPSRFSGSGSGTDFTLTISSLQPEDFATYYCQQANHLPFTFGGGTKVEIK

SEQ ID NO: 16 (HC of Antibody A – S228P IgG4)
QVQLVQSGAEVKKPGSSVKVSCKASGGTFSSYAISWVRQAPGQGLEWMGLIIPM
FGTAGYAQKFQGRVTITADESTSTAYMELSSLRSEDTAVYYCARAEYSSTGTFDY

WGQGTLVTVSSASTKGPSVFPLAPCSRSTSESTAALGCLVKDYFPEPVTVSWNSG
ALTSGVHTFPAVLQSSGLYSLSSVVTVPSSSLGTKTYTCNVDHKPSNTKVDKRVE
SKYGPPCPPCPAPEFLGGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSQEDPEVQF
NWYVDGVEVHNAKTKPREEQFNSTYRVVSVLTVLHQDWLNGKEYKCKVSNKG
LPSSIEKTISKAKGQPREPQVYTLPPSQEEMTKNQVSLTCLVKGFYPSDIAVEWES
NGQPENNYKTTPPVLDSDGSFFLYSRLTVDKSRWQEGNVFSCSVMHEALHNHYT
QKSLSLSLGK

SEQ ID NO: 17 (HC of Antibody D – S228P IgG4)
QVQLVQSGAEVKKPGSSVKVSCKASGGTFSSYAISWVRQAPGQGLEWMGLIIPM
FDTAGYAQKFQGRVAITVDESTSTAYMELSSLRSEDTAVYYCARAEHSSTGTFD
YWGQGTLVTVSSASTKGPSVFPLAPCSRSTSESTAALGCLVKDYFPEPVTVSWNS
GALTSGVHTFPAVLQSSGLYSLSSVVTVPSSSLGTKTYTCNVDHKPSNTKVDKRV
ESKYGPPCPPCPAPEFLGGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSQEDPEVQ
FNWYVDGVEVHNAKTKPREEQFNSTYRVVSVLTVLHQDWLNGKEYKCKVSNK
GLPSSIEKTISKAKGQPREPQVYTLPPSQEEMTKNQVSLTCLVKGFYPSDIAVEWE
SNGQPENNYKTTPPVLDSDGSFFLYSRLTVDKSRWQEGNVFSCSVMHEALHNHY
TQKSLSLSLGK

SEQ ID NO: 18 (HC of Antibody G – S228P IgG4)
QVQLVQSGAEVKKPGSSVRVSCKASGGTLSSYAISWVRQAPGQGLEWMGLIIPM
FGAAGYAQRFQGRVTITADESASTAYMELSSLRSEDTAVYYCARAEHSSTGTFD
YWGQGTLVTVSSASTKGPSVFPLAPCSRSTSESTAALGCLVKDYFPEPVTVSWNS
GALTSGVHTFPAVLQSSGLYSLSSVVTVPSSSLGTKTYTCNVDHKPSNTKVDKRV
ESKYGPPCPPCPAPEFLGGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSQEDPEVQ
FNWYVDGVEVHNAKTKPREEQFNSTYRVVSVLTVLHQDWLNGKEYKCKVSNK
GLPSSIEKTISKAKGQPREPQVYTLPPSQEEMTKNQVSLTCLVKGFYPSDIAVEWE
SNGQPENNYKTTPPVLDSDGSFFLYSRLTVDKSRWQEGNVFSCSVMHEALHNHY
TQKSLSLSLGK

SEQ ID NO: 19 (HC of Antibody B – PAA IgG4 des-Lys)

QVQLVQSGAEVKKPGSSVKVSCKASGGTFSSYAISWVRQAPGQGLEWMGLIIPM
FGTAGYAQKFQGRVTITADESTSTAYMELSSLRSEDTAVYYCARAEYSSTGTFDY
WGQGTLVTVSSASTKGPSVFPLAPCSRSTSESTAALGCLVKDYFPEPVTVSWNSG
ALTSGVHTFPAVLQSSGLYSLSSVVTVPSSSLGTKTYTCNVDHKPSNTKVDKRVE
SKYGPPCPPCPAPEAAGGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSQEDPEVQF
NWYVDGVEVHNAKTKPREEQFNSTYRVVSVLTVLHQDWLNGKEYKCKVSNKG
LPSSIEKTISKAKGQPREPQVYTLPPSQEEMTKNQVSLTCLVKGFYPSDIAVEWES
NGQPENNYKTTPPVLDSDGSFFLYSRLTVDKSRWQEGNVFSCSVMHEALHNHYT
QKSLSLSLG

SEQ ID NO: 20 (HC of Antibody E – PAA IgG4 des-Lys)
QVQLVQSGAEVKKPGSSVKVSCKASGGTFSSYAISWVRQAPGQGLEWMGLIIPM
FDTAGYAQKFQGRVAITVDESTSTAYMELSSLRSEDTAVYYCARAEHSSTGTFD
YWGQGTLVTVSSASTKGPSVFPLAPCSRSTSESTAALGCLVKDYFPEPVTVSWNS
GALTSGVHTFPAVLQSSGLYSLSSVVTVPSSSLGTKTYTCNVDHKPSNTKVDKRV
ESKYGPPCPPCPAPEAAGGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSQEDPEVQ
FNWYVDGVEVHNAKTKPREEQFNSTYRVVSVLTVLHQDWLNGKEYKCKVSNK
GLPSSIEKTISKAKGQPREPQVYTLPPSQEEMTKNQVSLTCLVKGFYPSDIAVEWE
SNGQPENNYKTTPPVLDSDGSFFLYSRLTVDKSRWQEGNVFSCSVMHEALHNHY
TQKSLSLSLG

SEQ ID NO: 21 (HC of Antibody H – PAA IgG4 des-Lys)
QVQLVQSGAEVKKPGSSVRVSCKASGGTLSSYAISWVRQAPGQGLEWMGLIIPM
FGAAGYAQRFQGRVTITADESASTAYMELSSLRSEDTAVYYCARAEHSSTGTFD
YWGQGTLVTVSSASTKGPSVFPLAPCSRSTSESTAALGCLVKDYFPEPVTVSWNS
GALTSGVHTFPAVLQSSGLYSLSSVVTVPSSSLGTKTYTCNVDHKPSNTKVDKRV
ESKYGPPCPPCPAPEAAGGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSQEDPEVQ
FNWYVDGVEVHNAKTKPREEQFNSTYRVVSVLTVLHQDWLNGKEYKCKVSNK
GLPSSIEKTISKAKGQPREPQVYTLPPSQEEMTKNQVSLTCLVKGFYPSDIAVEWE
SNGQPENNYKTTPPVLDSDGSFFLYSRLTVDKSRWQEGNVFSCSVMHEALHNHY
TQKSLSLSLG

SEQ ID NO: 22 (LC of Antibody A – Antibody I)
DIQMTQSPSSVSASVGDRVTITCRASQGISSWLAWYQQKPGKAPKLLISAASSLQS
GVPSRFSGSGSGTDFTLTISSLQPEDFATYYCQQANHLPFTFGGGTKVEIKRTVAA
PSVFIFPPSDEQLKSGTASVVCLLNNFYPREAKVQWKVDNALQSGNSQESVTEQD
SKDSTYSLSSTLTLSKADYEKHKVYACEVTHQGLSSPVTKSFNRGEC

SEQ ID NO: 23 (HC of Antibody C – VK1-12 IgG4)
QVQLVQSGAEVKKPGSSVKVSCKASGGTFSSYAISWVRQAPGQGLEWMGLIIPM
FGTAGYAQKFQGRVTITADESTSTAYMELSSLRSEDTAVYYCARAEYSSTGTFDY
WGQGTLVTVSSASTKGPSVFPLAPCSRSTSESTAALGCLVKDYFPEPVTVSWNSG
ALTSGVHTFPAVLQSSGLYSLSSVVTVPSSSLGTKTYTCNVDHKPSNTKVDKRVE
SKYGPPCPSCPAPEFLGGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSQEDPEVQF
NWYVDGVEVHNAKTKPREEQFNSTYRVVSVLTVLHQDWLNGKEYKCKVSNKG
LPSSIEKTISKAKGQPREPQVYTLPPSQEEMTKNQVSLTCLVKGFYPSDIAVEWES
NGQPENNYKTTPPVLDSDGSFFLYSRLTVDKSRWQEGNVFSCSVMHEALHNHYT
QKSLSLSLGK

SEQ ID NO: 24 (HC of Antibody F – VK1-12 IgG4)
QVQLVQSGAEVKKPGSSVKVSCKASGGTFSSYAISWVRQAPGQGLEWMGLIIPM
FDTAGYAQKFQGRVAITVDESTSTAYMELSSLRSEDTAVYYCARAEHSSTGTFD
YWGQGTLVTVSSASTKGPSVFPLAPCSRSTSESTAALGCLVKDYFPEPVTVSWNS
GALTSGVHTFPAVLQSSGLYSLSSVVTVPSSSLGTKTYTCNVDHKPSNTKVDKRV
ESKYGPPCPSCPAPEFLGGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSQEDPEVQ
FNWYVDGVEVHNAKTKPREEQFNSTYRVVSVLTVLHQDWLNGKEYKCKVSNK
GLPSSIEKTISKAKGQPREPQVYTLPPSQEEMTKNQVSLTCLVKGFYPSDIAVEWE
SNGQPENNYKTTPPVLDSDGSFFLYSRLTVDKSRWQEGNVFSCSVMHEALHNHY
TQKSLSLSLGK

SEQ ID NO: 25 (HC of Antibody I – VK1-12 IgG4)

QVQLVQSGAEVKKPGSSVRVSCKASGGTLSSYAISWVRQAPGQGLEWMGLIIPM
FGAAGYAQRFQGRVTITADESASTAYMELSSLRSEDTAVYYCARAEHSSTGTFD
YWGQGTLVTVSSASTKGPSVFPLAPCSRSTSESTAALGCLVKDYFPEPVTVSWNS
GALTSGVHTFPAVLQSSGLYSLSSVVTVPSSSLGTKTYTCNVDHKPSNTKVDKRV
ESKYGPPCPSCPAPEFLGGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSQEDPEVQ
FNWYVDGVEVHNAKTKPREEQFNSTYRVVSVLTVLHQDWLNGKEYKCKVSNK
GLPSSIEKTISKAKGQPREPQVYTLPPSQEEMTKNQVSLTCLVKGFYPSDIAVEWE
SNGQPENNYKTTPPVLDSDGSFFLYSRLTVDKSRWQEGNVFSCSVMHEALHNHY
TQKSLSLSLGK

SEQ ID NO: 26 (DNA of HC of Antibody A - S228P IgG4)

CAGGTGCAGCTGGTGCAGAGCGGCGCTGAGGTGAAGAAGCCTGGCTCCAGCG
TGAAGGTGTCCTGCAAAGCCTCCGGCGGCACCTTCAGCTCCTACGCTATCAGC
TGGGTGAGGCAGGCTCCTGGCCAGGGACTGGAGTGGATGGGCCTGATCATCC
CCATGTTCGGCACCGCTGGCTACGCCCAGAAGTTCCAGGGCAGGGTGACCAT
CACCGCCGACGAGTCCACCTCCACCGCCTACATGGAGCTGTCCTCCCTGAGGT
CCGAGGACACCGCCGTGTACTACTGTGCCAGGGCCGAGTACTCCTCCACCGG
CACCTTCGACTACTGGGGCCAGGGCACACTCGTGACCGTCAGCTCCGCCAGC
ACAAAGGGCCCCAGCGTGTTTCCCCTGGCCCCTTGCAGCAGGAGCACATCCG
AGAGCACCGCTGCCCTGGGATGTCTGGTGAAGGACTATTTCCCCGAGCCCGT
GACAGTGAGCTGGAACAGCGGAGCCCTGACCTCCGGAGTGCACACCTTCCCT
GCCGTGCTGCAGAGCAGCGGACTGTACAGCCTGTCCAGCGTGGTGACAGTGC
CTTCCTCCAGCCTCGGCACAAAGACCTACACCTGCAACGTGGACCACAAGCC
CTCCAACACCAAAGTGGACAAGCGGGTGGAAAGCAAGTATGGACCCCCTTGC
CCTCCTTGTCCCGCCCCTGAGTTCCTGGGAGGCCCTTCCGTCTTCCTGTTTCCC
CCCAAGCCCAAGGACACACTCATGATTTCCAGGACCCCCGAGGTGACCTGCG
TCGTGGTCGACGTGAGCCAGGAGGACCCCGAGGTGCAGTTTAACTGGTATGT
GGACGGCGTGGAGGTCCACAATGCCAAAACCAAGCCCAGGGAGGAACAGTT
CAACTCCACCTATAGGGTGGTCAGCGTGCTGACCGTCCTGCACCAGGACTGG
CTGAACGGAAAGGAGTATAAGTGCAAAGTCTCCAACAAGGGCCTGCCTAGCA
GCATCGAGAAGACCATCTCCAAAGCCAAGGGCCAGCCCAGGGAGCCCCAGGT

TTATACTCTGCCCCCTTCCCAGGAGGAGATGACCAAGAATCAGGTGTCCCTGA
CCTGCCTGGTGAAAGGCTTTTACCCCTCCGACATCGCTGTGGAGTGGGAGAGC
AATGGCCAGCCCGAGAACAACTACAAGACCACCCCCCCCGTGCTGGATAGCG
ATGGCAGCTTCTTCCTCTACAGCAGGCTGACCGTGGATAAGAGCAGGTGGCA
GGAGGGCAACGTGTTTTCCTGCTCCGTGATGCATGAGGCCCTCCACAACCATT
ACACACAGAAAAGCCTGAGCCTGAGCCTGGGCAAGTGATGA

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

CAAGTGCAGCTGGTGCAGTCCGGCGCTGAGGTGAAAAAACCCGGATCCTCCG
TCAAGGTGTCCTGTAAAGCCAGCGGCGGCACATTCAGCAGCTACGCCATCTC
CTGGGTGAGGCAAGCTCCTGGACAGGGCCTGGAATGGATGGGCCTGATCATC
CCCATGTTCGACACCGCCGGCTACGCTCAGAAATTCCAGGGCCGGGTCGCCA
TTACAGTGGATGAGAGCACCAGCACAGCCTACATGGAGCTCAGCTCCCTGAG
GAGCGAAGATACCGCCGTCTACTATTGTGCCCGGGCTGAGCATAGCAGCACC
GGCACCTTCGACTATTGGGGCCAGGGAACCCTGGTCACAGTGAGCTCCGCTTC
CACAAAAGGCCCCAGCGTGTTTCCCCTGGCCCCTTGTAGCAGGTCCACCTCCG
AAAGCACAGCCGCTCTGGGCTGCCTGGTCAAGGATTACTTCCCCGAGCCCGT
GACCGTGTCCTGGAATAGCGGCGCTCTCACATCCGGAGTGCATACCTTTCCTG
CCGTGCTCCAGTCCTCCGGCCTGTACTCCCTGAGCTCCGTGGTGACCGTCCCT
TCCAGCTCCCTGGGCACCAAGACCTATACCTGTAACGTGGACCACAAGCCCTC
CAATACCAAGGTGGATAAGCGGGTCGAGTCCAAGTACGGACCCCCTTGCCCT
CCTTGTCCTGCTCCTGAATTCCTCGGCGGACCTAGCGTCTTTCTCTTCCCCCCC
AAGCCCAAGGATACCCTGATGATCTCCAGGACCCCCGAGGTGACATGCGTCG
TGGTCGATGTGTCCCAGGAGGATCCTGAAGTGCAGTTCAACTGGTACGTGGA
CGGCGTCGAAGTGCATAACGCCAAGACCAAGCCCAGGGAGGAGCAGTTCAA
CTCCACCTATCGGGTGGTGAGCGTGCTGACCGTGCTGCATCAGGACTGGCTCA
ACGGCAAAGAGTACAAGTGCAAGGTCTCCAACAAGGGACTCCCCTCCAGCAT
CGAGAAGACCATTAGCAAGGCCAAAGGCCAACCCAGGGAGCCTCAGGTATAT
ACGCTGCCCCCCAGCCAGGAGGAGATGACCAAAAACCAGGTCAGCCTCACCT
GTCTGGTCAAGGGCTTCTACCCTAGCGACATTGCTGTCGAGTGGGAGAGCAA
CGGCCAGCCCGAGAACAACTATAAAACCACCCCCCCTGTCCTGGACTCCGAC

GGATCCTTCTTCCTGTACTCCAGGCTGACAGTCGACAAGTCCCGGTGGCAAGA
GGGAAACGTCTTCTCCTGCTCCGTGATGCACGAAGCTCTCCACAACCACTACA
CCCAGAAGAGCCTCAGCCTGTCCCTGGGCAAATGATGA

SEQ ID NO: 28 (DNA of HC of Antibody G - S228P IgG4)

CAAGTCCAGCTCGTGCAAAGCGGAGCCGAGGTGAAGAAACCCGGCAGCTCCG
TGCGGGTGAGCTGTAAGGCCTCCGGAGGCACCCTGTCCAGCTATGCTATCAG
CTGGGTGAGGCAGGCCCCCGGACAGGGCCTGGAATGGATGGGACTGATCATC
CCTATGTTTGGAGCCGCCGGCTATGCTCAGAGGTTCCAGGGCCGGGTCACCAT
CACCGCTGACGAGAGCGCCAGCACCGCCTATATGGAGCTGTCCTCCCTGAGG
AGCGAGGATACCGCTGTCTACTACTGTGCCAGGGCCGAGCACTCCTCCACAG
GAACCTTCGACTACTGGGGCCAGGGCACCCTGGTGACAGTGTCCTCCGCCTCC
ACCAAGGGCCCTTCCGTGTTTCCTCTGGCTCCTTGCTCCCGGTCCACCAGCGA
GTCCACAGCCGCTCTGGGCTGTCTGGTGAAGGACTATTTCCCCGAGCCTGTGA
CCGTCAGCTGGAATAGCGGCGCCCTGACCTCCGGAGTGCACACATTCCCCGC
CGTCCTGCAGAGCAGCGGACTCTACTCCCTGAGCTCCGTGGTGACCGTGCCTT
CCAGCAGCCTGGGAACCAAGACCTACACCTGCAATGTGGACCACAAACCCAG
CAACACCAAGGTGGATAAGCGGGTGGAATCCAAGTACGGCCCTCCCTGTCCC
CCTTGTCCCGCTCCCGAATTCCTGGGCGGACCTAGCGTGTTCCTGTTTCCCCCT
AAGCCCAAGGATACCCTGATGATCTCCAGGACCCCCGAAGTCACCTGCGTCG
TCGTGGACGTGTCCCAGGAGGACCCTGAAGTCCAGTTTAATTGGTACGTCGAC
GGCGTGGAGGTGCACAACGCCAAGACAAAGCCTCGGGAGGAGCAGTTCAAC
AGCACCTACAGGGTGGTGAGCGTGCTGACCGTGCTGCACCAGGACTGGCTGA
ACGGCAAAGAGTACAAGTGCAAGGTGAGCAACAAGGGCCTGCCCTCCTCCAT
CGAGAAGACCATCTCCAAGGCCAAAGGCCAGCCGAGGGAGCCCCAGGTGTA
CACCCTGCCCCCTAGCCAGGAGGAGATGACCAAGAACCAGGTCTCCCTGACC
TGCCTGGTGAAGGGATTCTATCCCAGCGACATTGCCGTGGAGTGGGAGTCCA
ACGGCCAGCCCGAGAATAACTACAAGACCACCCCCCCTGTGCTGGACAGCGA
CGGGAGCTTCTTCCTGTATTCCCGGCTGACCGTCGACAAGTCCCGGTGGCAGG
AGGGCAACGTGTTTAGCTGCAGCGTGATGCACGAAGCCCTCCACAACCACTA
TACCCAGAAGAGCCTGTCCCTGTCCCTGGGCAAGTGATGA

SEQ ID NO: 29 (DNA of HC of Antibody E - PAA IgG4 des-Lys)

CAGGTGCAGCTGGTCCAGTCAGGGGCTGAAGTGAAGAAGCCCGGCAGCTCCG
TGAAGGTGTCTTGCAAGGCCAGCGGCGGAACATTCTCCAGTTACGCCATCTCT
TGGGTGCGGCAGGCTCCAGGCCAGGGCCTGGAGTGGATGGGCCTGATCATCC
CCATGTTCGACACCGCCGGGTATGCCCAGAAGTTTCAGGGCAGAGTGGCAAT
CACAGTGGACGAGAGCACCTCCACAGCCTACATGGAGCTGTCTAGCCTGAGA
TCCGAGGATACCGCCGTGTATTATTGTGCCCGGGCCGAACACAGCTCTACAG
GGACTTTCGACTACTGGGGCCAGGGCACCCTGGTGACAGTGTCCTCTGCTAGC
ACCAAGGGCCCATCGGTCTTCCCGCTCGCGCCCTGCTCCAGGAGCACCTCCGA
GAGCACAGCCGCCCTGGGCTGCCTGGTCAAGGACTACTTCCCCGAACCGGTG
ACGGTGTCGTGGAACTCAGGCGCCCTGACCAGCGGCGTGCACACCTTCCCGG
CTGTCCTACAGTCCTCAGGACTCTACTCCCTCAGCAGCGTGGTGACCGTGCCC
TCCAGCAGCTTGGGCACGAAGACCTACACCTGCAACGTAGATCACAAGCCCA
GCAACACCAAGGTGGACAAGAGAGTTGAGTCCAAATATGGTCCCCCATGCCC
ACCCTGCCCAGCACCTGAGGCCGCCGGGGGACCATCAGTCTTCCTGTTCCCCC
CAAAACCCAAGGACACTCTCATGATCTCCCGGACCCCTGAGGTCACGTGCGT
GGTGGTGGACGTGAGCCAGGAAGACCCCGAGGTCCAGTTCAACTGGTATGTT
GATGGCGTGGAGGTGCATAATGCCAAGACAAAGCCGCGGGAGGAGCAGTTC
AACAGCACGTACCGTGTGGTCAGCGTCCTCACCGTCCTGCACCAGGACTGGCT
GAACGGCAAGGAGTACAAGTGCAAGGTCTCCAACAAAGGCCTCCCGTCCTCC
ATCGAGAAAACCATCTCCAAAGCCAAAGGGCAGCCCCGAGAGCCACAGGTGT
ACACCCTGCCCCCATCCCAAGAAGAAATGACCAAAAACCAAGTCAGCCTGAC
CTGCCTGGTCAAAGGCTTCTACCCCAGCGACATCGCCGTGGAGTGGGAAAGC
AATGGGCAGCCGGAGAACAACTACAAGACCACGCCTCCCGTGCTGGACTCCG
ACGGCTCCTTCTTCCTCTACTCCCGTCTAACCGTGGACAAGAGCAGGTGGCAG
GAGGGGAATGTCTTCTCATGCTCCGTGATGCATGAGGCTCTGCACAACCACTA
CACACAGAAGAGCCTCTCCCTGTCTCTGGGT

SEQ ID NO: 30 (DNA of LC of Antibody A – Antibody I)

GACATCCAGATGACACAGTCCCCTAGCTCCGTGTCCGCTTCCGTGGGAGACA
GGGTGACAATCACATGCAGGGCTTCCCAGGGCATCAGCAGCTGGCTGGCTTG
GTATCAGCAGAAGCCCGGCAAGGCCCCCAAGCTGCTGATCAGCGCTGCTAGC
TCCCTGCAGTCCGGAGTGCCTTCCAGGTTCTCCGGCTCCGGAAGCGGCACCGA
CTTCACCCTGACCATCTCCAGCCTGCAGCCCGAGGACTTCGCCACCTACTACT
GCCAACAGGCCAACCACCTGCCCTTCACCTTCGGCGGCGGCACCAAGGTGGA
GATCAAGAGGACCGTGGCCGCCCCCTCCGTGTTCATCTTTCCCCCCAGCGACG
AGCAGCTGAAGAGCGGCACCGCCTCCGTGGTGTGCCTGCTGAACAACTTCTA
TCCCCGGGAGGCCAAGGTGCAGTGGAAGGTCGACAATGCCCTGCAGAGCGGC
AACTCCCAGGAGAGCGTGACCGAGCAGGACAGCAAGGACTCCACCTACTCCC
TGAGCTCCACCCTGACACTGTCCAAGGCCGACTACGAGAAGCACAAGGTGTA
CGCCTGCGAGGTGACACACCAGGGCCTGAGCTCCCCCGTGACCAAGTCCTTC
AACAGGGGCGAGTGCTGATGA

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 RASQGISSWLA (SEQ ID NO: 9), SAASSLQS (SEQ ID NO: 10), and QQANHLPFT (SEQ ID NO: 11), respectively, and wherein the heavy chain comprises heavy chain complementarity determining regions HCDR1, HCDR2, and HCDR3, wherein(1) wherein LCDR1, LCDR2, and LCDR3 consist of the amino acid sequences RASQGISSWLA (SEQ ID NO: 9), SAASSLQS (SEQ ID NO: 10), and QQANHLPFT (SEQ ID NO: 11), respectively, and
wherein HCDR1, HCDR2, and HCDR3 consist of the amino acid sequences KASGGTFSSYAIS (SEQ ID NO: 2), LIIPMFGTAGYAQKFQG (SEQ ID NO: 4), and ARAEYSSTGTFDY (SEQ ID NO: 7), respectively; or
(2) wherein LCDR1, LCDR2, and LCDR3 consist of the amino acid sequences RASQGISSWLA (SEQ ID NO: 9), SAASSLQS (SEQ ID NO: 10), and QQANHLPFT (SEQ ID NO: 11), respectively, and wherein HCDR1, HCDR2, and HCDR3 consist of the amino acid sequences KASGGTFSSYAIS (SEQ ID NO: 2), LIIPMFDTAGYAQKFQG (SEQ ID NO: 5), and ARAEHSSTGTFDY (SEQ ID NO: 8), respectively; or

(3) wherein LCDR1, LCDR2, and LCDR3 consist of the amino acid sequences RASQGISSWLA (SEQ ID NO: 9), SAASSLQS (SEQ ID NO: 10), and QQANHLPFT (SEQ ID NO: 11), respectively, and wherein HCDR1, HCDR2, and HCDR3 consist of the amino acid sequences KASGGTLSSYAIS (SEQ ID NO: 3), LIIPMFGAAGYAQRFQG (SEQ ID NO: 6), and ARAEHSSTGTFDY (SEQ ID NO: 8), respectively.

2. 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: 15, and the HCVR has the amino acid sequence given in SEQ ID NO: 12, SEQ ID NO: 13, or SEQ ID NO: 14.

3. The antibody of Claim 2,

(1) wherein the LCVR has the amino acid sequence given in SEQ ID NO: 15, and the HCVR has the amino acid sequence given in SEQ ID NO: 12; or

(2) wherein the LCVR has the amino acid sequence given in SEQ ID NO: 15, and the HCVR has the amino acid sequence given in SEQ ID NO: 13; or

(3) wherein the LCVR has the amino acid sequence given in SEQ ID NO: 15, and the HCVR has the amino acid sequence given in SEQ ID NO: 14.

4. The antibody of Claim 2, wherein the LC has the amino acid sequence given in SEQ ID NO: 22, and the HC has the amino acid sequence given in SEQ ID NO: 16, SEQ ID NO: 17, SEQ ID NO: 18, SEQ ID NO: 19, SEQ ID NO: 20, or SEQ ID NO: 21.

5. The antibody of Claim 4,

(1) wherein the LC has the amino acid sequence given in SEQ ID NO: 22, and the HC has the amino acid sequence given in SEQ ID NO: 16; or

(2) wherein the LC has the amino acid sequence given in SEQ ID NO: 22, and the HC has the amino acid sequence given in SEQ ID NO: 17; or

(3) wherein the LC has the amino acid sequence given in SEQ ID NO: 22, and the HC has the amino acid sequence given in SEQ ID NO: 18; or

(4) wherein the LC has the amino acid sequence given in SEQ ID NO: 22, and the HC has the amino acid sequence given in SEQ ID NO: 19; or

(5) wherein the LC has the amino acid sequence given in SEQ ID NO: 22, and the HC has the amino acid sequence given in SEQ ID NO: 20; or

(6) wherein the LC has the amino acid sequence given in SEQ ID NO: 22, and the HC has the amino acid sequence given in SEQ ID NO: 21.

6. The antibody of Claim 4, comprising two light chains and two heavy chains, wherein each light chain has the amino acid sequence given in SEQ ID NO: 22, and each heavy chain has the amino acid sequence given in SEQ ID NO: 16, SEQ ID NO: 17, SEQ ID NO: 18, SEQ ID NO: 19, SEQ ID NO: 20, or SEQ ID NO: 21.

7. The antibody of Claim 6,

(1) wherein each light chain has the amino acid sequence given in SEQ ID NO: 22, and each heavy chain has the amino acid sequence given in SEQ ID NO: 16; or

(2) wherein each light chain has the amino acid sequence given in SEQ ID NO: 22, and each heavy chain has the amino acid sequence given in SEQ ID NO: 17; or

(3) wherein each light chain has the amino acid sequence given in SEQ ID NO: 22, and each heavy chain has the amino acid sequence given in SEQ ID NO: 18; or

(4) wherein each light chain has the amino acid sequence given in SEQ ID NO: 22, and each heavy chain has the amino acid sequence given in SEQ ID NO: 19; or

(5) wherein each light chain has the amino acid sequence given in SEQ ID NO: 22, and each heavy chain has the amino acid sequence given in SEQ ID NO: 20; or

(6) wherein each light chain has the amino acid sequence given in SEQ ID NO: 22, and each heavy chain has the amino acid sequence given in SEQ ID NO: 21.

8. The antibody of Claim 6 or 7, 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.

9. The antibody of any one of Claims 1-8, wherein the antibody is glycosylated.

10. A mammalian cell comprising a DNA molecule comprising a polynucleotide sequence encoding a polypeptide having an amino acid sequence of SEQ ID NO: 22 and a polynucleotide sequence encoding a polypeptide having an amino acid sequence of SEQ ID NO: 17 or SEQ ID NO: 20, wherein the cell is capable of expressing an antibody comprising a light chain having an amino acid sequence of SEQ ID NO: 22 and a heavy chain having an amino acid sequence of SEQ ID NO: 17 or SEQ ID NO: 20.

11. A process for producing an antibody comprising a light chain having an amino acid sequence of SEQ ID NO: 22 and a heavy chain having an amino acid sequence of SEQ ID NO: 17 or SEQ ID NO: 20, comprising cultivating the mammalian cell of Claim 10 under conditions such that the antibody is expressed, and recovering the expressed antibody.

12. An antibody produced by the process of Claim 11.

13. A pharmaceutical composition, comprising the antibody of any one of Claims 1-9, and an acceptable carrier, diluent, or excipient.

14. A use of the antibody of any one of Claims 1-9 in the manufacture of a pharmaceutical composition for treating cancer in a patient in need thereof.

15. The use of Claim 14, 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.

16. Use of an antibody according to any one of claims 1-9, and one or more anti-tumor agents in the manufacture of a medicament for treating cancer, wherein the antibody is to be administered simultaneously, separately, or sequentially with said one or more anti-tumor agents.

17. The use of claim 16, 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.

18. The antibody of any one of Claims 1-9, for use in therapy.

19. The antibody of any one of Claims 1-9, for use in the treatment of cancer.

20. The antibody for use of Claim 19, 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.

21. A combination of an antibody of any one of Claims 1-9 and one or more anti-tumor agents for simultaneous, separate, or sequential use in treating cancer.

22. The combination for use of Claim 21, 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.