(51) **International Patent Classification:**
C07K 16/28 (2006.01)   A61K 39/00 (2006.01)

(21) **International Application Number:**
PCT/US2016/049913

(22) **International Filing Date:**
1 September 2016 (01.09.2016)

(25) **Filing Language:** English

(26) **Publication Language:** English

(30) **Priority Data:**
62/212,851   1 September 2015 (01.09.2015)   US
62/216,043   9 September 2015 (09.09.2015)   US
62/257,195   18 November 2015 (18.11.2015)   US

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(81) **Designated States** *(unless otherwise indicated, for every kind of national protection available)*: AE, AG, AL, AM, AO, AT, AU, AZ, BA, BB, BG, BH, BN, BR, BW, BY, BZ, CA, CH, CL, CN, CO, CR, CU, CZ, DE, DK, DM, DO, DZ, EC, EE, EG, ES, FI, GB, GD, GE, GH, GM, GT, HN, HR, HU, ID, IL, IN, IR, IS, JP, KE, KG, KN, KP, KR, KZ, LA, LC, LK, LR, LS, LU, LY, MA, MD, ME, MG, MK, MN, MW, MX, MY, MZ, NA, NG, NI, NO, NZ, OM, PA, PE, PG, PH, PL, PT, QA, RO, RS, RU, RW, SA, SC, SD, SE, SG, SK, SL, SM, ST, SV, SY, TH, TJ, TM, TN, TR, TT, TZ, UA, UG, US, UZ, VC, VN, ZA, ZM, ZW.

(84) **Designated States** *(unless otherwise indicated, for every kind of regional protection available)*: ARIPO (BW, GH, GM, KE, LR, LS, MW, MZ, NA, RW, SD, SL, ST, SZ, TZ, UG, ZM, ZW), Eurasian (AM, AZ, BY, KG, KZ, RU, TJ, TM), European (AL, AT, BE, BG, CH, CY, CZ, DE, DK, EE, ES, FI, FR, GB, GR, HR, HU, IE, IS, IT, LT, LU, LV, MC, MK, MT, NL, NO, PL, PT, RO, RS, SE, SI, SK, SM, TR), OAPI (BF, BJ, CF, CG, CI, CM, GA, GN, GQ, GW, KM, ML, MR, NE, SN, TD, TG).

Published:

— *with international search report (Art. 21(3))*

— *before the expiration of the time limit for amending the claims and to be republished in the event of receipt of amendments (Rule 48.2(h))*

— *with sequence listing part of description (Rule 5.2(a))*

(54) **Title:** ANTI-PD-1 ANTIBODIES AND METHODS OF USE THEREOF

(57) **Abstract:** The instant disclosure provides antibodies that specifically bind to human PD-1 and antagonize PD-1 function. Also provided are pharmaceutical compositions comprising these antibodies, nucleic acids encoding these antibodies, expression vectors and host cells for making these antibodies, and methods of treating a subject using these antibodies.

ANTI-PD-1 ANTIBODIES AND METHODS OF USE THEREOF

RELATED APPLICATIONS

This application claims the benefit of U.S. Provisional Application Nos: 62/212,851, filed September 1, 2015; 62/216,043, filed September 9, 2015; and 62/257,195, filed November 18, 2015, each of which is incorporated by reference herein in its entirety.

1.   FIELD

[0001]   The instant disclosure relates to antibodies that specifically bind to human PD-1 and methods of using the same.

2.   BACKGROUND

[0002]   The protein programmed cell death protein 1 (PD-1) is an inhibitory member of the CD28 family of receptors. PD-1 is expressed on activated B cells, T cells, and myeloid cells (Agata et al. (1996) Int Immunol 8:765-72; Okazaki et al. (2002) Curr. Opin. Immunol. 14: 391779-82; Bennett et al. (2003) J Immunol 170:711-8). PD-1 is a 55 kDa type I transmembrane protein that is part of the Ig gene superfamily and contains a membrane proximal immunoreceptor tyrosine inhibitory motif (ITIM) and a membrane distal tyrosine-based switch motif (ITSM) (Thomas, M. L. (1995) J Exp Med 181:1953-6; Vivier, E and Daeron, M (1997) Immunol Today 18:286-91). Two ligands for PD-1 have been identified, PD-L1 and PD-L2, that have been shown to downregulate T cell activation upon binding to PD-1 (Freeman et al. (2000) J Exp Med 192:1027-34; Latchman et al. (2001) Nat Immunol 2:261-8; Carter et al. (2002) Eur J Immunol 32:634-43). PD-L1 is abundant in a variety of human cancers (Dong et al. (2002) Nat. Med. 8:787-9). The interaction between PD-1 and PD-L1 results in a decrease in tumor infiltrating lymphocytes, a decrease in T cell receptor mediated proliferation, and immune evasion by the cancerous cells (Dong et al. (2003) J. Mol. Med. 81:281-7; Blank et al. (2005) Cancer Immunol. Immunother. 54:307-314; Konishi et al. (2004) Clin. Cancer Res. 10:5094-100). This immune suppression can be reversed by inhibiting the local interaction of PD-1 with PD-L1, and the effect is additive when the interaction of PD-1 with PD-L2 is blocked as well (Iwai et al. (2002) Proc. Nat'l. Acad. Sci. USA 99:12293-7; Brown et al. (2003) J. Immunol. 170:1257-66).

[0003]   PD-1 is an immune cell inhibitory molecule. PD-1 deficient animals develop various autoimmune phenotypes, including autoimmune cardiomyopathy and a lupus-like syndrome with arthritis and nephritis (Nishimura et al. (1999) Immunity 11:141-51; Nishimura et al. (2001) Science 291:319-22). Additionally, PD-1 has been found to play a

role in autoimmune encephalomyelitis, systemic lupus erythematosus, graft-versus-host disease (GVHD), type I diabetes, and rheumatoid arthritis (Salama et al. (2003) J Exp Med 198:71-78; Prokunina and Alarcon-Riquelme (2004) Hum Mol Genet 13:R143; Nielsen et al. (2004) Lupus 13:510). In a murine B cell tumor line, the ITSM of PD-1 was shown to be essential to block BCR-mediated Ca^{2+}-flux and tyrosine phosphorylation of downstream effector molecules (Okazaki et al. (2001) PNAS 98:13866-71).

[0004]   Given the role of human PD-1 in modulating immune responses, therapeutic agents designed to antagonize PD-1 signaling hold great promise for the treatment of diseases that involve PD-1-mediated immune suppression.

3.   SUMMARY

[0005]   The instant disclosure provides antibodies that specifically bind to human PD-1 and antagonize PD-1 function, *e.g.*, PD-1-mediated immune suppression. Also provided are pharmaceutical compositions comprising these antibodies, nucleic acids encoding these antibodies, expression vectors and host cells for making these antibodies, and methods of treating a subject using these antibodies. The antibodies disclosed herein are particularly useful for increasing T cell activation in response to an antigen (*e.g.*, a tumor antigen or an infectious disease antigen) and/or decreasing Treg-mediated immune suppression, and hence for treating cancer in a subject or treating or preventing an infectious disease in a subject.

[0006]   Accordingly, in one aspect, the instant disclosure provides an antibody or isolated antibody comprising a heavy chain variable region comprising complementarity determining regions CDRH1, CDRH2, and CDRH3 and a light chain variable region comprising complementarity determining regions CDRL1, CDRL2, and CDRL3, wherein: (a) CDRH1 comprises the amino acid sequence of SYGMH (SEQ ID NO: 1); (b) CDRH2 comprises the amino acid sequence of $VIWX_1DGSNX_2YYADSVX_3G$ (SEQ ID NO: 32), wherein X_1 is Y or F; X_2 is K or E; and X_3 is K or M; (c) CDRH3 comprises the amino acid sequence of NX_1DX_2 (SEQ ID NO: 33), wherein X_1 is G or V; and X_2 is H or Y; (d) CDRL1 comprises the amino acid sequence of RASQSVSSNLA (SEQ ID NO: 4); (e) CDRL2 comprises the amino acid sequence of GASTRAT (SEQ ID NO: 5); and (f) CDRL3 comprises the amino acid sequence of QQYNNWPRT (SEQ ID NO: 6).

[0007]   In certain embodiments, CDRH2 comprises an amino acid sequence selected from the group consisting of SEQ ID NOs: 2 and 34-36.

[0008]   In certain embodiments, CDRH3 comprises an amino acid sequence selected from

the group consisting of SEQ ID NOs: 3, 7, and 37.

[0009]   In certain embodiments, CDRH1, CDRH2 and CDRH3 comprise the CDRH1, CDRH2 and CDRH3 amino acid sequences, respectively, set forth in SEQ ID NOs: 1, 2, and 3; 1, 2, and 7; 1, 2, and 37; 1, 34, and 7; 1, 35, and 7; or 1, 36, and 7.

[0010]   In certain embodiments, the antibody comprises a heavy chain variable region comprising complementarity determining regions CDRH1, CDRH2 and CDRH3, and a light chain variable region comprising complementarity determining regions CDRL1, CDRL2 and CDRL3, wherein CDRH1, CDRH2, CDRH3, CDRL1, CDRL2, and CDRL3 comprise the amino acid sequences set forth in SEQ ID NOs: 1, 2, 3, 4, 5, and 6, respectively.

[0011]   In certain embodiments, the antibody comprises a heavy chain variable region comprising complementarity determining regions CDRH1, CDRH2 and CDRH3, and a light chain variable region comprising complementarity determining regions CDRL1, CDRL2 and CDRL3, wherein CDRH1, CDRH2, CDRH3, CDRL1, CDRL2, and CDRL3 comprise the amino acid sequences set forth in SEQ ID NOs: 1, 2, 7, 4, 5, and 6, respectively.

[0012]   In certain embodiments, the antibody comprises a heavy chain variable region comprising the amino acid sequence of SEQ ID NO: 49.

[0013]   In certain embodiments, the antibody comprises a heavy chain variable region comprising an amino acid sequence which is at least 75%, 80%, 85%, 90%, 95%, or 100% identical to an amino acid sequence selected from the group consisting of SEQ ID NOs: 15, 17, and 26-31. In certain embodiments, the heavy chain variable region comprises an amino acid sequence selected from the group consisting of SEQ ID NOs: 15, 17, and 26-31. In certain embodiments, the heavy chain variable region comprises the amino acid sequence of SEQ ID NO: 15. In certain embodiments, the heavy chain variable region comprises the amino acid sequence of SEQ ID NO: 17. In certain embodiments, the heavy chain variable region comprises the amino acid sequence of SEQ ID NO: 26. In certain embodiments, the heavy chain variable region comprises the amino acid sequence of SEQ ID NO: 27. In certain embodiments, the heavy chain variable region comprises the amino acid sequence of SEQ ID NO: 28. In certain embodiments, the heavy chain variable region comprises the amino acid sequence of SEQ ID NO: 29. In certain embodiments, the heavy chain variable region comprises the amino acid sequence of SEQ ID NO: 30. In certain embodiments, the heavy chain variable region comprises the amino acid sequence of SEQ ID NO: 31. In certain embodiments, the antibody comprises a heavy chain comprising the amino acid sequence of SEQ ID NO: 18. In certain embodiments, the antibody comprises a heavy chain comprising the amino acid sequence of SEQ ID NO: 21. In certain embodiments, the

antibody comprises a heavy chain comprising the amino acid sequence of SEQ ID NO: 20. In certain embodiments, the antibody comprises a heavy chain comprising the amino acid sequence of SEQ ID NO: 22. In certain embodiments, the antibody comprises a heavy chain comprising the amino acid sequence of SEQ ID NO: 23. In certain embodiments, the antibody comprises a heavy chain comprising the amino acid sequence of SEQ ID NO: 24. In certain embodiments, the antibody comprises a heavy chain comprising the amino acid sequence of SEQ ID NO: 25. In certain embodiments, the antibody comprises a heavy chain comprising the amino acid sequence of SEQ ID NO: 52. In certain embodiments, the antibody comprises a heavy chain comprising the amino acid sequence of SEQ ID NO: 53. In certain embodiments, the antibody comprises a heavy chain comprising the amino acid sequence of SEQ ID NO: 54. In certain embodiments, the antibody comprises a heavy chain comprising the amino acid sequence of SEQ ID NO: 55. In certain embodiments, the antibody comprises a heavy chain comprising the amino acid sequence of SEQ ID NO: 56. In certain embodiments, the antibody comprises a heavy chain comprising the amino acid sequence of SEQ ID NO: 57. In certain embodiments, the antibody comprises a heavy chain comprising the amino acid sequence of SEQ ID NO: 58.

[0014] In certain embodiments, the antibody comprises a heavy chain variable region having an amino acid sequence derived from a human IGHV3-33 germline sequence (e.g., IGHV3-33*01, e.g., having amino acid sequence of SEQ ID NO: 50).

[0015] In certain embodiments, the antibody comprises a light chain variable region comprising an amino acid sequence which is at least 75%, 80%, 85%, 90%, 95%, or 100% identical to the amino acid sequence of SEQ ID NO: 16. In certain embodiments, the light chain variable region comprises the amino acid sequence of SEQ ID NO: 16. In certain embodiments, the antibody comprises a light chain comprising the amino acid sequence of SEQ ID NO: 19.

[0016] In certain embodiments, the antibody comprises a light chain variable region having an amino acid sequence derived from a human IGKV3-15 germline sequence (e.g., IGKV3-15*01, e.g., having amino acid sequence of SEQ ID NO: 51).

[0017] In certain embodiments, the antibody comprises a heavy chain variable region and a light chain variable region, wherein the heavy chain variable region and the light chain variable region, respectively, comprise the amino acid sequences set forth in SEQ ID NOs: 15 and 16; 17 and 16; 26 and 16; 27 and 16; 28 and 16; 29 and 16; 30 and 16; or 31 and 16.

[0018] In another aspect, the instant disclosure provides an antibody or isolated antibody comprising: (a) a heavy chain variable region comprising the amino acid sequence of SEQ ID

NO: 15, and (b) a light chain variable region comprising the amino acid sequence of SEQ ID NO: 16.

[0019]   In another aspect, the instant disclosure provides an antibody or isolated antibody comprising: (a) a heavy chain variable region comprising the amino acid sequence of SEQ ID NO: 17, and (b) a light chain variable region comprising the amino acid sequence of SEQ ID NO: 16.

[0020]   In another aspect, the instant disclosure provides an antibody or isolated antibody comprising: (a) a heavy chain variable region comprising the amino acid sequence of SEQ ID NO: 26, and (b) a light chain variable region comprising the amino acid sequence of SEQ ID NO: 16.

[0021]   In another aspect, the instant disclosure provides an antibody or isolated antibody comprising: (a) a heavy chain variable region comprising the amino acid sequence of SEQ ID NO: 27, and (b) a light chain variable region comprising the amino acid sequence of SEQ ID NO: 16.

[0022]   In another aspect, the instant disclosure provides an antibody or isolated antibody comprising: (a) a heavy chain variable region comprising the amino acid sequence of SEQ ID NO: 28, and (b) a light chain variable region comprising the amino acid sequence of SEQ ID NO: 16.

[0023]   In another aspect, the instant disclosure provides an antibody or isolated antibody comprising: (a) a heavy chain variable region comprising the amino acid sequence of SEQ ID NO: 29, and (b) a light chain variable region comprising the amino acid sequence of SEQ ID NO: 16.

[0024]   In another aspect, the instant disclosure provides an antibody or isolated antibody comprising: (a) a heavy chain variable region comprising the amino acid sequence of SEQ ID NO: 30, and (b) a light chain variable region comprising the amino acid sequence of SEQ ID NO: 16.

[0025]   In another aspect, the instant disclosure provides an antibody or isolated antibody comprising: (a) a heavy chain variable region having an amino acid sequence derived from a human IGHV3-33 germline sequence (*e.g.*, IGHV3-33*01, *e.g.*, having amino acid sequence of SEQ ID NO: 50), and (b) a light chain variable region having an amino acid sequence derived from a human IGKV3-15 germline sequence (*e.g.*, IGKV3-15*01, *e.g.*, having amino acid sequence of SEQ ID NO: 51).

[0026]   In another aspect, the instant disclosure provides an antibody or isolated antibody comprising: (a) a heavy chain variable region comprising the amino acid sequence of SEQ ID

NO: 31, and (b) a light chain variable region comprising the amino acid sequence of SEQ ID NO: 16.

[0027] In another aspect, the instant disclosure provides an antibody or isolated antibody comprising a heavy chain variable region comprising an amino acid sequence selected from the group consisting of SEQ ID NOs: 15, 17, and 26-31.

[0028] In another aspect, the instant disclosure provides an antibody or isolated antibody comprising a light chain variable region comprising the amino acid sequence of SEQ ID NO: 16.

[0029] In another aspect, the instant disclosure provides an antibody or isolated antibody comprising: (a) a heavy chain comprising the amino acid sequence of SEQ ID NO: 18; and (b) a light chain comprising the amino acid sequence of SEQ ID NO: 19.

[0030] In another aspect, the instant disclosure provides an antibody or isolated antibody comprising: (a) a heavy chain comprising the amino acid sequence of SEQ ID NO: 20; and (b) a light chain comprising the amino acid sequence of SEQ ID NO: 19.

[0031] In another aspect, the instant disclosure provides an antibody or isolated antibody comprising: (a) a heavy chain comprising the amino acid sequence of SEQ ID NO: 21; and (b) a light chain comprising the amino acid sequence of SEQ ID NO: 19.

[0032] In another aspect, the instant disclosure provides an antibody or isolated antibody comprising: (a) a heavy chain comprising the amino acid sequence of SEQ ID NO: 22; and (b) a light chain comprising the amino acid sequence of SEQ ID NO: 19.

[0033] In another aspect, the instant disclosure provides an antibody or isolated antibody comprising: (a) a heavy chain comprising the amino acid sequence of SEQ ID NO: 23; and (b) a light chain comprising the amino acid sequence of SEQ ID NO: 19.

[0034] In another aspect, the instant disclosure provides an antibody or isolated antibody comprising: (a) a heavy chain comprising the amino acid sequence of SEQ ID NO: 52; and (b) a light chain comprising the amino acid sequence of SEQ ID NO: 19.

[0035] In another aspect, the instant disclosure provides an antibody or isolated antibody comprising: (a) a heavy chain comprising the amino acid sequence of SEQ ID NO: 53; and (b) a light chain comprising the amino acid sequence of SEQ ID NO: 19.

[0036] In another aspect, the instant disclosure provides an antibody or isolated antibody comprising: (a) a heavy chain comprising the amino acid sequence of SEQ ID NO: 54; and (b) a light chain comprising the amino acid sequence of SEQ ID NO: 19.

[0037] In another aspect, the instant disclosure provides an antibody or isolated antibody comprising: (a) a heavy chain comprising the amino acid sequence of SEQ ID NO: 55; and

(b) a light chain comprising the amino acid sequence of SEQ ID NO: 19.

[0038]   In another aspect, the instant disclosure provides an antibody or isolated antibody comprising: (a) a heavy chain comprising the amino acid sequence of SEQ ID NO: 56; and (b) a light chain comprising the amino acid sequence of SEQ ID NO: 19.

[0039]   In another aspect, the instant disclosure provides an antibody or isolated antibody that specifically binds to human PD-1 comprising a heavy chain variable region comprising complementarity determining regions CDRH1, CDRH2, and CDRH3 and a light chain variable region comprising complementarity determining regions CDRL1, CDRL2, and CDRL3, wherein: (a) CDRH1 comprises the amino acid sequence of SYGMH (SEQ ID NO: 1); (b) CDRH2 comprises the amino acid sequence of VIWX_1DGSNX_2YYADSVX_3G (SEQ ID NO: 32), wherein X_1 is Y or F; X_2 is K or E; and X_3 is K or M; (c) CDRH3 comprises the amino acid sequence of NX_1DX_2 (SEQ ID NO: 33), wherein X_1 is G or V; and X_2 is H or Y; (d) CDRL1 comprises the amino acid sequence of RASQSVSSNLA (SEQ ID NO: 4); (e) CDRL2 comprises the amino acid sequence of GASTRAT (SEQ ID NO: 5); and (f) CDRL3 comprises the amino acid sequence of QQYNNWPRT (SEQ ID NO: 6).

[0040]   In certain embodiments, CDRH2 comprises an amino acid sequence selected from the group consisting of SEQ ID NOs: 2 and 34-36.

[0041]   In certain embodiments, CDRH3 comprises an amino acid sequence selected from the group consisting of SEQ ID NOs: 3, 7, and 37.

[0042]   In certain embodiments, CDRH1, CDRH2 and CDRH3 comprise the CDRH1, CDRH2 and CDRH3 amino acid sequences, respectively, set forth in SEQ ID NOs: 1, 2, and 3; 1, 2, and 7; 1, 2, and 37; 1, 34, and 7; 1, 35, and 7; or 1, 36, and 7.

[0043]   In certain embodiments, the antibody comprises a heavy chain variable region comprising complementarity determining regions CDRH1, CDRH2 and CDRH3, and a light chain variable region comprising complementarity determining regions CDRL1, CDRL2 and CDRL3, wherein CDRH1, CDRH2, CDRH3, CDRL1, CDRL2, and CDRL3 comprise the amino acid sequences set forth in SEQ ID NOs: 1, 2, 3, 4, 5, and 6, respectively.

[0044]   In certain embodiments, the antibody comprises a heavy chain variable region comprising complementarity determining regions CDRH1, CDRH2 and CDRH3, and a light chain variable region comprising complementarity determining regions CDRL1, CDRL2 and CDRL3, wherein CDRH1, CDRH2, CDRH3, CDRL1, CDRL2, and CDRL3 comprise the amino acid sequences set forth in SEQ ID NOs: 1, 2, 7, 4, 5, and 6, respectively.

[0045]   In certain embodiments, the antibody comprises a heavy chain variable region comprising the amino acid sequence of SEQ ID NO: 49.

[0046]   In certain embodiments, the antibody comprises a heavy chain variable region comprising an amino acid sequence which is at least 75%, 80%, 85%, 90%, 95%, or 100% identical to an amino acid sequence selected from the group consisting of SEQ ID NOs: 15, 17, and 26-31. In certain embodiments, the heavy chain variable region comprises an amino acid sequence selected from the group consisting of SEQ ID NOs: 15, 17, and 26-31. In certain embodiments, the heavy chain variable region comprises the amino acid sequence of SEQ ID NO: 15. In certain embodiments, the heavy chain variable region comprises the amino acid sequence of SEQ ID NO: 17. In certain embodiments, the heavy chain variable region comprises the amino acid sequence of SEQ ID NO: 26. In certain embodiments, the heavy chain variable region comprises the amino acid sequence of SEQ ID NO: 27. In certain embodiments, the heavy chain variable region comprises the amino acid sequence of SEQ ID NO: 28. In certain embodiments, the heavy chain variable region comprises the amino acid sequence of SEQ ID NO: 29. In certain embodiments, the heavy chain variable region comprises the amino acid sequence of SEQ ID NO: 30. In certain embodiments, the heavy chain variable region comprises the amino acid sequence of SEQ ID NO: 31. In certain embodiments, the antibody comprises a heavy chain comprising the amino acid sequence of SEQ ID NO: 18. In certain embodiments, the antibody comprises a heavy chain comprising the amino acid sequence of SEQ ID NO: 21. In certain embodiments, the antibody comprises a heavy chain comprising the amino acid sequence of SEQ ID NO: 20. In certain embodiments, the antibody comprises a heavy chain comprising the amino acid sequence of SEQ ID NO: 22. In certain embodiments, the antibody comprises a heavy chain comprising the amino acid sequence of SEQ ID NO: 23. In certain embodiments, the antibody comprises a heavy chain comprising the amino acid sequence of SEQ ID NO: 24. In certain embodiments, the antibody comprises a heavy chain comprising the amino acid sequence of SEQ ID NO: 25. In certain embodiments, the antibody comprises a heavy chain comprising the amino acid sequence of SEQ ID NO: 52. In certain embodiments, the antibody comprises a heavy chain comprising the amino acid sequence of SEQ ID NO: 53. In certain embodiments, the antibody comprises a heavy chain comprising the amino acid sequence of SEQ ID NO: 54. In certain embodiments, the antibody comprises a heavy chain comprising the amino acid sequence of SEQ ID NO: 55. In certain embodiments, the antibody comprises a heavy chain comprising the amino acid sequence of SEQ ID NO: 56. In certain embodiments, the antibody comprises a heavy chain comprising the amino acid sequence of SEQ ID NO: 57. In certain embodiments, the antibody comprises a heavy chain comprising the amino acid sequence of SEQ ID NO: 58.

[0047]   In certain embodiments, the antibody comprises a heavy chain variable region having an amino acid sequence derived from a human IGHV3-33 germline sequence (*e.g.*, IGHV3-33*01, *e.g.*, having amino acid sequence of SEQ ID NO: 50).

[0048]   In certain embodiments, the antibody comprises a light chain variable region comprising an amino acid sequence which is at least 75%, 80%, 85%, 90%, 95%, or 100% identical to the amino acid sequence of SEQ ID NO: 16. In certain embodiments, the light chain variable region comprises the amino acid sequence of SEQ ID NO: 16. In certain embodiments, the antibody comprises a light chain comprising the amino acid sequence of SEQ ID NO: 19.

[0049]   In certain embodiments, the antibody comprises a light chain variable region having an amino acid sequence derived from a human IGKV3-15 germline sequence (*e.g.*, IGKV3-15*01, *e.g.*, having amino acid sequence of SEQ ID NO: 51).

[0050]   In certain embodiments, the antibody comprises a heavy chain variable region and a light chain variable region, wherein the heavy chain variable region and the light chain variable region, respectively, comprise the amino acid sequences set forth in SEQ ID NOs: 15 and 16; 17 and 16; 26 and 16; 27 and 16; 28 and 16; 29 and 16; 30 and 16; or 31 and 16.

[0051]   In another aspect, the instant disclosure provides an antibody or isolated antibody that specifically binds to human PD-1, comprising: (a) a heavy chain variable region comprising the amino acid sequence of SEQ ID NO: 15, and (b) a light chain variable region comprising the amino acid sequence of SEQ ID NO: 16.

[0052]   In another aspect, the instant disclosure provides an antibody or isolated antibody that specifically binds to human PD-1, comprising: (a) a heavy chain variable region comprising the amino acid sequence of SEQ ID NO: 17, and (b) a light chain variable region comprising the amino acid sequence of SEQ ID NO: 16.

[0053]   In another aspect, the instant disclosure provides an antibody or isolated antibody that specifically binds to human PD-1, comprising: (a) a heavy chain variable region comprising the amino acid sequence of SEQ ID NO: 26, and (b) a light chain variable region comprising the amino acid sequence of SEQ ID NO: 16.

[0054]   In another aspect, the instant disclosure provides an antibody or isolated antibody that specifically binds to human PD-1, comprising: (a) a heavy chain variable region comprising the amino acid sequence of SEQ ID NO: 27, and (b) a light chain variable region comprising the amino acid sequence of SEQ ID NO: 16.

[0055]   In another aspect, the instant disclosure provides an antibody or isolated antibody that specifically binds to human PD-1, comprising: (a) a heavy chain variable region

comprising the amino acid sequence of SEQ ID NO: 28, and (b) a light chain variable region comprising the amino acid sequence of SEQ ID NO: 16.

[0056]   In another aspect, the instant disclosure provides an antibody or isolated antibody that specifically binds to human PD-1, comprising: (a) a heavy chain variable region comprising the amino acid sequence of SEQ ID NO: 29, and (b) a light chain variable region comprising the amino acid sequence of SEQ ID NO: 16.

[0057]   In another aspect, the instant disclosure provides an antibody or isolated antibody that specifically binds to human PD-1, comprising: (a) a heavy chain variable region comprising the amino acid sequence of SEQ ID NO: 30, and (b) a light chain variable region comprising the amino acid sequence of SEQ ID NO: 16.

[0058]   In another aspect, the instant disclosure provides an antibody or isolated antibody that specifically binds to human PD-1, comprising: (a) a heavy chain variable region comprising the amino acid sequence of SEQ ID NO: 31, and (b) a light chain variable region comprising the amino acid sequence of SEQ ID NO: 16.

[0059]   In another aspect, the instant disclosure provides an antibody or isolated antibody that specifically binds to human PD-1, comprising: (a) a heavy chain variable region having an amino acid sequence derived from a human IGHV3-33 germline sequence (*e.g.*, IGHV3-33*01, *e.g.*, having amino acid sequence of SEQ ID NO: 50), and (b) a light chain variable region having an amino acid sequence derived from a human IGKV3-15 germline sequence (*e.g.*, IGKV3-15*01, *e.g.*, having amino acid sequence of SEQ ID NO: 51).

[0060]   In another aspect, the instant disclosure provides an antibody or isolated antibody that specifically binds to human PD-1, comprising a heavy chain variable region comprising an amino acid sequence selected from the group consisting of SEQ ID NOs: 15, 17, and 26-31.

[0061]   In another aspect, the instant disclosure provides an antibody or isolated antibody that specifically binds to human PD-1, comprising a light chain variable region comprising the amino acid sequence of SEQ ID NO: 16.

[0062]   In another aspect, the instant disclosure provides an antibody or isolated antibody that specifically binds to human PD-1, comprising: (a) a heavy chain comprising the amino acid sequence of SEQ ID NO: 18; and (b) a light chain comprising the amino acid sequence of SEQ ID NO: 19.

[0063]   In another aspect, the instant disclosure provides an antibody or isolated antibody that specifically binds to human PD-1, comprising: (a) a heavy chain comprising the amino acid sequence of SEQ ID NO: 20; and (b) a light chain comprising the amino acid sequence

of SEQ ID NO: 19.

[0064]   In another aspect, the instant disclosure provides an antibody or isolated antibody that specifically binds to human PD-1, comprising: (a) a heavy chain comprising the amino acid sequence of SEQ ID NO: 21; and (b) a light chain comprising the amino acid sequence of SEQ ID NO: 19.

[0065]   In another aspect, the instant disclosure provides an antibody or isolated antibody that specifically binds to human PD-1, comprising: (a) a heavy chain comprising the amino acid sequence of SEQ ID NO: 22; and (b) a light chain comprising the amino acid sequence of SEQ ID NO: 19.

[0066]   In another aspect, the instant disclosure provides an antibody or isolated antibody that specifically binds to human PD-1, comprising: (a) a heavy chain comprising the amino acid sequence of SEQ ID NO: 23; and (b) a light chain comprising the amino acid sequence of SEQ ID NO: 19.

[0067]   In another aspect, the instant disclosure provides an antibody or isolated antibody that specifically binds to human PD-1, comprising: (a) a heavy chain comprising the amino acid sequence of SEQ ID NO: 52; and (b) a light chain comprising the amino acid sequence of SEQ ID NO: 19.

[0068]   In another aspect, the instant disclosure provides an antibody or isolated antibody that specifically binds to human PD-1, comprising: (a) a heavy chain comprising the amino acid sequence of SEQ ID NO: 53; and (b) a light chain comprising the amino acid sequence of SEQ ID NO: 19.

[0069]   In another aspect, the instant disclosure provides an antibody or isolated antibody that specifically binds to human PD-1, comprising: (a) a heavy chain comprising the amino acid sequence of SEQ ID NO: 54; and (b) a light chain comprising the amino acid sequence of SEQ ID NO: 19.

[0070]   In another aspect, the instant disclosure provides an antibody or isolated antibody that specifically binds to human PD-1, comprising: (a) a heavy chain comprising the amino acid sequence of SEQ ID NO: 55; and (b) a light chain comprising the amino acid sequence of SEQ ID NO: 19.

[0071]   In another aspect, the instant disclosure provides an antibody or isolated antibody that specifically binds to human PD-1, comprising: (a) a heavy chain comprising the amino acid sequence of SEQ ID NO: 56; and (b) a light chain comprising the amino acid sequence of SEQ ID NO: 19.

[0072]   In another aspect, the instant disclosure provides an antibody or isolated antibody

that cross-competes for binding to human PD-1 with any antibody disclosed herein. In another aspect the instant disclosure provides an antibody or isolated antibody that binds, *e.g.*, specifically binds, to the same epitope of human PD-1 as any antibody disclosed herein.

[0073]   In another aspect, the instant disclosure provides an antibody that binds, *e.g.*, specifically binds, to an epitope of human PD-1. In certain embodiments, the antibody binds to an epitope of human PD-1 comprising, consisting essentially of, or consisting of residues 107-122 of SEQ ID NO: 74. In certain embodiments, the antibody binds to an epitope of human PD-1 consisting of residues 107-122 of SEQ ID NO: 74. In certain embodiments, the antibody binds to an epitope of human PD-1 comprising, consisting essentially of, or consisting of residues 5-22 of SEQ ID NO: 74. In certain embodiments, the antibody binds to an epitope of human PD-1 consisting of residues 5-22 of SEQ ID NO: 74. In certain embodiments, the antibody binds to an epitope of human PD-1 comprising, consisting essentially of, or consisting of residues 6-15 of SEQ ID NO: 74. In certain embodiments, the antibody binds to an epitope of human PD-1 consisting of residues 6-15 of SEQ ID NO: 74. In certain embodiments, the antibody binds to an epitope of human PD-1 comprising, consisting essentially of, or consisting of residues 130-138 of SEQ ID NO: 74. In certain embodiments, the antibody binds to an epitope of human PD-1 consisting of residues 130-138 of SEQ ID NO: 74. In certain embodiments, the antibody binds to an epitope of human PD-1 comprising, consisting essentially of, or consisting of residues 106-113 of SEQ ID NO: 74. In certain embodiments, the antibody binds to an epitope of human PD-1 consisting of residues 106-113 of SEQ ID NO: 74.

[0074]   In another aspect, the instant disclosure provides an antibody for which, upon binding of the antibody to human PD-1 protein followed by addition of deuterium, the exchange of hydrogen in the human PD-1 protein with deuterium in a region comprising residues 107-122 of SEQ ID NO: 74 is substantially reduced relative to the exchange of hydrogen in the human PD-1 protein with deuterium in the same region in the absence of the antibody, as determined by hydrogen/deuterium exchange. In another aspect, the instant disclosure provides an antibody for which, upon binding of the antibody to human PD-1 protein followed by addition of deuterium, the exchange of hydrogen in the human PD-1 protein with deuterium in a region comprising residues 5-22 of SEQ ID NO: 74 is substantially reduced relative to the exchange of hydrogen in the human PD-1 protein with deuterium in the same region in the absence of the antibody, as determined by hydrogen/deuterium exchange.

[0075]   In another aspect, the instant disclosure provides an antibody or isolated antibody

that binds, *e.g.*, specifically binds, to the same epitope of human PD-1 as any antibody of the present invention. In a preferred embodiment, the antibody binds to an epitope of human PD-1 comprising, consisting essentially of, or consisting of residues 107-122 of SEQ ID NO: 74. In another preferred embodiment, the antibody binds to an epitope of human PD-1 consisting of residues 107-122 of SEQ ID NO: 74. In another preferred embodiment, the antibody binds to an epitope of human PD-1 comprising, consisting essentially of, or consisting of residues 5-22 of SEQ ID NO: 74. In another preferred embodiment, the antibody binds to an epitope of human PD-1 consisting of residues 5-22 of SEQ ID NO: 74. In another preferred embodiment, the antibody binds to an epitope of human PD-1 comprising, consisting essentially of, or consisting of residues 6-15 of SEQ ID NO: 74. In another preferred embodiment, the antibody binds to an epitope of human PD-1 consisting of residues 6-15 of SEQ ID NO: 74. In another preferred embodiment, the antibody binds to an epitope of human PD-1 comprising, consisting essentially of, or consisting of residues 130-138 of SEQ ID NO: 74. In another preferred embodiment, the antibody binds to an epitope of human PD-1 consisting of residues 130-138 of SEQ ID NO: 74. In another preferred embodiment, the antibody binds to an epitope of human PD-1 comprising, consisting essentially of, or consisting of residues 106-113 of SEQ ID NO: 74. In another preferred embodiment, the antibody binds to an epitope of human PD-1 consisting of residues 106-113 of SEQ ID NO: 74. In a further preferred embodiment, the antibody binds to an epitope of human PD-1 comprising, consisting essentially of, or consisting of residues 6-15 of SEQ ID NO: 74, and/or an epitope of human PD-1 comprising, consisting essentially of, or consisting of residues 130-138 of SEQ ID NO: 74, and/or comprising, consisting essentially of, or consisting of residues 106-113 of SEQ ID NO: 74. The binding to an epitope is preferably determined by Pepscan analysis, in particular as described in the Examples. For example, binding to more than one above epitope sequences of human PD-1 may occur in the case of discontinuous epitopes.

[0076]   In another aspect, the instant disclosure provides an antibody or isolated antibody that binds, *e.g.*, specifically binds, to the same epitope of human PD-1 as any antibody of the present invention, for which, upon binding of the antibody to human PD-1 protein followed by addition of deuterium, the exchange of hydrogen in the human PD-1 protein with deuterium in a region comprising residues 107-122 of SEQ ID NO: 74 is substantially reduced relative to the exchange of hydrogen in the human PD-1 protein with deuterium in the same region in the absence of the antibody, as determined by hydrogen/deuterium exchange. In another aspect, the instant disclosure provides an antibody or isolated antibody

that binds, *e.g.*, specifically binds, to the same epitope of human PD-1 as any antibody of the present invention, and for which, upon binding of the antibody to human PD-1 protein followed by addition of deuterium, the exchange of hydrogen in the human PD-1 protein with deuterium in a region comprising residues 5-22 of SEQ ID NO: 74 is substantially reduced relative to the exchange of hydrogen in the human PD-1 protein with deuterium in the same region in the absence of the antibody, as determined by hydrogen/deuterium exchange. In a more preferred embodiment, upon binding of the antibody to human PD-1 protein followed by addition of deuterium, the exchange of hydrogen in the human PD-1 protein with deuterium in a region comprising residues 107-122 of SEQ ID NO: 74 is substantially reduced relative to the exchange of hydrogen in the human PD-1 protein with deuterium in the same region in the absence of the antibody, as determined by hydrogen/deuterium exchange, and, upon binding of the antibody to human PD-1 protein followed by addition of deuterium, the exchange of hydrogen in the human PD-1 protein with deuterium in a region comprising residues 5-22 of SEQ ID NO: 74 is substantially reduced relative to the exchange of hydrogen in the human PD-1 protein with deuterium in the same region in the absence of the antibody, as determined by hydrogen/deuterium exchange. For example, binding to more than one above epitope sequences of human PD-1 may occur in the case of discontinuous epitopes.

[0077]   In another aspect, the instant disclosure provides an antibody or isolated antibody that binds, *e.g.*, specifically binds, to the same epitope of human PD-1 as any antibody of the present invention, wherein the epitope is determined by hydrogen-deuterium exchange (HDX), in particular as described in the examples, or by Pepscan analysis, in particular as described in the examples, more preferably by hydrogen-deuterium exchange.

[0078]   The following embodiments apply to all of the foregoing aspects.

[0079]   In certain embodiments, the antibody comprises a heavy chain constant region selected from the group consisting of human IgG_1, IgG_2, IgG_3, IgG_4, IgA_1, and IgA_2. In certain embodiments, the antibody comprises a human IgG_1 heavy chain constant region. In certain embodiments, the antibody comprises a human IgG_1 heavy chain constant region that lacks a glycan moiety at position N297, according to the EU numbering system. In certain embodiments, the antibody comprises a human IgG_1 heavy chain constant region comprising an N297A mutation, according to the EU numbering system. In certain embodiments, the antibody comprises a human IgG_1 heavy chain constant region comprising an N297Q mutation, according to the EU numbering system. In certain embodiments, the antibody comprises a human IgG_1 heavy chain constant region comprising a D265A mutation,

according to the EU numbering system. In certain embodiments, the antibody comprises a human IgG$_4$ heavy chain constant region comprising an S228P mutation, according to the EU numbering system.

[0080] In certain embodiments, the antibody comprises a human IgG heavy chain constant region that is a variant of a wild type human IgG heavy chain constant region, wherein the variant human IgG heavy chain constant region binds to human Fc receptor with lower affinity than the wild type human IgG heavy chain constant region binds to the human Fc receptor. In certain embodiments, the human Fc receptor is an FcγR. In certain embodiments, the FcγR is FcγRIIB. In certain embodiments, the FcγR is expressed on a cell selected from the group consisting of dendritic cells, monocytes, macrophages, neutrophils, granulocytes, B cells, and natural killer cells. In certain embodiments, the variant human IgG heavy chain constant region is a variant human IgG$_1$, a variant human IgG$_2$, or a variant human IgG$_4$ heavy chain constant region. In certain embodiments, the antibody comprises a light chain constant region selected from the group consisting of human IgGκ and IgGλ.

[0081] In certain embodiments, the antibody is a human antibody. In certain embodiments, the antibody is antagonistic to human PD-1. In certain embodiments, the antibody deactivates, reduces, or inhibits an activity of human PD-1. In certain embodiments, the antibody inhibits binding of human PD-1 to human PD-L1 or to human PD-L2. In certain embodiments, the antibody increases IL-2 production by peripheral blood mononuclear cells (PBMCs) stimulated with staphylococcal enterotoxin A (SEA). In certain embodiments, the antibody increases IFNγ production of a co-culture of human T cells and allogenic dendritic cells. In certain embodiments, the antibody increases proliferation of anti-CD3-antibody-stimulated CD4+ or CD8+ T cells co-cultured with ovarian cancer ascites fluid. In certain embodiments, the antibody increases NFAT signaling in PD-1-expressing NFAT-luciferase reporter cells co-cultured with PD-L1-expressing target cells.

[0082] In certain embodiments, an antibody disclosed herein is conjugated to a cytotoxic agent, cytostatic agent, toxin, radionuclide, or detectable label.

[0083] In another aspect, the instant disclosure provides a pharmaceutical composition comprising an antibody disclosed herein and a pharmaceutically acceptable carrier or excipient.

[0084] In another aspect, the instant disclosure provides a polynucleotide or isolated polynucleotide encoding a heavy and/or light chain of an antibody disclosed herein. In another aspect, the instant disclosure provides a vector comprising the polynucleotide. In yet another aspect, the instant disclosure provides a recombinant host cell comprising the

polynucleotide or the vector. In a further aspect, the instant disclosure provides a method of producing an antibody that binds to human PD-1, the method comprising culturing the host cell so that the polynucleotide is expressed and the antibody is produced. In a preferred embodiment, the method is an *in vitro* method.

[0085]   In one embodiment, the present invention relates to an antibody of the invention, or a pharmaceutical composition of the invention, or a polynucleotide of the invention, or a vector of the invention, or a recombinant host cell of the invention for use as a medicament.

[0086]   In one embodiment, the present invention relates to an antibody of the invention, or a pharmaceutical composition of the invention, or a polynucleotide of the invention, or a vector of the invention, or a recombinant host cell of the invention for use as a diagnostic.

[0087]   In one embodiment, the present invention relates to the use of an antibody of the present invention for preparing pharmaceutical compositions or medicaments for immunotherapy. Preferably, the immunotherapy is for increasing the activity of T cells, optionally for treating cancer or treating or preventing an infectious disease.

[0088]   In another aspect, the instant disclosure provides a method of increasing T cell activation in response to an antigen in a subject, the method comprising administering to the subject an effective amount of an antibody or pharmaceutical composition disclosed herein.

[0089]   In another aspect, the instant disclosure provides a method of treating cancer in a subject, the method comprising administering to the subject an effective amount of an antibody or pharmaceutical composition disclosed herein. In certain embodiments, the cancer is selected from the group consisting of melanoma, head and neck cancer (*e.g.*, head and neck squamous cancer), lung cancer (*e.g.*, non-small cell lung cancer and small cell lung cancer), breast cancer (*e.g.*, herceptin resistant breast cancer and trastuzumab-DM1 (T-DM1) resistant breast cancer), prostate cancer, glioblastoma multiforme, colorectal cancer, sarcoma, bladder cancer, cervical cancer, HPV-associated cancers, cancers of the vagina, cancers of the vulva, cancers of the penis, cancers of the anus, cancers of the rectum, cancers of the oropharynx, multiple myeloma, renal cell carcinoma, ovarian cancer, hepatocellular cancer, endometrial cancer, pancreatic cancer, lymphoma, and leukemia (*e.g.*, elderly leukemia, acute myeloid leukemia (AML), and elderly AML). In certain embodiments, the antibody or pharmaceutical composition is administered subcutaneously or intravenously. In certain embodiments, the antibody or pharmaceutical composition is administered intratumorally. In certain embodiments, the antibody or pharmaceutical composition is delivered to a tumor draining lymph node. In certain embodiments, the antibody or pharmaceutical composition is administered intra-arterially.

[0090]   In one aspect, the present invention relates to an antibody, polynucleotide, vector, recombinant host cell, and/or pharmaceutical composition of the present invention for use in a method for increasing T cell activation in response to an antigen.

[0091]   In one aspect, the present invention relates to an antibody, polynucleotide, vector, recombinant host cell, and/or pharmaceutical composition of the present invention for use in a method for increasing T cell activation in response to an antigen in a subject.

[0092]   In one aspect, the present invention relates to the use of an antibody, polynucleotide, vector, recombinant host cell, and/or pharmaceutical composition of the present invention for preparing a pharmaceutical composition for increasing T cell activation in response to an antigen in a subject.

[0093]   In one aspect, the present invention relates to an antibody, polynucleotide, vector, recombinant host cell, and/or pharmaceutical composition of the present invention for use in a method for increasing T cell activation in response to an antigen in a subject comprising administering to the subject an effective amount of an antibody, polynucleotide, vector, recombinant host cell, and/or pharmaceutical composition of the invention.

[0094]   In one aspect, the present invention relates to an antibody, polynucleotide, vector, recombinant host cell, and/or pharmaceutical composition of the present invention for use in a method for the treatment of cancer, preferably wherein the cancer is selected from the group consisting of melanoma, head and neck cancer (*e.g.*, head and neck squamous cancer), lung cancer (*e.g.*, non-small cell lung cancer and small cell lung cancer), breast cancer (*e.g.*, herceptin resistant breast cancer and trastuzumab-DM1 (T-DM1) resistant breast cancer), prostate cancer, glioblastoma multiforme, colorectal cancer, sarcoma, bladder cancer, cervical cancer, HPV-associated cancers, cancers of the vagina, cancers of the vulva, cancers of the penis, cancers of the anus, cancers of the rectum, cancers of the oropharynx, multiple myeloma, renal cell carcinoma, ovarian cancer, hepatocellular cancer, endometrial cancer, pancreatic cancer, lymphoma, and leukemia (*e.g.*, elderly leukemia, acute myeloid leukemia (AML), and elderly AML).

[0095]   In one aspect, the present invention relates to an antibody, polynucleotide, vector, recombinant host cell, and/or pharmaceutical composition of the present invention for use in a method for the treatment of cancer in a subject, preferably wherein the cancer is selected from the group consisting of melanoma, head and neck cancer (*e.g.*, head and neck squamous cancer), lung cancer (*e.g.*, non-small cell lung cancer and small cell lung cancer), breast cancer (*e.g.*, herceptin resistant breast cancer and trastuzumab-DM1 (T-DM1) resistant breast cancer), prostate cancer, glioblastoma multiforme, colorectal cancer, sarcoma, bladder

cancer, cervical cancer, HPV-associated cancers, cancers of the vagina, cancers of the vulva, cancers of the penis, cancers of the anus, cancers of the rectum, cancers of the oropharynx, multiple myeloma, renal cell carcinoma, ovarian cancer, hepatocellular cancer, endometrial cancer, pancreatic cancer, lymphoma, and leukemia (*e.g.*, elderly leukemia, acute myeloid leukemia (AML), and elderly AML).

[0096]   In one aspect, the present invention relates to an antibody, polynucleotide, vector, recombinant host cell, and/or pharmaceutical composition of the present invention for use in a method for the treatment of cancer in a subject comprising administering to the subject an effective amount of an antibody, polynucleotide, vector, recombinant host cell, and/or pharmaceutical composition of the invention, preferably wherein the cancer is selected from the group consisting of melanoma, head and neck cancer (*e.g.*, head and neck squamous cancer), lung cancer (*e.g.*, non-small cell lung cancer and small cell lung cancer), breast cancer (*e.g.*, herceptin resistant breast cancer and trastuzumab-DM1 (T-DM1) resistant breast cancer), prostate cancer, glioblastoma multiforme, colorectal cancer, sarcoma, bladder cancer, cervical cancer, HPV-associated cancers, cancers of the vagina, cancers of the vulva, cancers of the penis, cancers of the anus, cancers of the rectum, cancers of the oropharynx, multiple myeloma, renal cell carcinoma, ovarian cancer, hepatocellular cancer, endometrial cancer, pancreatic cancer, lymphoma, and leukemia (*e.g.*, elderly leukemia, acute myeloid leukemia (AML), and elderly AML).

[0097]   In a preferred embodiment of an antibody, polynucleotide, vector, recombinant host cell, and/or pharmaceutical composition for use of the present invention, the antibody, polynucleotide, vector, recombinant host cell, and/or pharmaceutical composition, more preferably the antibody or pharmaceutical composition, is administered subcutaneously or intravenously. In another preferred embodiment of an antibody, polynucleotide, vector, recombinant host cell, and/or pharmaceutical composition for use of the present invention, the antibody, polynucleotide, vector, recombinant host cell, and/or pharmaceutical composition, more preferably the antibody or pharmaceutical composition, is administered intratumorally or intra-arterially.

[0098]   In certain embodiments, the foregoing methods further comprise administering an additional therapeutic agent to the subject. Therefore, in one preferred embodiment of an antibody, polynucleotide, vector, recombinant host cell, and/or pharmaceutical composition for use in a method of the present invention, the method further comprises administering an additional therapeutic agent to the subject.

[0099]   In one aspect, the present invention relates to (a) an antibody, polynucleotide,

vector, recombinant host cell, and/or pharmaceutical composition of the present invention and (b) an additional therapeutic agent for use as a medicament.

[00100]   In one aspect, the present invention relates to (a) an antibody, polynucleotide, vector, recombinant host cell, and/or pharmaceutical composition of the present invention and (b) an additional therapeutic agent for use in a method for the treatment of cancer.

[00101]   In one aspect, the present invention relates to a pharmaceutical composition, kit or kit-of-parts comprising (a) an antibody, polynucleotide, vector, recombinant host cell, and/or pharmaceutical composition of the present invention and (b) an additional therapeutic agent.

[00102]   In certain embodiments, the additional therapeutic agent is a chemotherapeutic or a checkpoint targeting agent. In certain embodiments, the checkpoint targeting agent is selected from the group consisting of an antagonist anti-PD-1 antibody, an antagonist anti-PD-L1 antibody, an antagonist anti-PD-L2 antibody, an antagonist anti-CTLA-4 antibody, an antagonist anti-TIM-3 antibody, an antagonist anti-LAG-3 antibody, an antagonist anti-CEACAM1 antibody, an antagonist anti-TIGIT antibody, an agonist anti-CD137 antibody, an agonist anti-ICOS antibody, an agonist anti-GITR antibody, and an agonist anti-OX40 antibody. In certain embodiments, the additional therapeutic agent is an inhibitor of indoleamine-2,3-dioxygenase (IDO). In certain embodiments, the inhibitor is selected from the group consisting of epacadostat, F001287, indoximod, and NLG919. In certain embodiments, the additional therapeutic agent is a vaccine. In certain embodiments, the vaccine comprises a heat shock protein peptide complex (HSPPC) comprising a heat shock protein complexed with an antigenic peptide. In certain embodiments, the heat shock protein is hsc70 and is complexed with a tumor-associated antigenic peptide. In certain embodiments, the heat shock protein is gp96 protein and is complexed with a tumor-associated antigenic peptide, wherein the HSPPC is derived from a tumor obtained from a subject. In certain embodiments, the additional therapeutic agent comprises a TCR. In certain embodiments, the additional therapeutic agent is a soluble TCR. In certain embodiments, the additional therapeutic agent is a cell expressing a TCR. In certain embodiments, the additional therapeutic agent is a cell expressing a chimeric antigen receptor. In certain embodiments, the additional therapeutic agent is an antibody that specifically binds to a peptide-MHC complex. In certain embodiments, the additional therapeutic agent is an adjuvant. In one aspect, the present invention relates to (a) an antibody, polynucleotide, vector, recombinant host cell, and/or pharmaceutical composition of the present invention, as well as the use thereof for preparing medicines, and (b) a vaccine for use as a medicament, in particular, for use in a method for the treatment of cancer,

preferably wherein the vaccine comprises a heat shock protein peptide complex (HSPPC) comprising a heat shock protein complexed with an antigenic peptide. In one aspect, the present invention relates to a pharmaceutical composition, kit or kit-of-parts comprising (a) an antibody, polynucleotide, vector, recombinant host cell, and/or pharmaceutical composition of the present invention and (b) a vaccine, preferably wherein the vaccine comprises a heat shock protein peptide complex (HSPPC) comprising a heat shock protein complexed with an antigenic peptide.

4.   BRIEF DESCRIPTION OF THE DRAWINGS

[00103]   **Figures 1A, 1B, 1C, and 1D** are graphs showing the binding of anti-PD-1 antibodies to activated primary human or cynomolgus T cells as measured by flow cytometry. The mean fluorescence intensity (MFI) was calculated and plotted against a range of antibody concentrations. In Figure 1A, AGEN2046w, AGEN2047w, and a human IgG_1 isotype control were measured for binding to Staphylococcus Enterotoxin A (SEA) stimulated human CD4+ T cells. AGEN2034w and a human IgG_4 isotype control were tested against SEA stimulated human CD4+ T cells (Figures 1B and 1D) and Staphylococcus Enterotoxin B (SEB) stimulated cynomolgus CD4+ T cells (Figure 1C).

[00104]   **Figure 2** is a graph showing the binding of AGEN2034w to human PD-1-Fc, human ROBO2-Fc, human B7-H7-Fc, or SIRPγ-His. The interaction was measured by suspension array technology and the median fluorescent intensity (MFI) is plotted against antibody concentrations.

[00105]   **Figures 3A, 3B, 3C, 3D, 3E, and 3F** are graphs showing the percent of recombinant PD-L1-Fc and/or PD-L2-Fc binding to PD-1 coupled beads in the presence of a dose titration of anti-PD-1 antibodies. In Figures 3A, 3B, 3C, and 3D, the anti-PD-1 antibodies tested are AGEN2033w, AGEN2034w, AGEN2046w, and AGEN2047w, respectively. In Figures 3E and 3F, similar results are shown for AGEN2034w and an isotype control antibody.

[00106]   **Figures 4A, 4B, 4C, 4D, 4E, and 4F** are graphs depicting the functional activity of anti-PD-1 antibodies on cultures of primary human PBMCs following SEA stimulation. Figure 4A is a graph showing IL-2 production induced by anti-PD-1 antibodies AGEN2033w, AGEN2034w, AGEN2046w, AGEN2047w, and an IgG_1 isotype control. The mean values (bar) of secreted IL-2 are shown. Figure 4B is a graph showing IL-2 production in the presence of a dose titration of AGEN2034w as compared with an isotype control.

Error bars represent one standard deviation. AGEN2034w or an isotype control antibody was tested in the presence or absence of anti-CTLA-4 antibody Ipilimumab (Figure 4C), anti-TIGIT antibody pab2197 or pab2196 (Figure 4D), anti-CD137 antibody pab2225 (Figure 4E), or anti-OX40 antibody pab1928 (Figure 4F).

[00107]   **Figures 5A, 5B, and 5C** are results from studies examining the impact of Fc gamma receptor (FcγR) engagement on the antagonistic activity of anti-PD-1 antibodies on primary human PBMCs following SEA stimulation. Figure 5A is a graph showing IL-2 production induced by an anti-PD-1 reference antibody or an isotype control in the presence or absence of an anti-CD16 antibody. Figure 5B is the result from a similar study examining IL-2 secretion induced by AGEN2034w or an isotype control in the presence of an isotype control, an anti-CD16 antibody, anti-CD32 antibody, or anti-CD64 antibody. Figure 5C is a graph showing IL-2 production induced by AGEN2047w with wild type human IgG_1 Fc region, three corresponding Fc mutants (N297A, S267E/L328F, and S239D/A330L/I332E), and their respective isotype controls. The mean values (bar) of secreted IL-2 are shown.

[00108]   **Figure 6** is a graph showing IFNγ production of a co-culture of human T cells and allogenic dendritic cells in the absence of any antibody or in the presence of an isotype control antibody or the anti-PD-1 antibody AGEN2034w. The mean values (bar) of IFNγ are shown.

[00109]   **Figures 7A, 7B, and 7C** are results from assays measuring proliferation of anti-CD3-antibody-stimulated T cells after co-culturing with ovarian cancer ascites fluid in the presence of AGEN2034w or an isotype control antibody. Figures 7A and 7B are representative histograms showing CFSE fluorescence from CD4+ and CD8+ T cells, respectively, in the presence of AGEN2034w or an isotype control antibody at 10 µg/ml. Figure 7C is a graph showing results from a similar study. In Figure 7C, proliferation of CD4+ T cells, as measured by CFSE dilution, was normalized to proliferation of CD4+ T cells in the absence of ovarian cancer ascites fluid and plotted against antibody concentrations.

[00110]   **Figure 8** is a graph showing response in a Jurkat NFAT-luciferase reporter assay induced by the anti-PD-1 antibody AGEN2034w or an IgG_4 isotype control antibody. Response, as measured by luciferase expression, was normalized to the response induced in the presence of the isotype control antibody at the lowest concentration tested (fold induction) and plotted against antibody concentrations.

[00111]   **Figures 9A, 9B, 9C, 9D, 9E, and 9F** are graphs showing the percent of recombinant PD-L1-Fc and PD-L2-Fc binding to PD-1 coupled beads in the presence of a

dose titration of anti-PD-1 antibodies AGEN2001w (Figure 9A), AGEN2002w (Figure 9B), EP11_pl1_B03 (Figure 9C), EP11_pl1_B05 (Figure 9D), EP11_pl1_C02 (Figure 9E), or EP11_pl1_C03 (Figure 9F).

[00112]   **Figure 10** is a graph depicting the functional activity of the anti-PD-1 antibody AGEN2002w or an IgG$_1$ isotype control on cultures of primary human PBMCs following SEA stimulation, as demonstrated by IL-2 production. The mean values (bar) of secreted IL-2 are shown.

5.   DETAILED DESCRIPTION

[00113]   The instant disclosure provides antibodies that specifically bind to human PD-1 and antagonize PD-1 function, *e.g.*, PD-1-mediated immune suppression. Also provided are pharmaceutical compositions comprising these antibodies, nucleic acids encoding these antibodies, expression vectors and host cells for making these antibodies, and methods of treating a subject using these antibodies. The antibodies disclosed herein are particularly useful for increasing T cell activation in response to an antigen (*e.g.*, a tumor antigen or an infectious disease antigen), and hence for treating cancer in a subject or treating or preventing an infectious disease in a subject. All instances of "isolated antibodies" described herein are additionally contemplated as antibodies that may be, but need not be, isolated. All instances of "isolated polynucleotides" described herein are additionally contemplated as polynucleotides that may be, but need not be, isolated. All instances of "antibodies" described herein are additionally contemplated as antibodies that may be, but need not be, isolated. All instances of "polynucleotides" described herein are additionally contemplated as polynucleotides that may be, but need not be, isolated.

5.1   Definitions

[00114]   As used herein, the terms "about" and "approximately," when used to modify a numeric value or numeric range, indicate that deviations of up to 5% to 10% above and up to 5% to 10% below the value or range remain within the intended meaning of the recited value or range.

[00115]   As used herein, the term "PD-1" refers to the protein programmed cell death protein 1. PD-1 nucleotide and amino acid sequences are well known in the art. An exemplary human PD-1 amino acid sequence is set forth in GenBank deposit GI: 167857792.

[00116]   As used herein, the terms "antibody" and "antibodies" include full length

antibodies, antigen-binding fragments of full length antibodies, and molecules comprising antibody CDRs, VH regions or VL regions. Examples of antibodies include monoclonal antibodies, recombinantly produced antibodies, monospecific antibodies, multispecific antibodies (including bispecific antibodies), human antibodies, humanized antibodies, chimeric antibodies, immunoglobulins, synthetic antibodies, tetrameric antibodies comprising two heavy chain and two light chain molecules, an antibody light chain monomer, an antibody heavy chain monomer, an antibody light chain dimer, an antibody heavy chain dimer, an antibody light chain- antibody heavy chain pair, intrabodies, heteroconjugate antibodies, antibody-drug conjugates, single domain antibodies, monovalent antibodies, single chain antibodies or single-chain Fvs (scFv), camelized antibodies, affybodies, Fab fragments, F(ab')$_2$ fragments, disulfide-linked Fvs (sdFv), anti-idiotypic (anti-Id) antibodies (including, *e.g.*, anti-anti-Id antibodies), and antigen-binding fragments of any of the above. In certain embodiments, antibodies described herein refer to polyclonal antibody populations. Antibodies can be of any type (*e.g.*, IgG, IgE, IgM, IgD, IgA or IgY), any class (*e.g.*, IgG$_1$, IgG$_2$, IgG$_3$, IgG$_4$, IgA$_1$ or IgA$_2$), or any subclass (*e.g.*, IgG$_{2a}$ or IgG$_{2b}$) of immunoglobulin molecule. In certain embodiments, antibodies described herein are IgG antibodies, or a class (*e.g.*, human IgG$_1$ or IgG$_4$) or subclass thereof. In a specific embodiment, the antibody is a humanized monoclonal antibody. In another specific embodiment, the antibody is a human monoclonal antibody. In certain embodiments, an antibody described herein is an IgG$_1$ or IgG$_2$ antibody.

[00117]   As used herein, the terms "VH region" and "VL region" refer to single antibody heavy and light chain variable regions, respectively, comprising FR (Framework Regions) 1, 2, 3 and 4 and CDR (Complementarity Determining Regions) 1, 2 and 3 (see Kabat *et al.*, (1991) Sequences of Proteins of Immunological Interest (NIH Publication No. 91-3242, Bethesda), which is herein incorporated by reference in its entirety).

[00118]   As used herein, the term "CDR" or "complementarity determining region" means the noncontiguous antigen combining sites found within the variable region of both heavy and light chain polypeptides. These particular regions have been described by Kabat *et al.*, J. Biol. Chem. 252, 6609-6616 (1977) and Kabat *et al.*, Sequences of protein of immunological interest. (1991), by Chothia *et al.*, J. Mol. Biol. 196:901-917 (1987), and by MacCallum *et al.*, J. Mol. Biol. 262:732-745 (1996), all of which are herein incorporated by reference in their entireties, where the definitions include overlapping or subsets of amino acid residues when compared against each other. Preferably, the term "CDR" is a CDR as defined by Kabat, based on sequence comparisons. CDRH1, CDRH2 and CDRH3 denote the heavy

chain CDRs, and CDRL1, CDRL2 and CDRL3 denote the light chain CDRs.

[00119]   As used herein, the term "EU numbering system" refers to the EU numbering convention for the constant regions of an antibody, as described in Edelman, G.M. *et al.*, Proc. Natl. Acad. USA, 63, 78-85 (1969) and Kabat *et al.*, in "Sequences of Proteins of Immunological Interest", U.S. Dept. Health and Human Services, 5th edition, 1991, each of which is herein incorporated by reference in its entirety.

[00120]   As used herein the term "framework (FR) amino acid residues" refers to those amino acids in the framework region of an immunoglobulin chain. The term "framework region" or "FR region" as used herein, includes the amino acid residues that are part of the variable region, but are not part of the CDRs (*e.g.*, using the Kabat definition of CDRs).

[00121]   As used herein, the terms "variable region" and "variable domain" are used interchangeably and are common in the art. The variable region typically refers to a portion of an antibody, generally, a portion of a light or heavy chain, typically about the amino-terminal 110 to 125 amino acids in the mature heavy chain and about 90 to 115 amino acids in the mature light chain, which differ extensively in sequence among antibodies and are used in the binding and specificity of a particular antibody for its particular antigen. The variability in sequence is concentrated in those regions called complementarity determining regions (CDRs) while the more highly conserved regions in the variable domain are called framework regions (FR). In certain embodiments, the variable region is a human variable region. In certain embodiments, the variable region comprises rodent or murine CDRs and human framework regions (FRs). In particular embodiments, the variable region is a primate (*e.g.*, non-human primate) variable region. In certain embodiments, the variable region comprises rodent or murine CDRs and primate (*e.g.*, non-human primate) framework regions (FRs).

[00122]   The terms "VL" and "VL domain" are used interchangeably to refer to the light chain variable region of an antibody.

[00123]   The terms "VH" and "VH domain" are used interchangeably to refer to the heavy chain variable region of an antibody.

[00124]   As used herein, the terms "constant region" and "constant domain" are interchangeable and are common in the art. The constant region is an antibody portion, *e.g.*, a carboxyl terminal portion of a light and/or heavy chain which is not directly involved in binding of an antibody to antigen but which can exhibit various effector functions, such as interaction with an Fc receptor (*e.g.*, Fc gamma receptor). The constant region of an immunoglobulin molecule generally has a more conserved amino acid sequence relative to an

immunoglobulin variable domain.

[00125]   As used herein, the term "heavy chain" when used in reference to an antibody can refer to any distinct type, *e.g.*, alpha (α), delta (δ), epsilon (ε), gamma (γ), and mu (μ), based on the amino acid sequence of the constant domain, which give rise to IgA, IgD, IgE, IgG, and IgM classes of antibodies, respectively, including subclasses of IgG, *e.g.*, IgG_1, IgG_2, IgG_3, and IgG_4.

[00126]   As used herein, the term "light chain" when used in reference to an antibody can refer to any distinct type, *e.g.*, kappa (κ) or lambda (λ) based on the amino acid sequence of the constant domains. Light chain amino acid sequences are well known in the art. In specific embodiments, the light chain is a human light chain.

[00127]   "Binding affinity" generally refers to the strength of the sum total of non-covalent interactions between a single binding site of a molecule (*e.g.*, an antibody) and its binding partner (*e.g.*, an antigen). Unless indicated otherwise, as used herein, "binding affinity" refers to intrinsic binding affinity which reflects a 1:1 interaction between members of a binding pair (*e.g.*, antibody and antigen). The affinity of a molecule X for its partner Y can generally be represented by the dissociation constant (K_D). Affinity can be measured and/or expressed in a number of ways known in the art, including, but not limited to, equilibrium dissociation constant (K_D), and equilibrium association constant (K_A). The K_D is calculated from the quotient of k_{off}/k_{on}, whereas K_A is calculated from the quotient of k_{on}/k_{off}. k_{on} refers to the association rate constant of, *e.g.*, an antibody to an antigen, and k_{off} refers to the dissociation rate constant of, *e.g.*, an antibody to an antigen. The k_{on} and k_{off} can be determined by techniques known to one of ordinary skill in the art, such as BIAcore$^{®}$ or KinExA.

[00128]   As used herein, the term "specifically binds to" refers to the ability of an antibody to bind to an antigen with a dissociation constant (K_d) of at least about 1×10^{-6} M, 1×10^{-7} M, 1×10^{-8} M, 1×10^{-9} M, 1×10^{-10} M, 1×10^{-11} M, 1×10^{-12} M, or less, and/or bind to an antigen with an affinity that is at least two-fold greater than its affinity for a nonspecific antigen. In one embodiment, a molecule that specifically binds to an antigen can bind to other peptides or polypeptides, generally with lower affinity as determined by, *e.g.*, immunoassays, BIAcore$^{®}$, KinExA 3000 instrument (Sapidyne Instruments, Boise, ID), or other assays known in the art. In one embodiment, a molecule that specifically binds to an antigen binds to the antigen with an association constant (K_A) that is at least 2 logs, 2.5 logs, 3 logs, 4 logs or greater than the K_A when the molecule binds non-specifically to another antigen. In one embodiment, a molecule that specifically binds to an antigen binds to the antigen with a K_d of

1 x 10^{-6} M or less, 1 x 10^{-7} M or less, 1 x 10^{-8} M or less, 1 x 10^{-9} M or less, 1 x 10^{-10} M or less, 1 x 10^{-11} M or less, or 1 x 10^{-12} M or less.

[00129]   In another specific embodiment, molecules that specifically bind to an antigen do not cross react with other proteins under similar binding conditions.   In another specific embodiment, molecules that specifically bind to PD-1 do not cross react with other non-PD-1 proteins. In a specific embodiment, provided herein is an antibody that binds to PD-1 (*e.g.*, human PD-1) with higher affinity than to another unrelated antigen. In certain embodiments, provided herein is an antibody that binds to PD-1 (*e.g.*, human PD-1) with a 20%, 25%, 30%, 35%, 40%, 45%, 50%, 55%, 60%, 65%, 70%, 75%, 80%, 85%, 90%, 95% or higher affinity than to another, unrelated antigen as measured by, *e.g.*, a radioimmunoassay, surface plasmon resonance, or kinetic exclusion assay. In a specific embodiment, the extent of binding of an anti-PD-1 antibody described herein to an unrelated, non-PD-1 protein is less than 10%, 15%, or 20% of the binding of the antibody to PD-1 protein as measured by, *e.g.*, a radioimmunoassay.

[00130]   As used herein, an "epitope" is a term in the art and refers to a localized region of an antigen to which an antibody can specifically bind. An epitope can be, for example, contiguous amino acids of a polypeptide (linear or contiguous epitope) or an epitope can, for example, come together from two or more non-contiguous regions of a polypeptide or polypeptides (conformational, non-linear, discontinuous, or non-contiguous epitope). In certain embodiments, the epitope to which an antibody binds can be determined by, *e.g.*, NMR spectroscopy, X-ray diffraction crystallography studies, ELISA assays, hydrogen/deuterium exchange coupled with mass spectrometry (*e.g.*, liquid chromatography electrospray mass spectrometry), array-based oligo-peptide scanning assays (*e.g.*, constraining peptides using CLIPS (Chemical Linkage of Peptides onto Scaffolds) to map discontinuous or conformational epitopes), and/or mutagenesis mapping (*e.g.*, site-directed mutagenesis mapping). For X-ray crystallography, crystallization may be accomplished using any of the known methods in the art (*e.g.*, Giegé R *et al.*, (1994) Acta Crystallogr D Biol Crystallogr 50(Pt 4): 339-350; McPherson A (1990) Eur J Biochem 189: 1-23; Chayen NE (1997) Structure 5: 1269-1274; McPherson A (1976) J Biol Chem 251: 6300-6303, each of which is herein incorporated by reference in its entirety). Antibody:antigen crystals may be studied using well known X-ray diffraction techniques and may be refined using computer software such as X-PLOR (Yale University, 1992, distributed by Molecular Simulations, Inc.; see, *e.g.*, Meth Enzymol (1985) volumes 114 & 115, eds Wyckoff HW *et al.*,; U.S. 2004/0014194), and BUSTER (Bricogne G (1993) Acta Crystallogr D Biol Crystallogr 49(Pt

1): 37-60; Bricogne G (1997) Meth Enzymol 276A: 361-423, ed Carter CW; Roversi P *et al.,* (2000) Acta Crystallogr D Biol Crystallogr 56(Pt 10): 1316-1323, each of which is herein incorporated by reference in its entirety). Mutagenesis mapping studies may be accomplished using any method known to one of skill in the art. *See, e.g.,* Champe M *et al.,* (1995) J Biol Chem 270: 1388-1394 and Cunningham BC & Wells JA (1989) Science 244: 1081-1085, each of which is herein incorporated by reference in its entirety, for a description of mutagenesis techniques, including alanine scanning mutagenesis techniques. CLIPS (Chemical Linkage of Peptides onto Scaffolds) is a technology to present one or more peptides in a structurally constrained configuration to behave as functional mimics of complex protein domains. *See, e.g.,* U.S. Publication Nos. US 2008/0139407 A1 and US 2007/099240 A1, and US Patent No. 7,972,993, all of which are herein incorporated by reference in their entireties. In a specific embodiment, the epitope of an antibody is determined using alanine scanning mutagenesis studies. In a specific embodiment, the epitope of an antibody is determined using hydrogen/deuterium exchange coupled with mass spectrometry. In a specific embodiment, the epitope of an antibody is determined using CLIPS Epitope Mapping Technology from Pepscan Therapeutics.

[001]   As used herein, the terms "T cell receptor" and "TCR" are used interchangeably and refer to full length heterodimeric αβ or γδ TCRs, antigen-binding fragments of full length TCRs, and molecules comprising TCR CDRs or variable regions. Examples of TCRs include, but are not limited to, full length TCRs, antigen-binding fragments of full length TCRs, soluble TCRs lacking transmembrane and cytoplasmic regions, single-chain TCRs containing variable regions of TCRs attached by a flexible linker, TCR chains linked by an engineered disulfide bond, monospecific TCRs, multi-specific TCRs (including bispecific TCRs), TCR fusions, human TCRs, humanized TCRs, chimeric TCRs, recombinantly produced TCRs, and synthetic TCRs. The term encompasses wild-type TCRs and genetically engineered TCRs (*e.g.,* a chimeric TCR comprising a chimeric TCR chain which includes a first portion from a TCR of a first species and a second portion from a TCR of a second species).

[002]   As used herein, the terms "major histocompatibility complex" and "MHC" are used interchangeably and refer to an MHC class I molecule and/or an MHC class II molecule.

[003]   As used herein, the term "peptide-MHC complex" refers to an MHC molecule (MHC class I or MHC class II) with a peptide bound in the art-recognized peptide binding pocket of the MHC.

[00131] As used herein, the term "treat," "treating," and "treatment" refer to therapeutic or preventative measures described herein. The methods of "treatment" employ administration of an antibody to a subject having a disease or disorder, or predisposed to having such a disease or disorder, in order to prevent, cure, delay, reduce the severity of, or ameliorate one or more symptoms of the disease or disorder or recurring disease or disorder, or in order to prolong the survival of a subject beyond that expected in the absence of such treatment.

[00132] As used herein, the term "effective amount" in the context of the administration of a therapy to a subject refers to the amount of a therapy that achieves a desired prophylactic or therapeutic effect.

[00133] As used herein, the term "subject" includes any human or non-human animal. In a preferred embodiment, the subject is a human or non-human mammal, more preferably a human.

[00134] The determination of "percent identity" between two sequences (e.g., amino acid sequences or nucleic acid sequences) can be accomplished using a mathematical algorithm. A specific, non-limiting example of a mathematical algorithm utilized for the comparison of two sequences is the algorithm of Karlin S & Altschul SF (1990) PNAS 87: 2264-2268, modified as in Karlin S & Altschul SF (1993) PNAS 90: 5873-5877, each of which is herein incorporated by reference in its entirety. Such an algorithm is incorporated into the NBLAST and XBLAST programs of Altschul SF et al., (1990) J Mol Biol 215: 403, which is herein incorporated by reference in its entirety. BLAST nucleotide searches can be performed with the NBLAST nucleotide program parameters set, e.g., for score=100, wordlength=12 to obtain nucleotide sequences homologous to a nucleic acid molecules described herein. BLAST protein searches can be performed with the XBLAST program parameters set, e.g., to score 50, wordlength=3 to obtain amino acid sequences homologous to a protein molecule described herein. To obtain gapped alignments for comparison purposes, Gapped BLAST can be utilized as described in Altschul SF et al., (1997) Nuc Acids Res 25: 3389-3402, which is herein incorporated by reference in its entirety. Alternatively, PSI BLAST can be used to perform an iterated search which detects distant relationships between molecules (Id.). When utilizing BLAST, Gapped BLAST, and PSI Blast programs, the default parameters of the respective programs (e.g., of XBLAST and NBLAST) can be used (see, e.g., National Center for Biotechnology Information (NCBI) on the worldwide web, ncbi.nlm.nih.gov). Another specific, non-limiting example of a mathematical algorithm utilized for the comparison of sequences is the algorithm of Myers and Miller, 1988, CABIOS 4:11-17, which is herein incorporated by reference in its entirety. Such an

algorithm is incorporated in the ALIGN program (version 2.0) which is part of the GCG sequence alignment software package. When utilizing the ALIGN program for comparing amino acid sequences, a PAM120 weight residue table, a gap length penalty of 12, and a gap penalty of 4 can be used.

[00135]   The percent identity between two sequences can be determined using techniques similar to those described above, with or without allowing gaps. In calculating percent identity, typically only exact matches are counted.

5.2   Anti-PD-1 Antibodies

[00136]   In one aspect the instant disclosure provides antibodies that specifically bind to human PD-1 and antagonize PD-1 function. The amino acid sequences of exemplary antibodies are set forth in Tables 1-6, herein.

Table 1. Sequences of variable regions, CDRs, and FRs of exemplary anti-PD-1 antibodies

SEQ ID NO:	Description	Amino acid Sequence
1	AGEN2033w Kabat CDRH1	SYGMH
2	AGEN2033w Kabat CDRH2	VIWYDGSNKYYADSVKG
3	AGEN2033w Kabat CDRH3	NVDY
4	AGEN2033w Kabat CDRL1	RASQSVSSNLA
5	AGEN2033w Kabat CDRL2	GASTRAT
6	AGEN2033w Kabat CDRL3	QQYNNWPRT
7	AGEN2034w Kabat CDRH3	NGDH
8	AGEN2033w IMGT CDRH1	GFTFSSYG
9	AGEN2033w IMGT CDRH2	IWYDGSNK
10	AGEN2033w IMGT CDRH3	ASNVDY
11	AGEN2033w IMGT CDRL1	QSVSSN
12	AGEN2033w IMGT CDRL2	GAS
13	AGEN2033w IMGT CDRL3	QQYNNWPRT
14	AGEN2034w IMGT CDRH3	ASNGDH

SEQ ID NO:	Description	Amino acid Sequence
15	AGEN2033w, AGEN2046w VH	QVQLVESGGGVVQPGRSLRLSCAASGFTFSSYGMHWV RQAPGKGLEWVAVIWYDGSNKYYADSVKGRFTISRDN SKNTLYLQMNSLRAEDTAVYYCASNVDYWGQGTLVTV SS
16	AGEN2033w, AGEN2034w, AGEN2046w, AGEN2047w VL	EIVMTQSPATLSVSPGERATLSCRASQSVSSNLAWYQ QKPGQAPRLLIYGASTRATGIPARFSGSGSGTEFTLT ISSLQSEDFAVYYCQQYNNWPRTFGQGTKVEIK
17	AGEN2034w, AGEN2047w VH	QVQLVESGGGVVQPGRSLRLSCAASGFTFSSYGMHWV RQAPGKGLEWVAVIWYDGSNKYYADSVKGRFTISRDN SKNTLYLQMNSLRAEDTAVYYCASNGDHWGQGTLVTV SS
18	AGEN2033w heavy chain IgG4 S228P	QVQLVESGGGVVQPGRSLRLSCAASGFTFSSYGMHWV RQAPGKGLEWVAVIWYDGSNKYYADSVKGRFTISRDN SKNTLYLQMNSLRAEDTAVYYCASNVDYWGQGTLVTV SSASTKGPSVFPLAPCSRSTSESTAALGCLVKDYFPE PVTVSWNSGALTSGVHTFPAVLQSSGLYSLSSVVTVP SSSLGTKTYTCNVDHKPSNTKVDKRVESKYGPPCPPC PAPEFLGGPSVFLFPPKPKDTLMISRTPEVTCVVVDV SQEDPEVQFNWYVDGVEVHNAKTKPREEQFNSTYRVV SVLTVLHQDWLNGKEYKCKVSNKGLPSSIEKTISKAK GQPREPQVYTLPPSQEEMTKNQVSLTCLVKGFYPSDI AVEWESNGQPENNYKTTPPVLDSDGSFFLYSRLTVDK SRWQEGNVFSCSVMHEALHNHYTQKSLSLSLGK
52	AGEN2033w heavy chain IgG4 S228P (without C-terminal lysine)	QVQLVESGGGVVQPGRSLRLSCAASGFTFSSYGMHWV RQAPGKGLEWVAVIWYDGSNKYYADSVKGRFTISRDN SKNTLYLQMNSLRAEDTAVYYCASNVDYWGQGTLVTV SSASTKGPSVFPLAPCSRSTSESTAALGCLVKDYFPE PVTVSWNSGALTSGVHTFPAVLQSSGLYSLSSVVTVP SSSLGTKTYTCNVDHKPSNTKVDKRVESKYGPPCPPC PAPEFLGGPSVFLFPPKPKDTLMISRTPEVTCVVVDV SQEDPEVQFNWYVDGVEVHNAKTKPREEQFNSTYRVV SVLTVLHQDWLNGKEYKCKVSNKGLPSSIEKTISKAK GQPREPQVYTLPPSQEEMTKNQVSLTCLVKGFYPSDI AVEWESNGQPENNYKTTPPVLDSDGSFFLYSRLTVDK SRWQEGNVFSCSVMHEALHNHYTQKSLSLSLG
19	AGEN2033w, AGEN2034w, AGEN2046w, AGEN2047w light chain	EIVMTQSPATLSVSPGERATLSCRASQSVSSNLAWYQ QKPGQAPRLLIYGASTRATGIPARFSGSGSGTEFTLT ISSLQSEDFAVYYCQQYNNWPRTFGQGTKVEIKRTVA APSVFIFPPSDEQLKSGTASVVCLLNNFYPREAKVQW KVDNALQSGNSQESVTEQDSKDSTYSLSSTLTLSKAD YEKHKVYACEVTHQGLSSPVTKSFNRGEC

SEQ ID NO:	Description	Amino acid Sequence
20	AGEN2034w heavy chain IgG4 S228P	QVQLVESGGGVVQPGRSLRLSCAASGFTFSSYGMHWV RQAPGKGLEWVAVIWYDGSNKYYADSVKGRFTISRDN SKNTLYLQMNSLRAEDTAVYYCASNGDHWGQGTLVTV SSASTKGPSVFPLAPCSRSTSESTAALGCLVKDYFPE PVTVSWNSGALTSGVHTFPAVLQSSGLYSLSSVVTVP SSSLGTKTYTCNVDHKPSNTKVDKRVESKYGPPCPPC PAPEFLGGPSVFLFPPKPKDTLMISRTPEVTCVVVDV SQEDPEVQFNWYVDGVEVHNAKTKPREEQFNSTYRVV SVLTVLHQDWLNGKEYKCKVSNKGLPSSIEKTISKAK GQPREPQVYTLPPSQEEMTKNQVSLTCLVKGFYPSDI AVEWESNGQPENNYKTTPPVLDSDGSFFLYSRLTVDK SRWQEGNVFSCSVMHEALHNHYTQKSLSLSLGK
53	AGEN2034w heavy chain IgG4 S228P (without C-terminal lysine)	QVQLVESGGGVVQPGRSLRLSCAASGFTFSSYGMHWV RQAPGKGLEWVAVIWYDGSNKYYADSVKGRFTISRDN SKNTLYLQMNSLRAEDTAVYYCASNGDHWGQGTLVTV SSASTKGPSVFPLAPCSRSTSESTAALGCLVKDYFPE PVTVSWNSGALTSGVHTFPAVLQSSGLYSLSSVVTVP SSSLGTKTYTCNVDHKPSNTKVDKRVESKYGPPCPPC PAPEFLGGPSVFLFPPKPKDTLMISRTPEVTCVVVDV SQEDPEVQFNWYVDGVEVHNAKTKPREEQFNSTYRVV SVLTVLHQDWLNGKEYKCKVSNKGLPSSIEKTISKAK GQPREPQVYTLPPSQEEMTKNQVSLTCLVKGFYPSDI AVEWESNGQPENNYKTTPPVLDSDGSFFLYSRLTVDK SRWQEGNVFSCSVMHEALHNHYTQKSLSLSLG
21	AGEN2046w heavy chain IgG1	QVQLVESGGGVVQPGRSLRLSCAASGFTFSSYGMHWV RQAPGKGLEWVAVIWYDGSNKYYADSVKGRFTISRDN SKNTLYLQMNSLRAEDTAVYYCASNVDYWGQGTLVTV SSASTKGPSVFPLAPSSKSTSGGTAALGCLVKDYFPE PVTVSWNSGALTSGVHTFPAVLQSSGLYSLSSVVTVP SSSLGTQTYICNVNHKPSNTKVDKRVEPKSCDKTHTC PPCPAPELLGGPSVFLFPPKPKDTLMISRTPEVTCVV VDVSHEDPEVKFNWYVDGVEVHNAKTKPREEQYNSTY RVVSVLTVLHQDWLNGKEYKCKVSNKALPAPIEKTIS KAKGQPREPQVYTLPPSREEMTKNQVSLTCLVKGFYP SDIAVEWESNGQPENNYKTTPPVLDSDGSFFLYSKLT VDKSRWQQGNVFSCSVMHEALHNHYTQKSLSLSPGK
54	AGEN2046w heavy chain IgG1 (without C-terminal lysine)	QVQLVESGGGVVQPGRSLRLSCAASGFTFSSYGMHWV RQAPGKGLEWVAVIWYDGSNKYYADSVKGRFTISRDN SKNTLYLQMNSLRAEDTAVYYCASNVDYWGQGTLVTV SSASTKGPSVFPLAPSSKSTSGGTAALGCLVKDYFPE PVTVSWNSGALTSGVHTFPAVLQSSGLYSLSSVVTVP SSSLGTQTYICNVNHKPSNTKVDKRVEPKSCDKTHTC PPCPAPELLGGPSVFLFPPKPKDTLMISRTPEVTCVV VDVSHEDPEVKFNWYVDGVEVHNAKTKPREEQYNSTY RVVSVLTVLHQDWLNGKEYKCKVSNKALPAPIEKTIS KAKGQPREPQVYTLPPSREEMTKNQVSLTCLVKGFYP SDIAVEWESNGQPENNYKTTPPVLDSDGSFFLYSKLT VDKSRWQQGNVFSCSVMHEALHNHYTQKSLSLSPG

SEQ ID NO:	Description	Amino acid Sequence
22	AGEN2047w heavy chain IgG1	QVQLVESGGGVVQPGRSLRLSCAASGFTFSSYGMHWV RQAPGKGLEWVAVIWYDGSNKYYADSVKGRFTISRDN SKNTLYLQMNSLRAEDTAVYYCASNGDHWGQGTLVTV SSASTKGPSVFPLAPSSKSTSGGTAALGCLVKDYFPE PVTVSWNSGALTSGVHTFPAVLQSSGLYSLSSVVTVP SSSLGTQTYICNVNHKPSNTKVDKRVEPKSCDKTHTC PPCPAPELLGGPSVFLFPPKPKDTLMISRTPEVTCVV VDVSHEDPEVKFNWYVDGVEVHNAKTKPREEQYNSTY RVVSVLTVLHQDWLNGKEYKCKVSNKALPAPIEKTIS KAKGQPREPQVYTLPPSREEMTKNQVSLTCLVKGFYP SDIAVEWESNGQPENNYKTTPPVLDSDGSFFLYSKLT VDKSRWQQGNVFSCSVMHEALHNHYTQKSLSLSPGK
55	AGEN2047w heavy chain IgG1 (without C-terminal lysine)	QVQLVESGGGVVQPGRSLRLSCAASGFTFSSYGMHWV RQAPGKGLEWVAVIWYDGSNKYYADSVKGRFTISRDN SKNTLYLQMNSLRAEDTAVYYCASNGDHWGQGTLVTV SSASTKGPSVFPLAPSSKSTSGGTAALGCLVKDYFPE PVTVSWNSGALTSGVHTFPAVLQSSGLYSLSSVVTVP SSSLGTQTYICNVNHKPSNTKVDKRVEPKSCDKTHTC PPCPAPELLGGPSVFLFPPKPKDTLMISRTPEVTCVV VDVSHEDPEVKFNWYVDGVEVHNAKTKPREEQYNSTY RVVSVLTVLHQDWLNGKEYKCKVSNKALPAPIEKTIS KAKGQPREPQVYTLPPSREEMTKNQVSLTCLVKGFYP SDIAVEWESNGQPENNYKTTPPVLDSDGSFFLYSKLT VDKSRWQQGNVFSCSVMHEALHNHYTQKSLSLSPG
23	AGEN2047w heavy chain IgG1 N297A	QVQLVESGGGVVQPGRSLRLSCAASGFTFSSYGMHWV RQAPGKGLEWVAVIWYDGSNKYYADSVKGRFTISRDN SKNTLYLQMNSLRAEDTAVYYCASNGDHWGQGTLVTV SSASTKGPSVFPLAPSSKSTSGGTAALGCLVKDYFPE PVTVSWNSGALTSGVHTFPAVLQSSGLYSLSSVVTVP SSSLGTQTYICNVNHKPSNTKVDKRVEPKSCDKTHTC PPCPAPELLGGPSVFLFPPKPKDTLMISRTPEVTCVV VDVSHEDPEVKFNWYVDGVEVHNAKTKPREEQYASTY RVVSVLTVLHQDWLNGKEYKCKVSNKALPAPIEKTIS KAKGQPREPQVYTLPPSREEMTKNQVSLTCLVKGFYP SDIAVEWESNGQPENNYKTTPPVLDSDGSFFLYSKLT VDKSRWQQGNVFSCSVMHEALHNHYTQKSLSLSPGK
56	AGEN2047w heavy chain IgG1 N297A (without C-terminal lysine)	QVQLVESGGGVVQPGRSLRLSCAASGFTFSSYGMHWV RQAPGKGLEWVAVIWYDGSNKYYADSVKGRFTISRDN SKNTLYLQMNSLRAEDTAVYYCASNGDHWGQGTLVTV SSASTKGPSVFPLAPSSKSTSGGTAALGCLVKDYFPE PVTVSWNSGALTSGVHTFPAVLQSSGLYSLSSVVTVP SSSLGTQTYICNVNHKPSNTKVDKRVEPKSCDKTHTC PPCPAPELLGGPSVFLFPPKPKDTLMISRTPEVTCVV VDVSHEDPEVKFNWYVDGVEVHNAKTKPREEQYASTY RVVSVLTVLHQDWLNGKEYKCKVSNKALPAPIEKTIS KAKGQPREPQVYTLPPSREEMTKNQVSLTCLVKGFYP SDIAVEWESNGQPENNYKTTPPVLDSDGSFFLYSKLT VDKSRWQQGNVFSCSVMHEALHNHYTQKSLSLSPG

SEQ ID NO:	Description	Amino acid Sequence
24	AGEN2047w heavy chain IgG1 S267E/L328F	QVQLVESGGGVVQPGRSLRLSCAASGFTFSSYGMHWV RQAPGKGLEWVAVIWYDGSNKYYADSVKGRFTISRDN SKNTLYLQMNSLRAEDTAVYYCASNGDHWGQGTLVTV SSASTKGPSVFPLAPSSKSTSGGTAALGCLVKDYFPE PVTVSWNSGALTSGVHTFPAVLQSSGLYSLSSVVTVP SSSLGTQTYICNVNHKPSNTKVDKRVEPKSCDKTHTC PPCPAPELLGGPSVFLFPPKPKDTLMISRTPEVTCVV VDVEHEDPEVKFNWYVDGVEVHNAKTKPREEQYNSTY RVVSVLTVLHQDWLNGKEYKCKVSNKAFPAPIEKTIS KAKGQPREPQVYTLPPSREEMTKNQVSLTCLVKGFYP SDIAVEWESNGQPENNYKTTPPVLDSDGSFFLYSKLT VDKSRWQQGNVFSCSVMHEALHNHYTQKSLSLSPGK
57	AGEN2047w heavy chain IgG1 S267E/L328F (without C-terminal lysine)	QVQLVESGGGVVQPGRSLRLSCAASGFTFSSYGMHWV RQAPGKGLEWVAVIWYDGSNKYYADSVKGRFTISRDN SKNTLYLQMNSLRAEDTAVYYCASNGDHWGQGTLVTV SSASTKGPSVFPLAPSSKSTSGGTAALGCLVKDYFPE PVTVSWNSGALTSGVHTFPAVLQSSGLYSLSSVVTVP SSSLGTQTYICNVNHKPSNTKVDKRVEPKSCDKTHTC PPCPAPELLGGPSVFLFPPKPKDTLMISRTPEVTCVV VDVEHEDPEVKFNWYVDGVEVHNAKTKPREEQYNSTY RVVSVLTVLHQDWLNGKEYKCKVSNKAFPAPIEKTIS KAKGQPREPQVYTLPPSREEMTKNQVSLTCLVKGFYP SDIAVEWESNGQPENNYKTTPPVLDSDGSFFLYSKLT VDKSRWQQGNVFSCSVMHEALHNHYTQKSLSLSPG
25	AGEN2047w heavy chain IgG1 S239D/A330L/I332E	QVQLVESGGGVVQPGRSLRLSCAASGFTFSSYGMHWV RQAPGKGLEWVAVIWYDGSNKYYADSVKGRFTISRDN SKNTLYLQMNSLRAEDTAVYYCASNGDHWGQGTLVTV SSASTKGPSVFPLAPSSKSTSGGTAALGCLVKDYFPE PVTVSWNSGALTSGVHTFPAVLQSSGLYSLSSVVTVP SSSLGTQTYICNVNHKPSNTKVDKRVEPKSCDKTHTC PPCPAPELLGGPDVFLFPPKPKDTLMISRTPEVTCVV VDVSHEDPEVKFNWYVDGVEVHNAKTKPREEQYNSTY RVVSVLTVLHQDWLNGKEYKCKVSNKALPLPEEKTIS KAKGQPREPQVYTLPPSREEMTKNQVSLTCLVKGFYP SDIAVEWESNGQPENNYKTTPPVLDSDGSFFLYSKLT VDKSRWQQGNVFSCSVMHEALHNHYTQKSLSLSPGK
58	AGEN2047w heavy chain IgG1 S239D/A330L/I332E (without C-terminal lysine)	QVQLVESGGGVVQPGRSLRLSCAASGFTFSSYGMHWV RQAPGKGLEWVAVIWYDGSNKYYADSVKGRFTISRDN SKNTLYLQMNSLRAEDTAVYYCASNGDHWGQGTLVTV SSASTKGPSVFPLAPSSKSTSGGTAALGCLVKDYFPE PVTVSWNSGALTSGVHTFPAVLQSSGLYSLSSVVTVP SSSLGTQTYICNVNHKPSNTKVDKRVEPKSCDKTHTC PPCPAPELLGGPDVFLFPPKPKDTLMISRTPEVTCVV VDVSHEDPEVKFNWYVDGVEVHNAKTKPREEQYNSTY RVVSVLTVLHQDWLNGKEYKCKVSNKALPLPEEKTIS KAKGQPREPQVYTLPPSREEMTKNQVSLTCLVKGFYP SDIAVEWESNGQPENNYKTTPPVLDSDGSFFLYSKLT VDKSRWQQGNVFSCSVMHEALHNHYTQKSLSLSPG

SEQ ID NO:	Description	Amino acid Sequence
26	AGEN2001w VH (BADD426-2614)	QVQLVESGGGVVQPGRSLRLSCAASGFTFSSYGMHWV RQAPGKGLEWVAVIWYDGSNKYYADSVKGRFTISRDN SKNTLYLQMNSLRAEDTAVYYCATNGDYWGQGTLVTV SS
27	AGEN2002w VH (BADD426-2615)	QVQLVESGGGVVQPGRSLRLSCAASGFTFSSYGMHWV RQAPGKGLEWVAVIWYDGSNKYYADSVKGRFTISRDN SKNTLYLQMNSLRAEDTAVYYCASNGDYWGQGTLVTV SS
28	EP11_pl1_B03 (BADD438-2743)	QVQLVESGGGVVQPGRSLRLSCAASGFTFSSYGMHWV RQAPGKGLEWVAVIWYDGSNEYYADSVKGRFTISRDN SKNTLYLQMNSLRAEDTAVYYCASNGDHWGQGTLVTV SS
29	EP11_pl1_B05 (BADD438-2745)	QVQLVESGGGMVQPGRSLRLSCAASGFTFSSYGMHWV RQAPGKGLEWVAVIWFDGSNKYYADSVKGRFTISRDN SKNTLYLQMNSLRAEDTAVYYCASNGDHWGQGTLVTV SS
30	EP11_pl1_C02 (BADD438-2746)	QVQLVESGGGVVQPGRSLRLSCAASGFTFSSYGMHWV RQAPGKGLEWVAVIWYDGSNKYYADSVKGRFTISRDN SKNTLYLQMNSLRAEDTAVYYCASNGDHWGHGTLVTV SS
31	EP11_pl1_C03 (BADD438-2747)	QVQLVESGGGVVQPGRSLRLSCAASGFTFSSYGMHWV RQAPGKGLEWVAVIWYDGSNKYYADSVMGRFTISRDN SKNTLYLQMNSLRAEDTAVYYCASNGDHWGQGTLVTV SS
32	CDRH2 consensus	VIWX$_1$DGSNX$_2$YYADSVX$_3$G X$_1$ is Y or F; X$_2$ is K or E; and X$_3$ is K or M
33	CDRH3 consensus	NX$_1$DX$_2$ X1 is G or V; and X2 is H or Y
34	CDRH2	VIWYDGSNEYYADSVKG
35	CDRH2	VIWFDGSNKYYADSVKG
36	CDRH2	VIWYDGSNKYYADSVMG
37	CDRH3	NGDY
38	VH FR1	QVQLVESGGGVVQPGRSLRLSCAASGFTFS
39	VH FR1	QVQLVESGGGMVQPGRSLRLSCAASGFTFS
40	VH FR2	WVRQAPGKGLEWVA
41	VH FR3	RFTISRDNSKNTLYLQMNSLRAEDTAVYYCAS
42	VH FR3	RFTISRDNSKNTLYLQMNSLRAEDTAVYYCAT
43	VH FR4	WGQGTLVTVSS
44	VH FR4	WGHGTLVTVSS
45	VL FR1	EIVMTQSPATLSVSPGERATLSC
46	VL FR2	WYQQKPGQAPRLLIY
47	VL FR3	GIPARFSGSGSGTEFTLTISSLQSEDFAVYYC
48	VL FR4	FGQGTKVEIK

SEQ ID NO:	Description	Amino acid Sequence
49	VH consensus sequence	QVQLVESGGGX_1VQPGRSLRLSCAASGFTFSSYGMHW VRQAPGKGLEWVAVIWX_2DGSNX_3YYADSVX_4GRFTIS RDNSKNTLYLQMNSLRAEDTAVYYCAX_5NX_6DX_7WGX_8 GTLVTVSS X_1 is V or M, X_2 is Y or F, X_3 is K or E, X_4 is K or M, X_5 is S or T, X_6 is G or V, X_7 is H or Y, and X_8 is Q or H
50	IGHV3-33*01 germline sequence	QVQLVESGGGVVQPGRSLRLSCAASGFTFSSYGMHWV RQAPGKGLEWVAVIWYDGSNKYYADSVKGRFTISRDN SKNTLYLQMNSLRAEDTAVYYCAR
51	IGKV3-15*01 germline sequence	EIVMTQSPATLSVSPGERATLSCRASQSVSSNLAWYQ QKPGQAPRLLIYGASTRATGIPARFSGSGSGTEFTLT ISSLQSEDFAVYYCQQYNNWP
59	Human IgG1 G1m3 allotype (without C-terminal lysine)	ASTKGPSVFPLAPSSKSTSGGTAALGCLVKDYFPEPV TVSWNSGALTSGVHTFPAVLQSSGLYSLSSVVTVPSS SLGTQTYICNVNHKPSNTKVDKRVEPKSCDKTHTCPP CPAPELLGGPSVFLFPPKPKDTLMISRTPEVTCVVVD VSHEDPEVKFNWYVDGVEVHNAKTKPREEQYNSTYRV VSVLTVLHQDWLNGKEYKCKVSNKALPAPIEKTISKA KGQPREPQVYTLPPSREEMTKNQVSLTCLVKGFYPSD IAVEWESNGQPENNYKTTPPVLDSDGSFFLYSKLTVD KSRWQQGNVFSCSVMHEALHNHYTQKSLSLSPG
60	Human IgG1 G1m3 allotype	ASTKGPSVFPLAPSSKSTSGGTAALGCLVKDYFPEPV TVSWNSGALTSGVHTFPAVLQSSGLYSLSSVVTVPSS SLGTQTYICNVNHKPSNTKVDKRVEPKSCDKTHTCPP CPAPELLGGPSVFLFPPKPKDTLMISRTPEVTCVVVD VSHEDPEVKFNWYVDGVEVHNAKTKPREEQYNSTYRV VSVLTVLHQDWLNGKEYKCKVSNKALPAPIEKTISKA KGQPREPQVYTLPPSREEMTKNQVSLTCLVKGFYPSD IAVEWESNGQPENNYKTTPPVLDSDGSFFLYSKLTVD KSRWQQGNVFSCSVMHEALHNHYTQKSLSLSPGK
61	IgG1 N297A (without C-terminal lysine)	ASTKGPSVFPLAPSSKSTSGGTAALGCLVKDYFPEPV TVSWNSGALTSGVHTFPAVLQSSGLYSLSSVVTVPSS SLGTQTYICNVNHKPSNTKVDKRVEPKSCDKTHTCPP CPAPELLGGPSVFLFPPKPKDTLMISRTPEVTCVVVD VSHEDPEVKFNWYVDGVEVHNAKTKPREEQYASTYRV VSVLTVLHQDWLNGKEYKCKVSNKALPAPIEKTISKA KGQPREPQVYTLPPSREEMTKNQVSLTCLVKGFYPSD IAVEWESNGQPENNYKTTPPVLDSDGSFFLYSKLTVD KSRWQQGNVFSCSVMHEALHNHYTQKSLSLSPG

SEQ ID NO:	Description	Amino acid Sequence
62	IgG1 N297A	ASTKGPSVFPLAPSSKSTSGGTAALGCLVKDYFPEPV TVSWNSGALTSGVHTFPAVLQSSGLYSLSSVVTVPSS SLGTQTYICNVNHKPSNTKVDKRVEPKSCDKTHTCPP CPAPELLGGPSVFLFPPKPKDTLMISRTPEVTCVVVD VSHEDPEVKFNWYVDGVEVHNAKTKPREEQYASTYRV VSVLTVLHQDWLNGKEYKCKVSNKALPAPIEKTISKA KGQPREPQVYTLPPSREEMTKNQVSLTCLVKGFYPSD IAVEWESNGQPENNYKTTPPVLDSDGSFFLYSKLTVD KSRWQQGNVFSCSVMHEALHNHYTQKSLSLSPGK
63	IgG4 S228P (without C-terminal lysine)	ASTKGPSVFPLAPCSRSTSESTAALGCLVKDYFPEPV TVSWNSGALTSGVHTFPAVLQSSGLYSLSSVVTVPSS SLGTKTYTCNVDHKPSNTKVDKRVESKYGPPCPPCPA PEFLGGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSQ EDPEVQFNWYVDGVEVHNAKTKPREEQFNSTYRVVSV LTVLHQDWLNGKEYKCKVSNKGLPSSIEKTISKAKGQ PREPQVYTLPPSQEEMTKNQVSLTCLVKGFYPSDIAV EWESNGQPENNYKTTPPVLDSDGSFFLYSRLTVDKSR WQEGNVFSCSVMHEALHNHYTQKSLSLSLG
64	IgG4 S228P	ASTKGPSVFPLAPCSRSTSESTAALGCLVKDYFPEPV TVSWNSGALTSGVHTFPAVLQSSGLYSLSSVVTVPSS SLGTKTYTCNVDHKPSNTKVDKRVESKYGPPCPPCPA PEFLGGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSQ EDPEVQFNWYVDGVEVHNAKTKPREEQFNSTYRVVSV LTVLHQDWLNGKEYKCKVSNKGLPSSIEKTISKAKGQ PREPQVYTLPPSQEEMTKNQVSLTCLVKGFYPSDIAV EWESNGQPENNYKTTPPVLDSDGSFFLYSRLTVDKSR WQEGNVFSCSVMHEALHNHYTQKSLSLSLGK
65	Human kappa light chain constant region IGKC*01 Km3 allotype	RTVAAPSVFIFPPSDEQLKSGTASVVCLLNNFYPREA KVQWKVDNALQSGNSQESVTEQDSKDSTYSLSSTLTL SKADYEKHKVYACEVTHQGLSSPVTKSFNRGEC

Table 2. Heavy chain CDR sequences of exemplary anti-PD-1 antibodies [1]

Antibody	CDRH1 (SEQ ID NO:)	CDRH2 (SEQ ID NO:)	CDRH3 (SEQ ID NO:)
AGEN2033w	SYGMH (1)	VIWYDGSNKYYADSVKG (2)	NVDY (3)
AGEN2034w	SYGMH (1)	VIWYDGSNKYYADSVKG (2)	NGDH (7)
AGEN2001w	SYGMH (1)	VIWYDGSNKYYADSVKG (2)	NGDY (37)
AGEN2002w	SYGMH (1)	VIWYDGSNKYYADSVKG (2)	NGDY (37)
EP11_pl1_B03	SYGMH (1)	VIWYDGSNEYYADSVKG (34)	NGDH (7)
EP11_pl1_B05	SYGMH (1)	VIWFDGSNKYYADSVKG (35)	NGDH (7)
EP11_pl1_C02	SYGMH (1)	VIWYDGSNKYYADSVKG (2)	NGDH (7)

| EP11_pl1_C03 | SYGMH (1) | VIWYDGSNKYYADSVMG (36) | NGDH (7) |

[1] The VH CDRs in Table 2 are determined according to Kabat.

Table 3. Light chain CDR sequences of exemplary anti-PD-1 antibodies [2]

Antibody	CDRL1 (SEQ ID NO:)	CDRL2 (SEQ ID NO:)	CDRL3 (SEQ ID NO:)
AGEN2033w	RASQSVSSNLA (4)	GASTRAT (5)	QQYNNWPRT (6)
AGEN2034w	RASQSVSSNLA (4)	GASTRAT (5)	QQYNNWPRT (6)
AGEN2001w	RASQSVSSNLA (4)	GASTRAT (5)	QQYNNWPRT (6)
AGEN2002w	RASQSVSSNLA (4)	GASTRAT (5)	QQYNNWPRT (6)
EP11_pl1_B03	RASQSVSSNLA (4)	GASTRAT (5)	QQYNNWPRT (6)
EP11_pl1_B05	RASQSVSSNLA (4)	GASTRAT (5)	QQYNNWPRT (6)
EP11_pl1_C02	RASQSVSSNLA (4)	GASTRAT (5)	QQYNNWPRT (6)
EP11_pl1_C03	RASQSVSSNLA (4)	GASTRAT (5)	QQYNNWPRT (6)

[2] The VL CDRs in Table 3 are determined according to Kabat.

Table 4. VH framework (FR) sequences of exemplary anti-PD-1 antibodies [3]

Antibody	VH FR1 (SEQ ID NO:)	VH FR2 (SEQ ID NO:)	VH FR3 (SEQ ID NO:)	VH FR4 (SEQ ID NO:)
AGEN2033w	QVQLVESGGGVVQPGRSLRLSCAASGFTFS (38)	WVRQAPGKGLEWVA (40)	RFTISRDNSKNTLYLQMNSLRAEDTAVYYCAS (41)	WGQGTLVTVSS (43)
AGEN2034w	QVQLVESGGGVVQPGRSLRLSCAASGFTFS (38)	WVRQAPGKGLEWVA (40)	RFTISRDNSKNTLYLQMNSLRAEDTAVYYCAS (41)	WGQGTLVTVSS (43)
AGEN2001w	QVQLVESGGGVVQPGRSLRLSCAASGFTFS (38)	WVRQAPGKGLEWVA (40)	RFTISRDNSKNTLYLQMNSLRAEDTAVYYCAT (42)	WGQGTLVTVSS (43)
AGEN2002w	QVQLVESGGGVVQPGRSLRLSCAASGFTFS (38)	WVRQAPGKGLEWVA (40)	RFTISRDNSKNTLYLQMNSLRAEDTAVYYCAS (41)	WGQGTLVTVSS (43)
EP11_pl1_B03	QVQLVESGGGVVQPGRSLRLSCAASGFTFS (38)	WVRQAPGKGLEWVA (40)	RFTISRDNSKNTLYLQMNSLRAEDTAVYYCAS (41)	WGQGTLVTVSS (43)
EP11_pl1_B05	QVQLVESGGGMVQPGRSLRLSCAASGFTFS (39)	WVRQAPGKGLEWVA (40)	RFTISRDNSKNTLYLQMNSLRAEDTAVYYCAS (41)	WGQGTLVTVSS (43)
EP11_pl1_C02	QVQLVESGGGVVQPGRSLRLSCAASGFTFS (38)	WVRQAPGKGLEWVA (40)	RFTISRDNSKNTLYLQMNSLRAEDTAVYYCAS (41)	WGHGTLVTVSS (44)

| EP11_pl1_C 03 | QVQLVESGGGVVQPGR SLRLSCAASGFTFS (38) | WVRQAPGKG LEWVA (40) | RFTISRDNSKNTLYLQMN SLRAEDTAVYYCAS (41) | WGQGTLVT VSS (43) |

[3] The VH framework regions described in Table 4 are determined based upon the boundaries of the Kabat numbering system for CDRs. In other words, the VH CDRs are determined by Kabat and the framework regions are the amino acid residues surrounding the CDRs in the variable region in the format FR1, CDRH1, FR2, CDRH2, FR3, CDRH3, and FR4.

Table 5. VL framework (FR) sequences of exemplary anti-PD-1 antibodies [4]

Antibody	VL FR1 (SEQ ID NO:)	VL FR2 (SEQ ID NO:)	VL FR3 (SEQ ID NO:)	VL FR4 (SEQ ID NO:)
AGEN2033w	EIVMTQSPATLSVS PGERATLSC (45)	WYQQKPGQA PRLLIY (46)	GIPARFSGSGSGTEFTLT ISSLQSEDFAVYYC (47)	FGQGTKVEIK (48)
AGEN2034w	EIVMTQSPATLSVS PGERATLSC (45)	WYQQKPGQA PRLLIY (46)	GIPARFSGSGSGTEFTLT ISSLQSEDFAVYYC (47)	FGQGTKVEIK (48)
AGEN2001w	EIVMTQSPATLSVS PGERATLSC (45)	WYQQKPGQA PRLLIY (46)	GIPARFSGSGSGTEFTLT ISSLQSEDFAVYYC (47)	FGQGTKVEIK (48)
AGEN2002w	EIVMTQSPATLSVS PGERATLSC (45)	WYQQKPGQA PRLLIY (46)	GIPARFSGSGSGTEFTLT ISSLQSEDFAVYYC (47)	FGQGTKVEIK (48)
EP11_pl1_B03	EIVMTQSPATLSVS PGERATLSC (45)	WYQQKPGQA PRLLIY (46)	GIPARFSGSGSGTEFTLT ISSLQSEDFAVYYC (47)	FGQGTKVEIK (48)
EP11_pl1_B05	EIVMTQSPATLSVS PGERATLSC (45)	WYQQKPGQA PRLLIY (46)	GIPARFSGSGSGTEFTLT ISSLQSEDFAVYYC (47)	FGQGTKVEIK (48)
EP11_pl1_C02	EIVMTQSPATLSVS PGERATLSC (45)	WYQQKPGQA PRLLIY (46)	GIPARFSGSGSGTEFTLT ISSLQSEDFAVYYC (47)	FGQGTKVEIK (48)
EP11_pl1_C03	EIVMTQSPATLSVS PGERATLSC (45)	WYQQKPGQA PRLLIY (46)	GIPARFSGSGSGTEFTLT ISSLQSEDFAVYYC (47)	FGQGTKVEIK (48)

[4] The VL framework regions described in Table 5 are determined based upon the boundaries of the Kabat numbering system for CDRs. In other words, the VL CDRs are determined by Kabat and the framework regions are the amino acid residues surrounding the CDRs in the variable region in the format FR1, CDRL1, FR2, CDRL2, FR3, CDRL3, and FR4.

Table 6. VH and VL sequences of exemplary anti-PD-1 antibodies

Antibody	Heavy chain variable region	SEQ ID NO:	Light chain variable region	SEQ ID NO:
AGEN2033w	BADD438-2742	15	3738	16
AGEN2034w	BADD438-2744	17	3738	16

AGEN2001w	BADD426-2614	26	3738	16
AGEN2002w	BADD426-2615	27	3738	16
EP11_pl1_B03	BADD438-2743	28	3738	16
EP11_pl1_B05	BADD438-2745	29	3738	16
EP11_pl1_C02	BADD438-2746	30	3738	16
EP11_pl1_C03	BADD438-2747	31	3738	16

Table 7. Exemplary sequences of PD-1

SEQ ID NO:	Description	Amino acid Sequence
74	Exemplary mature PD-1 sequence	PGWFLDSPDRPWNPPTFSPALLVVTEGDNATFTCSFSNTS ESFVLNWYRMSPSNQTDKLAAFPEDRSQPGQDCRFRVTQ LPNGRDFHMSVVRARRNDSGTYLCGAISLAPKAQIKESLR AELRVTERRAEVPTAHPSPSPRPAGQFQTLVVGVVGGLL GSLVLLVWVLAVICSRAARGTIGARRTGQPLKEDPSAVPV FSVDYGELDFQWREKTPEPPVPCVPEQTEYATIVFPSGMG TSSPARRGSADGPRSAQPLRPEDGHCSWPL
75	PD-1 epitope (residues 107-122)	SLAPKAQIKESLRAEL
76	PD-1 epitope (residues 5-22)	LDSPDRPWNPPTFSPALL
77	PD-1 epitope (residues 6-15)	DSPDRPWNPP
78	PD-1 epitope (residues 130-138)	EVPTAHPSP
79	PD-1 epitope (residues 106-113)	ISLAPKAQ

[00137]   In certain embodiments, the instant disclosure provides an isolated antibody that specifically binds to human PD-1, comprising:

(a)   CDRH1 comprises the amino acid sequence of SYGMH (SEQ ID NO: 1); and/or

(b)   CDRH2 comprises the amino acid sequence of VIWX$_1$DGSNX$_2$YYADSVX$_3$G (SEQ ID NO: 32), wherein

X$_1$ is Y or F;

X$_2$ is K or E; and

X$_3$ is K or M; and/or

(c)   CDRH3 comprises the amino acid sequence of NX$_1$DX$_2$ (SEQ ID NO: 33), wherein

X$_1$ is G or V; and

X$_2$ is H or Y; and/or

(d)   CDRL1 comprises the amino acid sequence of RASQSVSSNLA (SEQ ID NO: 4);

and/or

(e)   CDRL2 comprises the amino acid sequence of GASTRAT (SEQ ID NO: 5); and/or

(f)   CDRL3 comprises the amino acid sequence of QQYNNWPRT (SEQ ID NO: 6).

[00138]   In certain embodiments, the antibody comprises one, two, or all three of the VH CDRs above. In certain embodiments, the antibody comprises the CDRH1 of one of the antibodies in Table 2. In certain embodiments, the antibody comprises the CDRH2 of one of the antibodies in Table 2. In certain embodiments, the antibody comprises the CDRH3 of one of the antibodies in Table 2. In certain embodiments, the antibody comprises one, two, or all three of VH CDRs of one of the antibodies in Table 2 (*e.g.*, the VH CDRs in one row of Table 2, for example, all of the VH CDRs from AGEN2033w or AGEN2034w). In certain embodiments, the antibody comprises the VH frameworks described herein. In certain embodiments, the antibody comprises the VH framework regions of an antibody set forth in Table 4 (*e.g.*, one, two, three, or four of the framework regions in one row of Table 4).

[00139]   In certain embodiments, the antibody comprises one, two, or all three of the VL CDRs above. In certain embodiments, the antibody comprises the CDRL1 of one of the antibodies in Table 3. In certain embodiments, the antibody comprises the CDRL2 of one of the antibodies in Table 3. In certain embodiments, the antibody comprises the CDRL3 of one of the antibodies in Table 3. In certain embodiments, the antibody comprises one, two, or all three of the VL CDRs of one of the antibodies in Table 3 (*e.g.*, the VL CDRs in one row of Table 3, for example, all of the VL CDRs from AGEN2033w or AGEN2034w). In certain embodiments, the antibody comprises the VL framework regions described herein. In certain embodiments, the antibody comprises the VL framework regions (FRs) of an antibody set forth in Table 5 (*e.g.*, one, two, three, or four of the framework regions in one row of Table 5).

[00140]   In certain embodiments, the instant disclosure provides an isolated antibody that specifically binds to human PD-1, comprising a heavy chain variable region comprising complementarity determining regions CDRH1, CDRH2 and CDRH3 and a light chain variable region comprising complementarity determining regions CDRL1, CDRL2 and CDRL3, wherein:

(a)   CDRH1 comprises the amino acid sequence of SYGMH (SEQ ID NO: 1);

(b)   CDRH2 comprises the amino acid sequence of VIWX$_1$DGSNX$_2$YYADSVX$_3$G (SEQ ID NO: 32), wherein

X$_1$ is Y or F;

X$_2$ is K or E; and

X_3 is K or M;

(c)   CDRH3 comprises the amino acid sequence of NX_1DX_2 (SEQ ID NO: 33), wherein

X_1 is G or V; and

X_2 is H or Y;

(d)   CDRL1 comprises the amino acid sequence of RASQSVSSNLA (SEQ ID NO: 4);

(e)   CDRL2 comprises the amino acid sequence of GASTRAT (SEQ ID NO: 5); and

(f)   CDRL3 comprises the amino acid sequence of QQYNNWPRT (SEQ ID NO: 6).

[00141]   In certain embodiments, the CDRH2 comprises an amino acid sequence selected from the group consisting of SEQ ID NOs: 2 and 34-36. In certain embodiments, the CDRH3 comprises an amino acid sequence selected from the group consisting of SEQ ID NOs: 3, 7, and 37.

[00142]   In certain embodiments, the instant disclosure provides an isolated antibody that specifically binds to human PD-1, comprising a heavy chain variable region comprising complementarity determining regions CDRH1, CDRH2 and CDRH3, wherein the CDRH1, CDRH2 and CDRH3 comprise the CDRH1, CDRH2 and CDRH3 amino acid sequences, respectively, set forth in SEQ ID NOs: 1, 2, and 3; 1, 2, and 7; 1, 2, and 37; 1, 34, and 7; 1, 35, and 7; or 1, 36, and 7.

[00143]   In certain embodiments, the instant disclosure provides an isolated antibody that specifically binds to human PD-1, comprising a heavy chain variable region comprising complementarity determining regions CDRH1, CDRH2 and CDRH3, and a light chain variable region comprising complementarity determining regions CDRL1, CDRL2 and CDRL3, wherein CDRH1, CDRH2, CDRH3, CDRL1, CDRL2 and CDRL3 comprise the CDRH1, CDRH2, CDRH3, CDRL1, CDRL2 and CDRL3 amino acid sequences, respectively, set forth in SEQ ID NOs: 1, 2, 3, 4, 5, and 6; 1, 2, 7, 4, 5, and 6; 1, 2, 37, 4, 5, and 6; 1, 34, 7, 4, 5, and 6; 1, 35, 7, 4, 5, and 6; or 1, 36, 7, 4, 5, and 6, respectively.

[00144]   In certain embodiments, the instant disclosure provides an isolated antibody that specifically binds to human PD-1, comprising:

(a)   a CDRH1 comprising the amino acid sequence of SYGMH (SEQ ID NO: 1); and/or

(b)   a CDRH2 comprising the amino acid sequence of VIWYDGSNKYYADSVKG (SEQ ID NO: 2); and/or

(c)   a CDRH3 comprising the amino acid sequence of NVDY (SEQ ID NO: 3) or NGDH (SEQ ID NO: 7); and/or

(d)   a CDRL1 comprising the amino acid sequence of RASQSVSSNLA (SEQ ID NO: 4); and/or

(e)   a CDRL2 comprising the amino acid sequence of GASTRAT (SEQ ID NO: 5); and/or

(f)   a CDRL3 comprising the amino acid sequence of QQYNNWPRT (SEQ ID NO: 6).

[00145]   In certain embodiments, the antibody comprises one, two, or all three of the VH CDRs set forth in SEQ ID NOs: 1, 2, and 3. In certain embodiments, the antibody comprises one, two, or all three of the VH CDRs set forth in SEQ ID NOs: 1, 2, and 7. In certain embodiments, the antibody comprises one, two, or all three of the VL CDRs set forth in SEQ ID NOs: 4, 5, and 6.

[00146]   In certain embodiments, the instant disclosure provides an isolated antibody that specifically binds to human PD-1, comprising a heavy chain variable region comprising complementarity determining regions CDRH1, CDRH2 and CDRH3, wherein the CDRH1, CDRH2 and CDRH3 comprise the CDRH1, CDRH2 and CDRH3 amino acid sequences set forth in SEQ ID NOs: 1, 2, and 3, respectively.

[00147]   In certain embodiments, the instant disclosure provides an isolated antibody that specifically binds to human PD-1, comprising a heavy chain variable region comprising complementarity determining regions CDRH1, CDRH2 and CDRH3, wherein the CDRH1, CDRH2 and CDRH3 comprise the CDRH1, CDRH2 and CDRH3 amino acid sequences set forth in SEQ ID NOs: 1, 2, and 7, respectively.

[00148]   In certain embodiments, the instant disclosure provides an isolated antibody that specifically binds to human PD-1, comprising a light chain variable region comprising complementarity determining regions CDRL1, CDRL2 and CDRL3, wherein the CDRL1, CDRL2 and CDRL3 comprise the CDRL1, CDRL2 and CDRL3 amino acid sequences set forth in SEQ ID NOs: 4, 5, and 6, respectively.

[00149]   In certain embodiments, the antibody comprises a heavy chain variable region comprising CDRH1, CDRH2, and CDRH3 regions, and a light chain variable region comprising CDRL1, CDRL2, and CDRL3 regions, wherein the CDRH1, CDRH2, CDRH3, CDRL1, CDRL2, and CDRL3 regions comprise the amino acid sequences set forth in SEQ ID NOs: 1, 2, 3, 4, 5, and 6, respectively.

[00150]   In certain embodiments, the antibody comprises a heavy chain variable region comprising CDRH1, CDRH2, and CDRH3 regions, and a light chain variable region comprising CDRL1, CDRL2, and CDRL3 regions, wherein the CDRH1, CDRH2, CDRH3, CDRL1, CDRL2, and CDRL3 regions comprise the amino acid sequences set forth in SEQ ID NOs: 1, 2, 7, 4, 5, and 6, respectively.

[00151]   In certain embodiments, the CDRs of an antibody can be determined according to the Chothia numbering scheme, which refers to the location of immunoglobulin structural

loops (*see, e.g.*, Chothia C & Lesk AM, (1987), J Mol Biol 196: 901-917; Al-Lazikani B *et al.*, (1997) J Mol Biol 273: 927-948; Chothia C *et al.*, (1992) J Mol Biol 227: 799-817; Tramontano A *et al.*, (1990) J Mol Biol 215(1): 175-82; and U.S. Patent No. 7,709,226, all of which are herein incorporated by reference in their entireties). Typically, when using the Kabat numbering convention, the Chothia CDRH1 loop is present at heavy chain amino acids 26 to 32, 33, or 34, the Chothia CDRH2 loop is present at heavy chain amino acids 52 to 56, and the Chothia CDRH3 loop is present at heavy chain amino acids 95 to 102, while the Chothia CDRL1 loop is present at light chain amino acids 24 to 34, the Chothia CDRL2 loop is present at light chain amino acids 50 to 56, and the Chothia CDRL3 loop is present at light chain amino acids 89 to 97. The end of the Chothia CDRH1 loop when numbered using the Kabat numbering convention varies between H32 and H34 depending on the length of the loop (this is because the Kabat numbering scheme places the insertions at H35A and H35B; if neither 35A nor 35B is present, the loop ends at 32; if only 35A is present, the loop ends at 33; if both 35A and 35B are present, the loop ends at 34).

[00152] In certain embodiments, the instant disclosure provides an isolated antibody that specifically binds to human PD-1, the antibody comprising the Chothia VL CDRs of a VL of an antibody disclosed in Table 6 herein (*e.g.*, AGEN2033w or AGEN2034w). In certain embodiments, the instant disclosure provides an isolated antibody that specifically binds to human PD-1, the antibody comprising the Chothia VH CDRs of an antibody disclosed in Table 6 herein (*e.g.*, AGEN2033w or AGEN2034w). In certain embodiments, the instant disclosure provides an isolated antibody that specifically binds to human PD-1, the antibody comprising the Chothia VH CDRs and Chothia VL CDRs of an antibody disclosed in Table 6 herein (*e.g.*, AGEN2033w or AGEN2034w). In certain embodiments, antibodies that specifically bind to human PD-1 comprise one or more CDRs, in which the Chothia and Kabat CDRs have the same amino acid sequence. In certain embodiments, the instant disclosure provides an isolated antibody that specifically binds to human PD-1 and comprises combinations of Kabat CDRs and Chothia CDRs.

[00153] In certain embodiments, the CDRs of an antibody can be determined according to the IMGT numbering system as described in Lefranc M-P, (1999) The Immunologist 7: 132-136 and Lefranc M-P *et al.*, (1999) Nucleic Acids Res 27: 209-212, each of which is herein incorporated by reference in its entirety. According to the IMGT numbering scheme, CDRH1 is at positions 26 to 35, CDRH2 is at positions 51 to 57, CDRH3 is at positions 93 to 102, CDRL1 is at positions 27 to 32, CDRL2 is at positions 50 to 52, and CDRL3 is at positions 89 to 97. In a particular embodiment, the instant disclosure provides antibodies that

specifically bind to human PD-1 and comprise CDRs of an antibody disclosed in Table 6 herein (*e.g.*, AGEN2033w or AGEN2034w) as determined by the IMGT numbering system, for example, as described in Lefranc M-P (1999) *supra* and Lefranc M-P *et al.*, (1999) *supra*.

[00154]   In certain embodiments, the CDRs of an antibody can be determined according to MacCallum RM *et al.*, (1996) J Mol Biol 262: 732-745, herein incorporated by reference in its entirety. *See also*, *e.g.*, Martin A. "Protein Sequence and Structure Analysis of Antibody Variable Domains," in *Antibody Engineering*, Kontermann and Dübel, eds., Chapter 31, pp. 422-439, Springer-Verlag, Berlin (2001), herein incorporated by reference in its entirety. In a particular embodiment, the instant disclosure provides antibodies that specifically bind to human PD-1 and comprise CDRs of an antibody disclosed in Table 6 herein (*e.g.*, AGEN2033w or AGEN2034w) as determined by the method in MacCallum RM *et al.*, (1996) *supra*.

[00155]   In certain embodiments, the CDRs of an antibody can be determined according to the AbM numbering scheme, which refers to AbM hypervariable regions, which represent a compromise between the Kabat CDRs and Chothia structural loops, and are used by Oxford Molecular's AbM antibody modeling software (Oxford Molecular Group, Inc.), herein incorporated by reference in its entirety. In a particular embodiment, the instant disclosure provides antibodies that specifically bind to human PD-1 and comprise CDRs of an antibody disclosed in Table 6 herein (*e.g.*, AGEN2033w or AGEN2034w) as determined by the AbM numbering scheme.

[00156]   Accordingly, in certain embodiments, the instant disclosure provides an isolated antibody that specifically binds to human PD-1, wherein the antibody comprises a heavy chain variable region comprising the CDRH1, CDRH2, and CDRH3 region amino acid sequences set forth in SEQ ID NO: 15, and the CDRL1, CDRL2, and CDRL3 region amino acid sequences set forth in SEQ ID NO: 16, wherein each CDR is defined in accordance with the Kabat definition, the Chothia definition, the combination of the Kabat definition and the Chothia definition, the IMGT numbering system, the AbM definition, or the contact definition of CDR.

[00157]   Accordingly, in certain embodiments, the instant disclosure provides an isolated antibody that specifically binds to human PD-1, wherein the antibody comprises a heavy chain variable region comprising the CDRH1, CDRH2, and CDRH3 region amino acid sequences set forth in SEQ ID NO: 17, and the CDRL1, CDRL2, and CDRL3 region amino acid sequences set forth in SEQ ID NO: 16, wherein each CDR is defined in accordance with the Kabat definition, the Chothia definition, the combination of the Kabat definition and the

Chothia definition, the IMGT numbering system, the AbM definition, or the contact definition of CDR.

[00158]   In certain embodiments, the instant disclosure provides an isolated antibody that specifically binds to human PD-1, the antibody comprising a heavy chain variable region comprising CDRH1, CDRH2, and CDRH3 regions, and a light chain variable region comprising CDRL1, CDRL2, and CDRL3 regions, wherein the CDRH1, CDRH2, CDRH3, CDRL1, CDRL2, and CDRL3 regions comprise the amino acid sequences set forth in SEQ ID NOs: 8, 9, 10, 11, 12, and 13, respectively.

[00159]   In certain embodiments, the instant disclosure provides an isolated antibody that specifically binds to human PD-1, the antibody comprising a heavy chain variable region comprising CDRH1, CDRH2, and CDRH3 regions, and a light chain variable region comprising CDRL1, CDRL2, and CDRL3 regions, wherein the CDRH1, CDRH2, CDRH3, CDRL1, CDRL2, and CDRL3 regions comprise the amino acid sequences set forth in SEQ ID NOs: 8, 9, 14, 11, 12, and 13, respectively.

[00160]   In certain embodiments, the instant disclosure provides an isolated antibody that specifically binds to human PD-1, comprising a heavy chain variable region comprising the amino acid sequence of SEQ ID NO: 49.

[00161]   In certain embodiments, the instant disclosure provides an isolated antibody that specifically binds to human PD-1, comprising a heavy chain variable region having an amino acid sequence derived from a human IGHV3-33 germline sequence.   One or more regions selected from framework 1, framework 2, framework 3, CDRH1, and CDRH2 (*e.g.*, two, three, four or five of these regions) can be derived from a human IGHV3-33 germline sequence.   In one embodiment, framework 1, framework 2, framework 3, CDRH1, and CDRH2 are all derived from a human IGHV3-33 germline sequence.   In certain embodiments, the instant disclosure provides an isolated antibody that specifically binds to human PD-1, comprising a heavy chain variable region comprising an amino acid sequence that is at least 75%, 80%, 85%, 90%, 95%, or 100% (*e.g.*, at least 86, 87, 88, 89, 90, 91, 92, 93, 94, 95, 96, 97, 98 or 99%) identical to an amino acid sequence selected from the group consisting of SEQ ID NOs: 15, 17, and 26-31.   In certain embodiments, the antibody comprises a heavy chain variable region having an amino acid sequence selected from the group consisting of SEQ ID NOs: 15, 17, and 26-31.   In certain embodiments, the antibody comprises a heavy chain variable region having the amino acid sequence set forth in SEQ ID NO: 15.   In certain embodiments, the antibody comprises a heavy chain variable region having the amino acid sequence set forth in SEQ ID NO: 17.   In certain embodiments, the

antibody comprises a heavy chain variable region having the amino acid sequence set forth in SEQ ID NO: 26. In certain embodiments, the antibody comprises a heavy chain variable region having the amino acid sequence set forth in SEQ ID NO: 27. In certain embodiments, the antibody comprises a heavy chain variable region having the amino acid sequence set forth in SEQ ID NO: 28. In certain embodiments, the antibody comprises a heavy chain variable region having the amino acid sequence set forth in SEQ ID NO: 29. In certain embodiments, the antibody comprises a heavy chain variable region having the amino acid sequence set forth in SEQ ID NO: 30. In certain embodiments, the antibody comprises a heavy chain variable region having the amino acid sequence set forth in SEQ ID NO: 31. In certain embodiments, the antibody comprises a heavy chain having the amino acid sequence set forth in SEQ ID NO: 18. In certain embodiments, the antibody comprises a heavy chain having the amino acid sequence set forth in SEQ ID NO: 21. In certain embodiments, the antibody comprises a heavy chain having the amino acid sequence set forth in SEQ ID NO: 20. In certain embodiments, the antibody comprises a heavy chain having the amino acid sequence set forth in SEQ ID NO: 22. In certain embodiments, the antibody comprises a heavy chain having the amino acid sequence set forth in SEQ ID NO: 23. In certain embodiments, the antibody comprises a heavy chain having the amino acid sequence set forth in SEQ ID NO: 24. In certain embodiments, the antibody comprises a heavy chain having the amino acid sequence set forth in SEQ ID NO: 25. In certain embodiments, the antibody comprises a heavy chain having the amino acid sequence set forth in SEQ ID NO: 52. In certain embodiments, the antibody comprises a heavy chain having the amino acid sequence set forth in SEQ ID NO: 53. In certain embodiments, the antibody comprises a heavy chain having the amino acid sequence set forth in SEQ ID NO: 54. In certain embodiments, the antibody comprises a heavy chain having the amino acid sequence set forth in SEQ ID NO: 55. In certain embodiments, the antibody comprises a heavy chain having the amino acid sequence set forth in SEQ ID NO: 56. In certain embodiments, the antibody comprises a heavy chain having the amino acid sequence set forth in SEQ ID NO: 57. In certain embodiments, the antibody comprises a heavy chain having the amino acid sequence set forth in SEQ ID NO: 58.

[00162] In certain embodiments, the instant disclosure provides an isolated antibody that specifically binds to human PD-1, comprising a light chain variable region having an amino acid sequence derived from a human IGKV3-15 germline sequence. One or more regions selected from framework 1, framework 2, framework 3, CDRL1, and CDRL2 (*e.g.*, two, three, four or five of these regions) can be derived from a human IGKV3-15 germline

sequence. In one embodiment, framework 1, framework 2, framework 3, CDRL1, and CDRL2 are all derived from a human IGKV3-15 germline sequence. In certain embodiments, the instant disclosure provides an isolated antibody that specifically binds to human PD-1, comprising a light chain variable region comprising an amino acid sequence that is at least 75%, 80%, 85%, 90%, 95%, or 100% (*e.g.*, at least 86, 87, 88, 89, 90, 91, 92, 93, 94, 95, 96, 97, 98 or 99%) identical to the amino acid sequence set forth in SEQ ID NO: 16. In certain embodiments, the antibody comprises a light chain variable region having the amino acid sequence set forth in SEQ ID NO: 16. In certain embodiments, the antibody comprises a light chain having the amino acid sequence set forth in SEQ ID NO: 19.

[00163]   In certain embodiments, the instant disclosure provides an isolated antibody that specifically binds to human PD-1, comprising a heavy chain variable region comprising an amino acid sequence that is at least 75%, 80%, 85%, 90%, 95%, or 100% (*e.g.*, at least 86, 87, 88, 89, 90, 91, 92, 93, 94, 95, 96, 97, 98 or 99%) identical to the amino acid sequence set forth in SEQ ID NO: 15 or SEQ ID NO: 17, and a light chain variable region comprising an amino acid sequence that is at least 75%, 80%, 85%, 90%, 95%, or 100% (*e.g.*, at least 86, 87, 88, 89, 90, 91, 92, 93, 94, 95, 96, 97, 98 or 99%) identical to the amino acid sequence set forth in SEQ ID NO: 16. In certain embodiments, the antibody comprises a heavy chain variable region having an amino acid sequence selected from the group consisting of SEQ ID NO: 15, 17, and 26-31, and a light chain variable region having the amino acid sequence set forth in SEQ ID NO: 16.

[00164]   In certain embodiments, the antibody comprises the heavy chain variable region and light chain variable region amino acid sequences set forth in SEQ ID NOs: 15 and 16; 17 and 16; 26 and 16; 27 and 16; 28 and 16; 29 and 16; 30 and 16; or 31 and 16, respectively. In certain embodiments, the antibody comprises a heavy chain variable region having the amino acid sequence set forth in SEQ ID NO: 15, and a light chain variable region having the amino acid sequence set forth in SEQ ID NO: 16. In certain embodiments, the antibody comprises a heavy chain variable region having the amino acid sequence set forth in SEQ ID NO: 17, and a light chain variable region having the amino acid sequence set forth in SEQ ID NO: 16. In certain embodiments, the antibody comprises a heavy chain variable region having the amino acid sequence set forth in SEQ ID NO: 26, and a light chain variable region having the amino acid sequence set forth in SEQ ID NO: 16. In certain embodiments, the antibody comprises a heavy chain variable region having the amino acid sequence set forth in SEQ ID NO: 27, and a light chain variable region having the amino acid sequence set forth in SEQ ID NO: 16. In certain embodiments, the antibody comprises a heavy chain variable region having the amino

acid sequence set forth in SEQ ID NO: 28, and a light chain variable region having the amino acid sequence set forth in SEQ ID NO: 16. In certain embodiments, the antibody comprises a heavy chain variable region having the amino acid sequence set forth in SEQ ID NO: 29, and a light chain variable region having the amino acid sequence set forth in SEQ ID NO: 16. In certain embodiments, the antibody comprises a heavy chain variable region having the amino acid sequence set forth in SEQ ID NO: 30, and a light chain variable region having the amino acid sequence set forth in SEQ ID NO: 16. In certain embodiments, the antibody comprises a heavy chain variable region having the amino acid sequence set forth in SEQ ID NO: 31, and a light chain variable region having the amino acid sequence set forth in SEQ ID NO: 16. In certain embodiments, the antibody comprises a heavy chain comprising the amino acid sequence of SEQ ID NO: 18; and a light chain comprising the amino acid sequence of SEQ ID NO: 19. In certain embodiments, the antibody comprises a heavy chain comprising the amino acid sequence of SEQ ID NO: 21; and a light chain comprising the amino acid sequence of SEQ ID NO: 19. In certain embodiments, the antibody comprises a heavy chain comprising the amino acid sequence of SEQ ID NO: 20; and a light chain comprising the amino acid sequence of SEQ ID NO: 19. In certain embodiments, the antibody comprises a heavy chain comprising the amino acid sequence of SEQ ID NO: 22; and a light chain comprising the amino acid sequence of SEQ ID NO: 19. In certain embodiments, the antibody comprises a heavy chain comprising the amino acid sequence of SEQ ID NO: 23; and a light chain comprising the amino acid sequence of SEQ ID NO: 19. In certain embodiments, the antibody comprises a heavy chain comprising the amino acid sequence of SEQ ID NO: 24; and a light chain comprising the amino acid sequence of SEQ ID NO: 19. In certain embodiments, the antibody comprises a heavy chain comprising the amino acid sequence of SEQ ID NO: 25; and a light chain comprising the amino acid sequence of SEQ ID NO: 19. In certain embodiments, the antibody comprises a heavy chain comprising the amino acid sequence of SEQ ID NO: 52; and a light chain comprising the amino acid sequence of SEQ ID NO: 19. In certain embodiments, the antibody comprises a heavy chain comprising the amino acid sequence of SEQ ID NO: 53; and a light chain comprising the amino acid sequence of SEQ ID NO: 19. In certain embodiments, the antibody comprises a heavy chain comprising the amino acid sequence of SEQ ID NO: 54; and a light chain comprising the amino acid sequence of SEQ ID NO: 19. In certain embodiments, the antibody comprises a heavy chain comprising the amino acid sequence of SEQ ID NO: 55; and a light chain comprising the amino acid sequence of SEQ ID NO: 19. In certain embodiments, the antibody comprises a heavy chain comprising the amino acid sequence of

SEQ ID NO: 56; and a light chain comprising the amino acid sequence of SEQ ID NO: 19. In certain embodiments, the antibody comprises a heavy chain comprising the amino acid sequence of SEQ ID NO: 57; and a light chain comprising the amino acid sequence of SEQ ID NO: 19. In certain embodiments, the antibody comprises a heavy chain comprising the amino acid sequence of SEQ ID NO: 58; and a light chain comprising the amino acid sequence of SEQ ID NO: 19.

[00165] In certain embodiments, the instant disclosure provides an isolated antibody that cross-competes for binding to human PD-1 with an antibody comprising the heavy and light chain variable region amino acid sequences set forth in SEQ ID NOs: 15 and 16, respectively.

[00166] In certain embodiments, the instant disclosure provides an isolated antibody that binds to the same epitope on human PD-1 as an antibody comprising the heavy and light chain variable region amino acid sequences set forth in SEQ ID NOs: 15 and 16, respectively.

[00167] In certain embodiments, the instant disclosure provides an isolated antibody that cross-competes for binding to human PD-1 with an antibody comprising the heavy and light chain variable region amino acid sequences set forth in SEQ ID NOs: 17 and 16, respectively.

[00168] In certain embodiments, the instant disclosure provides an isolated antibody that binds to the same epitope on human PD-1 as an antibody comprising the heavy and light chain variable region amino acid sequences set forth in SEQ ID NOs: 17 and 16, respectively.

[00169] In certain embodiments, the instant disclosure provides an isolated antibody that cross-competes for binding to human PD-1 with an antibody comprising the heavy and light chain variable region amino acid sequences set forth in SEQ ID NOs: 26 and 16, respectively.

[00170] In certain embodiments, the instant disclosure provides an isolated antibody that binds to the same epitope on human PD-1 as an antibody comprising the heavy and light chain variable region amino acid sequences set forth in SEQ ID NOs: 26 and 16, respectively.

[00171] In certain embodiments, the instant disclosure provides an isolated antibody that cross-competes for binding to human PD-1 with an antibody comprising the heavy and light chain variable region amino acid sequences set forth in SEQ ID NOs: 27 and 16, respectively.

[00172] In certain embodiments, the instant disclosure provides an isolated antibody that binds to the same epitope on human PD-1 as an antibody comprising the heavy and light chain variable region amino acid sequences set forth in SEQ ID NOs: 27 and 16, respectively.

[00173] In certain embodiments, the instant disclosure provides an isolated antibody that cross-competes for binding to human PD-1 with an antibody comprising the heavy and light chain variable region amino acid sequences set forth in SEQ ID NOs: 28 and 16, respectively.

[00174] In certain embodiments, the instant disclosure provides an isolated antibody that

binds to the same epitope on human PD-1 as an antibody comprising the heavy and light chain variable region amino acid sequences set forth in SEQ ID NOs: 28 and 16, respectively.

[00175] In certain embodiments, the instant disclosure provides an isolated antibody that cross-competes for binding to human PD-1 with an antibody comprising the heavy and light chain variable region amino acid sequences set forth in SEQ ID NOs: 29 and 16, respectively.

[00176] In certain embodiments, the instant disclosure provides an isolated antibody that binds to the same epitope on human PD-1 as an antibody comprising the heavy and light chain variable region amino acid sequences set forth in SEQ ID NOs: 29 and 16, respectively.

[00177] In certain embodiments, the instant disclosure provides an isolated antibody that cross-competes for binding to human PD-1 with an antibody comprising the heavy and light chain variable region amino acid sequences set forth in SEQ ID NOs: 30 and 16, respectively.

[00178] In certain embodiments, the instant disclosure provides an isolated antibody that binds to the same epitope on human PD-1 as an antibody comprising the heavy and light chain variable region amino acid sequences set forth in SEQ ID NOs: 30 and 16, respectively.

[00179] In certain embodiments, the instant disclosure provides an isolated antibody that cross-competes for binding to human PD-1 with an antibody comprising the heavy and light chain variable region amino acid sequences set forth in SEQ ID NOs: 31 and 16, respectively.

[00180] In certain embodiments, the instant disclosure provides an isolated antibody that binds to the same epitope on human PD-1 as an antibody comprising the heavy and light chain variable region amino acid sequences set forth in SEQ ID NOs: 31 and 16, respectively.

[00181] Any Ig constant region can be used in the antibodies disclosed herein. In certain embodiments, the Ig region is a human IgG_1, IgG_2, IgG_3, IgG_4, IgA_1, and IgA_2 heavy chain constant region. In certain embodiments, the Ig region is a human IgG_1. In certain embodiments, the Ig region is a human IgG_4. In certain embodiments, the Ig (e.g., IgG_1) lacks the N-linked glycan moiety that is normally present at position N297 (according to the EU numbering system) in mature wild-type IgG_1 antibodies *in vivo*. The lack of N297 glycan results in a substantial loss of effector function. Elimination of the N297 glycan can be achieved using any methods known in the art. For example, in certain embodiments, the elimination of the N297 glycan is achieved by mutation of the N297 residue to remove the glycosylation site. Accordingly, in certain embodiments, the antibodies disclosed herein comprise a heavy chain constant region (e.g., a human IgG_1 heavy chain constant region) comprising an N297A mutation, according to the EU numbering system. In certain embodiments, the antibodies disclosed herein comprise a heavy chain constant region (e.g., an IgG_1 heavy chain constant region) comprising an N297Q mutation, according to the EU

numbering system. In certain embodiments, the antibodies disclosed herein comprise an IgG$_1$ heavy chain constant region (*e.g.*, a human IgG$_1$ heavy chain constant region) comprising a D265A mutation, according to the EU numbering system. In certain embodiments, the antibodies disclosed herein comprise an IgG$_4$ heavy chain constant region (*e.g.*, a human IgG$_4$ heavy chain constant region) comprising an S228P mutation, according to the EU numbering system.

[00182] In certain embodiments, the instant disclosure provides an isolated antibody that specifically binds to human PD-1, the antibody comprising a heavy chain constant region comprising the amino acid sequence of SEQ ID NO: 59, 60, 61, 62, 63, or 64. In certain embodiments, the instant disclosure provides an isolated antibody that specifically binds to human PD-1, the antibody comprising a heavy chain constant region comprising the amino acid sequence of SEQ ID NO: 59. In certain embodiments, the instant disclosure provides an isolated antibody that specifically binds to human PD-1, the antibody comprising a heavy chain constant region comprising the amino acid sequence of SEQ ID NO: 60. In certain embodiments, the instant disclosure provides an isolated antibody that specifically binds to human PD-1, the antibody comprising a heavy chain constant region comprising the amino acid sequence of SEQ ID NO: 61. In certain embodiments, the instant disclosure provides an isolated antibody that specifically binds to human PD-1, the antibody comprising a heavy chain constant region comprising the amino acid sequence of SEQ ID NO: 62. In certain embodiments, the instant disclosure provides an isolated antibody that specifically binds to human PD-1, the antibody comprising a heavy chain constant region comprising the amino acid sequence of SEQ ID NO: 63. In certain embodiments, the instant disclosure provides an isolated antibody that specifically binds to human PD-1, the antibody comprising a heavy chain constant region comprising the amino acid sequence of SEQ ID NO: 64. In certain embodiments, the instant disclosure provides an isolated antibody that specifically binds to human PD-1, the antibody comprising a light chain constant region comprising the amino acid sequence of SEQ ID NO: 65.

[00183] In certain embodiments, the IgG regions of the antibodies described herein have an increased affinity for CD32B (also known as FcγRIIB or FCGR2B), *e.g.*, as compared with an antibody with a wild-type Fc region, *e.g.*, an IgG$_1$ Fc. In certain embodiments, antibodies described herein have a selectively increased affinity for CD32B (FcγRIIB) over both CD32A (FcγRIIA) and CD16 (FcγRIIIA). Sequence alterations that result in increased affinity for CD32B are known in the art, for example, in Mimoto *et al.*, *Protein Engineering, Design & Selection 10*: 589-598 (2013), Chu *et al.*, *Molecular Immunology 45*: 3926-3933

(2008), and Strohl, *Current Opinion in Biology* 20: 685-691 (2009), each of which is herein incorporated by reference in its entirety. In certain embodiments, the antibody comprises a heavy chain constant region, *e.g.*, an IgG$_1$ constant region, or fragment thereof comprising a mutation selected from the group consisting of: G236D, P238D, S239D, S267E, L328F, and L328E, and combinations thereof, numbered according to the EU numbering system. In certain embodiments, the antibody comprises a heavy chain constant region, *e.g.*, an IgG1 constant region, or fragment thereof comprising S267E and L328F substitutions, numbered according to the EU numbering system. In certain embodiments, the antibody comprises a heavy chain constant region, *e.g.*, an IgG$_1$ constant region, or fragment thereof comprising P238D and L328E substitutions, numbered according to the EU numbering system. In certain embodiments, the antibody comprises a heavy chain constant region, *e.g.*, an IgG$_1$ constant region, or fragment thereof comprising a P238D substitution and substitution selected from the group consisting of E233D, G237D, H268D, P271G, A330R, and combinations thereof, numbered according to the EU numbering system. In certain embodiments, the antibody comprises a heavy chain constant region, *e.g.*, an IgG$_1$ constant region, or fragment thereof comprising P238D, E233D, G237D, H268D, P271G, and A330R substitutions, numbered according to the EU numbering system. In certain embodiments, the antibody comprises a heavy chain constant region, *e.g.*, an IgG$_1$ constant region, or fragment thereof comprising G236D and S267E, numbered according to the EU numbering system. In certain embodiments, the antibody comprises a heavy chain constant region, *e.g.*, an IgG$_1$ constant region, or fragment thereof comprising S239D and S267E, numbered according to the EU numbering system. In certain embodiments, the antibody comprises a heavy chain constant region, *e.g.*, an IgG$_1$ constant region, or fragment thereof comprising V262E, S267E, and L328F, numbered according to the EU numbering system. In certain embodiments, the antibody comprises a heavy chain constant region, *e.g.*, an IgG$_1$ constant region, or fragment thereof comprising V264E, S267E, and L328F, numbered according to the EU numbering system.

[00184]   In certain embodiments, one, two, or more mutations (*e.g.*, amino acid substitutions) are introduced into the Fc region of an antibody described herein (*e.g.*, CH2 domain (residues 231-340 of human IgG$_1$) and/or CH3 domain (residues 341-447 of human IgG$_1$) and/or the hinge region, numbered according to the EU numbering system, to alter one or more functional properties of the antibody, such as serum half-life, complement fixation, Fc receptor binding and/or antigen-dependent cellular cytotoxicity.

[00185]   In certain embodiments, one, two, or more mutations (*e.g.*, amino acid

substitutions) are introduced into the hinge region of the Fc region (CH1 domain) such that the number of cysteine residues in the hinge region are altered (*e.g.*, increased or decreased) as described in, *e.g.*, U.S. Patent No. 5,677,425, herein incorporated by reference in its entirety. The number of cysteine residues in the hinge region of the CH1 domain may be altered to, *e.g.*, facilitate assembly of the light and heavy chains, or to alter (*e.g.*, increase or decrease) the stability of the antibody.

[00186]   In a specific embodiment, one, two, or more amino acid mutations (*e.g.*, substitutions, insertions or deletions) are introduced into an IgG constant domain, or FcRn-binding fragment thereof (preferably an Fc or hinge-Fc domain fragment) to alter (*e.g.*, decrease or increase) half-life of the antibody *in vivo*. *See, e.g.*, International Publication Nos. WO 02/060919; WO 98/23289; and WO 97/34631; and U.S. Patent Nos. 5,869,046, 6,121,022, 6,277,375 and 6,165,745, all of which are herein incorporated by reference in their entireties, for examples of mutations that will alter (*e.g.*, decrease or increase) the half-life of an antibody *in vivo*. In some embodiments, one, two or more amino acid mutations (*e.g.*, substitutions, insertions, or deletions) are introduced into an IgG constant domain, or FcRn-binding fragment thereof (preferably an Fc or hinge-Fc domain fragment) to decrease the half-life of the antibody *in vivo*. In other embodiments, one, two or more amino acid mutations (*e.g.*, substitutions, insertions or deletions) are introduced into an IgG constant domain, or FcRn-binding fragment thereof (preferably an Fc or hinge-Fc domain fragment) to increase the half-life of the antibody *in vivo*. In a specific embodiment, the antibodies may have one or more amino acid mutations (*e.g.*, substitutions) in the second constant (CH2) domain (residues 231-340 of human IgG$_1$) and/or the third constant (CH3) domain (residues 341-447 of human IgG$_1$), numbered according to the EU numbering system. In a specific embodiment, the constant region of the IgG$_1$ of an antibody described herein comprises a methionine (M) to tyrosine (Y) substitution in position 252, a serine (S) to threonine (T) substitution in position 254, and a threonine (T) to glutamic acid (E) substitution in position 256, numbered according to the EU numbering system. *See* U.S. Patent No. 7,658,921, which is herein incorporated by reference in its entirety. This type of mutant IgG, referred to as "YTE mutant" has been shown to display fourfold increased half-life as compared to wild-type versions of the same antibody (*see* Dall'Acqua WF *et al.*, (2006) J Biol Chem 281: 23514-24, which is herein incorporated by reference in its entirety). In certain embodiments, an antibody comprises an IgG constant domain comprising one, two, three or more amino acid substitutions of amino acid residues at positions 251-257, 285-290, 308-314, 385-389, and 428-436, numbered according to the EU numbering system.

[00187] In some embodiments, one, two, or more mutations (*e.g.*, amino acid substitutions) are introduced into the Fc region of an antibody described herein (*e.g.*, CH2 domain (residues 231-340 of human IgG_1) and/or CH3 domain (residues 341-447 of human IgG_1) and/or the hinge region, numbered according to the EU numbering system, to increase or decrease the affinity of the antibody for an Fc receptor (*e.g.*, an activated Fc receptor) on the surface of an effector cell. Mutations in the Fc region of an antibody that decrease or increase the affinity of an antibody for an Fc receptor and techniques for introducing such mutations into the Fc receptor or fragment thereof are known to one of skill in the art. Examples of mutations in the Fc receptor of an antibody that can be made to alter the affinity of the antibody for an Fc receptor are described in, *e.g.*, Smith P *et al.*, (2012) PNAS 109: 6181-6186, U.S. Patent No. 6,737,056, and International Publication Nos. WO 02/060919; WO 98/23289; and WO 97/34631, all of which are herein incorporated by reference in their entireties.

[00188] In a further embodiment, one, two, or more amino acid substitutions are introduced into an IgG constant domain Fc region to alter the effector function(s) of the antibody. For example, one or more amino acids selected from amino acid residues 234, 235, 236, 237, 297, 318, 320 and 322, numbered according to the EU numbering system, can be replaced with a different amino acid residue such that the antibody has an altered affinity for an effector ligand but retains the antigen-binding ability of the parent antibody. The effector ligand to which affinity is altered can be, for example, an Fc receptor or the C1 component of complement. This approach is described in further detail in U.S. Patent Nos. 5,624,821 and 5,648,260, each of which is herein incorporated by reference in its entirety. In some embodiments, the deletion or inactivation (through point mutations or other means) of a constant region domain may reduce Fc receptor binding of the circulating antibody thereby increasing tumor localization. *See, e.g.*, U.S. Patent Nos. 5,585,097 and 8,591,886, each of which is herein incorporated by reference in its entirety, for a description of mutations that delete or inactivate the constant domain and thereby increase tumor localization. In certain embodiments, one or more amino acid substitutions may be introduced into the Fc region of an antibody described herein to remove potential glycosylation sites on Fc region, which may reduce Fc receptor binding (*see, e.g.*, Shields RL *et al.*, (2001) J Biol Chem 276: 6591-604, which is herein incorporated by reference in its entirety). In various embodiments, one or more of the following mutations in the constant region of an antibody described herein may be made: an N297A substitution; an N297Q substitution; a L235A substitution and a L237A substitution; a L234A substitution and a L235A substitution; a E233P substitution; a L234V

substitution; a L235A substitution; a C236 deletion; a P238A substitution; a D265A substitution; a A327Q substitution; or a P329A substitution, numbered according to the EU numbering system. In certain embodiments, a mutation selected from the group consisting of D265A, P329A, and a combination thereof, numbered according to the EU numbering system, may be made in the constant region of an antibody described herein.

[00189] In a specific embodiment, an antibody described herein comprises the constant domain of an IgG_1 with an N297Q or N297A amino acid substitution, numbered according to the EU numbering system. In one embodiment, an antibody described herein comprises the constant domain of an IgG_1 with a mutation selected from the group consisting of D265A, P329A, and a combination thereof, numbered according to the EU numbering system. In another embodiment, an antibody described herein comprises the constant domain of an IgG_1 with a mutation selected from the group consisting of L234A, L235A, and a combination thereof, numbered according to the EU numbering system. In certain embodiments, amino acid residues in the constant region of an antibody described herein in the positions corresponding to positions L234, L235, and D265 in a human IgG_1 heavy chain, numbered according to the EU numbering system, are not L, L, and D, respectively. This approach is described in detail in International Publication No. WO 14/108483, which is herein incorporated by reference in its entirety. In a particular embodiment, the amino acids corresponding to positions L234, L235, and D265 in a human IgG_1 heavy chain are F, E, and A; or A, A, and A, respectively, numbered according to the EU numbering system.

[00190] In certain embodiments, one or more amino acids selected from amino acid residues 329, 331, and 322 in the constant region of an antibody described herein, numbered according to the EU numbering system, can be replaced with a different amino acid residue such that the antibody has altered C1q binding and/or reduced or abolished complement dependent cytotoxicity (CDC). This approach is described in further detail in U.S. Patent No. 6,194,551 (Idusogie *et al*), which is herein incorporated by reference in its entirety. In some embodiments, one or more amino acid residues within amino acid positions 231 to 238 in the N-terminal region of the CH2 domain of an antibody described herein are altered to thereby alter the ability of the antibody to fix complement, numbered according to the EU numbering system. This approach is described further in International Publication No. WO 94/29351, which is herein incorporated by reference in its entirety. In certain embodiments, the Fc region of an antibody described herein is modified to increase the ability of the antibody to mediate antibody dependent cellular cytotoxicity (ADCC) and/or to increase the affinity of the antibody for an Fcγ receptor by mutating one or more amino acids (*e.g.*, introducing

amino acid substitutions) at the following positions: 238, 239, 248, 249, 252, 254, 255, 256, 258, 265, 267, 268, 269, 270, 272, 276, 278, 280, 283, 285, 286, 289, 290, 292, 293, 294, 295, 296, 298, 301, 303, 305, 307, 309, 312, 315, 320, 322, 324, 326, 327, 328, 329, 330, 331, 333, 334, 335, 337, 338, 340, 360, 373, 376, 378, 382, 388, 389, 398, 414, 416, 419, 430, 434, 435, 437, 438, or 439, numbered according to the EU numbering system. This approach is described further in International Publication No. WO 00/42072, which is herein incorporated by reference in its entirety.

[00191]   In certain embodiments, an antibody described herein comprises the constant region of an IgG$_4$ antibody and the serine at amino acid residue 228 of the heavy chain, numbered according to the EU numbering system, is substituted for proline. In certain embodiments, the instant disclosure provides an isolated antibody that specifically binds to human PD-1, the antibody comprising a heavy chain constant region comprising the amino acid sequence of SEQ ID NO: 63. In certain embodiments, the instant disclosure provides an isolated antibody that specifically binds to human PD-1, the antibody comprising a heavy chain constant region comprising the amino acid sequence of SEQ ID NO: 64.

[00192]   In certain embodiments, any of the constant region mutations or modifications described herein can be introduced into one or both heavy chain constant regions of an antibody described herein having two heavy chain constant regions.

[00193]   In certain embodiments, the instant disclosure provides an isolated antibody that specifically binds to human PD-1 and functions as an antagonist.

[00194]   In certain embodiments, the instant disclosure provides an isolated antibody that specifically binds to human PD-1 and decreases PD-1 activity by at least 5%, 10%, 15%, 20%, 25%, 30%, 35%, 40%, 45%, 50%, 55%, 60%, 65%, 70%, 75%, 80%, 85%, 90%, 95%, 98%, or 99% as assessed by methods described herein and/or known to one of skill in the art, relative to PD-1 activity without any antibody or with an unrelated antibody (*e.g.*, an antibody that does not specifically bind to human PD-1). In certain embodiments, the instant disclosure provides an isolated antibody that specifically binds to human PD-1 and decreases PD-1 activity by at least about 1.2 fold, 1.3 fold, 1.4 fold, 1.5 fold, 2 fold, 2.5 fold, 3 fold, 3.5 fold, 4 fold, 4.5 fold, 5 fold, 6 fold, 7 fold, 8 fold, 9 fold, 10 fold, 15 fold, 20 fold, 30 fold, 40 fold, 50 fold, 60 fold, 70 fold, 80 fold, 90 fold, or 100 fold as assessed by methods described herein and/or known to one of skill in the art, relative to PD-1 activity without any antibody or with an unrelated antibody (*e.g.*, an antibody that does not specifically bind to human PD-1). Non-limiting examples of PD-1 activity can include PD-1 signaling, PD-1 binding to PD-1 ligand (*e.g.*, PD-L1 or PD-L2), inhibition of cytokine production (*e.g.*, IL-2 or IFNγ), and

inhibition of T cell proliferation. In certain embodiments, the instant disclosure provides an isolated antibody that specifically binds to human PD-1 and deactivates, reduces, or inhibits a PD-1 activity. In specific embodiments, a decrease in a PD-1 activity is assessed as described in the Examples, *infra.*

[00195] In specific embodiments, the instant disclosure provides an isolated antibody that specifically binds to human PD-1 and reduces PD-1 binding to its ligand (*e.g.*, PD-L1 or PD-L2) by at least about 5%, 10%, 15%, 20%, 25%, 30%, 35%, 40%, 45%, 50%, 55%, 60%, 65%, 70%, 75%, 80%, 85%, 90%, 95%, 98%, or 99%, as assessed by methods described herein (*see* the Examples, *infra*) or known to one of skill in the art, relative to PD-1 binding to its ligand (*e.g.*, PD-L1 or PD-L2) without any antibody or with an unrelated antibody (*e.g.*, an antibody that does not specifically bind to human PD-1). In specific embodiments, the instant disclosure provides an isolated antibody that specifically binds to human PD-1 and reduces PD-1 binding to its ligand (*e.g.*, PD-L1 or PD-L2) by at least about 1.2 fold, 1.3 fold, 1.4 fold, 1.5 fold, 2 fold, 2.5 fold, 3 fold, 3.5 fold, 4 fold, 4.5 fold, 5 fold, 6 fold, 7 fold, 8 fold, 9 fold, 10 fold, 15 fold, 20 fold, 30 fold, 40 fold, 50 fold, 60 fold, 70 fold, 80 fold, 90 fold, or 100 fold, as assessed by methods described herein (*see* the Examples, *infra*) or known to one of skill in the art, relative to PD-1 binding to its ligand (*e.g.*, PD-L1 or PD-L2) without any antibody or with an unrelated antibody (*e.g.*, an antibody that does not specifically bind to human PD-1).

[00196] In specific embodiments, the instant disclosure provides an isolated antibody that specifically binds to human PD-1 and increases cytokine production (*e.g.*, IL-2 or IFNγ) by at least about 5%, 10%, 15%, 20%, 25%, 30%, 35%, 40%, 45%, 50%, 55%, 60%, 65%, 70%, 75%, 80%, 85%, 90%, 95%, 98%, or 99%, as assessed by methods described herein (*see* the Examples, *infra*) or known to one of skill in the art, relative to cytokine production without any antibody or with an unrelated antibody (*e.g.*, an antibody that does not specifically bind to human PD-1). In specific embodiments, the instant disclosure provides an isolated antibody that specifically binds to human PD-1 and increases cytokine production (*e.g.*, IL-2 or IFNγ) by at least about 1.2 fold, 1.3 fold, 1.4 fold, 1.5 fold, 2 fold, 2.5 fold, 3 fold, 3.5 fold, 4 fold, 4.5 fold, 5 fold, 6 fold, 7 fold, 8 fold, 9 fold, 10 fold, 15 fold, 20 fold, 30 fold, 40 fold, 50 fold, 60 fold, 70 fold, 80 fold, 90 fold, or 100 fold, as assessed by methods described herein (*see* the Examples, *infra*) or known to one of skill in the art, relative to cytokine production without any antibody or with an unrelated antibody (*e.g.*, an antibody that does not specifically bind to human PD-1).

[00197] In specific embodiments, the instant disclosure provides an isolated antibody that

specifically binds to human PD-1 and either alone or in combination with an anti-CTLA-4 antibody (*e.g.*, ipilimumab or tremelimumab), an anti-TIGIT antibody, an anti-CD137 antibody (*e.g.*, urelumab or utomilumab), or an anti-OX40 antibody (*e.g.*, pogalizumab or tavolixizumab) increases IL-2 production in human peripheral blood mononuclear cells (PBMCs) in response to Staphylococcus Enterotoxin A (SEA) stimulation by at least about 1.2 fold, 1.3 fold, 1.4 fold, 1.5 fold, 2 fold, 2.5 fold, 3 fold, 3.5 fold, 4 fold, 4.5 fold, 5 fold, 6 fold, 7 fold, 8 fold, 9 fold, 10 fold, 15 fold, 20 fold, 30 fold, 40 fold, 50 fold, 60 fold, 70 fold, 80 fold, 90 fold, or 100 fold, as assessed by methods described herein (*see* the Examples, *infra*) or known to one of skill in the art, relative to IL-2 production without any antibody or with an unrelated antibody (*e.g.*, an antibody that does not specifically bind to human PD-1).

[00198]   In certain embodiments, human peripheral blood mononuclear cells (PBMCs) stimulated with Staphylococcus Enterotoxin A (SEA) in the presence of an antibody described herein, which specifically binds to human PD-1, either alone or in combination with an anti-CTLA-4 antibody (*e.g.*, ipilimumab or tremelimumab), an anti-TIGIT antibody, an anti-CD137 antibody (*e.g.*, urelumab or utomilumab), or an anti-OX40 antibody (*e.g.*, pogalizumab or tavolixizumab), have increased IL-2 production by at least about 1.2 fold, 1.3 fold, 1.4 fold, 1.5 fold, 2 fold, 2.5 fold, 3 fold, 3.5 fold, 4 fold, 4.5 fold, 5 fold, 6 fold, 7 fold, 8 fold, 9 fold, 10 fold, 15 fold, 20 fold, 30 fold, 40 fold, 50 fold, 60 fold, 70 fold, 80 fold, 90 fold, or 100 fold relative to PBMCs only stimulated with SEA without any antibody or with an unrelated antibody (*e.g.*, an antibody that does not specifically bind to human PD-1), as assessed by methods described herein (*see* the Examples, *infra*) or known to one of skill in the art.

[00199]   In specific embodiments, the instant disclosure provides an isolated antibody that specifically binds to human PD-1 and increases IFNγ production of a co-culture of human T cells and allogenic dendritic cells by at least about 1.2 fold, 1.3 fold, 1.4 fold, 1.5 fold, 2 fold, 2.5 fold, 3 fold, 3.5 fold, 4 fold, 4.5 fold, 5 fold, 6 fold, 7 fold, 8 fold, 9 fold, 10 fold, 15 fold, 20 fold, 30 fold, 40 fold, 50 fold, 60 fold, 70 fold, 80 fold, 90 fold, or 100 fold, as assessed by methods described herein (*see* the Examples, *infra*) or known to one of skill in the art, relative to IFNγ production of a co-culture of human T cells and allogenic dendritic cells without any antibody or with an unrelated antibody (*e.g.*, an antibody that does not specifically bind to human PD-1).

[00200]   In certain embodiments, a co-culture of human T cells and allogenic dendritic cells in the presence of an antibody described herein, which specifically binds to human PD-1, has increased IFNγ production by at least about 1.2 fold, 1.3 fold, 1.4 fold, 1.5 fold, 2 fold,

2.5 fold, 3 fold, 3.5 fold, 4 fold, 4.5 fold, 5 fold, 6 fold, 7 fold, 8 fold, 9 fold, 10 fold, 15 fold, 20 fold, 30 fold, 40 fold, 50 fold, 60 fold, 70 fold, 80 fold, 90 fold, or 100 fold relative to a co-culture of human T cells and allogenic dendritic cells without any antibody or with an unrelated antibody (*e.g.*, an antibody that does not specifically bind to human PD-1), as assessed by methods described herein (*see* the Examples, *infra*) or known to one of skill in the art.

[00201]   In specific embodiments, the instant disclosure provides an isolated antibody that specifically binds to human PD-1 and increases T cell proliferation by at least about 5%, 10%, 15%, 20%, 25%, 30%, 35%, 40%, 45%, 50%, 55%, 60%, 65%, 70%, 75%, 80%, 85%, 90%, 95%, 98%, or 99%, as assessed by methods described herein (*see* the Examples, *infra*) or known to one of skill in the art, relative to T cell proliferation without any antibody or with an unrelated antibody (*e.g.*, an antibody that does not specifically bind to human PD-1). In specific embodiments, the instant disclosure provides an isolated antibody that specifically binds to human PD-1 and increases T cell proliferation by at least about 1.2 fold, 1.3 fold, 1.4 fold, 1.5 fold, 2 fold, 2.5 fold, 3 fold, 3.5 fold, 4 fold, 4.5 fold, 5 fold, 6 fold, 7 fold, 8 fold, 9 fold, 10 fold, 15 fold, 20 fold, 30 fold, 40 fold, 50 fold, 60 fold, 70 fold, 80 fold, 90 fold, or 100 fold, as assessed by methods described herein (*see* the Examples, *infra*) or known to one of skill in the art, relative to T cell proliferation without any antibody or with an unrelated antibody (*e.g.*, an antibody that does not specifically bind to human PD-1).

[00202]   In specific embodiments, the instant disclosure provides an isolated antibody that specifically binds to human PD-1 and increases proliferation of anti-CD3-antibody-stimulated CD4+ or CD8+ T cells co-cultured with ovarian cancer ascites fluid by at least about 1.2 fold, 1.3 fold, 1.4 fold, 1.5 fold, 2 fold, 2.5 fold, 3 fold, 3.5 fold, 4 fold, 4.5 fold, 5 fold, 6 fold, 7 fold, 8 fold, 9 fold, 10 fold, 15 fold, 20 fold, 30 fold, 40 fold, 50 fold, 60 fold, 70 fold, 80 fold, 90 fold, or 100 fold, as assessed by methods described herein (*see* the Examples, *infra*) or known to one of skill in the art, relative to proliferation of anti-CD3-antibody-stimulated CD4+ or CD8+ T cells co-cultured with ovarian cancer ascites fluid without any antibody or with an unrelated antibody (*e.g.*, an antibody that does not specifically bind to human PD-1).

[00203]   In specific embodiments, the instant disclosure provides an isolated antibody that specifically binds to human PD-1 and increases NFAT signaling in PD-1-expressing NFAT-luciferase reporter cells co-cultured with PD-L1-expressing target cells by at least about 1.2 fold, 1.3 fold, 1.4 fold, 1.5 fold, 2 fold, 2.5 fold, 3 fold, 3.5 fold, 4 fold, 4.5 fold, 5 fold, 6 fold, 7 fold, 8 fold, 9 fold, 10 fold, 15 fold, 20 fold, 30 fold, 40 fold, 50 fold, 60 fold, 70 fold,

80 fold, 90 fold, or 100 fold, as assessed by methods described herein (*see* the Examples, *infra*) or known to one of skill in the art, relative to NFAT signaling in PD-1-expressing NFAT-luciferase reporter cells co-cultured with PD-L1-expressing target cells without any antibody or with an unrelated antibody (*e.g.*, an antibody that does not specifically bind to human PD-1).

5.3   Pharmaceutical Compositions

[00204]   Provided herein are compositions comprising an anti-PD-1 antibody described herein having the desired degree of purity in a physiologically acceptable carrier, excipient or stabilizer (Remington's Pharmaceutical Sciences (1990) Mack Publishing Co., Easton, PA). Acceptable carriers, excipients, or stabilizers are nontoxic to recipients at the dosages and concentrations employed, and include buffers such as phosphate, citrate, and other organic acids; antioxidants including ascorbic acid and methionine; preservatives (such as octadecyldimethylbenzyl ammonium chloride; hexamethonium chloride; benzalkonium chloride, benzethonium chloride; phenol, butyl or benzyl alcohol; alkyl parabens such as methyl or propyl paraben; catechol; resorcinol; cyclohexanol; 3-pentanol; and m-cresol); low molecular weight (less than about 10 residues) polypeptides; proteins, such as serum albumin, gelatin, or immunoglobulins; hydrophilic polymers such as polyvinylpyrrolidone; amino acids such as glycine, glutamine, asparagine, histidine, arginine, or lysine; monosaccharides, disaccharides, and other carbohydrates including glucose, mannose, or dextrins; chelating agents such as EDTA; sugars such as sucrose, mannitol, trehalose or sorbitol; salt-forming counter-ions such as sodium; metal complexes (*e.g.*, Zn-protein complexes); and/or non-ionic surfactants such as TWEEN™, PLURONICS™ or polyethylene glycol (PEG).

[00205]   In a specific embodiment, pharmaceutical compositions comprise an anti-PD-1 antibody described herein, and optionally one or more additional prophylactic or therapeutic agents, in a pharmaceutically acceptable carrier. In a specific embodiment, pharmaceutical compositions comprise an effective amount of an antibody described herein, and optionally one or more additional prophylactic or therapeutic agents, in a pharmaceutically acceptable carrier. In some embodiments, the antibody is the only active ingredient included in the pharmaceutical composition. Pharmaceutical compositions described herein can be useful in inhibiting PD-1 activity and treating a condition, such as cancer or an infectious disease. In a preferred embodiment, the present invention relates to a pharmaceutical composition of the present invention comprising an anti-PD-1 antibody of the present invention for use as a medicament. In another preferred embodiment, the present invention relates to a

pharmaceutical composition of the present invention for use in a method for the treatment of cancer or an infectious disease. In another preferred embodiment, the present invention relates to the use of an anti-PD-1 antibody of the present invention for preparing a pharmaceutical composition for treating cancer or an infectious disease.

[00206] Pharmaceutically acceptable carriers used in parenteral preparations include aqueous vehicles, nonaqueous vehicles, antimicrobial agents, isotonic agents, buffers, antioxidants, local anesthetics, suspending and dispersing agents, emulsifying agents, sequestering or chelating agents and other pharmaceutically acceptable substances. Examples of aqueous vehicles include Sodium Chloride Injection, Ringers Injection, Isotonic Dextrose Injection, Sterile Water Injection, Dextrose and Lactated Ringers Injection. Nonaqueous parenteral vehicles include fixed oils of vegetable origin, cottonseed oil, corn oil, sesame oil and peanut oil. Antimicrobial agents in bacteriostatic or fungistatic concentrations can be added to parenteral preparations packaged in multiple-dose containers which include phenols or cresols, mercurials, benzyl alcohol, chlorobutanol, methyl and propyl p-hydroxybenzoic acid esters, thimerosal, benzalkonium chloride and benzethonium chloride. Isotonic agents include sodium chloride and dextrose. Buffers include phosphate and citrate. Antioxidants include sodium bisulfate. Local anesthetics include procaine hydrochloride. Suspending and dispersing agents include sodium carboxymethylcelluose, hydroxypropyl methylcellulose and polyvinylpyrrolidone. Emulsifying agents include Polysorbate 80 (TWEEN® 80). A sequestering or chelating agent of metal ions includes EDTA. Pharmaceutical carriers also include ethyl alcohol, polyethylene glycol and propylene glycol for water miscible vehicles; and sodium hydroxide, hydrochloric acid, citric acid or lactic acid for pH adjustment.

[00207] A pharmaceutical composition may be formulated for any route of administration to a subject. Specific examples of routes of administration include intranasal, oral, pulmonary, transdermal, intradermal, and parenteral. Parenteral administration, characterized by either subcutaneous, intramuscular or intravenous injection, is also contemplated herein. Injectables can be prepared in conventional forms, either as liquid solutions or suspensions, solid forms suitable for solution or suspension in liquid prior to injection, or as emulsions. The injectables, solutions and emulsions also contain one or more excipients. Suitable excipients are, for example, water, saline, dextrose, glycerol or ethanol. In addition, if desired, the pharmaceutical compositions to be administered can also contain minor amounts of non-toxic auxiliary substances such as wetting or emulsifying agents, pH buffering agents, stabilizers, solubility enhancers, and other such agents, such as for example, sodium acetate,

sorbitan monolaurate, triethanolamine oleate and cyclodextrins.

[00208]   Preparations for parenteral administration of an antibody include sterile solutions ready for injection, sterile dry soluble products, such as lyophilized powders, ready to be combined with a solvent just prior to use, including hypodermic tablets, sterile suspensions ready for injection, sterile dry insoluble products ready to be combined with a vehicle just prior to use and sterile emulsions. The solutions may be either aqueous or nonaqueous.

[00209]   If administered intravenously, suitable carriers include physiological saline or phosphate buffered saline (PBS), and solutions containing thickening and solubilizing agents, such as glucose, polyethylene glycol, and polypropylene glycol and mixtures thereof.

[00210]   Topical mixtures comprising an antibody are prepared as described for the local and systemic administration. The resulting mixture can be a solution, suspension, emulsions or the like and can be formulated as creams, gels, ointments, emulsions, solutions, elixirs, lotions, suspensions, tinctures, pastes, foams, aerosols, irrigations, sprays, suppositories, bandages, dermal patches or any other formulations suitable for topical administration.

[00211]   An anti-PD-1 antibody described herein can be formulated as an aerosol for topical application, such as by inhalation (see, *e.g.*, U.S. Patent Nos. 4,044,126, 4,414,209 and 4,364,923, which describe aerosols for delivery of a steroid useful for treatment of inflammatory diseases, particularly asthma and are incorporated by reference in their entireties). These formulations for administration to the respiratory tract can be in the form of an aerosol or solution for a nebulizer, or as a microfine powder for insufflations, alone or in combination with an inert carrier such as lactose. In such a case, the particles of the formulation will, in one embodiment, have diameters of less than 50 microns, in one embodiment less than 10 microns.

[00212]   An anti-PD-1 antibody described herein can be formulated for local or topical application, such as for topical application to the skin and mucous membranes, such as in the eye, in the form of gels, creams, and lotions and for application to the eye or for intracisternal or intraspinal application. Topical administration is contemplated for transdermal delivery and also for administration to the eyes or mucosa, or for inhalation therapies. Nasal solutions of the antibody alone or in combination with other pharmaceutically acceptable excipients can also be administered.

[00213]   Transdermal patches, including iontophoretic and electrophoretic devices, are well known to those of skill in the art, and can be used to administer an antibody. For example, such patches are disclosed in U.S. Patent Nos. 6,267,983, 6,261,595, 6,256,533, 6,167,301, 6,024,975, 6,010715, 5,985,317, 5,983,134, 5,948,433, and 5,860,957, all of which are herein

incorporated by reference in their entireties.

[00214] In certain embodiments, a pharmaceutical composition comprising an antibody described herein is a lyophilized powder, which can be reconstituted for administration as solutions, emulsions and other mixtures. It may also be reconstituted and formulated as solids or gels. The lyophilized powder is prepared by dissolving an antibody described herein, or a pharmaceutically acceptable derivative thereof, in a suitable solvent. In some embodiments, the lyophilized powder is sterile. The solvent may contain an excipient which improves the stability or other pharmacological component of the powder or reconstituted solution, prepared from the powder. Excipients that may be used include, but are not limited to, dextrose, sorbitol, fructose, corn syrup, xylitol, glycerin, glucose, sucrose or other suitable agent. The solvent may also contain a buffer, such as citrate, sodium or potassium phosphate or other such buffer known to those of skill in the art at, in one embodiment, about neutral pH. Subsequent sterile filtration of the solution followed by lyophilization under standard conditions known to those of skill in the art provides the desired formulation. In one embodiment, the resulting solution will be apportioned into vials for lyophilization. Each vial will contain a single dosage or multiple dosages of the compound. The lyophilized powder can be stored under appropriate conditions, such as at about 4°C to room temperature. Reconstitution of this lyophilized powder with water for injection provides a formulation for use in parenteral administration. For reconstitution, the lyophilized powder is added to sterile water or other suitable carrier. The precise amount depends upon the selected compound. Such amount can be empirically determined.

[00215] The anti-PD-1 antibodies described herein and other compositions provided herein can also be formulated to be targeted to a particular tissue, receptor, or other area of the body of the subject to be treated. Many such targeting methods are well known to those of skill in the art. All such targeting methods are contemplated herein for use in the instant compositions. For non-limiting examples of targeting methods, see, *e.g.*, U.S. Patent Nos. 6,316,652, 6,274,552, 6,271,359, 6,253,872, 6,139,865, 6,131,570, 6,120,751, 6,071,495, 6,060,082, 6,048,736, 6,039,975, 6,004,534, 5,985,307, 5,972,366, 5,900,252, 5,840,674, 5,759,542 and 5,709,874, all of which are herein incorporated by reference in their entireties. In a specific embodiment, an antibody described herein is targeted to a tumor.

[00216] The compositions to be used for *in vivo* administration can be sterile. This is readily accomplished by filtration through, *e.g.*, sterile filtration membranes.

5.4   Methods of Use and Uses

[00217]   In another aspect, the instant disclosure provides a method of treating a subject using the anti-PD-1 antibodies disclosed herein. Any disease or disorder in a subject that would benefit from inhibition of PD-1 function can be treated using the anti-PD-1 antibodies disclosed herein. The anti-PD-1 antibodies disclosed herein are particularly useful for inhibiting immune system tolerance to tumors, and accordingly can be used as an immunotherapy for subjects with cancer. For example, in certain embodiments, the instant disclosure provides a method of increasing T cell activation in response to an antigen in a subject, the method comprising administering to the subject an effective amount of an anti-PD-1 antibody or pharmaceutical composition thereof, as disclosed herein. In certain embodiments, the instant disclosure provides a method of treating cancer in a subject, the method comprising administering to the subject an effective amount of the antibody or pharmaceutical composition, as disclosed herein. Cancers that can be treated with the anti-PD-1 antibodies or pharmaceutical compositions disclosed herein include, without limitation, melanoma, head and neck cancer (*e.g.*, head and neck squamous cancer), lung cancer (*e.g.*, non-small cell lung cancer and small cell lung cancer), breast cancer (*e.g.*, herceptin resistant breast cancer and trastuzumab-DM1 (T-DM1) resistant breast cancer), prostate cancer, glioblastoma multiforme, colorectal cancer, sarcoma, bladder cancer, cervical cancer, HPV-associated cancers, cancers of the vagina, cancers of the vulva, cancers of the penis, cancers of the anus, cancers of the rectum, cancers of the oropharynx, multiple myeloma, renal cell carcinoma, ovarian cancer, hepatocellular cancer, endometrial cancer, pancreatic cancer, lymphoma, and leukemia (*e.g.*, elderly leukemia, acute myeloid leukemia (AML), and elderly AML). Therefore, the present invention relates in one embodiment to an antibody and/or pharmaceutical composition of the present invention for use as a medicament. In a preferred embodiment, the present invention relates to the use of an antibody and/or pharmaceutical composition of the present invention for preparing a medicine for use in a method of treating cancer in a subject, and/or for use for inhibiting immune system tolerance to tumors and/or for use in immunotherapy for subjects with cancer, and/or for use in a method of increasing T cell activation in response to an antigen in a subject. In a preferred embodiment, the cancer is selected from the group consisting of melanoma, head and neck cancer (*e.g.*, head and neck squamous cancer), lung cancer (*e.g.*, non-small cell lung cancer and small cell lung cancer), breast cancer (*e.g.*, herceptin resistant breast cancer and trastuzumab-DM1 (T-DM1) resistant breast cancer), prostate cancer, glioblastoma multiforme, colorectal cancer, sarcoma, bladder

cancer, cervical cancer, HPV-associated cancers, cancers of the vagina, cancers of the vulva, cancers of the penis, cancers of the anus, cancers of the rectum, cancers of the oropharynx, multiple myeloma, renal cell carcinoma, ovarian cancer, hepatocellular cancer, endometrial cancer, pancreatic cancer, lymphoma, and leukemia (*e.g.*, elderly leukemia, acute myeloid leukemia (AML), and elderly AML).

[00218] Additional cancers that can be treated with the anti-PD-1 antibodies or pharmaceutical compositions disclosed herein include, without limitation, melanoma (*e.g.*, metastatic malignant melanoma and cutaneous or intraocular malignant melanoma), renal cancer (*e.g.*, clear cell carcinoma), prostate cancer (*e.g.*, hormone refractory prostate adenocarcinoma), breast cancer, colon cancer, lung cancer (*e.g.*, non-small cell lung cancer), bone cancer, pancreatic cancer, skin cancer, cancer of the head or neck, uterine cancer, ovarian cancer, rectal cancer, cancer of the anal region, stomach cancer, testicular cancer, uterine cancer, carcinoma of the fallopian tubes, carcinoma of the endometrium, carcinoma of the cervix, carcinoma of the vagina, carcinoma of the vulva, Hodgkin's Disease, non-Hodgkin's lymphoma, cancer of the esophagus, cancer of the small intestine, cancer of the endocrine system, cancer of the thyroid gland, cancer of the parathyroid gland, cancer of the adrenal gland, sarcoma of soft tissue, cancer of the urethra, cancer of the penis, chronic or acute leukemias including acute myeloid leukemia, chronic myeloid leukemia, acute lymphoblastic leukemia, chronic lymphocytic leukemia, solid tumors of childhood, lymphocytic lymphoma, cancer of the bladder, cancer of the kidney or ureter, carcinoma of the renal pelvis, neoplasm of the central nervous system (CNS), primary CNS lymphoma, tumor angiogenesis, spinal axis tumor, brain stem glioma, glioma, pituitary adenoma, Kaposi's sarcoma, epidermoid cancer, squamous cell cancer, T-cell lymphoma, environmentally induced cancers including those induced by asbestos, esophageal cancer, liver cancer, refractory or recurrent malignancies, metastatic cancers, cancers that express PD-L1, and combinations of said cancers.

[00219] In certain embodiments, the instant disclosure provides a method of preventing or treating an infectious disease in a subject, the method comprising administering to the subject an effective amount of an anti-PD-1 antibody or pharmaceutical composition thereof, as disclosed herein. In one embodiment, provided herein are methods for preventing and/or treating an infection (*e.g.*, a viral infection, a bacterial infection, a fungal infection, a protozoal infection, or a parasitic infection). The infection prevented and/or treated in accordance with the methods can be caused by an infectious agent identified herein. In a specific embodiment, an anti-PD-1 antibody described herein or a composition thereof is the

only active agent administered to a subject. In some embodiments, an anti-PD-1 antibody described herein or a composition thereof is used in combination with anti-infective interventions (*e.g.*, antivirals, antibacterials, antifungals, or anti-helminthics) for the treatment of infectious diseases. Therefore, in a preferred embodiment, the present invention relates to an antibody, the use of such antibody for preparing pharmaceutical compositions, and/or pharmaceutical compositions of the present invention for use in a method of preventing and/or treating an infectious disease, more preferably wherein the antibody or pharmaceutical composition is the only active agent administered to a subject, or wherein the antibody or pharmaceutical composition is used in combination with anti-infective interventions.

[00220] Infectious diseases that can be treated and/or prevented by anti-PD-1 antibodies or pharmaceutical compositions disclosed herein are caused by infectious agents including but not limited to bacteria, parasites, fungi, protozae, and viruses. In a specific embodiment, the infectious disease treated and/or prevented by anti-PD-1 antibodies or pharmaceutical compositions disclosed herein is caused by a virus. Viral diseases or viral infections that can be prevented and/or treated in accordance with the methods described herein include, but are not limited to, those caused by hepatitis type A, hepatitis type B, hepatitis type C, influenza (*e.g.*, influenza A or influenza B), varicella, adenovirus, herpes simplex type I (HSV-I), herpes simplex type II (HSV-II), rinderpest, rhinovirus, echovirus, rotavirus, respiratory syncytial virus, papilloma virus, papova virus, cytomegalovirus, echinovirus, arbovirus, huntavirus, coxsackie virus, mumps virus, measles virus, rubella virus, polio virus, small pox, Epstein Barr virus, human immunodeficiency virus type I (HIV-I), human immunodeficiency virus type II (HIV-II), and agents of viral diseases such as viral meningitis, encephalitis, dengue or small pox.

[00221] Bacterial infections that can be prevented and/or treated include infections caused by Escherichia coli, Klebsiella pneumoniae, Staphylococcus aureus, Enterococcus faecalis, Proteus vulgaris, Staphylococcus viridans, and Pseudomonas aeruginosa. Bacterial diseases caused by bacteria (*e.g.*, Escherichia coli, Klebsiella pneumoniae, Staphylococcus aureus, Enterococcus faecalis, Proteus vulgaris, Staphylococcus viridans, and Pseudomonas aeruginosa) that can be prevented and/or treated in accordance with the methods described herein include, but are not limited to, Mycobacteria rickettsia, Mycoplasma, Neisseria, S. pneumonia, Borrelia burgdorferi (Lyme disease), Bacillus antracis (anthrax), tetanus, Streptococcus, Staphylococcus, mycobacterium, pertissus, cholera, plague, diptheria, chlamydia, S. aureus and legionella.

[00222] Protozoal diseases or protozoal infections caused by protozoa that can be

prevented and/or treated in accordance with the methods described herein include, but are not limited to, leishmania, coccidiosis, trypanosoma schistosoma or malaria. Parasitic diseases or parasitic infections caused by parasites that can be prevented and/or treated in accordance with the methods described herein include, but are not limited to, chlamydia and rickettsia.

[00223]   Fungal diseases or fungal infections that can be prevented and/or treated in accordance with the methods described herein include, but are not limited to, those caused by *Candida* infections, zygomycosis, *Candida* mastitis, progressive disseminated trichosporonosis with latent trichosporonemia, disseminated candidiasis, pulmonary paracoccidioidomycosis, pulmonary aspergillosis, *Pneumocystis carinii* pneumonia, cryptococcal meningitis, *coccidioidal* meningoencephalitis and cerebrospinal vasculitis, *Aspergillus niger* infection, *Fusarium keratitis*, paranasal sinus mycoses, *Aspergillus fumigatus* endocarditis, tibial dyschondroplasia, *Candida glabrata* vaginitis, oropharyngeal candidiasis, X-linked chronic granulomatous disease, tinea pedis, cutaneous candidiasis, mycotic placentitis, disseminated trichosporonosis, allergic bronchopulmonary aspergillosis, mycotic keratitis, *Cryptococcus neoformans* infection, fungal peritonitis, *Curvularia geniculata* infection, staphylococcal endophthalmitis, sporotrichosis, and dermatophytosis.

[00224]   In certain embodiments, these methods further comprise administering an additional therapeutic agent to the subject. In certain embodiments, the additional therapeutic agent is a chemotherapeutic, a radiotherapeutic, or a checkpoint targeting agent. In certain embodiments, the checkpoint targeting agent is selected from the group consisting of an antagonist anti-CTLA-4 antibody, an antagonist anti-PD-L1 antibody, an antagonist anti-PD-L2 antibody, an antagonist anti-PD-1 antibody, an antagonist anti-TIM-3 antibody, an antagonist anti-LAG-3 antibody, an antagonist anti-CEACAM1 antibody, an antagonist anti-TIGIT antibody, an agonist anti-CD137 antibody, an agonist anti-ICOS antibody, an agonist anti-GITR antibody, and an agonist anti-OX40 antibody. In certain embodiments, an anti-PD-1 antibody disclosed herein is administered to a subject in combination with an antagonist anti-CTLA-4 antibody and an agonist anti-ICOS antibody. In certain embodiments, an anti-PD-1 antibody disclosed herein is administered to a subject in combination with an antagonist anti-CTLA-4 antibody. In certain embodiments, the instant disclosure provides a method of treating cancer in a subject, the method comprising administering to the subject an anti-PD-1 antibody or pharmaceutical composition thereof, as disclosed herein, in combination with an antagonist anti-CTLA-4 antibody or pharmaceutical composition thereof, wherein the cancer is selected from the group consisting of lung cancer (*e.g.*, non-small cell lung cancer (NSCLC), *e.g.*, first-line NSCLC), melanoma (*e.g.*, first-line melanoma), and head and neck

cancer (*e.g.*, squamous cell carcinoma of the head and neck (SCCHN), *e.g.*, first-line SCCHN).

[00225]   In a preferred embodiment, the present invention relates to an antibody, the use of such antibody for preparing pharmaceutical compositions and/or pharmaceutical composition of the present invention for use in a method of the present invention, wherein the method further comprises administering an additional therapeutic agent to the subject.   In another preferred embodiment, the present invention relates to (a) an antibody and/or pharmaceutical composition of the present invention and (b) an additional therapeutic agent for use as a medicament.   In another preferred embodiment, the present invention relates to (a) an antibody and/or pharmaceutical composition of the present invention, and (b) an additional therapeutic agent for use in a method for the treatment of cancer.   In a further embodiment, the present invention relates to a pharmaceutical composition, kit or kit-of-parts comprising (a) an antibody and/or pharmaceutical composition of the present invention and (b) an additional therapeutic agent.   In one more preferred embodiment, the additional therapeutic agent is a chemotherapeutic, a radiotherapeutic, or a checkpoint targeting agent.

[00226]   In certain embodiments, an anti-CTLA-4 antibody is used in methods disclosed herein.   In certain embodiments, the anti-CTLA-4 antibody is Ipilimumab developed by Bristol-Myers Squibb.   In certain embodiments, the anti-CTLA-4 antibody is Tremelimumab developed by Pfizer and Medimmune.   In certain embodiments, the anti-CTLA-4 antibody is a Probody targeting CTLA-4 developed by CytomX and Bristol-Myers Squibb.

[00227]   Non-limiting examples of anti-CTLA-4 antibodies that may be used in treatment methods disclosed herein are disclosed in the following patents and patent applications, all of which are herein incorporated by reference in their entireties: US Patent No. 6,984,720; US Patent No. 7,411,057; US Patent No. 7,034,121; US Patent No. 8,697,845; US Patent No. 8,518,404; U.S. Publication No. US 2009/0123477 A1; U.S. Publication No. US 2014/0105914 A1; U.S. Publication No. US 2013/0267688 A1; U.S. Publication No. US 2016/0145355 A1; PCT Publication No. WO 2014/207064 A1; and PCT Publication No. WO 2016/015675 A1.

[00228]   In certain embodiments, an anti-PD-1 antibody disclosed herein is administered to a subject in combination with a compound that targets an immunomodulatory enzyme(s) such as IDO (indoleamine-(2,3)-dioxygenase) and/or TDO (tryptophan 2,3-dioxygenase). Therefore, in another more preferred embodiment, the additional therapeutic agent is a compound that targets an immunomodulatory enzyme(s), even more preferably an inhibitor of indoleamine-(2,3)-dioxygenase (IDO).   In certain embodiments, such compound is

selected from the group consisting of epacadostat (Incyte Corp; see, *e.g.*, WO 2010/005958 which is incorporated by reference herein in its entirety), F001287 (Flexus Biosciences/Bristol-Myers Squibb), indoximod (NewLink Genetics), and NLG919 (NewLink Genetics). In one embodiment, the compound is epacadostat. In another embodiment, the compound is F001287. In another embodiment, the compound is indoximod. In another embodiment, the compound is NLG919.

[00229]   In certain embodiments, an anti-PD-1 antibody disclosed herein is administered to a subject in combination with a vaccine. The vaccine can be, *e.g.*, a peptide vaccine, a DNA vaccine, or an RNA vaccine. In certain embodiments, the vaccine is a heat shock protein based tumor vaccine or a heat shock protein based pathogen vaccine. In a specific embodiment, an anti-PD-1 antibody disclosed herein is administered to a subject in combination with a heat shock protein based tumor-vaccine. Heat shock proteins (HSPs) are a family of highly conserved proteins found ubiquitously across all species. Their expression can be powerfully induced to much higher levels as a result of heat shock or other forms of stress, including exposure to toxins, oxidative stress or glucose deprivation. Five families have been classified according to molecular weight: HSP-110, -90, -70, -60 and -28. HSPs deliver immunogenic peptides through the cross-presentation pathway in antigen presenting cells (APCs) such as macrophages and dendritic cells (DCs), leading to T cell activation. HSPs function as chaperone carriers of tumor-associated antigenic peptides forming complexes able to induce tumor-specific immunity. Upon release from dying tumor cells, the HSP-antigen complexes are taken up by antigen-presenting cells (APCs) wherein the antigens are processed into peptides that bind MHC class I and class II molecules leading to the activation of anti-tumor CD8+ and CD4+ T cells. The immunity elicited by HSP complexes derived from tumor preparations is specifically directed against the unique antigenic peptide repertoire expressed by the cancer of each subject. Therefore, in a further preferred embodiment, the present invention relates to (a) an antibody and/or pharmaceutical composition of the present invention and (b) a vaccine for use as a medicament, in particular for use in a method for the treatment of cancer. In another preferred embodiment, the present invention relates to a pharmaceutical composition, kit or kit-of-parts comprising (a) an antibody and/or pharmaceutical composition of the present invention and (b) a vaccine. In a further preferred embodiment, the vaccine is a heat shock protein based tumor vaccine or a heat shock protein based pathogen vaccine, more preferably a heat shock protein based tumor vaccine.

[00230]   A heat shock protein peptide complex (HSPPC) is a protein peptide complex

consisting of a heat shock protein non-covalently complexed with antigenic peptides. HSPPCs elicit both innate and adaptive immune responses. In a specific embodiment, the antigenic peptide(s) displays antigenicity for the cancer being treated. HSPPCs are efficiently seized by APCs via membrane receptors (mainly CD91) or by binding to Toll-like receptors. HSPPC internalization results in functional maturation of the APCs with chemokine and cytokine production leading to activation of natural killer cells (NK), monocytes and Th1 and Th-2-mediated immune responses. In certain embodiments, HSPPCs used in methods disclosed herein comprise one or more heat shock proteins from the hsp60, hsp70, or hsp90 family of stress proteins complexed with antigenic peptides. In certain embodiments, HSPPCs comprise hsc70, hsp70, hsp90, hsp110, grp170, gp96, calreticulin, or combinations of two or more thereof.

[00231] In a specific embodiment, the heat shock protein peptide complex (HSPPC) comprises recombinant heat shock proteins (*e.g.*, hsp70 or hsc70) or a peptide-binding domain thereof complexed with recombinant antigenic peptides. Recombinant heat shock proteins can be produced by recombinant DNA technology, for example, using human hsc70 sequence as described in Dworniczak and Mirault, Nucleic Acids Res. 15:5181-5197 (1987) and GenBank accession no. P11142 and/or Y00371, each of which is incorporated herein by reference in its entirety. In certain embodiments, Hsp70 sequences are as described in Hunt and Morimoto Proc. Natl. Acad. Sci. U.S.A. 82 (19), 6455-6459 (1985) and GenBank accession no. P0DMV8 and/or M11717, each of which is incorporated herein by reference in its entirety. Antigenic peptides can also be prepared by recombinant DNA methods known in the art.

[00232] In certain embodiments, the antigenic peptides comprise a modified amino acid. In certain embodiments, the modified amino acid comprises a post-translational modification. In certain embodiments, the modified amino acid comprises a mimetic of a post-translational modification. In certain embodiments, the modified amino acid is a Tyr, Ser, Thr, Arg, Lys, or His that has been phosphorylated on a side chain hydroxyl or amine. In certain embodiments, the modified amino acid is a mimetic of a Tyr, Ser, Thr, Arg, Lys, or His amino acid that has been phosphorylated on a side chain hydroxyl or amine.

[00233] In a specific embodiment, an anti-PD-1 antibody disclosed herein is administered to a subject in combination with a heat shock protein peptide complex (HSPPC), *e.g.*, heat shock protein peptide complex-96 (HSPPC-96), to treat cancer. HSPPC-96 comprises a 96 kDa heat shock protein (Hsp), gp96, complexed to antigenic peptides. HSPPC-96 is a cancer immunotherapy manufactured from a subject's tumor and contains the cancer's antigenic

"fingerprint." In certain embodiments, this fingerprint contains unique antigens that are present only in that particular subject's specific cancer cells and injection of the vaccine is intended to stimulate the subject's immune system to recognize and attack any cells with the specific cancer fingerprint. Therefore, in another preferred embodiment, the present invention relates to an antibody and/or pharmaceutical composition of the present invention in combination with a heat shock protein peptide complex (HSPPC) for use as a medicament and/or for use in a method for the treatment of cancer.

[00234]   In certain embodiments, the HSPPC, *e.g.*, HSPPC-96, is produced from the tumor tissue of a subject. In a specific embodiment, the HSPPC (*e.g.*, HSPPC-96) is produced from a tumor of the type of cancer or metastasis thereof being treated. In another specific embodiment, the HSPPC (*e.g.*, HSPPC-96) is autologous to the subject being treated. In certain embodiments, the tumor tissue is non-necrotic tumor tissue. In certain embodiments, at least 1 gram (*e.g.*, at least 1, at least 2, at least 3, at least 4, at least 5, at least 6, at least 7, at least 8, at least 9, or at least 10 grams) of non-necrotic tumor tissue is used to produce a vaccine regimen. In certain embodiments, after surgical resection, non-necrotic tumor tissue is frozen prior to use in vaccine preparation. In some embodiments, the HSPPC, *e.g.*, HSPPC-96, is isolated from the tumor tissue by purification techniques, filtered and prepared for an injectable vaccine. In certain embodiments, a subject is administered 6-12 doses of the HSPPC, *e.g.*, HSPCC-96. In such embodiments, the HSPPC, *e.g.*, HSPPC-96, doses may be administered weekly for the first 4 doses and then biweekly for the 2-8 additional doses.

[00235]   Further examples of HSPPCs that may be used in accordance with the methods described herein are disclosed in the following patents and patent applications, U.S. Patent Nos. 6,391,306, 6,383,492, 6,403,095, 6,410,026, 6,436,404, 6,447,780, 6,447,781 and 6,610,659, all of which are herein incorporated by reference in their entireties.

[00236]   In certain embodiments, an anti-PD-1 antibody disclosed herein is administered to a subject in combination with an adjuvant. Various adjuvants can be used depending on the treatment context. Non-limiting examples of appropriate adjuvants include, but not limited to, Complete Freund's Adjuvant (CFA), Incomplete Freund's Adjuvant (IFA), montanide ISA (incomplete Seppic adjuvant), the Ribi adjuvant system (RAS), Titer Max, muramyl peptides, Syntex Adjuvant Formulation (SAF), alum (aluminum hydroxide and/or aluminum phosphate), aluminum salt adjuvants, Gerbu® adjuvants, nitrocellulose absorbed antigen, encapsulated or entrapped antigen, 3 De-O-acylated monophosphoryl lipid A (3 D-MPL), immunostimulatory oligonucleotides, toll-like receptor (TLR) ligands, mannan-binding lectin (MBL) ligands, STING agonists, immuno-stimulating complexes such as saponins, Quil A,

QS-21, QS-7, ISCOMATRIX, and others. Other adjuvants include CpG oligonucleotides and double stranded RNA molecules, such as poly(A) and poly(U). Combinations of the above adjuvants may also be used. See, *e.g.*, U.S. Patent Nos. 6,645,495; 7,029,678; and 7,858,589, all of which are incorporated herein by reference in their entireties. In one embodiment, the adjuvant used herein is QS-21 STIMULON.

[00237]   In certain embodiments, an anti-PD-1 antibody disclosed herein is administered to a subject in combination with an additional therapeutic agent comprising a TCR. In certain embodiments, the additional therapeutic agent is a soluble TCR. In certain embodiments, the additional therapeutic agent is a cell expressing a TCR. Therefore, in another preferred embodiment, the present invention relates to an antibody and/or pharmaceutical composition of the present invention in combination with an additional therapeutic agent comprising a TCR for use as a medicament and/or for use in a method for the treatment of cancer.

[00238]   In certain embodiments, an anti-PD-1 antibody disclosed herein is administered to a subject in combination with a cell expressing a chimeric antigen receptor (CAR). In certain embodiments, the cell is a T cell.

[00239]   In certain embodiments, an anti-PD-1 antibody disclosed herein is administered to a subject in combination with a TCR mimic antibody. In certain embodiments, the TCR mimic antibody is an antibody that specifically binds to a peptide-MHC complex. For non-limiting examples of TCR mimic antibodies, see, *e.g.*, U.S. Patent No. 9,074,000 and U.S. Publication Nos. US 2009/0304679 A1 and US 2014/0134191 A1, all of which are incorporated herein by reference in their entireties.

[00240]   The anti-PD-1 antibody and the additional therapeutic agent (*e.g.*, chemotherapeutic, radiotherapeutic, checkpoint targeting agent, IDO inhibitor, vaccine, adjuvant, a soluble TCR, a cell expressing a TCR, a cell expressing a chimeric antigen receptor, and/or a TCR mimic antibody) can be administered separately, sequentially or concurrently as separate dosage forms. In one embodiment, an anti-PD-1 antibody is administered parenterally, and an IDO inhibitor is administered orally.

[00241]   An antibody or pharmaceutical composition described herein may be delivered to a subject by a variety of routes. These include, but are not limited to, parenteral, intranasal, intratracheal, oral, intradermal, topical, intramuscular, intraperitoneal, transdermal, intravenous, intratumoral, conjunctival, intra-arterial, and subcutaneous routes. Pulmonary administration can also be employed, *e.g.*, by use of an inhaler or nebulizer, and formulation with an aerosolizing agent for use as a spray. In certain embodiments, the antibody or pharmaceutical composition described herein is delivered subcutaneously or intravenously.

In certain embodiments, the antibody or pharmaceutical composition described herein is delivered intra-arterially. In certain embodiments, the antibody or pharmaceutical composition described herein is delivered intratumorally. In certain embodiments, the antibody or pharmaceutical composition described herein is delivered to a tumor draining lymph node.

[00242] The amount of an antibody or composition which will be effective in the treatment and/or prevention of a condition will depend on the nature of the disease, and can be determined by standard clinical techniques.

[00243] The precise dose to be employed in a composition will also depend on the route of administration, and the seriousness of the infection or disease caused by it, and should be decided according to the judgment of the practitioner and each subject's circumstances. For example, effective doses may also vary depending upon means of administration, target site, physiological state of the patient (including age, body weight and health), whether the patient is human or an animal, other medications administered, or whether treatment is prophylactic or therapeutic. Usually, the patient is a human but non-human mammals including transgenic mammals can also be treated. Treatment dosages are optimally titrated to optimize safety and efficacy.

[00244] An anti-PD-1 antibody described herein can also be used to assay PD-1 protein levels in a biological sample using classical immunohistological methods known to those of skill in the art, including immunoassays, such as the enzyme linked immunosorbent assay (ELISA), immunoprecipitation, or Western blotting. Suitable antibody assay labels are known in the art and include enzyme labels, such as, glucose oxidase; radioisotopes, such as iodine (^{125}I, ^{121}I), carbon (^{14}C), sulfur (^{35}S), tritium (^{3}H), indium (^{121}In), and technetium (^{99}Tc); luminescent labels, such as luminol; and fluorescent labels, such as fluorescein and rhodamine, and biotin. Such labels can be used to label an antibody described herein. Alternatively, a second antibody that recognizes an anti-PD-1 antibody described herein can be labeled and used in combination with an anti-PD-1 antibody to detect PD-1 protein levels. Therefore, in one embodiment, the present invention relates to the use of an antibody of the present invention for *in vitro* detection of human PD-1 protein in a biological sample. In a further embodiment, the present invention relates to the use of an anti-PD-1 antibody of the invention, for assaying and/or detecting human PD-1 protein levels in a biological sample *in vitro*, preferably wherein the anti-PD-1 antibody is conjugated to a radionuclide or detectable label, and/or carries a label described herein, and/or wherein an immunohistological method is used.

[00245]   Assaying for the expression level of PD-1 protein is intended to include qualitatively or quantitatively measuring or estimating the level of PD-1 protein in a first biological sample either directly (*e.g.,* by determining or estimating absolute protein level) or relatively (*e.g.,* by comparing to the disease associated protein level in a second biological sample). PD-1 polypeptide expression level in the first biological sample can be measured or estimated and compared to a standard PD-1 protein level, the standard being taken from a second biological sample obtained from an individual not having the disorder or being determined by averaging levels from a population of individuals not having the disorder. As will be appreciated in the art, once the "standard" PD-1 polypeptide level is known, it can be used repeatedly as a standard for comparison. Therefore, in a further embodiment, the present invention relates to an *in vitro* method for assaying and/or detecting PD-1 protein levels, in particular human PD-1 protein levels, in a biological sample, comprising qualitatively or quantitatively measuring or estimating the level of PD-1 protein, in particular of human PD-1 protein, in a biological sample, by an immunohistological method.

[00246]   As used herein, the term "biological sample" refers to any biological sample obtained from a subject, cell line, tissue, or other source of cells potentially expressing PD-1. Methods for obtaining tissue biopsies and body fluids from animals (*e.g.,* humans) are well known in the art. Biological samples include peripheral mononuclear blood cells.

[00247]   An anti-PD-1 antibody described herein can be used for prognostic, diagnostic, monitoring and screening applications, including *in vitro* and *in vivo* applications well known and standard to the skilled artisan and based on the present description. Prognostic, diagnostic, monitoring and screening assays and kits for *in vitro* assessment and evaluation of immune system status and/or immune response may be utilized to predict, diagnose and monitor to evaluate patient samples including those known to have or suspected of having an immune system-dysfunction or with regard to an anticipated or desired immune system response, antigen response or vaccine response. The assessment and evaluation of immune system status and/or immune response is also useful in determining the suitability of a patient for a clinical trial of a drug or for the administration of a particular chemotherapeutic agent, a radiotherapeutic agent, or an antibody, including combinations thereof, versus a different agent or antibody. This type of prognostic and diagnostic monitoring and assessment is already in practice utilizing antibodies against the HER2 protein in breast cancer (HercepTest™, Dako) where the assay is also used to evaluate patients for antibody therapy using Herceptin®. *In vivo* applications include directed cell therapy and immune system

modulation and radio imaging of immune responses. Therefore, in one embodiment, the present invention relates to an anti-PD-1 antibody, the use of such antibody for preparing a pharmaceutical composition and/or pharmaceutical compositions of the present invention for use as a diagnostic. In a preferred embodiment, the present invention relates to an anti-PD-1 antibody, the use of such antibody for preparing a pharmaceutical composition and/or pharmaceutical compositions of the present invention for use in a method for the prediction, diagnosis and/or monitoring of a subject having or suspected to have an immune system-dysfunction and/or with regard to an anticipated or desired immune system response, antigen response or vaccine response. In another embodiment, the present invention relates to the use of anti-PD-1 antibody of the invention, for predicting, diagnosing and/or monitoring of a subject having or suspected to have an immune system-dysfunction and/or with regard to an anticipated or desired immune system response, antigen response or vaccine response by assaying and/or detecting human PD-1 protein levels in a biological sample of the subject *in vitro*.

[00248] In one embodiment, an anti-PD-1 antibody can be used in immunohistochemistry of biopsy samples. Preferably, the method is an *in vitro* method. In another embodiment, an anti-PD-1 antibody can be used to detect levels of PD-1, or levels of cells which contain PD-1 on their membrane surface, which levels can then be linked to certain disease symptoms. Anti-PD-1 antibodies described herein may carry a detectable or functional label and/or may be conjugated to a radionuclide or detectable label. When fluorescence labels are used, currently available microscopy and fluorescence-activated cell sorter analysis (FACS) or combination of both methods procedures known in the art may be utilized to identify and to quantitate the specific binding members. Anti-PD-1 antibodies described herein may carry or may be conjugated to a fluorescence label. Exemplary fluorescence labels include, for example, reactive and conjugated probes, *e.g.*, Aminocoumarin, Fluorescein and Texas red, Alexa Fluor dyes, Cy dyes and DyLight dyes. An anti-PD-1 antibody may carry or may be conjugated to a radioactive label or radionuclide, such as the isotopes ^{3}H, ^{14}C, ^{32}P, ^{35}S, ^{36}Cl, ^{51}Cr, ^{57}Co, ^{58}Co, ^{59}Fe, ^{67}Cu, ^{90}Y, ^{99}Tc, ^{111}In, ^{117}Lu, ^{121}I, ^{124}I, ^{125}I, ^{131}I, ^{198}Au, ^{211}At, ^{213}Bi, ^{225}Ac and ^{186}Re. When radioactive labels are used, currently available counting procedures known in the art may be utilized to identify and quantitate the specific binding of anti-PD-1 antibody to PD-1 (*e.g.*, human PD-1). In the instance where the label is an enzyme, detection may be accomplished by any of the presently utilized colorimetric, spectrophotometric, fluorospectrophotometric, amperometric or gasometric techniques as known in the art. This can be achieved by contacting a sample or a control sample with an anti-PD-1 antibody under

conditions that allow for the formation of a complex between the antibody and PD-1. Any complexes formed between the antibody and PD-1 are detected and compared in the sample and the control. In light of the specific binding of the antibodies described herein for PD-1, the antibodies can be used to specifically detect PD-1 expression on the surface of cells. The antibodies described herein can also be used to purify PD-1 via immunoaffinity purification. Also included herein is an assay system which may be prepared in the form of a test kit, kit or kit-of-parts for the quantitative analysis of the extent of the presence of, for instance, PD-1 or PD-1/PD-1 ligand complexes. The system, test kit, kit or kit-of-parts may comprise a labeled component, *e.g.*, a labeled antibody, and one or more additional immunochemical reagents.

5.5   Polynucleotides, Vectors and Methods of Producing Anti-PD-1 Antibodies

[00249]   In another aspect, provided herein are polynucleotides comprising a nucleotide sequence encoding an antibody described herein or a fragment thereof (*e.g.*, a light chain variable region and/or heavy chain variable region) that specifically binds to a PD-1 (*e.g.*, human PD-1) antigen, and vectors, *e.g.*, vectors comprising such polynucleotides for recombinant expression in host cells (*e.g.*, *E. coli* and mammalian cells). Provided herein are polynucleotides comprising nucleotide sequences encoding a heavy and/or light chain of any of the antibodies provided herein, as well as vectors comprising such polynucleotide sequences, *e.g.*, expression vectors for their efficient expression in host cells, *e.g.*, mammalian cells.

[00250]   As used herein, an "isolated" polynucleotide or nucleic acid molecule is one which is separated from other nucleic acid molecules which are present in the natural source (*e.g.*, in a mouse or a human) of the nucleic acid molecule. Moreover, an "isolated" nucleic acid molecule, such as a cDNA molecule, can be substantially free of other cellular material, or culture medium when produced by recombinant techniques, or substantially free of chemical precursors or other chemicals when chemically synthesized. For example, the language "substantially free" includes preparations of polynucleotide or nucleic acid molecule having less than about 15%, 10%, 5%, 2%, 1%, 0.5%, or 0.1% (in particular less than about 10%) of other material, *e.g.*, cellular material, culture medium, other nucleic acid molecules, chemical precursors and/or other chemicals. In a specific embodiment, a nucleic acid molecule(s) encoding an antibody described herein is isolated or purified.

[00251]   In particular aspects, provided herein are polynucleotides comprising nucleotide sequences encoding antibodies, which specifically bind to a PD-1 polypeptide (*e.g.*, human PD-1) and comprises an amino acid sequence as described herein, as well as antibodies which

compete with such antibodies for binding to a PD-1 polypeptide (*e.g.*, in a dose-dependent manner), or which binds to the same epitope as that of such antibodies.

[00252]   In certain aspects, provided herein are polynucleotides comprising a nucleotide sequence encoding the light chain or heavy chain of an antibody described herein.   The polynucleotides can comprise nucleotide sequences encoding a light chain comprising the VL FRs and CDRs of antibodies described herein (see, *e.g.*, Tables 3 and 5) or nucleotide sequences encoding a heavy chain comprising the VH FRs and CDRs of antibodies described herein (see, *e.g.*, Tables 2 and 4).

[00253]   Also provided herein are polynucleotides encoding an anti-PD-1 antibody that are optimized, *e.g.*, by codon/RNA optimization, replacement with heterologous signal sequences, and elimination of mRNA instability elements.   Methods to generate optimized nucleic acids encoding an anti-PD-1 antibody or a fragment thereof (*e.g.*, light chain, heavy chain, VH domain, or VL domain) for recombinant expression by introducing codon changes and/or eliminating inhibitory regions in the mRNA can be carried out by adapting the optimization methods described in, *e.g.*, U.S. Patent Nos. 5,965,726; 6,174,666; 6,291,664; 6,414,132; and 6,794,498, accordingly, all of which are herein incorporated by reference in their entireties.   For example, potential splice sites and instability elements (*e.g.*, A/T or A/U rich elements) within the RNA can be mutated without altering the amino acids encoded by the nucleic acid sequences to increase stability of the RNA for recombinant expression.   The alterations utilize the degeneracy of the genetic code, *e.g.*, using an alternative codon for an identical amino acid.   In some embodiments, it can be desirable to alter one or more codons to encode a conservative mutation, *e.g.*, a similar amino acid with similar chemical structure and properties and/or function as the original amino acid.   Such methods can increase expression of an anti-PD-1 antibody or fragment thereof by at least 1 fold, 2 fold, 3 fold, 4 fold, 5 fold, 10 fold, 20 fold, 30 fold, 40 fold, 50 fold, 60 fold, 70 fold, 80 fold, 90 fold, or 100 fold or more relative to the expression of an anti-PD-1 antibody encoded by polynucleotides that have not been optimized.

[00254]   In certain embodiments, an optimized polynucleotide sequence encoding an anti-PD-1 antibody described herein or a fragment thereof (*e.g.*, VL domain and/or VH domain) can hybridize to an antisense (*e.g.*, complementary) polynucleotide of an unoptimized polynucleotide sequence encoding an anti-PD-1 antibody described herein or a fragment thereof (*e.g.*, VL domain and/or VH domain).   In specific embodiments, an optimized nucleotide sequence encoding an anti-PD-1 antibody described herein or a fragment hybridizes under high stringency conditions to antisense polynucleotide of an unoptimized

polynucleotide sequence encoding an anti-PD-1 antibody described herein or a fragment thereof. In a specific embodiment, an optimized nucleotide sequence encoding an anti-PD-1 antibody described herein or a fragment thereof hybridizes under high stringency, intermediate or lower stringency hybridization conditions to an antisense polynucleotide of an unoptimized nucleotide sequence encoding an anti-PD-1 antibody described herein or a fragment thereof. Information regarding hybridization conditions has been described, see, *e.g.*, U.S. Patent Application Publication No. US 2005/0048549 (*e.g.*, paragraphs 72-73), which is incorporated herein by reference in its entirety.

[00255] The polynucleotides can be obtained, and the nucleotide sequence of the polynucleotides determined, by any method known in the art. Nucleotide sequences encoding antibodies described herein, *e.g.*, antibodies described in Tables 1-6, and modified versions of these antibodies can be determined using methods well known in the art, *i.e.*, nucleotide codons known to encode particular amino acids are assembled in such a way to generate a nucleic acid that encodes the antibody. Such a polynucleotide encoding the antibody can be assembled from chemically synthesized oligonucleotides (*e.g.*, as described in Kutmeier G *et al.*, (1994), BioTechniques 17: 242-6, which is herein incorporated by reference in its entirety), which, briefly, involves the synthesis of overlapping oligonucleotides containing portions of the sequence encoding the antibody, annealing and ligating of those oligonucleotides, and then amplification of the ligated oligonucleotides by PCR.

[00256] Alternatively, a polynucleotide encoding an antibody described herein can be generated from nucleic acid from a suitable source (*e.g.*, a hybridoma) using methods well known in the art (*e.g.*, PCR and other molecular cloning methods). For example, PCR amplification using synthetic primers hybridizable to the 3' and 5' ends of a known sequence can be performed using genomic DNA obtained from hybridoma cells producing the antibody of interest. Such PCR amplification methods can be used to obtain nucleic acids comprising the sequence encoding the light chain and/or heavy chain of an antibody. Such PCR amplification methods can be used to obtain nucleic acids comprising the sequence encoding the variable light chain region and/or the variable heavy chain region of an antibody. The amplified nucleic acids can be cloned into vectors for expression in host cells and for further cloning, for example, to generate chimeric and humanized antibodies.

[00257] If a clone containing a nucleic acid encoding a particular antibody is not available, but the sequence of the antibody molecule is known, a nucleic acid encoding the immunoglobulin can be chemically synthesized or obtained from a suitable source (*e.g.*, an

antibody cDNA library or a cDNA library generated from, or nucleic acid, preferably poly A+ RNA, isolated from, any tissue or cells expressing the antibody, such as hybridoma cells selected to express an antibody described herein) by PCR amplification using synthetic primers hybridizable to the 3' and 5' ends of the sequence or by cloning using an oligonucleotide probe specific for the particular gene sequence to identify, *e.g.*, a cDNA clone from a cDNA library that encodes the antibody. Amplified nucleic acids generated by PCR can then be cloned into replicable cloning vectors using any method well known in the art.

[00258]   DNA encoding anti-PD-1 antibodies described herein can be readily isolated and sequenced using conventional procedures (*e.g.*, by using oligonucleotide probes that are capable of binding specifically to genes encoding the heavy and light chains of the anti-PD-1 antibodies). Hybridoma cells can serve as a source of such DNA. Once isolated, the DNA can be placed into expression vectors, which are then transfected into host cells such as *E. coli* cells, simian COS cells, Chinese hamster ovary (CHO) cells (*e.g.*, CHO cells from the CHO GS System™ (Lonza)), or myeloma cells that do not otherwise produce immunoglobulin protein, to obtain the synthesis of anti-PD-1 antibodies in the recombinant host cells.

[00259]   To generate whole antibodies, PCR primers including VH or VL nucleotide sequences, a restriction site, and a flanking sequence to protect the restriction site can be used to amplify the VH or VL sequences in scFv clones. Utilizing cloning techniques known to those of skill in the art, the PCR amplified VH domains can be cloned into vectors expressing a heavy chain constant region, *e.g.*, the human gamma 4 constant region, and the PCR amplified VL domains can be cloned into vectors expressing a light chain constant region, *e.g.*, human kappa or lambda constant regions. In certain embodiments, the vectors for expressing the VH or VL domains comprise an EF-1α promoter, a secretion signal, a cloning site for the variable region, constant domains, and a selection marker such as neomycin. The VH and VL domains can also be cloned into one vector expressing the necessary constant regions. The heavy chain conversion vectors and light chain conversion vectors are then co-transfected into cell lines to generate stable or transient cell lines that express full-length antibodies, *e.g.*, IgG, using techniques known to those of skill in the art.

[00260]   The DNA also can be modified, for example, by substituting the coding sequence for human heavy and light chain constant domains in place of the murine sequences, or by covalently joining to the immunoglobulin coding sequence all or part of the coding sequence for a non-immunoglobulin polypeptide.

[00261]   Also provided are polynucleotides that hybridize under high stringency, intermediate or lower stringency hybridization conditions to polynucleotides that encode an antibody described herein.   In specific embodiments, polynucleotides described herein hybridize under high stringency, intermediate or lower stringency hybridization conditions to polynucleotides encoding a VH domain and/or VL domain provided herein.

[00262]   Hybridization conditions have been described in the art and are known to one of skill in the art.   For example, hybridization under stringent conditions can involve hybridization to filter-bound DNA in 6x sodium chloride/sodium citrate (SSC) at about 45° C followed by one or more washes in 0.2xSSC/0.1% SDS at about 50-65° C; hybridization under highly stringent conditions can involve hybridization to filter-bound nucleic acid in 6xSSC at about 45° C followed by one or more washes in 0.1xSSC/0.2% SDS at about 68° C. Hybridization under other stringent hybridization conditions are known to those of skill in the art and have been described, see, for example, Ausubel FM *et al.*, eds., (1989) Current Protocols in Molecular Biology, Vol. I, Green Publishing Associates, Inc. and John Wiley & Sons, Inc., New York at pages 6.3.1-6.3.6 and 2.10.3, which is herein incorporated by reference in its entirety.

[00263]   In certain aspects, provided herein are cells (*e.g.*, host cells) expressing (*e.g.*, recombinantly) antibodies described herein which specifically bind to PD-1 (*e.g.*, human PD-1) and related polynucleotides and expression vectors.   Provided herein are vectors (*e.g.*, expression vectors) comprising polynucleotides comprising nucleotide sequences encoding anti-PD-1 antibodies or a fragment for recombinant expression in host cells, preferably in mammalian cells.   Also provided herein are host cells comprising such vectors for recombinantly expressing anti-PD-1 antibodies described herein (*e.g.*, human or humanized antibody).   In a particular aspect, provided herein are methods for producing an antibody described herein, comprising expressing such antibody from a host cell.

[00264]   Recombinant expression of an antibody described herein (*e.g.*, a full-length antibody, heavy and/or light chain of an antibody, or a single chain antibody described herein) that specifically binds to PD-1 (*e.g.*, human PD-1) involves construction of an expression vector containing a polynucleotide that encodes the antibody.   Once a polynucleotide encoding an antibody molecule, heavy and/or light chain of an antibody, or a fragment thereof (*e.g.*, heavy and/or light chain variable regions) described herein has been obtained, the vector for the production of the antibody molecule can be produced by recombinant DNA technology using techniques well known in the art.   Thus, methods for preparing a protein by expressing a polynucleotide containing an antibody or antibody

fragment (*e.g.*, light chain or heavy chain) encoding nucleotide sequence are described herein. Methods which are well known to those skilled in the art can be used to construct expression vectors containing antibody or antibody fragment (*e.g.*, light chain or heavy chain) coding sequences and appropriate transcriptional and translational control signals. These methods include, for example, *in vitro* recombinant DNA techniques, synthetic techniques, and *in vivo* genetic recombination. Also provided are replicable vectors comprising a nucleotide sequence encoding an antibody molecule described herein, a heavy or light chain of an antibody, a heavy or light chain variable region of an antibody or a fragment thereof, or a heavy or light chain CDR, operably linked to a promoter. Such vectors can, for example, include the nucleotide sequence encoding the constant region of the antibody molecule (see, *e.g.*, International Publication Nos. WO 86/05807 and WO 89/01036; and U.S. Patent No. 5,122,464, which are herein incorporated by reference in their entireties) and variable regions of the antibody can be cloned into such a vector for expression of the entire heavy, the entire light chain, or both the entire heavy and light chains.

[00265]   An expression vector can be transferred to a cell (*e.g.*, host cell) by conventional techniques and the resulting cells can then be cultured by conventional techniques to produce an antibody described herein or a fragment thereof. Thus, provided herein are host cells containing a polynucleotide encoding an antibody described herein or fragments thereof, or a heavy or light chain thereof, or fragment thereof, or a single chain antibody described herein, operably linked to a promoter for expression of such sequences in the host cell. In certain embodiments, for the expression of double-chained antibodies, vectors encoding both the heavy and light chains, individually, can be co-expressed in the host cell for expression of the entire immunoglobulin molecule, as detailed below. In certain embodiments, a host cell contains a vector comprising a polynucleotide encoding both the heavy chain and light chain of an antibody described herein, or a fragment thereof. In specific embodiments, a host cell contains two different vectors, a first vector comprising a polynucleotide encoding a heavy chain or a heavy chain variable region of an antibody described herein, or a fragment thereof, and a second vector comprising a polynucleotide encoding a light chain or a light chain variable region of an antibody described herein, or a fragment thereof. In other embodiments, a first host cell comprises a first vector comprising a polynucleotide encoding a heavy chain or a heavy chain variable region of an antibody described herein, or a fragment thereof, and a second host cell comprises a second vector comprising a polynucleotide encoding a light chain or a light chain variable region of an antibody described herein. In specific embodiments, a heavy chain/heavy chain variable region expressed by a first cell

associated with a light chain/light chain variable region of a second cell to form an anti-PD-1 antibody described herein. In certain embodiments, provided herein is a population of host cells comprising such first host cell and such second host cell.

[00266]   In a particular embodiment, provided herein is a population of vectors comprising a first vector comprising a polynucleotide encoding a light chain/light chain variable region of an anti-PD-1 antibody described herein, and a second vector comprising a polynucleotide encoding a heavy chain/heavy chain variable region of an anti-PD-1 antibody described herein.

[00267]   A variety of host-expression vector systems can be utilized to express antibody molecules described herein (see, *e.g.*, U.S. Patent No. 5,807,715, which is herein incorporated by reference in its entirety). Such host-expression systems represent vehicles by which the coding sequences of interest can be produced and subsequently purified, but also represent cells which can, when transformed or transfected with the appropriate nucleotide coding sequences, express an antibody molecule described herein *in situ*. These include but are not limited to microorganisms such as bacteria (*e.g.*, *E. coli* and *B. subtilis*) transformed with recombinant bacteriophage DNA, plasmid DNA or cosmid DNA expression vectors containing antibody coding sequences; yeast (*e.g.*, *Saccharomyces Pichia*) transformed with recombinant yeast expression vectors containing antibody coding sequences; insect cell systems infected with recombinant virus expression vectors (*e.g.*, baculovirus) containing antibody coding sequences; plant cell systems (*e.g.*, green algae such as *Chlamydomonas reinhardtii*) infected with recombinant virus expression vectors (*e.g.*, cauliflower mosaic virus, CaMV; tobacco mosaic virus, TMV) or transformed with recombinant plasmid expression vectors (*e.g.*, Ti plasmid) containing antibody coding sequences; or mammalian cell systems (*e.g.*, COS (*e.g.*, COS1 or COS), CHO, BHK, MDCK, HEK 293, NS0, PER.C6, VERO, CRL7O3O, HsS78Bst, HeLa, and NIH 3T3, HEK-293T, HepG2, SP210, R1.1, B-W, L-M, BSC1, BSC40, YB/20 and BMT10 cells) harboring recombinant expression constructs containing promoters derived from the genome of mammalian cells (*e.g.*, metallothionein promoter) or from mammalian viruses (*e.g.*, the adenovirus late promoter; the vaccinia virus 7.5K promoter). In a specific embodiment, cells for expressing antibodies described herein are CHO cells, for example CHO cells from the CHO GS System™ (Lonza). In a particular embodiment, cells for expressing antibodies described herein are human cells, *e.g.*, human cell lines. In a specific embodiment, a mammalian expression vector is pOptiVEC™ or pcDNA3.3. In a particular embodiment, bacterial cells such as *Escherichia coli*, or eukaryotic cells (*e.g.*, mammalian cells), especially for the expression of whole recombinant

antibody molecule, are used for the expression of a recombinant antibody molecule. For example, mammalian cells such as Chinese hamster ovary (CHO) cells, in conjunction with a vector such as the major intermediate early gene promoter element from human cytomegalovirus is an effective expression system for antibodies (Foecking MK & Hofstetter H (1986) Gene 45: 101-5; and Cockett MI *et al.*, (1990) Biotechnology 8(7): 662-7, each of which is herein incorporated by reference in its entirety). In certain embodiments, antibodies described herein are produced by CHO cells or NS0 cells. In a specific embodiment, the expression of nucleotide sequences encoding antibodies described herein which specifically bind PD-1 (*e.g.*, human PD-1) is regulated by a constitutive promoter, inducible promoter or tissue specific promoter.

[00268]   In bacterial systems, a number of expression vectors can be advantageously selected depending upon the use intended for the antibody molecule being expressed. For example, when a large quantity of such an antibody is to be produced, for the generation of pharmaceutical compositions of an antibody molecule, vectors which direct the expression of high levels of fusion protein products that are readily purified can be desirable. Such vectors include, but are not limited to, the *E. coli* expression vector pUR278 (Ruether U & Mueller-Hill B (1983) EMBO J 2: 1791-1794), in which the antibody coding sequence can be ligated individually into the vector in frame with the lac Z coding region so that a fusion protein is produced; pIN vectors (Inouye S & Inouye M (1985) Nuc Acids Res 13: 3101-3109; Van Heeke G & Schuster SM (1989) J Biol Chem 24: 5503-5509); and the like, all of which are herein incorporated by reference in their entireties. For example, pGEX vectors can also be used to express foreign polypeptides as fusion proteins with glutathione 5-transferase (GST). In general, such fusion proteins are soluble and can easily be purified from lysed cells by adsorption and binding to matrix glutathione agarose beads followed by elution in the presence of free glutathione. The pGEX vectors are designed to include thrombin or factor Xa protease cleavage sites so that the cloned target gene product can be released from the GST moiety.

[00269]   In an insect system, *Autographa californica* nuclear polyhedrosis virus (AcNPV), for example, can be used as a vector to express foreign genes. The virus grows in *Spodoptera frugiperda* cells. The antibody coding sequence can be cloned individually into non-essential regions (for example the polyhedrin gene) of the virus and placed under control of an AcNPV promoter (for example the polyhedrin promoter).

[00270]   In mammalian host cells, a number of viral-based expression systems can be utilized. In cases where an adenovirus is used as an expression vector, the antibody coding

sequence of interest can be ligated to an adenovirus transcription/translation control complex, *e.g.*, the late promoter and tripartite leader sequence. This chimeric gene can then be inserted in the adenovirus genome by *in vitro* or *in vivo* recombination. Insertion in a non-essential region of the viral genome (*e.g.*, region El or E3) will result in a recombinant virus that is viable and capable of expressing the antibody molecule in infected hosts (*e.g.*, see Logan J & Shenk T (1984) PNAS 81(12): 3655-9, which is herein incorporated by reference in its entirety). Specific initiation signals can also be required for efficient translation of inserted antibody coding sequences. These signals include the ATG initiation codon and adjacent sequences. Furthermore, the initiation codon must be in phase with the reading frame of the desired coding sequence to ensure translation of the entire insert. These exogenous translational control signals and initiation codons can be of a variety of origins, both natural and synthetic. The efficiency of expression can be enhanced by the inclusion of appropriate transcription enhancer elements, transcription terminators, *etc.* (see, *e.g.*, Bitter G *et al.*, (1987) Methods Enzymol. 153: 516-544, which is herein incorporated by reference in its entirety).

[00271]   In addition, a host cell strain can be chosen which modulates the expression of the inserted sequences, or modifies and processes the gene product in the specific fashion desired. Such modifications (*e.g.*, glycosylation) and processing (*e.g.*, cleavage) of protein products can be important for the function of the protein. Different host cells have characteristic and specific mechanisms for the post-translational processing and modification of proteins and gene products. Appropriate cell lines or host systems can be chosen to ensure the correct modification and processing of the foreign protein expressed. To this end, eukaryotic host cells which possess the cellular machinery for proper processing of the primary transcript, glycosylation, and phosphorylation of the gene product can be used. Such mammalian host cells include but are not limited to CHO, VERO, BHK, Hela, MDCK, HEK 293, NIH 3T3, W138, BT483, Hs578T, HTB2, BT2O and T47D, NS0 (a murine myeloma cell line that does not endogenously produce any immunoglobulin chains), CRL7O3O, COS (*e.g.*, COS1 or COS), PER.C6, VERO, HsS78Bst, HEK-293T, HepG2, SP210, R1.1, B-W, L-M, BSC1, BSC40, YB/20, BMT10 and HsS78Bst cells. In certain embodiments, anti-PD-1 antibodies described herein are produced in mammalian cells, such as CHO cells.

[00272]   In a specific embodiment, the antibodies described herein have reduced fucose content or no fucose content. Such antibodies can be produced using techniques known one skilled in the art. For example, the antibodies can be expressed in cells deficient or lacking the ability of to fucosylate. In a specific example, cell lines with a knockout of both alleles of

α1,6-fucosyltransferase can be used to produce antibodies with reduced fucose content. The Potelligent® system (Lonza) is an example of such a system that can be used to produce antibodies with reduced fucose content.

[00273]   For long-term, high-yield production of recombinant proteins, stable expression cells can be generated. For example, cell lines which stably express an anti-PD-1 antibody described herein can be engineered. In specific embodiments, a cell provided herein stably expresses a light chain/light chain variable region and a heavy chain/heavy chain variable region which associate to form an antibody described herein.

[00274]   In certain aspects, rather than using expression vectors which contain viral origins of replication, host cells can be transformed with DNA controlled by appropriate expression control elements (*e.g.*, promoter, enhancer, sequences, transcription terminators, polyadenylation sites, *etc.*), and a selectable marker. Following the introduction of the foreign DNA/polynucleotide, engineered cells can be allowed to grow for 1-2 days in an enriched media, and then are switched to a selective media. The selectable marker in the recombinant plasmid confers resistance to the selection and allows cells to stably integrate the plasmid into their chromosomes and grow to form foci which in turn can be cloned and expanded into cell lines. This method can advantageously be used to engineer cell lines which express an anti-PD-1 antibody described herein or a fragment thereof. Such engineered cell lines can be particularly useful in screening and evaluation of compositions that interact directly or indirectly with the antibody molecule.

[00275]   A number of selection systems can be used, including but not limited to the herpes simplex virus thymidine kinase (Wigler M *et al.*, (1977) Cell 11(1): 223-32), hypoxanthineguanine phosphoribosyltransferase (Szybalska EH & Szybalski W (1962) PNAS 48(12): 2026-2034) and adenine phosphoribosyltransferase (Lowy I *et al.*, (1980) Cell 22(3): 817-23) genes in tk-, hgprt- or aprt-cells, respectively, all of which are herein incorporated by reference in their entireties. Also, antimetabolite resistance can be used as the basis of selection for the following genes: *dhfr*, which confers resistance to methotrexate (Wigler M *et al.*, (1980) PNAS 77(6): 3567-70; O'Hare K *et al.*, (1981) PNAS 78: 1527-31); *gpt*, which confers resistance to mycophenolic acid (Mulligan RC & Berg P (1981) PNAS 78(4): 2072-6); neo, which confers resistance to the aminoglycoside G-418 (Wu GY & Wu CH (1991) Biotherapy 3: 87-95; Tolstoshev P (1993) Ann Rev Pharmacol Toxicol 32: 573-596; Mulligan RC (1993) Science 260: 926-932; and Morgan RA & Anderson WF (1993) Ann Rev Biochem 62: 191-217; Nabel GJ & Felgner PL (1993) Trends Biotechnol 11(5): 211-5); and *hygro*, which confers resistance to hygromycin (Santerre RF *et al.*, (1984) Gene

30(1-3): 147-56), all of which are herein incorporated by reference in their entireties. Methods commonly known in the art of recombinant DNA technology can be routinely applied to select the desired recombinant clone and such methods are described, for example, in Ausubel FM *et al.*, (eds.), Current Protocols in Molecular Biology, John Wiley & Sons, NY (1993); Kriegler M, Gene Transfer and Expression, A Laboratory Manual, Stockton Press, NY (1990); and in Chapters 12 and 13, Dracopoli NC *et al.*, (eds.), Current Protocols in Human Genetics, John Wiley & Sons, NY (1994); Colbère-Garapin F *et al.*, (1981) J Mol Biol 150: 1-14, which are incorporated by reference herein in their entireties.

[00276]   The expression levels of an antibody molecule can be increased by vector amplification (for a review, see Bebbington CR & Hentschel CCG, The use of vectors based on gene amplification for the expression of cloned genes in mammalian cells in DNA cloning, Vol. 3 (Academic Press, New York, 1987), which is herein incorporated by reference in its entirety).   When a marker in the vector system expressing antibody is amplifiable, increase in the level of inhibitor present in culture of host cell will increase the number of copies of the marker gene.   Since the amplified region is associated with the antibody gene, production of the antibody will also increase (Crouse GF *et al.*, (1983) Mol Cell Biol 3: 257-66, which is herein incorporated by reference in its entirety).

[00277]   The host cell can be co-transfected with two or more expression vectors described herein, the first vector encoding a heavy chain derived polypeptide and the second vector encoding a light chain derived polypeptide.   The two vectors can contain identical selectable markers which enable equal expression of heavy and light chain polypeptides.   The host cells can be co-transfected with different amounts of the two or more expression vectors.   For example, host cells can be transfected with any one of the following ratios of a first expression vector and a second expression vector: 1:1, 1:2, 1:3, 1:4, 1:5, 1:6, 1:7, 1:8, 1:9, 1:10, 1:12, 1:15, 1:20, 1:25, 1:30, 1:35, 1:40, 1:45, or 1:50.

[00278]   Alternatively, a single vector can be used which encodes, and is capable of expressing, both heavy and light chain polypeptides.   In such situations, the light chain should be placed before the heavy chain to avoid an excess of toxic free heavy chain (Proudfoot NJ (1986) Nature 322: 562-565; and Köhler G (1980) PNAS 77: 2197-2199, each of which is herein incorporated by reference in its entirety).   The coding sequences for the heavy and light chains can comprise cDNA or genomic DNA.   The expression vector can be monocistronic or multicistronic.   A multicistronic nucleic acid construct can encode 2, 3, 4, 5, 6, 7, 8, 9, 10 or more, or in the range of 2-5, 5-10 or 10-20 genes/nucleotide sequences.   For example, a bicistronic nucleic acid construct can comprise in the following order a promoter,

a first gene (*e.g.*, heavy chain of an antibody described herein), and a second gene and (*e.g.*, light chain of an antibody described herein). In such an expression vector, the transcription of both genes can be driven by the promoter, whereas the translation of the mRNA from the first gene can be by a cap-dependent scanning mechanism and the translation of the mRNA from the second gene can be by a cap-independent mechanism, *e.g.*, by an IRES.

[00279] Once an antibody molecule described herein has been produced by recombinant expression, it can be purified by any method known in the art for purification of an immunoglobulin molecule, for example, by chromatography (*e.g.*, ion exchange, affinity, particularly by affinity for the specific antigen after Protein A, and sizing column chromatography), centrifugation, differential solubility, or by any other standard technique for the purification of proteins. Further, the antibodies described herein can be fused to heterologous polypeptide sequences described herein or otherwise known in the art to facilitate purification.

[00280] In specific embodiments, an antibody described herein is isolated or purified. Generally, an isolated antibody is one that is substantially free of other antibodies with different antigenic specificities than the isolated antibody. For example, in a particular embodiment, a preparation of an antibody described herein is substantially free of cellular material and/or chemical precursors. The language "substantially free of cellular material" includes preparations of an antibody in which the antibody is separated from cellular components of the cells from which it is isolated or recombinantly produced. Thus, an antibody that is substantially free of cellular material includes preparations of antibody having less than about 30%, 20%, 10%, 5%, 2%, 1%, 0.5%, or 0.1% (by dry weight) of heterologous protein (also referred to herein as a "contaminating protein") and/or variants of an antibody, for example, different post-translational modified forms of an antibody or other different versions of an antibody (*e.g.*, antibody fragments). When the antibody is recombinantly produced, it is also generally substantially free of culture medium, *i.e.*, culture medium represents less than about 20%, 10%, 2%, 1%, 0.5%, or 0.1% of the volume of the protein preparation. When the antibody is produced by chemical synthesis, it is generally substantially free of chemical precursors or other chemicals, *i.e.*, it is separated from chemical precursors or other chemicals which are involved in the synthesis of the protein. Accordingly, such preparations of the antibody have less than about 30%, 20%, 10%, or 5% (by dry weight) of chemical precursors or compounds other than the antibody of interest. In a specific embodiment, antibodies described herein are isolated or purified.

[00281] Antibodies or fragments thereof that specifically bind to PD-1 (*e.g.*, human PD-1)

can be produced by any method known in the art for the synthesis of antibodies, for example, by chemical synthesis or by recombinant expression techniques. The methods described herein employs, unless otherwise indicated, conventional techniques in molecular biology, microbiology, genetic analysis, recombinant DNA, organic chemistry, biochemistry, PCR, oligonucleotide synthesis and modification, nucleic acid hybridization, and related fields within the skill of the art. These techniques are described, for example, in the references cited herein and are fully explained in the literature. See, *e.g.*, Maniatis T *et al.*, (1982) Molecular Cloning: A Laboratory Manual, Cold Spring Harbor Laboratory Press; Sambrook J *et al.*, (1989), Molecular Cloning: A Laboratory Manual, Second Edition, Cold Spring Harbor Laboratory Press; Sambrook J *et al.*, (2001) Molecular Cloning: A Laboratory Manual, Cold Spring Harbor Laboratory Press, Cold Spring Harbor, NY; Ausubel FM *et al.*, Current Protocols in Molecular Biology, John Wiley & Sons (1987 and annual updates); Current Protocols in Immunology, John Wiley & Sons (1987 and annual updates) Gait (ed.) (1984) Oligonucleotide Synthesis: A Practical Approach, IRL Press; Eckstein (ed.) (1991) Oligonucleotides and Analogues: A Practical Approach, IRL Press; Birren B *et al.*, (eds.) (1999) Genome Analysis: A Laboratory Manual, Cold Spring Harbor Laboratory Press, all of which are herein incorporated by reference in their entireties.

[00282]   In a specific embodiment, an antibody described herein is an antibody (*e.g.*, recombinant antibody) prepared, expressed, created or isolated by any means that involves creation, *e.g.*, via synthesis, genetic engineering of DNA sequences. In certain embodiments, such antibody comprises sequences (*e.g.*, DNA sequences or amino acid sequences) that do not naturally exist within the antibody germline repertoire of an animal or mammal (*e.g.*, human) *in vivo*.

[00283]   In one aspect, provided herein is a method of making an antibody which specifically binds to PD-1 (*e.g.*, human PD-1) comprising culturing a cell or host cell described herein. Preferably, the method is performed *in vitro*. In a certain aspect, provided herein is a method of making an antibody which specifically binds to PD-1 (*e.g.*, human PD-1) comprising expressing (*e.g.*, recombinantly expressing) the antibody using a cell or host cell described herein (*e.g.*, a cell or a host cell comprising polynucleotides encoding an antibody described herein). In a particular embodiment, the cell is an isolated cell. In a particular embodiment, the exogenous polynucleotides have been introduced into the cell. In a particular embodiment, the method further comprises the step of purifying the antibody obtained from the cell or host cell.

[00284]   Methods for producing polyclonal antibodies are known in the art (see, for

example, Chapter 11 in: Short Protocols in Molecular Biology, (2002) 5th Ed., Ausubel FM *et al.*, eds., John Wiley and Sons, New York, which is herein incorporated by reference in its entirety).

[00285]   Monoclonal antibodies can be prepared using a wide variety of techniques known in the art including the use of hybridoma, recombinant, and phage display technologies, or a combination thereof. For example, monoclonal antibodies can be produced using hybridoma techniques including those known in the art and taught, for example, in Harlow E & Lane D, Antibodies: A Laboratory Manual, (Cold Spring Harbor Laboratory Press, 2nd ed. 1988); Hammerling GJ *et al.*, in: Monoclonal Antibodies and T-Cell Hybridomas 563 681 (Elsevier, N.Y., 1981), each of which is herein incorporated by reference in its entirety. The term "monoclonal antibody" as used herein is not limited to antibodies produced through hybridoma technology. For example, monoclonal antibodies can be produced recombinantly from host cells exogenously expressing an antibody described herein or a fragment thereof, for example, light chain and/or heavy chain of such antibody.

[00286]   In specific embodiments, a "monoclonal antibody," as used herein, is an antibody produced by a single cell (*e.g.*, hybridoma or host cell producing a recombinant antibody), wherein the antibody specifically binds to PD-1 (*e.g.*, human PD-1) as determined, *e.g.*, by ELISA or other antigen-binding or competitive binding assay known in the art or in the examples provided herein. In particular embodiments, a monoclonal antibody can be a chimeric antibody or a humanized antibody. In certain embodiments, a monoclonal antibody is a monovalent antibody or multivalent (*e.g.*, bivalent) antibody. In particular embodiments, a monoclonal antibody is a monospecific or multispecific antibody (*e.g.*, bispecific antibody). Monoclonal antibodies described herein can, for example, be made by the hybridoma method as described in Kohler G & Milstein C (1975) Nature 256: 495, which is herein incorporated by reference in its entirety, or can, *e.g.*, be isolated from phage libraries using the techniques as described herein, for example. Other methods for the preparation of clonal cell lines and of monoclonal antibodies expressed thereby are well known in the art (see, for example, Chapter 11 in: Short Protocols in Molecular Biology, (2002) 5th Ed., Ausubel FM *et al.*, *supra*).

[00287]   Methods for producing and screening for specific antibodies using hybridoma technology are routine and well known in the art. For example, in the hybridoma method, a mouse or other appropriate host animal, such as a sheep, goat, rabbit, rat, hamster or macaque monkey, is immunized to elicit lymphocytes that produce or are capable of producing antibodies that will specifically bind to the protein (*e.g.*, PD-1 (*e.g.*, human PD-1)) used for

immunization. Alternatively, lymphocytes may be immunized *in vitro*. Lymphocytes then are fused with myeloma cells using a suitable fusing agent, such as polyethylene glycol, to form a hybridoma cell (Goding JW (Ed), Monoclonal Antibodies: Principles and Practice, pp. 59-103 (Academic Press, 1986), herein incorporated by reference in its entirety). Additionally, a RIMMS (repetitive immunization multiple sites) technique can be used to immunize an animal (Kilpatrick KE *et al.*, (1997) Hybridoma 16:381-9, herein incorporated by reference in its entirety).

[00288] In some embodiments, mice (or other animals, such as rats, monkeys, donkeys, pigs, sheep, hamster, or dogs) can be immunized with an antigen (*e.g.*, PD-1 (*e.g.*, human PD-1)) and once an immune response is detected, *e.g.*, antibodies specific for the antigen are detected in the mouse serum, the mouse spleen is harvested and splenocytes isolated. The splenocytes are then fused by well-known techniques to any suitable myeloma cells, for example cells from cell line SP20 available from the American Type Culture Collection (ATCC®) (Manassas, VA), to form hybridomas. Hybridomas are selected and cloned by limited dilution. In certain embodiments, lymph nodes of the immunized mice are harvested and fused with NS0 myeloma cells.

[00289] The hybridoma cells thus prepared are seeded and grown in a suitable culture medium that preferably contains one or more substances that inhibit the growth or survival of the unfused, parental myeloma cells. For example, if the parental myeloma cells lack the enzyme hypoxanthine guanine phosphoribosyl transferase (HGPRT or HPRT), the culture medium for the hybridomas typically will include hypoxanthine, aminopterin, and thymidine (HAT medium), which substances prevent the growth of HGPRT-deficient cells.

[00290] Specific embodiments employ myeloma cells that fuse efficiently, support stable high-level production of antibody by the selected antibody-producing cells, and are sensitive to a medium such as HAT medium. Among these myeloma cell lines are murine myeloma lines, such as NS0 cell line or those derived from MOPC-21 and MPC-11 mouse tumors available from the Salk Institute Cell Distribution Center, San Diego, CA, USA, and SP-2 or X63-Ag8.653 cells available from the American Type Culture Collection, Rockville, MD, USA. Human myeloma and mouse-human heteromyeloma cell lines also have been described for the production of human monoclonal antibodies (Kozbor D (1984) J Immunol 133: 3001-5; Brodeur *et al.*, Monoclonal Antibody Production Techniques and Applications, pp. 51-63 (Marcel Dekker, Inc., New York, 1987), each of which is herein incorporated by reference in its entirety).

[00291] Culture medium in which hybridoma cells are growing is assayed for production

of monoclonal antibodies directed against PD-1 (*e.g.*, human PD-1). The binding specificity of monoclonal antibodies produced by hybridoma cells is determined by methods known in the art, for example, immunoprecipitation or by an *in vitro* binding assay, such as radioimmunoassay (RIA) or enzyme-linked immunoabsorbent assay (ELISA).

[00292]   After hybridoma cells are identified that produce antibodies of the desired specificity, affinity, and/or activity, the clones may be subcloned by limiting dilution procedures and grown by standard methods (Goding JW (Ed), Monoclonal Antibodies: Principles and Practice, *supra*). Suitable culture media for this purpose include, for example, D-MEM or RPMI 1640 medium. In addition, the hybridoma cells may be grown *in vivo* as ascites tumors in an animal.

[00293]   The monoclonal antibodies secreted by the subclones are suitably separated from the culture medium, ascites fluid, or serum by conventional immunoglobulin purification procedures such as, for example, protein A-Sepharose, hydroxylapatite chromatography, gel electrophoresis, dialysis, or affinity chromatography.

[00294]   Antibodies described herein include antibody fragments which recognize specific PD-1 (*e.g.*, human PD-1) and can be generated by any technique known to those of skill in the art. For example, Fab and F(ab')$_2$ fragments described herein can be produced by proteolytic cleavage of immunoglobulin molecules, using enzymes such as papain (to produce Fab fragments) or pepsin (to produce F(ab')$_2$ fragments). A Fab fragment corresponds to one of the two identical arms of an antibody molecule and contains the complete light chain paired with the VH and CH1 domains of the heavy chain. A F(ab')$_2$ fragment contains the two antigen-binding arms of an antibody molecule linked by disulfide bonds in the hinge region.

[00295]   Further, the antibodies described herein can also be generated using various phage display methods known in the art. In phage display methods, functional antibody domains are displayed on the surface of phage particles which carry the polynucleotide sequences encoding them. In particular, DNA sequences encoding VH and VL domains are amplified from animal cDNA libraries (*e.g.*, human or murine cDNA libraries of affected tissues). The DNA encoding the VH and VL domains are recombined together with a scFv linker by PCR and cloned into a phagemid vector. The vector is electroporated in *E. coli* and the *E. coli* is infected with helper phage. Phage used in these methods are typically filamentous phage including fd and M13, and the VH and VL domains are usually recombinantly fused to either the phage gene III or gene VIII. Phage expressing an antigen binding domain that binds to a particular antigen can be selected or identified with antigen, *e.g.*, using labeled antigen or

antigen bound or captured to a solid surface or bead. Examples of phage display methods that can be used to make the antibodies described herein include those disclosed in Brinkman U *et al.,* (1995) J Immunol Methods 182: 41-50; Ames RS *et al.,* (1995) J Immunol Methods 184: 177-186; Kettleborough CA *et al.,* (1994) Eur J Immunol 24: 952-958; Persic L *et al.,* (1997) Gene 187: 9-18; Burton DR & Barbas CF (1994) Advan Immunol 57: 191-280; PCT Application No. PCT/GB91/001134; International Publication Nos. WO 90/02809, WO 91/10737, WO 92/01047, WO 92/18619, WO 93/1 1236, WO 95/15982, WO 95/20401, and WO 97/13844; and U.S. Patent Nos. 5,698,426, 5,223,409, 5,403,484, 5,580,717, 5,427,908, 5,750,753, 5,821,047, 5,571,698, 5,427,908, 5,516,637, 5,780,225, 5,658,727, 5,733,743 and 5,969,108, all of which are herein incorporated by reference in their entireties.

[00296] As described in the above references, after phage selection, the antibody coding regions from the phage can be isolated and used to generate whole antibodies, including human antibodies, or any other desired antigen binding fragment, and expressed in any desired host, including mammalian cells, insect cells, plant cells, yeast, and bacteria, *e.g.,* as described below. Techniques to recombinantly produce antibody fragments such as Fab, Fab' and F(ab')₂ fragments can also be employed using methods known in the art such as those disclosed in PCT publication No. WO 92/22324; Mullinax RL *et al.,* (1992) BioTechniques 12(6): 864-9; Sawai H *et al.,* (1995) Am J Reprod Immunol 34: 26-34; and Better M *et al.,* (1988) Science 240: 1041-1043, all of which are herein incorporated by reference in their entireties.

[00297] In certain embodiments, to generate whole antibodies, PCR primers including VH or VL nucleotide sequences, a restriction site, and a flanking sequence to protect the restriction site can be used to amplify the VH or VL sequences from a template, *e.g.,* scFv clones. Utilizing cloning techniques known to those of skill in the art, the PCR amplified VH domains can be cloned into vectors expressing a VH constant region, and the PCR amplified VL domains can be cloned into vectors expressing a VL constant region, *e.g.,* human kappa or lambda constant regions. The VH and VL domains can also be cloned into one vector expressing the necessary constant regions. The heavy chain conversion vectors and light chain conversion vectors are then co-transfected into cell lines to generate stable or transient cell lines that express full-length antibodies, *e.g.,* IgG, using techniques known to those of skill in the art.

[00298] A chimeric antibody is a molecule in which different portions of the antibody are derived from different immunoglobulin molecules. For example, a chimeric antibody can contain a variable region of a mouse or rat monoclonal antibody fused to a constant region of

a human antibody. Methods for producing chimeric antibodies are known in the art. See, *e.g.,* Morrison SL (1985) Science 229: 1202-7; Oi VT & Morrison SL (1986) BioTechniques 4: 214-221; Gillies SD *et al.,* (1989) J Immunol Methods 125: 191-202; and U.S. Patent Nos. 5,807,715, 4,816,567, 4,816,397, and 6,331,415, all of which are herein incorporated by reference in their entireties.

[00299]   A humanized antibody is capable of binding to a predetermined antigen and which comprises a framework region having substantially the amino acid sequence of a human immunoglobulin and CDRs having substantially the amino acid sequence of a non-human immunoglobulin (*e.g.,* a murine immunoglobulin). In particular embodiments, a humanized antibody also comprises at least a portion of an immunoglobulin constant region (Fc), typically that of a human immunoglobulin. The antibody also can include the CH1, hinge, CH2, CH3, and CH4 regions of the heavy chain. A humanized antibody can be selected from any class of immunoglobulins, including IgM, IgG, IgD, IgA and IgE, and any isotype, including IgG_1, IgG_2, IgG_3 and IgG_4. Humanized antibodies can be produced using a variety of techniques known in the art, including but not limited to, CDR-grafting (European Patent No. EP 239400; International Publication No. WO 91/09967; and U.S. Patent Nos. 5,225,539, 5,530,101, and 5,585,089), veneering or resurfacing (European Patent Nos. EP 592106 and EP 519596; Padlan EA (1991) Mol Immunol 28(4/5): 489-498; Studnicka GM *et al.,* (1994) Prot Engineering 7(6): 805-814; and Roguska MA *et al.,* (1994) PNAS 91: 969-973), chain shuffling (U.S. Patent No. 5,565,332), and techniques disclosed in, *e.g.,* U.S. Pat. No. 6,407,213, U.S. Pat. No. 5,766,886, International Publication No. WO 93/17105; Tan P *et al.,* (2002) J Immunol 169: 1119-25; Caldas C *et al.,* (2000) Protein Eng. 13(5): 353-60; Morea V *et al.,* (2000) Methods 20(3): 267-79; Baca M *et al.,* (1997) J Biol Chem 272(16): 10678-84; Roguska MA *et al.,* (1996) Protein Eng 9(10): 895 904; Couto JR *et al.,* (1995) Cancer Res. 55 (23 Supp): 5973s-5977s; Couto JR *et al.,* (1995) Cancer Res 55(8): 1717-22; Sandhu JS (1994) Gene 150(2): 409-10 and Pedersen JT *et al.,* (1994) J Mol Biol 235(3): 959-73, all of which are herein incorporated by reference in their entireties. See also U.S. Application Publication No. US 2005/0042664 A1 (Feb. 24, 2005), which is incorporated by reference herein in its entirety.

[00300]   Methods for making multispecific (*e.g.,* bispecific antibodies) have been described, see, for example, U.S. Patent Nos. 7,951,917; 7,183,076; 8,227,577; 5,837,242; 5,989,830; 5,869,620; 6,132,992 and 8,586,713, all of which are herein incorporated by reference in their entireties.

[00301]   Single domain antibodies, for example, antibodies lacking the light chains, can be

produced by methods well known in the art. See Riechmann L & Muyldermans S (1999) J Immunol 231: 25-38; Nuttall SD *et al.,* (2000) Curr Pharm Biotechnol 1(3): 253-263; Muyldermans S, (2001) J Biotechnol 74(4): 277-302; U.S. Patent No. 6,005,079; and International Publication Nos. WO 94/04678, WO 94/25591 and WO 01/44301, all of which are herein incorporated by reference in their entireties.

[00302]   Further, antibodies that specifically bind to a PD-1 antigen can, in turn, be utilized to generate anti-idiotype antibodies that "mimic" an antigen using techniques well known to those skilled in the art. See, *e.g.,* Greenspan NS & Bona CA (1989) FASEB J 7(5): 437-444; and Nissinoff A (1991) J Immunol 147(8): 2429-2438, each of which is herein incorporated by reference in its entirety.

[00303]   In particular embodiments, an antibody described herein, which binds to the same epitope of PD-1 (*e.g.,* human PD-1) as an anti-PD-1 antibody described herein, is a human antibody. In particular embodiments, an antibody described herein, which competitively blocks (*e.g.,* in a dose-dependent manner) any one of the antibodies described herein, from binding to PD-1 (*e.g.,* human PD-1), is a human antibody. Human antibodies can be produced using any method known in the art. For example, transgenic mice which are incapable of expressing functional endogenous immunoglobulins, but which can express human immunoglobulin genes, can be used. In particular, the human heavy and light chain immunoglobulin gene complexes can be introduced randomly or by homologous recombination into mouse embryonic stem cells. Alternatively, the human variable region, constant region, and diversity region can be introduced into mouse embryonic stem cells in addition to the human heavy and light chain genes. The mouse heavy and light chain immunoglobulin genes can be rendered non-functional separately or simultaneously with the introduction of human immunoglobulin loci by homologous recombination. In particular, homozygous deletion of the J_H region prevents endogenous antibody production. The modified embryonic stem cells are expanded and microinjected into blastocysts to produce chimeric mice. The chimeric mice are then bred to produce homozygous offspring which express human antibodies. The transgenic mice are immunized in the normal fashion with a selected antigen, *e.g.,* all or a portion of an antigen (*e.g.,* PD-1). Monoclonal antibodies directed against the antigen can be obtained from the immunized, transgenic mice using conventional hybridoma technology. The human immunoglobulin transgenes harbored by the transgenic mice rearrange during B cell differentiation, and subsequently undergo class switching and somatic mutation. Thus, using such a technique, it is possible to produce therapeutically useful IgG, IgA, IgM and IgE antibodies. For an overview of this technology

for producing human antibodies, see Lonberg N & Huszar D (1995) Int Rev Immunol 13:65-93, herein incorporated by reference in its entirety. For a detailed discussion of this technology for producing human antibodies and human monoclonal antibodies and protocols for producing such antibodies, see, *e.g.,* International Publication Nos. WO 98/24893, WO 96/34096 and WO 96/33735; and U.S. Patent Nos. 5,413,923, 5,625,126, 5,633,425, 5,569,825, 5,661,016, 5,545,806, 5,814,318 and 5,939,598, all of which are herein incorporated by reference in their entireties. Examples of mice capable of producing human antibodies include the Xenomouse™ (Abgenix, Inc.; U.S. Patent Nos. 6,075,181 and 6,150,184), the HuAb-Mouse™ (Mederex, Inc./Gen Pharm; U.S. Patent Nos. 5,545,806 and 5,569, 825), the Trans Chromo Mouse™ (Kirin) and the KM Mouse™ (Medarex/Kirin), all of which are herein incorporated by reference in their entireties.

[00304]   Human antibodies which specifically bind to PD-1 (*e.g.,* human PD-1) can be made by a variety of methods known in the art including phage display methods described above using antibody libraries derived from human immunoglobulin sequences. *See* also U.S. Patent Nos. 4,444,887, 4,716,111, and 5,885,793; and International Publication Nos. WO 98/46645, WO 98/50433, WO 98/24893, WO 98/16654, WO 96/34096, WO 96/33735, and WO 91/10741, all of which are herein incorporated by reference in their entireties.

[00305]   In some embodiments, human antibodies can be produced using mouse–human hybridomas. For example, human peripheral blood lymphocytes transformed with Epstein-Barr virus (EBV) can be fused with mouse myeloma cells to produce mouse–human hybridomas secreting human monoclonal antibodies, and these mouse–human hybridomas can be screened to determine ones which secrete human monoclonal antibodies that specifically bind to a target antigen (*e.g.,* PD-1 (*e.g.,* human PD-1)). Such methods are known and are described in the art, see, *e.g.,* Shinmoto H *et al.,* (2004) Cytotechnology 46: 19-23; Naganawa Y *et al.,* (2005) Human Antibodies 14: 27-31, each of which is herein incorporated by reference in its entirety.

5.6   Kits

[00306]   Also provided, are kits comprising one or more antibodies described herein, or pharmaceutical composition or conjugates thereof. In a specific embodiment, provided herein is a pharmaceutical pack or kit comprising one or more containers filled with one or more of the ingredients of the pharmaceutical compositions described herein, such as one or more antibodies provided herein. In some embodiments, the kits contain a pharmaceutical composition described herein and any prophylactic or therapeutic agent, such as those

described herein. In certain embodiments, the kits may contain a T cell mitogen, such as, *e.g.*, phytohaemagglutinin (PHA) and/or phorbol myristate acetate (PMA), or a TCR complex stimulating antibody, such as an anti-CD3 antibody and anti-CD28 antibody. Optionally associated with such container(s) can be a notice in the form prescribed by a governmental agency regulating the manufacture, use or sale of pharmaceuticals or biological products, which notice reflects approval by the agency of manufacture, use or sale for human administration.

[00307] Also provided, are kits that can be used in the above methods. In one embodiment, a kit comprises an antibody described herein, preferably a purified antibody, in one or more containers. In a specific embodiment, kits described herein contain a substantially isolated PD-1 antigen (*e.g.*, human PD-1) as a control. In another specific embodiment, the kits described herein further comprise a control antibody which does not react with a PD-1 antigen. In another specific embodiment, kits described herein contain one or more elements for detecting the binding of an antibody to a PD-1 antigen (*e.g.*, the antibody can be conjugated to a detectable substrate such as a fluorescent compound, an enzymatic substrate, a radioactive compound or a luminescent compound, or a second antibody which recognizes the first antibody can be conjugated to a detectable substrate). In specific embodiments, a kit provided herein can include a recombinantly produced or chemically synthesized PD-1 antigen. The PD-1 antigen provided in the kit can also be attached to a solid support. In a more specific embodiment, the detecting means of the above described kit includes a solid support to which a PD-1 antigen is attached. Such a kit can also include a non-attached reporter-labeled anti-human antibody or anti-mouse/rat antibody. In this embodiment, binding of the antibody to the PD-1 antigen can be detected by binding of the said reporter-labeled antibody. In one embodiment, the present invention relates to the use of a kit of the present invention for *in vitro* assaying and/or detecting PD-1 antigen (*e.g.*, human PD-1) in a biological sample.

6.   EXAMPLES

[00308] The examples in this Section (*i.e.*, Section 6) are offered by way of illustration, and not by way of limitation.

6.1   Example 1: Characterization of anti-PD-1 antibodies

[00309] This example describes the characterization of antibodies that specifically bind to human PD-1, in particular, antibodies designated AGEN2033w, AGEN2034w, AGEN2046w, and AGEN2047w. AGEN2033w and AGEN2046w share the same heavy chain variable

region amino acid sequence (SEQ ID NO: 15) and the same light chain variable region amino acid sequence (SEQ ID NO: 16). AGEN2034w and AGEN2047w share the same heavy chain variable region amino acid sequence (SEQ ID NO: 17) and the same light chain variable region amino acid sequence (SEQ ID NO: 16). AGEN2033w and AGEN2034w are human IgG$_4$ antibodies containing an S228P mutation (*i.e.*, substitution of serine with proline at position 228 relative to the wild type IgG$_4$ constant region) according to the EU numbering system, whereas AGEN2046w and AGEN2047w are human IgG$_1$ antibodies. In addition, three Fc mutants of AGEN2047w were also characterized: an N297A mutant, an S267E/L328F double mutant, and an S239D/A330L/I332E triple mutant, numbered according to the EU numbering system.

6.1.1 Antibody binding to PD-1 expressed by activated T cells

[00310] The anti-PD-1 antibodies AGEN2046w, AGEN2047w, and AGEN2034w were examined for binding to activated peripheral blood mononuclear cells (PBMCs) by flow cytometry. Cryopreserved human PBMCs prepared from unpurified buffy coats (Research Blood Components, Catalog number (Cat#) 002) or cryopreserved cynomolgus PBMCs (Worldwide Primates Inc., customer order) were plated at 10^5 cells/well in RPMI1640 medium supplemented with Normocin™ (InvivoGen, Cat# ant-nr-1) and 10% heat-inactivated FBS (Gibco, Cat# 16140063) in 96-well NUNCLON delta surface plates (NUNC™). The cells were cultured in the presence of 100 ng/ml of Staphylococcus Enterotoxin A (SEA; for human PBMCs) (Toxin Technologies, Cat# at101red) or Staphylococcus Enterotoxin B (SEB; for cynomolgus PBMCs) (Toxin Technology, Cat# bt202red) for 5 days at 37°C, 5% CO_2, and 97% humidity. The cells were then washed once with sample buffer (PBS + 2% FBS + 0.09% sodium azide) and incubated in the dark on ice with 100 µl of serially diluted antibodies or isotype controls (10, 1, 0.1, 0.01, 0.001, and 0.0001 µg/ml of AGEN2046w, AGEN2047w, or human IgG$_1$ isotype control (LifeTein LLC, Cat# LT12031); or 25, 5, 1, 0.2, 0.04, 0.008, 0.0016, 0.00032, and 0.000064 µg/ml of AGEN2034w or human IgG$_4$ isotype control (LifeTein LLC, Cat# LT12034)). After 45 minutes, the cells were washed twice with sample buffer and then incubated with LIVE/DEAD® Fixable Near-IR Dead Cell Stain (Life Technologies, Cat# L10119), CD4-BV421 (Biolegend, Cat# 317434), and goat F(ab')$_2$ anti-human IgG+A+M, R-PE (Life Technologies, Cat# AHI1707) for 30 minutes. The cells were washed twice with sample buffer and then resuspended in sample buffer and analyzed with a FACS Fortessa cytometer (Becton Dickinson). CD4+ T cells were gated and the mean fluorescence intensity (MFI)

was recorded.

[00311]   The anti-PD-1 antibodies AGEN2046w and AGEN2047w bound to activated human CD4+ T cells (Figure 1A). AGEN2034w bound to activated human and cynomolgus CD4+ T cells (Figures 1B and 1C).

[00312]   The binding of AGEN2034w to activated primary human T cells was measured again in a similar assay. Briefly, human PBMCs were cultured in the presence of 100 ng/ml of the SEA peptide for 5 days and then stained with serially diluted (50, 10, 2, 0.4, 0.080, 0.016, 0.0032, 0.00064, 0.000128, 0.0000256, 0.00000512, and 0.000001024 µg/ml) AGEN2034w or a human IgG$_4$ isotype control. Cells were analyzed with a FACS Fortessa cytometer (Becton Dickinson). CD4+ T cells were gated and the mean fluorescence intensity (MFI) of AGEN2034w-positive cells was determined.

[00313]   As shown in Figure 1D, AGEN2034w bound to activated primary human CD4+ T cells.

6.1.2   PD-1 antibody selectivity assay

[00314]   The selectivity of AGEN2034w for PD-1 was assessed against homologous proteins using suspension array technology.

[00315]   Based on their amino acid sequence homology with PD-1, Ig superfamily proteins roundabout homolog 2 (ROBO2), B7 homolog 7 (B7-H7), and signal regulatory protein gamma (SIRPγ) were selected for evaluation of binding by AGEN0234w using a suspension array assay. ROBO2, B7-H7 and SIRPγ were identified as homologs of PD-1 by protein alignment applying Basic Local Alignment Search Tool (BLAST; NCBI). The sequence homology of human PD-1 to its homologs was as follows: human PD-1 *versus* (*vs*) human ROBO2: 27.9%; human PD-1 *vs* human SIRPγ (SIRPG_HUMAN): 24.8%; and human PD-1 *vs* human B7-H7: 22.6%.

[00316]   Recombinant proteins, human ROBO2-Fc chimera (R&D systems, Cat# 3147-RB-050), human B7-H7-Fc chimera (R&D systems, Cat# 8084-B7-050), human SIRPγ-His chimera (Sino Biologicals, Cat# 11828-H08H), and human PD-1-Fc chimera (R&D systems, Cat# 1086-PD) were coupled to Luminex® microspheres (Luminex Corp, Cat# LC10005-01, LC10022-01, LC10046-01, LC10048-01, and LC10059-01) using N-hydroxysuccinimide (NHS) ester chemistry and incubated with a dose titration (7.5, 2.5, 0.833, 0.277, .0.0926, .0.0309, 0.0103, 0.0034, 0.0011, 0.0004, 0.0001, and 0.00004 µg/ml) of AGEN2034w. An anti-human IgG antibody labeled with phycoerythrin (PE) was then added to detect AGEN2034w. Binding was assessed by a Luminex® 200 detection system.

[00317]   The antibody AGEN2034w showed specific binding to human PD-1, and no significant binding to ROBO2, B7-H7, or SIRPγ was observed at tested concentrations (Figure 2).

6.1.3   Ligand blocking activity determined by suspension array technology

[00318]   To determine whether anti-PD-1 antibodies block binding of ligands PD-L1 and PD-L2, a ranking assay setup was performed using suspension array technology.   1200 Luminex® beads in 5 μl assay buffer (Luminex Corp, Cat# 48 LC10014-48) were added to each well of 96-well half area plates (Corning, Inc., Cat# 3884).   The beads were coupled with PD-1 antigen PD-1-Fc chimera (R&D systems, Cat# 1086-PD) via amine coupling with COOH bead surface.   The coupling reaction was performed using 50 μg/ml of PD-1 antigen and 1 x 10^7 Luminex beads per ml.   Standard NHS ester chemistry was used to form carbodiimide bonds between the primary amine groups of the antigen and the carboxyl groups on the bead surface (Luminex Xmap cookbook chapter 3).

[00319]   Antigen coupling for proteins is a simple two-step carbodiimide procedure during which microsphere carboxyl groups are first activated with EDC (1-Ethyl-3-[3-dimethylaminopropyl]carbodiimide hydrochloride) reagent in the presence of Sulfo-NHS (N-hydroxysulfosuccinimide) to form a sulfo-NHS-ester intermediate.   The reactive intermediate is then replaced by reaction with the primary amine of the target molecule (antibody, protein or peptide) to form a covalent amide bond. The coupled beads were incubated with different concentrations of anti-PD-1 antibodies in triplicates (final concentrations from 7.5 μg/ml to 0.01 μg/ml per well) for 1 hour at 20°C and 650 rpm.   The antibodies tested were AGEN2033w, AGEN2034w, AGEN2046w, and AGEN2047w, an IgG$_1$ isotype control, and an IgG$_4$ isotype control.   Subsequently, 30 μl of R-PE labeled PD-L1-Fc (R&D Systems, Cat# 156-B7) or PD-L2-Fc (R&D Systems, Cat# 1224-PL) at a concentration of 1 nM was added to each well, giving a total well volume of 60 μl (1200 beads per well and a final concentration of 0.5 nM of labeled PD-L1 or PD-L2).   The labeling of the ligand was conducted using R-PE labeling kits (AbDSerotec, LYNX Rapid RPE Antibody Conjugation Kit, Cat# LNK023RPE) according to the manufacturer's protocol.   Plates were analyzed using a Luminex® 200 system (Millipore).   100 beads were counted per well in 50 μl sample volume. Ligand blocking potential was calculated using the MFI values of the non-competed signal (100% binding) of the ligand only control.   A PE detectable signal indicated ligand binding to the antigen.

[00320]   All of the anti-PD-1 antibodies tested inhibited binding of PD-L1 and PD-L2 to

PD-1 (Figures 3A-3D).

[00321] The measurement of the ligand blocking activity of AGEN2034w was repeated in a similar assay. Briefly, recombinant PD-1-Fc chimera (R&D systems, Cat# 1086-PD) was coupled to Luminex® microspheres (Luminex Corp, Cat# LC10048-01) using N-hydroxysuccinimide (NHS) ester chemistry. The PD-1-coupled beads were incubated with a dose titration (4.0×10^{-5} – 7.5 μg/ml) of AGEN2034w or an isotype control antibody, followed by incubation of fluorescently labeled PD-L1-Fc (R&D Systems, Cat# 156-B7) or PD-L2-Fc (R&D Systems, Cat# 1224-PL). Subsequently, binding of PD-L1 or PD-L2 to the PD-1-coupled beads was assessed using a Luminex® 200 detection system and the median fluorescent intensity (MFI) was recorded.

[00322] The antibody AGEN2034w effectively blocked engagement of PD-1 with its ligands, PD-L1 (Figure 3E) and PD-L2 (Figure 3F).

6.1.4 Effect of anti-PD-1 antibodies on human PBMCs following Staphylococcus Enterotoxin A (SEA) stimulation

[00323] The functional activity of anti-PD-1 antibodies on primary human T cells was assessed following SEA stimulation. Cryopreserved human PBMCs prepared from unpurified buffy coats (Research Blood Components, Cat# 002) were plated at 10^5 cells/well in RPMI1640 medium supplemented with Normocin™ (InvivoGen, Cat# ant-nr-1) and 10% heat-inactivated FBS (Gibco, Cat# 16140063) in 96-well NUNCLON delta surface plates (NUNC™). The cells were cultured in the presence of a fixed concentration (10 μg/ml) or dose-range amounts of antibodies (50, 10, 2, 0.4, 0.08, 0.016, and 0.0032 μg/ml) and a fixed amount of SEA (100 ng/ml, Toxin Technology, Cat# at101red) for 5 days at 37°C, 5% CO_2, and 97% humidity. The antibodies tested were AGEN2033w, AGEN2034w, AGEN2046w, AGEN2047w, and an IgG_1 isotype control. Supernatant was collected and stored at -80°C until analysis. The titers of IL-2 were measured by electrochemiluminescence (MSD).

[00324] As shown in Figures 4A and 4B, the anti-PD-1 antibodies AGEN2033w, AGEN2034w, AGEN2046w, and AGEN2047w increased IL-2 production of PBMCs relative to isotype control in the presence of SEA stimulation.

[00325] Further, the antagonistic activity of AGEN2034w was examined either alone or in combination with anti-CTLA-4, anti-TIGIT, anti-CD137, or anti-OX40 antibodies in the primary PBMC assay described above. Briefly, cryopreserved human PBMCs prepared from unpurified buffy coats (Research Blood Components, Cat #002) were cultured with the SEA superantigen (Toxin Technology, Cat# at101red) (100 ng/ml in Figures 4C, 4D, and 4F; 200 ng/ml in Figure 4E) and AGEN2034w (10 μg/ml in Figures 4C and 4E; 5 μg/ml in Figure

4D; a dose range of 12, 6, 3, 0.3, 0.03, 0.003, 0.0003, and 0.0001 µg/ml in Figure 4F) or an isotype control antibody in the presence or absence of 5 µg/ml of anti-CTLA-4 antibody Ipilimumab (Myoderm) (Figures 4C), 10 µg/ml of anti-TIGIT antibody pab2197 or pab2196 (Figure 4D), 5 µg/ml of anti-CD137 antibody pab2225 (Figure 4E), or a dose range (12, 6, 3, 0.3, 0.03, 0.003, 0.0003, and 0.0001 µg/ml) of anti-OX40 antibody pab1928 for 5 days. Supernatants were collected and titers of IL-2 were measured using AlphaLISA (Perkin Elmer, Cat# AL221C). Anti-TIGIT antibodies pab2197 and pab2196 were generated based on the variable region sequences of antibodies 10A7 and 1F4, respectively, provided in U.S. Application Publication No. US2013/0251720 (herein incorporated by reference in its entirety). Anti-CD137 antibody pab2225 was generated based on the variable region sequences of antibody 20H4 provided in U.S. Patent No. 8,137,667 (herein incorporated by reference in its entirety). Anti-OX40 antibody pab1928 was generated based on the variable region sequences of antibody Hu106-122 provided in U.S. Patent Publication No. US 2013/0280275 (herein incorporated by reference in its entirety). The sequences of the anti-TIGIT, anti-CD137, and anti-OX40 antibodies are listed in Table 8.

Table 8. Sequences of anti-TIGIT, anti-CD137, and anti-OX40 antibodies

SEQ ID NO:	Description	Amino acid sequence
66	pab2197 heavy chain	EVQLVESGGGLTQPGKSLKLSCEASGFTFSSFTMHWVRQSPGKGLE WVAFIRSGSGIVFYADAVRGRFTISRDNAKNLLFLQMNDLKSEDTA MYYCARRPLGHNTFDSWGQGTLVTVSSASTKGPSVFPLAPSSKSTS GGTAALGCLVKDYFPEPVTVSWNSGALTSGVHTFPAVLQSSGLYSL SSVVTVPSSSLGTQTYICNVNHKPSNTKVDKRVEPKSCDKTHTCPP CPAPELLGGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSHEDPEVK FNWYVDGVEVHNAKTKPREEQYNSTYRVVSVLTVLHQDWLNGKEYK CKVSNKALPAPIEKTISKAKGQPREPQVYTLPPSREEMTKNQVSLT CLVKGFYPSDIAVEWESNGQPENNYKTTPPVLDSDGSFFLYSKLTV DKSRWQQGNVFSCSVMHEALHNHYTQKSLSLSPG

SEQ ID NO:	Description	Amino acid sequence
67	pab2197 light chain	DIVMTQSPSSLAVSPGEKVTMTCKSSQSLYYSGVKENLLAWYQQKP GQSPKLLIYYASIRFTGVPDRFTGSGSGTDYTLTITSVQAEDMGQY FCQQGINNPLTFGDGTKLEIKRTVAAPSVFIFPPSDEQLKSGTASV VCLLNNFYPREAKVQWKVDNALQSGNSQESVTEQDSKDSTYSLSST LTLSKADYEKHKVYACEVTHQGLSSPVTKSFNRGEC
68	pab2196 heavy chain	EVQLQQSGPELVKPGTSMKISCKASGYSFTGHLMNWVKQSHGKNLE WIGLIIPYNGGTSYNQKFKGKATLTVDKSSSTAYMELLSLTSDDSA VYFCSRGLRGFYAMDYWGQGTSVTVSSASTKGPSVFPLAPSSKSTS GGTAALGCLVKDYFPEPVTVSWNSGALTSGVHTFPAVLQSSGLYSL SSVVTVPSSSLGTQTYICNVNHKPSNTKVDKRVEPKSCDKTHTCPP CPAPELLGGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSHEDPEVK FNWYVDGVEVHNAKTKPREEQYNSTYRVVSVLTVLHQDWLNGKEYK CKVSNKALPAPIEKTISKAKGQPREPQVYTLPPSREEMTKNQVSLT CLVKGFYPSDIAVEWESNGQPENNYKTTPPVLDSDGSFFLYSKLTV DKSRWQQGNVFSCSVMHEALHNHYTQKSLSLSPG
69	pab2196 light chain	DVVLTQTPLSLSVSFGDQVSISCRSSQSLVNSYGNTFLSWYLHKPG QSPQLLIFGISNRFSGVPDRFSGSGSGTDFTLKISTIKPEDLGMYY CLQGTHQPPTFGPGTKLEVKRTVAAPSVFIFPPSDEQLKSGTASVV CLLNNFYPREAKVQWKVDNALQSGNSQESVTEQDSKDSTYSLSSTL TLSKADYEKHKVYACEVTHQGLSSPVTKSFNRGEC
70	pab2225 heavy chain	QVQLQQWGAGLLKPSETLSLTCAVYGGSFSGYYWSWIRQSPEKGLE WIGEINHGGYVTYNPSLESRVTISVDTSKNQFSLKLSSVTAADTAV YYCARDYGPGNYDWYFDLWGRGTLVTVSSASTKGPSVFPLAPSSKS TSGGTAALGCLVKDYFPEPVTVSWNSGALTSGVHTFPAVLQSSGLY SLSSVVTVPSSSLGTQTYICNVNHKPSNTKVDKRVEPKSCDKTHTC PPCPAPELLGGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSHEDPE VKFNWYVDGVEVHNAKTKPREEQYNSTYRVVSVLTVLHQDWLNGKE YKCKVSNKALPAPIEKTISKAKGQPREPQVYTLPPSREEMTKNQVS LTCLVKGFYPSDIAVEWESNGQPENNYKTTPPVLDSDGSFFLYSKL TVDKSRWQQGNVFSCSVMHEALHNHYTQKSLSLSPG

SEQ ID NO:	Description	Amino acid sequence
71	pab2225 light chain	EIVLTQSPATLSLSPGERATLSCRASQSVSSYLAWYQQKPGQAPRL LIYDASNRATGIPARFSGSGSGTDFTLTISSLEPEDFAVYYCQQRS NWPPALTFGGGTKVEIKRTVAAPSVFIFPPSDEQLKSGTASVVCLL NNFYPREAKVQWKVDNALQSGNSQESVTEQDSKDSTYSLSSTLTLS KADYEKHKVYACEVTHQGLSSPVTKSFNRGEC
72	pab1928 heavy chain	QVQLVQSGSELKKPGASVKVSCKASGYTFTDYSMHWVRQAPGQGLK WMGWINTETGEPTYADDFKGRFVFSLDTSVSTAYLQISSLKAEDTA VYYCANPYYDYVSYYAMDYWGQGTTVTVSSASTKGPSVFPLAPSSK STSGGTAALGCLVKDYFPEPVTVSWNSGALTSGVHTFPAVLQSSGL YSLSSVVTVPSSSLGTQTYICNVNHKPSNTKVDKRVEPKSCDKTHT CPPCPAPELLGGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSHEDP EVKFNWYVDGVEVHNAKTKPREEQYNSTYRVVSVLTVLHQDWLNGK EYKCKVSNKALPAPIEKTISKAKGQPREPQVYTLPPSREEMTKNQV SLTCLVKGFYPSDIAVEWESNGQPENNYKTTPPVLDSDGSFFLYSK LTVDKSRWQQGNVFSCSVMHEALHNHYTQKSLSLSPG
73	pab1928 light chain	DIQMTQSPSSLSASVGDRVTITCKASQDVSTAVAWYQQKPGKAPKL LIYSASYLYTGVPSRFSGSGSGTDFTFTISSLQPEDIATYYCQQHY STPRTFGQGTKLEIKRSVAAPSVFIFPPSDEQLKSGTASVVCLLNN FYPREAKVQWKVDNALQSGNSQESVTEQDSKDSTYSLSSTLTLSKA DYEKHKVYACEVTHQGLSSPVTKSFNRGEC

[00326]   The anti-PD-1 antibody AGEN2034w, either alone or in combination with anti-CTLA-4 antibody Ipilimumab (Figure 4C), anti-TIGIT antibody pab2197 or pab2196 (Figure 4D), anti-CD137 antibody pab2225 (Figure 4E), or anti-OX40 antibody pab1928 (Figure 4F), enhanced IL-2 production in human PBMCs in the presence of the SEA superantigen.

6.1.5   Effect of Fc gamma receptor binding on the antagonistic activity of anti-PD-1 antibodies

[00327]   In this example, the effect of FcγR binding on the antagonistic activity of anti-PD-1 antibodies was examined.

[00328]   First, the antagonistic activity of an anti-PD-1 reference antibody was examined in the presence or absence of FcγR blockers. Cryopreserved human PBMCs prepared from unpurified buffy coats (Research Blood Components, Cat# 002) were plated at 10^5 cells/well

in RPMI1640 medium supplemented with Normocin™ (InvivoGen, Cat# ant-nr-1) and 10% heat-inactivated FBS (Gibco, Cat# 16140063) in 96-well NUNCLON delta surface plates (NUNC™). The cells were cultured with 100 ng/ml of SEA peptide (Toxin Technology, Cat# at101red) and 10 μg/ml of an anti-PD-1 reference antibody or isotype control in the presence or absence of a fixed concentration of an Fc receptor blocking cocktail containing anti-CD16 antibody (1 μg/ml, R&D systems, Cat# AF1330) and irrelevant IgG$_1$ (25 μg/ml, LifeTein, Cat# LT12031) for 5 days at 37°C, 5% CO_2, and 97% humidity. Supernatant was collected and stored at -80°C until analysis. The titers of IL-2 were measured by electrochemiluminescence (MSD).

[00329]   FcγR blockade using an anti-CD16 antibody enhanced the ability of the anti-PD-1 reference antibody to induce IL-2 secretion in this primary human PBMC assay (Figure 5A).

[00330]   A similar study was conducted to examine the impact of FcγR blockade on the antagonistic activity of AGEN2034w. Briefly, human PBMCs were cultured with 100 ng/ml of SEA peptide (Toxin Technology, Cat# at101red), 10 μg/ml of AGEN2034w or an isotype control antibody (HEL IgG$_1$, LifeTein, Cat# LT12031), and 20 μg/ml of an anti-CD16 antibody (Biolegend, Cat# 302013), an anti-CD32 antibody (eBioscience, Cat# 16-0329-81) which binds to both CD32A and CD32B, an anti-CD64 antibody (Biolegend, Cat# 305016), or an isotype control (Biolegend, Cat# 400543) for 5 days at 37°C, 5% CO_2, and 97% humidity. Supernatants were collected and stored at -80°C until analysis. The titers of IL-2 were measured by electrochemiluminescence (MSD).

[00331]   Consistent with the result in Figure 5A, FcγR blockade using an anti-CD16 antibody, anti-CD32 antibody, or anti-CD64 antibody, increased IL-2 secretion induced by AGEN2034w in this human PBMC assay (Figure 5B).

[00332]   Next, three IgG$_1$ Fc mutants of AGEN2047w (an N297A mutant, an S267E/L328F double mutant, and an S239D/A330L/I332E triple mutant, numbered according to the EU numbering system) were generated and compared against AGEN2047w, which comprises a wild type IgG$_1$ constant region, in the human PBMC SEA assay described above. Briefly, cryopreserved human PBMCs were incubated with 100 ng/ml of SEA and 10 μg/ml of anti-PD-1 antibodies AGEN2047w, AGEN2047w-N297A, AGEN2047w-S267E/L328F, AGEN2047w-S239D/A330L/I332E, or isotype control antibodies with respective wild type or mutant Fc regions.

[00333]   As shown in Figure 5C, the N297A mutant with diminished FcγR binding further enhanced IL-2 secretion. In contrast, the S267E/L328F double mutant and the S239D/A330L/I332E triple mutant, both of which have enhanced FcγR binding, induced less

IL-2 production than that of wild type AGEN2047w.

6.1.6   Effect of anti-PD-1 antibody in mixed lymphocyte reaction

[00334]   Next, the anti-PD-1 antibody AGEN2034w was examined in a mixed lymphocyte reaction. Dendritic cells were derived from isolated CD14+ cells (Stemcell Technologies, Cat# 18058) obtained from cryopreserved HLA-A2+ human PBMCs and differentiated first in the presence of 500 U/ml IL-4 (Peprotech, Cat# 200-04-20UG) and 1000 U/ml GM-CSF (Peprotech, Cat# 300-03-20UG) for 24 hours, and then in the presence of 1000 U/ml TNFα (Peprotech, Cat# 300-01A-50UG), 10 ng/ml IL-1β (Peprotech, Cat# 200-01B-10UG), 10 ng/ml IL-6 (Peprotech, Cat# 200-06-20UG), and 1 μM PGE2 (Sigma, Cat# P0409-5MG) for additional 24 hours. 50,000 pan T cells purified from an allogeneic HLA-A2- human PBMC donor by MACS column purification (Miltenyi Biotec, Cat# 130-096-535) were co-cultured with 10,000 dendritic cells in the absence of any antibody or in the presence of 10 μg/ml human IgG₄ isotype control antibody (Biolegend, Cat# 403402) or 10 μg/ml AGEN2034w in RPMI (Corning, Cat# 10-040-CM) containing 5% human AB sera (Corning, Cat# 35-060-CI) and Penicillin/Streptomycin (Gibco, Cat# 15140-122). Cultures were incubated for 5 days at 37°C and 5% CO_2. Supernatant was assessed for steady-state concentrations of IFNγ using AlphaLISA (Perkin Elmer, Cat# AL217C).

[00335]   As shown in Figure 6, AGEN2034w induced IFNγ production in the co-culture of purified human T cells and *in vitro*-derived allogeneic dendritic cells.

6.1.7   Effect of anti-PD-1 antibody in an ascites fluid suppression assay

[00336]   In this example, the anti-PD-1 antibody AGEN2034w was examined for its ability to relieve suppression of T cell proliferation induced by ovarian cancer ascites fluid. Briefly, primary human PBMCs were labeled with CFSE (Biolegend, Cat# 423801) and then stimulated with 1 μg/ml anti-CD3 antibody (eBioscience, Cat# 16-0037-85) and 50% volume/volume of ovarian cancer ascites fluid in the presence of increasing concentrations (0.00000102-50 μg/ml) of AGEN2034w or an IgG₄ isotype control antibody (Biolegend, Cat# 317434) for 4 to 5 days. The cells were immunostained with anti-human CD4 antibody (Biolegend, Cat# 317434) or anti-human CD8 antibody (Biolegend, Cat# 344710) and LIVE-DEAD viability stain (Life Technologies, Cat# L10119). Proliferation of CD4+ or CD8+ T cells, as illustrated by CFSE dilution, was measured by flow cytometry using BD Fortessa (Becton Dickinson).

[00337]   As shown in Figures 7A-7C, co-culture with ovarian cancer ascites fluid reduced proliferation of anti-CD3-antibody-stimulated T cells and this reduction could be partially

relieved by the anti-PD-1 antibody AGEN2034w.

6.1.8   Effect of anti-PD-1 antibody in a Jurkat NFAT-luciferase reporter assay

[00338]   Further, a reporter assay was utilized to probe the antagonistic activity of AGEN2034w. Specifically, in this reporter assay, co-culture of PD-L1-expressing target cells and PD-1-expressing reporter cells inhibited expression of a NFAT-luciferase reporter gene in the reporter cells. Blockade of PD-1/PD-L1 interaction by an anti-PD-1 antibody could relieve the inhibitory signal, leading to luciferase expression.

[00339]   Briefly, PD-L1+ CHOK1 target cells (Promega, Cat# CS187108) were co-cultured with GloResponse™ NFAT-luc2/PD-1 Jurkat reporter cells (Promega, Cat# CS187102) in the presence of increasing concentrations (0 - 50 µg/ml) of AGEN2034w or an isotype control antibody in RPMI-1640 medium (Corning, Cat# 21-040-CV) supplemented with 2% heat-inactivated FBS (Gemini, Cat# 100-106). After 6 hours of incubation, the efficacy of AGEN2034w to relieve suppression of the reporter gene induced by PD-L1 binding to PD-1 was determined by measuring luciferase using Bio-Glo™ Luciferase Assay System (Promega, Cat# G7941).

[00340]   As shown in Figure 8, the anti-PD-1 antibody AGEN2034w enhanced TCR signaling in a dose-dependent manner in this Jurkat NFAT-luciferase reporter assay.

6.2   Example 2: Characterization of additional anti-PD-1 antibodies

[00341]   In this example, the following six additional anti-PD-1 antibodies were characterized: AGEN2001w, AGEN2002w, EP11_pl1_B03, EP11_pl1_B05, EP11_pl1_C02, and EP11_pl1_C03. The variable heavy chain and variable light chain sequences of these antibodies are disclosed in Table 6.

6.2.1   Binding and ligand blocking analysis of anti-PD-1 antibodies

[00342]   The affinity of the six anti-PD-1 antibodies described above was analyzed by surface plasmon resonance. All six antibodies bound to recombinant human PD-1 (data not shown).

[00343]   The ligand blocking activity of the six anti-PD-1 antibodies was examined using suspension array technology in an assay similar to the one described in Section 6.1.3. The coupled beads were incubated with different concentrations of anti-PD-1 antibodies in duplicates (final concentrations from 7.5 µg/ml to 0.01 µg/ml per well) for 1 hour at 20°C and 650 rpm. R-PE labeled PD-L1-Fc (R&D Systems, Cat# 156-B7) or PD-L2-Fc (R&D Systems, Cat# 1224-PL) was then added. The anti-PD-1 antibodies tested were AGEN2001w, AGEN2002w, EP11_pl1_B03, EP11_pl1_B05, EP11_pl1_C02, and

EP11_pl1_C03. As shown in Figures 9A-9F, all of the anti-PD-1 antibodies tested blocked binding of PD-1 to PD-L1 and PD-L2 in a dose-dependent manner.

6.2.2   Effect of anti-PD-1 antibody on human PBMCs following Staphylococcus Enterotoxin A (SEA) stimulation

[00344]   The functional activity of the anti-PD-1 antibody AGEN2002w on human PBMCs was tested in an assay similar to the one described in Section 6.1.4. Briefly, cryopreserved human PBMCs prepared from unpurified buffy coats (Research Blood Components, Cat# 002) were cultured in the presence of 10 µg/ml of the anti-PD-1 antibody AGEN2002w or an isotype control antibody (HEL IgG$_1$, LifeTein, Cat# LT12031) and 100 ng/ml of SEA peptide (Toxin Technologies, Cat# at101red) for 4 days at 37°C, 5% CO_2, and 97% humidity. Supernatant was collected and stored at -80°C until analysis. The titers of IL-2 were measured by electrochemiluminescence (MSD).

[00345]   As shown in Figure 10, the anti-PD-1 antibody AGEN2002w increased IL-2 production of primary human PBMCs in the presence of SEA stimulation.

6.3   Example 3: Epitope mapping of anti-PD-1 antibody

[00346]   The epitope of anti-PD-1 antibody AGEN2034w was characterized using hydrogen-deuterium exchange (HDX) mass spectrometry and a Pepscan analysis.

6.3.1   Epitope mapping of anti-PD-1 Fab using hydrogen-deuterium exchange (HDX) mass spectrometry

[00347]   The interaction of a Fab fragment of AGEN2034w (AGEN2034w-Fab) with the extracellular domain of human PD-1 was studied by hydrogen-deuterium exchange (HDX) mass spectrometry.

[00348]   Recombinant His-tagged human PD-1 was obtained from Sino Biological Inc (Cat# 10377-H08H). When used, deglycosylated PD-1 was prepared from 300 µg of recombinant His-tagged human PD-1 protein incubated with 6 µl of PNGase F at 37°C for 4 hours. Fab fragment of anti-PD-1 antibody was prepared from AGEN2034w by protease treatment.

[00349]   For pepsin/protease XVIII digestion, 4.0 µg of native or deglycosylated human PD-1 in 125 µl control buffer (50 mM phosphate, 100 mM sodium chloride at pH 7.4) was denatured by adding 135 µl of 4 M guanidine hydrochloride, 0.85 M TCEP buffer (final pH is 2.5), and incubating the mixture for 3 minutes at 11°C. Then, the mixture was subjected to on-column pepsin/protease XVIII digestion using an in-house packed pepsin/protease XVIII column and the resultant peptides were analyzed using a UPLC-MS system comprised of a

Waters Acquity UPLC coupled to a Q Exactive[TM] Hybrid Quadrupole-Orbitrap Mass Spectrometer (Thermo). The peptides were separated on a 50 mm × 1 mm C8 column with a 19 min gradient from 2-27% solvent B (0.2% formic acid in acetonitrile). Peptide identification was done through searching MS/MS data against the human PD-1 sequence with Mascot. The mass tolerance for the precursor and product ions was 10 ppm and 0.05 Da, respectively.

[00350] 10 µl human PD-1 (4.0 µg) or 10 µl human PD-1 and Fab mixture (4.0 µg: 4.0 µg) was incubated with 125 µl deuterium oxide labeling buffer (50 mM sodium phosphate, 100 mM sodium chloride at pD 7.4) for 0 second, 60 seconds, 600 seconds, and 3600 seconds at 11°C. Hydrogen/deuterium exchange was quenched by adding 135 µl of 4 M guanidine hydrochloride, 0.85 M TCEP buffer (final pH is 2.5). Subsequently, the quenched samples were subjected to on column pepsin/protease XVIII digestion and LC-MS analysis as described above. The mass spectra were recorded in MS only mode.

[00351] Raw MS data was processed using HDX WorkBench, software for the analysis of H/D exchange MS data (J. Am. Soc. Mass Spectrom. 2012, 23 (9), 1512-1521, incorporated herein by reference in its entirety). The deuterium levels were calculated using the average mass difference between the deuteriated peptide and its native form (t_0).

[00352] Sequence coverage of 85.4% was achieved for deglycosylated human PD-1 without His-tag. Most PD-1 peptides displayed identical or similar deuterium levels with and without the anti-human PD-1 Fab present. Several peptide segments, however, were found to have significantly decreased deuterium incorporation upon Fab binding. All the residues in this paragraph are numbered according to SEQ ID NO: 74. Deglycosylated human PD-1 showed strong reduction in deuterium uptake upon binding to anti-human PD-1 Fab at residues 107-122 (SLAPKAQIKESLRAEL) (SEQ ID NO: 75). In addition, a decrease in deuterium uptake was observed at residues 5-22 (LDSPDRPWNPPTFSPALL) (SEQ ID NO: 76) upon binding to anti-human PD-1 Fab.

6.3.2 Epitope mapping of anti-PD-1 antibody using a Pepscan analysis

[00353] The binding of anti-PD-1 antibody AGEN2034w was measured against synthesized PD-1 peptide fragments prepared as a chip-bound peptide array. Analysis was performed by Pepscan Presto BV, Lelystad, the Netherlands. Briefly, to reconstruct epitopes of human PD-1, a library of peptides was synthesized. An amino functionalized polypropylene support was obtained by grafting with a proprietary hydrophilic polymer formulation, followed by reaction with t-butyloxycarbonyl-hexamethylenediamine

(BocHMDA) using dicyclohexylcarbodiimide (DCC) with Nhydroxybenzotriazole (HOBt) and subsequent cleavage of the Boc-groups using trifluoroacetic acid (TFA). Standard Fmoc-peptide synthesis was used to synthesize peptides on the amino-functionalized solid support by custom modified JANUS liquid handling stations (Perkin Elmer). Synthesis of structural mimics was conducted using Pepscan's proprietary Chemically Linked Peptides on Scaffolds (CLIPS) technology. CLIPS technology allows to structure peptides into single loops, double loops, triple loops, sheet-like folds, helix-like folds and combinations thereof. The binding of antibody to each of the synthesized peptides was tested in a PEPSCAN-based ELISA. The peptide arrays were incubated with primary antibody solution overnight at 4°C. After washing, the peptide arrays were incubated with a goat anti-human HRP conjugate (Southern Biotech, Cat# 2010-05) for one hour at 25°C. After washing, the peroxidase substrate 2,2'-azino-di-3-ethylbenzthiazoline sulfonate (ABTS) and 20 µl/ml of 3% H_2O_2 were added. After one hour, the color development was measured and quantified with a charge coupled device (CCD) - camera and an image processing system.

[00354]   The Pepscan study showed that the anti-PD-1 antibody AGEN2034w recognized stretches of human PD-1 including residues 6-15 (DSPDRPWNPP) (SEQ ID NO: 77), residues 130-138 (EVPTAHPSP) (SEQ ID NO: 78), and residues 106-113 (ISLAPKAQ) (SEQ ID NO: 79), numbered according to SEQ ID NO: 74.

<div align="center">*   *   *</div>

[00355]   The invention is not to be limited in scope by the specific embodiments described herein. Indeed, various modifications of the invention in addition to those described will become apparent to those skilled in the art from the foregoing description and accompanying figures. Such modifications are intended to fall within the scope of the appended claims.

[00356]   All references (*e.g.*, publications or patents or patent applications) cited herein are incorporated herein by reference in their entirety and for all purposes to the same extent as if each individual reference (*e.g.*, publication or patent or patent application) was specifically and individually indicated to be incorporated by reference in its entirety for all purposes. Other embodiments are within the following claims.

WHAT IS CLAIMED:

1. An isolated antibody that specifically binds to human PD-1, comprising a heavy chain variable region comprising complementarity determining regions CDRH1, CDRH2, and CDRH3 and a light chain variable region comprising complementarity determining regions CDRL1, CDRL2, and CDRL3, wherein:

    (a) CDRH1 comprises the amino acid sequence of SYGMH (SEQ ID NO: 1);

    (b) CDRH2 comprises the amino acid sequence of VIWX$_1$DGSNX$_2$YYADSVX$_3$G (SEQ ID NO: 32), wherein

    X$_1$ is Y or F;

    X$_2$ is K or E; and

    X$_3$ is K or M;

    (c) CDRH3 comprises the amino acid sequence of NX$_1$DX$_2$ (SEQ ID NO: 33), wherein

    X$_1$ is G or V; and

    X$_2$ is H or Y;

    (d) CDRL1 comprises the amino acid sequence of RASQSVSSNLA (SEQ ID NO: 4);

    (e) CDRL2 comprises the amino acid sequence of GASTRAT (SEQ ID NO: 5); and

    (f) CDRL3 comprises the amino acid sequence of QQYNNWPRT (SEQ ID NO: 6).

2. The isolated antibody of claim 1, wherein CDRH2 comprises an amino acid sequence selected from the group consisting of SEQ ID NOs: 2 and 34-36.

3. The isolated antibody of claim 1 or 2, wherein CDRH3 comprises an amino acid sequence selected from the group consisting of SEQ ID NOs: 3, 7, and 37.

4. The isolated antibody of any one of claims 1-3, wherein CDRH1, CDRH2 and CDRH3 comprise the CDRH1, CDRH2 and CDRH3 amino acid sequences, respectively, set forth in SEQ ID NOs: 1, 2, and 3; 1, 2, and 7; 1, 2, and 37; 1, 34, and 7; 1, 35, and 7; or 1, 36, and 7.

5. An isolated antibody that specifically binds to human PD-1, comprising a heavy chain variable region comprising complementarity determining regions CDRH1, CDRH2 and CDRH3, and a light chain variable region comprising complementarity determining regions CDRL1, CDRL2 and CDRL3, wherein CDRH1, CDRH2, CDRH3, CDRL1, CDRL2, and CDRL3 comprise the amino acid sequences set forth in SEQ ID NOs: 1, 2, 3, 4, 5, and 6, respectively.

6. An isolated antibody that specifically binds to human PD-1, comprising a heavy chain variable region comprising complementarity determining regions CDRH1, CDRH2 and CDRH3, and a light chain variable region comprising complementarity determining regions CDRL1, CDRL2 and CDRL3, wherein CDRH1, CDRH2, CDRH3, CDRL1, CDRL2, and CDRL3 comprise the amino acid sequences set forth in SEQ ID NOs: 1, 2, 7, 4, 5, and 6, respectively.

7. The isolated antibody of any one of claims 1-6, wherein the antibody comprises a heavy chain variable region comprising the amino acid sequence of SEQ ID NO: 49.

8. The isolated antibody of any one of claims 1-6, wherein the antibody comprises a heavy chain variable region comprising an amino acid sequence which is at least 75%, 80%, 85%, 90%, 95%, or 100% identical to an amino acid sequence selected from the group consisting of SEQ ID NOs: 15, 17, and 26-31.

9. The isolated antibody of claim 8, wherein the heavy chain variable region comprises an amino acid sequence selected from the group consisting of SEQ ID NOs: 15, 17, and 26-31.

10. The isolated antibody of claim 9, wherein the heavy chain variable region comprises the amino acid sequence of SEQ ID NO: 15.

11. The isolated antibody of claim 9, wherein the heavy chain variable region comprises the amino acid sequence of SEQ ID NO: 17.

12. The isolated antibody of claim 10, wherein the antibody comprises a heavy chain comprising the amino acid sequence of SEQ ID NO: 52.

13. The isolated antibody of claim 10, wherein the antibody comprises a heavy chain comprising the amino acid sequence of SEQ ID NO: 54.

14. The isolated antibody of claim 11, wherein the antibody comprises a heavy chain comprising the amino acid sequence of SEQ ID NO: 53.

15. The isolated antibody of claim 11, wherein the antibody comprises a heavy chain comprising the amino acid sequence of SEQ ID NO: 55.

16. The isolated antibody of claim 11, wherein the antibody comprises a heavy chain comprising the amino acid sequence of SEQ ID NO: 56.

17. The isolated antibody of any one of claims 1-8, wherein the antibody comprises a heavy chain variable region having an amino acid sequence derived from a human IGHV3-33 germline sequence.

18. The isolated antibody of any one of claims 1-17, wherein the antibody comprises a light chain variable region comprising an amino acid sequence which is at least 75%, 80%, 85%, 90%, 95%, or 100% identical to the amino acid sequence of SEQ ID NO: 16.

19. The isolated antibody of claim 18, wherein the light chain variable region comprises the amino acid sequence of SEQ ID NO: 16.

20. The isolated antibody of claim 19, wherein the antibody comprises a light chain comprising the amino acid sequence of SEQ ID NO: 19.

21. The isolated antibody of any one of claims 1-18, wherein the antibody comprises a light chain variable region having an amino acid sequence derived from a human IGKV3-15 germline sequence.

22. The isolated antibody of claim 1, wherein the heavy chain variable region and the light chain variable region, respectively, comprise the amino acid sequences set forth in SEQ ID NOs: 15 and 16; 17 and 16; 26 and 16; 27 and 16; 28 and 16; 29 and 16; 30 and 16; or 31 and 16.

23. An isolated antibody that specifically binds to human PD-1, comprising:

(a) a heavy chain variable region comprising the amino acid sequence of SEQ ID NO: 15, and

(b) a light chain variable region comprising the amino acid sequence of SEQ ID NO: 16.

24. An isolated antibody that specifically binds to human PD-1, comprising:

(a) a heavy chain variable region comprising the amino acid sequence of SEQ ID NO: 17, and

(b) a light chain variable region comprising the amino acid sequence of SEQ ID NO: 16.

25. An isolated antibody that specifically binds to human PD-1, comprising a heavy chain variable region comprising an amino acid sequence selected from the group consisting of SEQ ID NOs: 15, 17, and 26-31.

26. An isolated antibody that specifically binds to human PD-1, comprising a light chain variable region comprising the amino acid sequence of SEQ ID NO: 16.

27. An isolated antibody that specifically binds to human PD-1, comprising:

(a) a heavy chain comprising the amino acid sequence of SEQ ID NO: 52; and

(b) a light chain comprising the amino acid sequence of SEQ ID NO: 19.

28. An isolated antibody that specifically binds to human PD-1, comprising:

(a) a heavy chain comprising the amino acid sequence of SEQ ID NO: 53; and

(b) a light chain comprising the amino acid sequence of SEQ ID NO: 19.

29. An isolated antibody that specifically binds to human PD-1, comprising:

(a) a heavy chain comprising the amino acid sequence of SEQ ID NO: 54; and

(b) a light chain comprising the amino acid sequence of SEQ ID NO: 19.

30. An isolated antibody that specifically binds to human PD-1, comprising:

(a) a heavy chain comprising the amino acid sequence of SEQ ID NO: 55; and

(b) a light chain comprising the amino acid sequence of SEQ ID NO: 19.

31. An isolated antibody that specifically binds to human PD-1, comprising:

(a) a heavy chain comprising the amino acid sequence of SEQ ID NO: 56; and

(b) a light chain comprising the amino acid sequence of SEQ ID NO: 19.

32. An isolated antibody that cross-competes for binding to human PD-1 with the antibody of any one of claims 1-31.

33. An isolated antibody that binds to the same epitope of human PD-1 as the antibody of any one of claims 1-31.

34. The isolated antibody of any one of claims 1-33, wherein the antibody binds to an epitope consisting of residues 107-122 of SEQ ID NO: 74.

35. The isolated antibody of any one of claims 1-34, wherein the antibody binds to an epitope consisting of residues 5-22 of SEQ ID NO: 74.

36. The isolated antibody of any one of claims 1-35, wherein the antibody binds to an epitope consisting of residues 6-15 of SEQ ID NO: 74.

37. The isolated antibody of any one of claims 1-36, wherein the antibody binds to an epitope consisting of residues 130-138 of SEQ ID NO: 74.

38. The isolated antibody of any one of claims 1-37, wherein the antibody binds to an epitope consisting of residues 106-113 of SEQ ID NO: 74.

39. The isolated antibody of any one of claims 1-11, 17-26, and 32-38, wherein the antibody comprises a heavy chain constant region selected from the group consisting of human IgG_1, IgG_2, IgG_3, IgG_4, IgA_1, and IgA_2.

40. The isolated antibody of any one of claims 1-11, 17-26, and 32-38, wherein the antibody comprises a human IgG_1 heavy chain constant region.

41. The isolated antibody of any one of claims 1-11, 17-26, and 32-38, wherein the antibody comprises a human IgG_1 heavy chain constant region that lacks a glycan moiety at position

N297, according to the EU numbering system.

42. The isolated antibody of any one of claims 1-11, 17-26, and 32-38, wherein the antibody comprises a human IgG$_1$ heavy chain constant region comprising an N297A mutation, according to the EU numbering system.

43. The isolated antibody of any one of claims 1-11, 17-26, and 32-38, wherein the antibody comprises a human IgG$_1$ heavy chain constant region comprising an N297Q mutation, according to the EU numbering system.

44. The isolated antibody of any one of claims 1-11, 17-26, and 32-38, wherein the antibody comprises a human IgG$_1$ heavy chain constant region comprising a D265A mutation, according to the EU numbering system.

45. The isolated antibody of any one of claims 1-11, 17-26, and 32-38, wherein the antibody comprises a human IgG$_4$ heavy chain constant region comprising an S228P mutation, according to the EU numbering system.

46. The isolated antibody of any one of claims 1-11, 17-26, and 32-38, wherein the antibody comprises a human IgG heavy chain constant region that is a variant of a wild type human IgG heavy chain constant region, wherein the variant human IgG heavy chain constant region binds to human Fc receptor with lower affinity than the wild type human IgG heavy chain constant region binds to the human Fc receptor.

47. The isolated antibody of claim 46, wherein the human Fc receptor is an FcγR.

48. The isolated antibody of claim 47, wherein the FcγR is FcγRIIB.

49. The isolated antibody of claim 47, wherein the FcγR is expressed on a cell selected from the group consisting of dendritic cells, monocytes, macrophages, neutrophils, granulocytes, B cells, and natural killer cells.

50. The isolated antibody of any one of claims 46-49, wherein the variant human IgG heavy chain constant region is a variant human IgG$_1$, a variant human IgG$_2$, or a variant human IgG$_4$ heavy chain constant region.

51. The isolated antibody of any one of claims 1-19, 21-26, and 32-50, wherein the antibody comprises a light chain constant region selected from the group consisting of human IgGκ and IgGλ.

52. The isolated antibody of any one of claims 1-51, wherein the antibody is a human antibody.

53. The isolated antibody of any one of claims 1-52, wherein the antibody is antagonistic to

human PD-1.

54. The isolated antibody of claim 53, wherein the antibody deactivates, reduces, or inhibits an activity of human PD-1.

55. The isolated antibody of claim 53, wherein the antibody inhibits binding of human PD-1 to human PD-L1 or to human PD-L2.

56. The isolated antibody of claim 53, wherein the antibody increases IL-2 production by peripheral blood mononuclear cells (PBMCs) stimulated with staphylococcal enterotoxin A (SEA).

57. The isolated antibody of claim 53, wherein the antibody increases IFNγ production of a co-culture of human T cells and allogenic dendritic cells.

58. The isolated antibody of claim 53, wherein the antibody increases proliferation of anti-CD3-antibody-stimulated CD4+ or CD8+ T cells co-cultured with ovarian cancer ascites fluid.

59. The isolated antibody of claim 53, wherein the antibody increases NFAT signaling in PD-1-expressing NFAT-luciferase reporter cells co-cultured with PD-L1-expressing target cells.

60. The isolated antibody of any one of claims 1-59 conjugated to a cytotoxic agent, cytostatic agent, toxin, radionuclide, or detectable label.

61. A pharmaceutical composition comprising the antibody of any one of claims 1-60 and a pharmaceutically acceptable carrier or excipient.

62. An isolated polynucleotide encoding a heavy and/or light chain of the antibody of any one of claims 1-60.

63. A vector comprising the polynucleotide of claim 62.

64. A recombinant host cell comprising the polynucleotide of claim 62 or the vector of claim 63.

65. A method of producing an antibody that binds to human PD-1, the method comprising culturing the host cell of claim 64 so that the polynucleotide is expressed and the antibody is produced.

66. A method of increasing T cell activation in response to an antigen in a subject, the method comprising administering to the subject an effective amount of the antibody or pharmaceutical composition of any one of claims 1-61.

67. A method of treating cancer in a subject, the method comprising administering to the subject an effective amount of the antibody or pharmaceutical composition of any one of claims 1-61.

68. The method of claim 67, wherein the cancer is selected from the group consisting of

melanoma, head and neck cancer, lung cancer, breast cancer, prostate cancer, glioblastoma multiforme, colorectal cancer, sarcoma, bladder cancer, cervical cancer, HPV-associated cancers, cancers of the vagina, cancers of the vulva, cancers of the penis, cancers of the anus, cancers of the rectum, cancers of the oropharynx, multiple myeloma, renal cell carcinoma, ovarian cancer, hepatocellular cancer, endometrial cancer, pancreatic cancer, lymphoma, and leukemia.

69. The method of any one of claims 66-68, wherein the antibody or pharmaceutical composition is administered subcutaneously or intravenously.

70. The method of any one of claims 66-68, wherein the antibody or pharmaceutical composition is administered intratumorally.

71. The method of any one of claims 66-70, further comprising administering an additional therapeutic agent to the subject.

72. The method of claim 71, wherein the additional therapeutic agent is a chemotherapeutic, a radiotherapeutic, or a checkpoint targeting agent.

73. The method of claim 72, wherein the checkpoint targeting agent is selected from the group consisting of an antagonist anti-PD-1 antibody, an antagonist anti-PD-L1 antibody, an antagonist anti-PD-L2 antibody, an antagonist anti-CTLA-4 antibody, an antagonist anti-TIM-3 antibody, an antagonist anti-LAG-3 antibody, an antagonist anti-CEACAM1 antibody, an antagonist anti-TIGIT antibody, an agonist anti-CD137 antibody, an agonist anti-ICOS antibody, an agonist anti-GITR antibody, and an agonist anti-OX40 antibody.

74. The method of claim 71, wherein the additional therapeutic agent is an inhibitor of indoleamine-2,3-dioxygenase (IDO).

75. The method of claim 74, wherein the inhibitor is selected from the group consisting of epacadostat, F001287, indoximod, and NLG919.

76. The method of claim 71, wherein the additional therapeutic agent is a vaccine.

77. The method of claim 76, wherein the vaccine comprises a heat shock protein peptide complex (HSPPC) comprising a heat shock protein complexed with an antigenic peptide.

78. The method of claim 77, wherein the heat shock protein is hsc70 and is complexed with a tumor-associated antigenic peptide.

79. The method of claim 77, wherein the heat shock protein is gp96 protein and is complexed with a tumor-associated antigenic peptide, wherein the HSPPC is derived from a tumor

obtained from a subject.

80. The method of claim 71, wherein the additional therapeutic agent comprises a TCR.

81. The method of claim 80, wherein the additional therapeutic agent is a soluble TCR.

82. The method of claim 80, wherein the additional therapeutic agent is a cell expressing a TCR.

83. The method of claim 71, wherein the additional therapeutic agent is a cell expressing a chimeric antigen receptor.

84. The method of claim 71, wherein the additional therapeutic agent is an antibody that specifically binds to a peptide-MHC complex.

85. The isolated antibody of any one of claims 1-60 for use in the treatment of cancer or an infectious disease.

86. Use of the isolated antibody of any one of claims 1-60 for preparing a pharmaceutical composition or medicine for treating cancer or an infectious disease.

Figure 1A
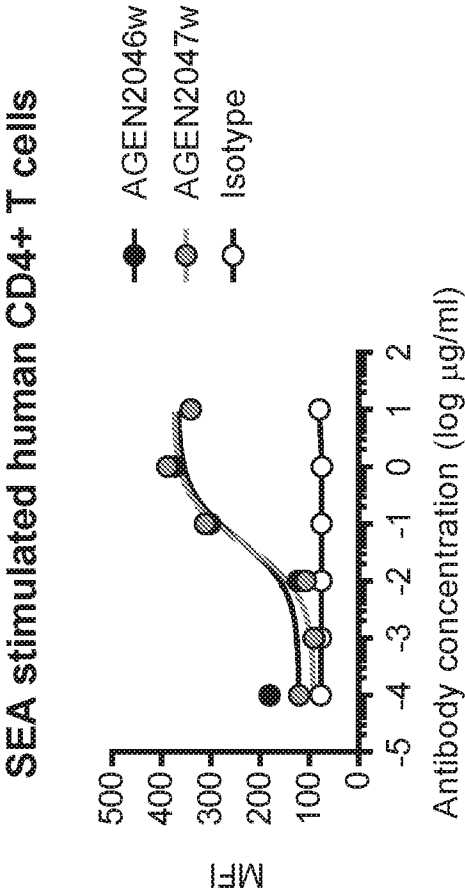
Figure 1B
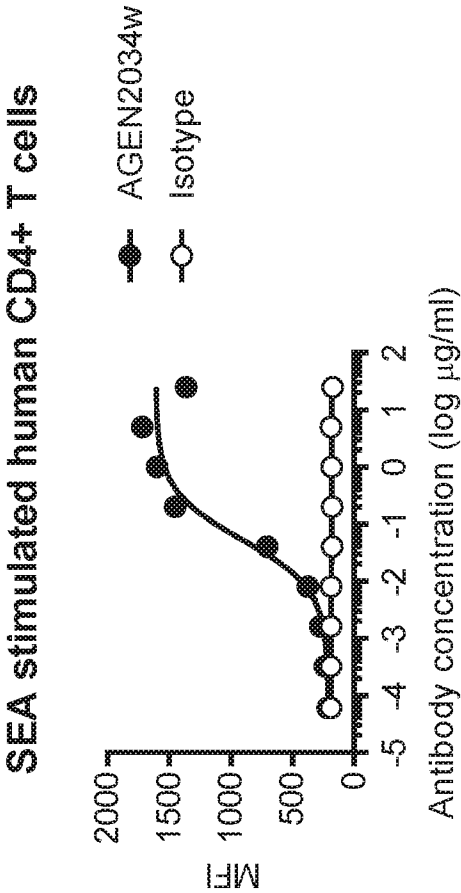
Figure 1C

SEB stimulated cynomolgus CD4+ T cells
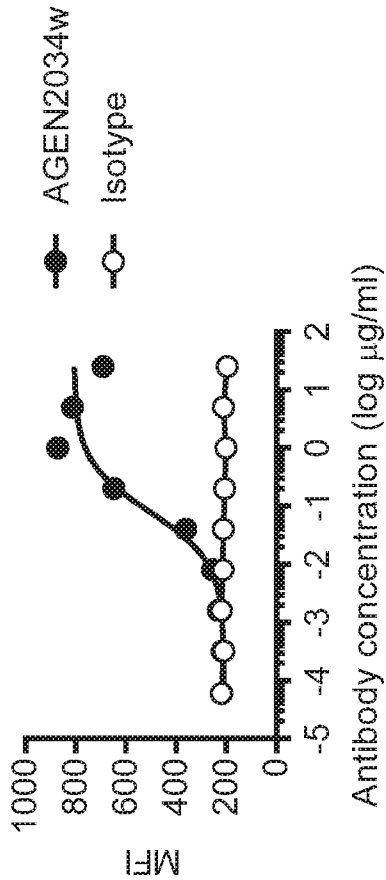
Figure 1D

SEA stimulated human CD4+ T cells
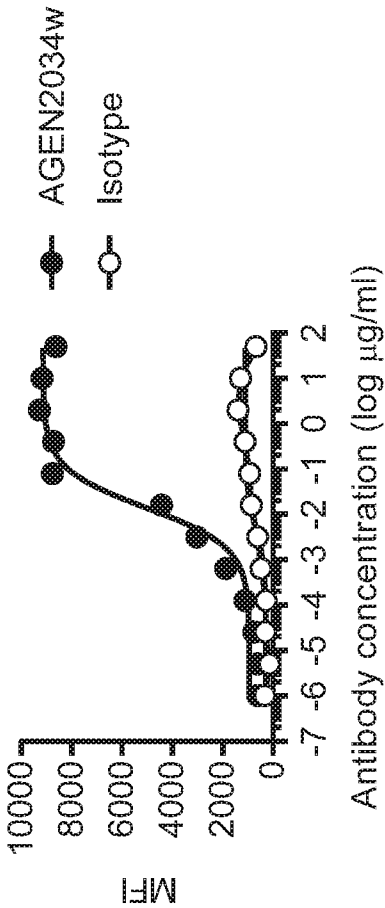
Figure 2
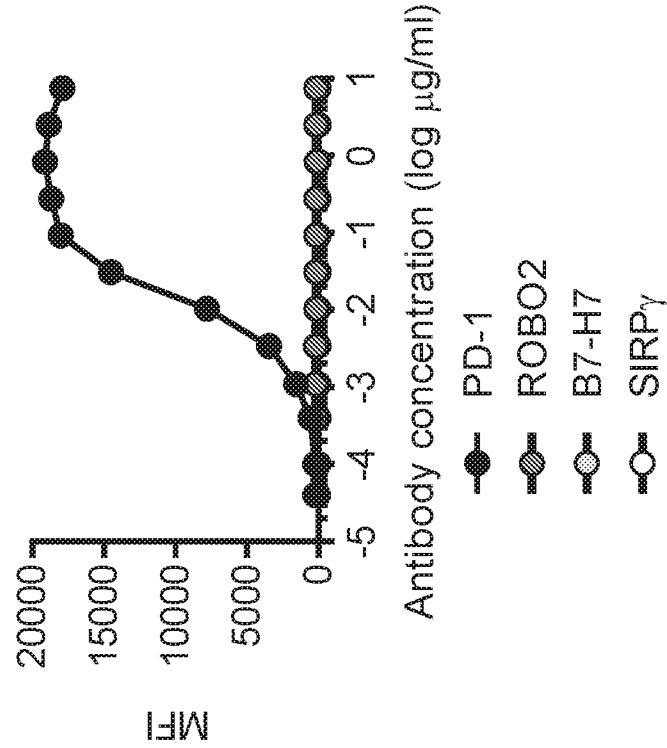
Figure 3B
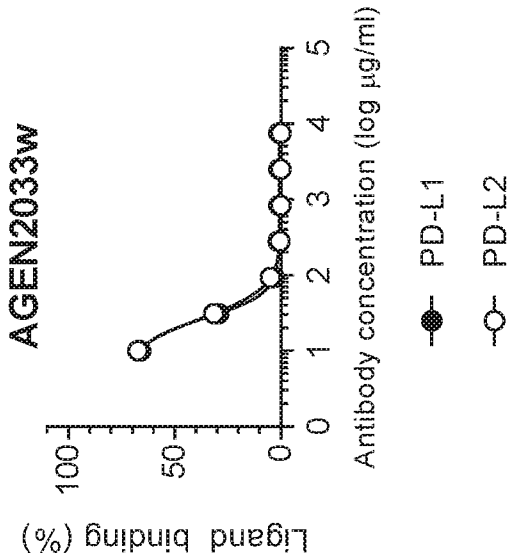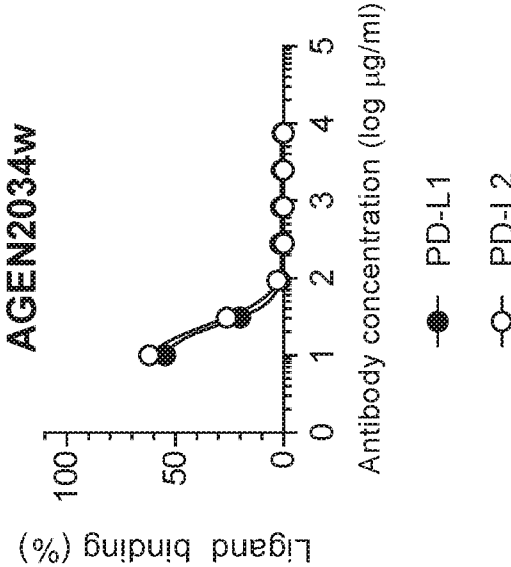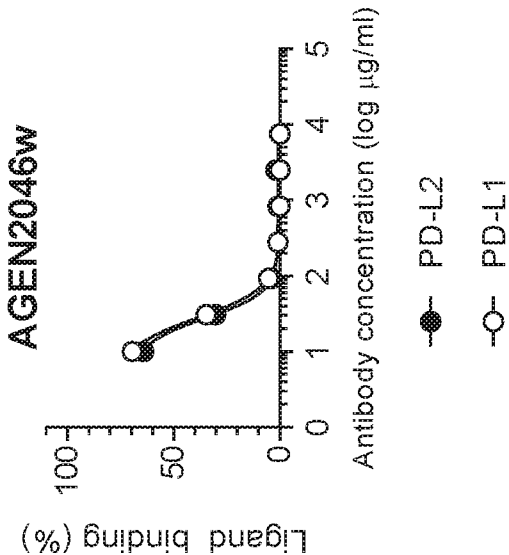
Figure 3D
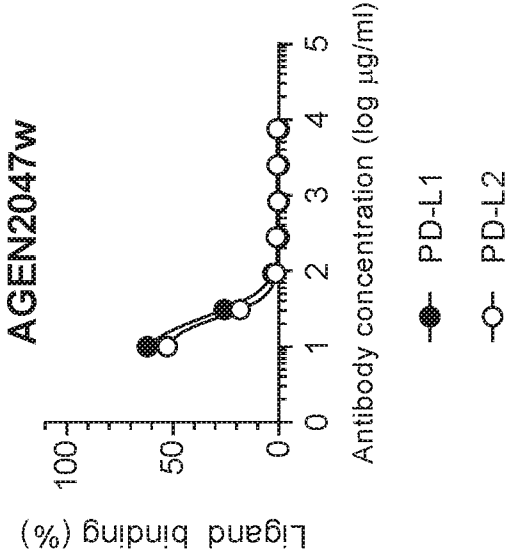
Figure 3A

Figure 3C

Figure 3E

Figure 3F

Figure 4B

SEA stimulated human PBMCs

Figure 4A

SEA stimulated human PBMCs
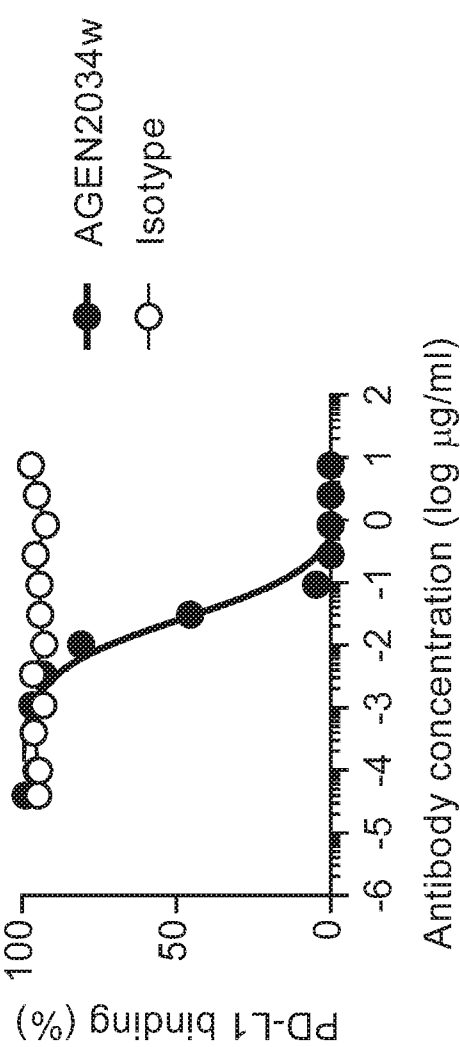
Figure 4D
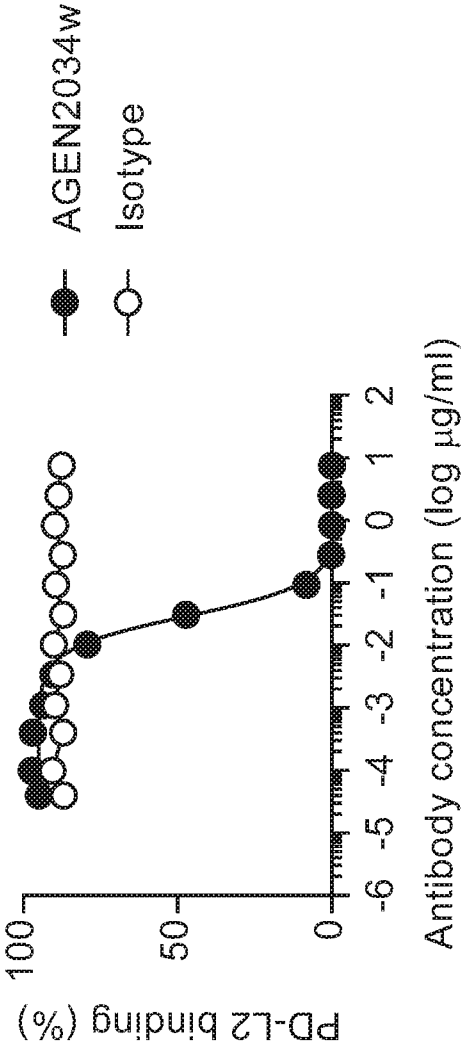
Combination with anti-TIGIT antibody
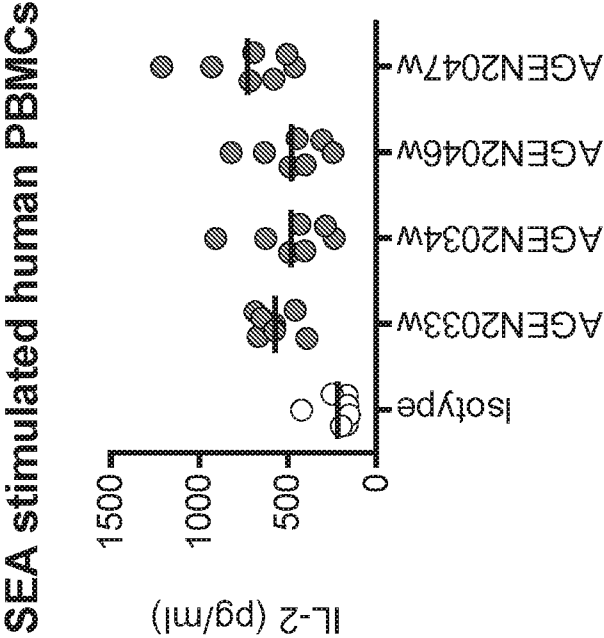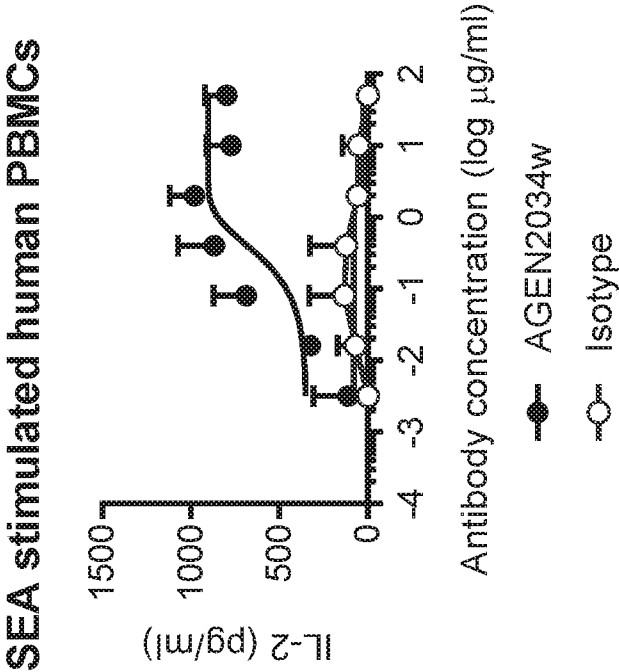
Figure 4C

Combination with anti-CTLA-4 antibody
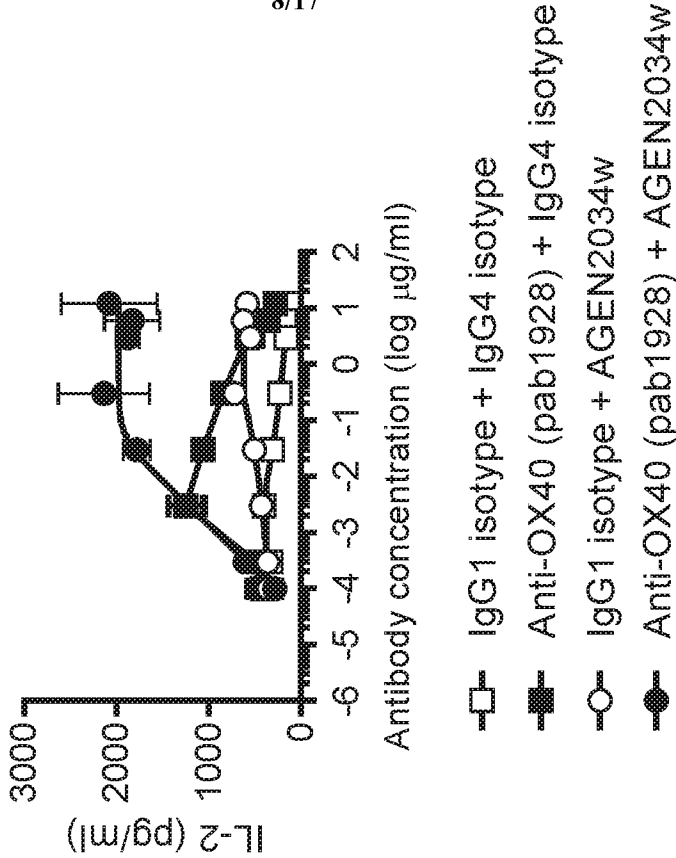
Figure 4F

Combination with anti-OX40 antibody

Figure 4E

Combination with anti-CD137 antibody

Figure 5B

SEA stimulated human PBMCs
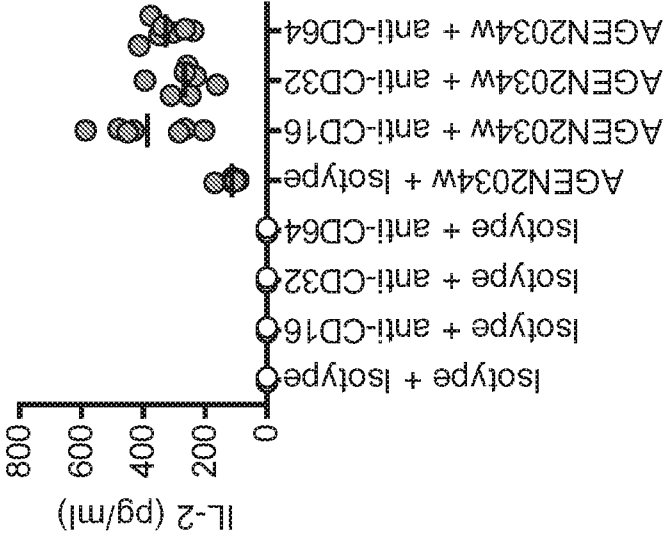
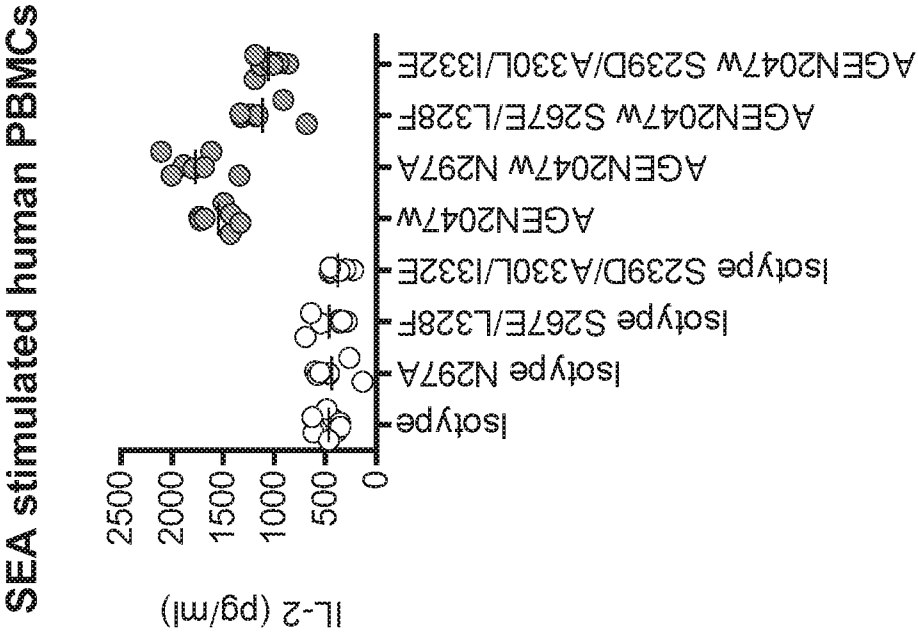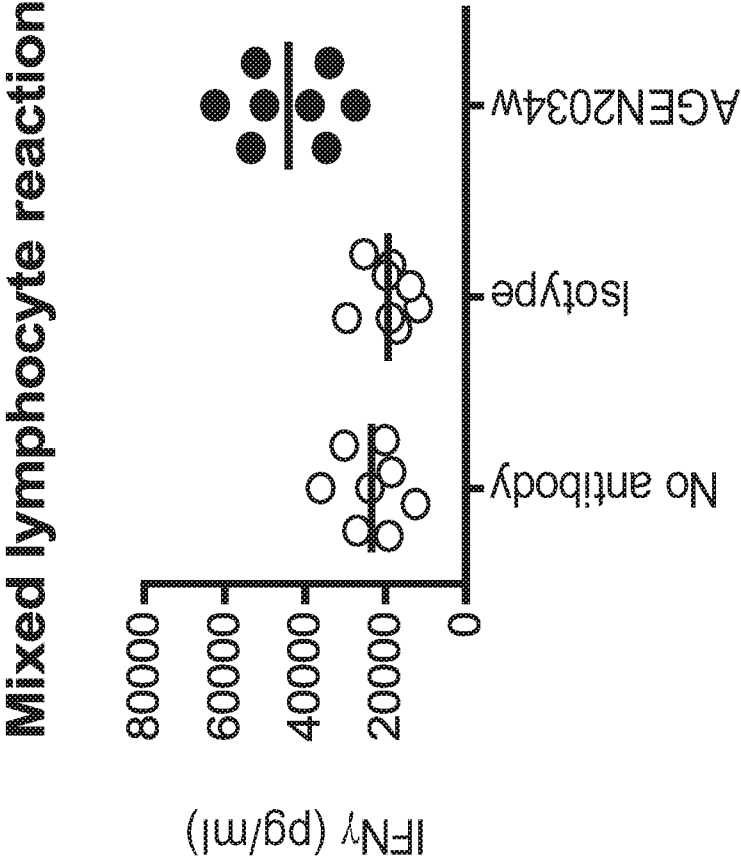Figure 5A

SEA stimulated human PBMCs

Figure 5C

SEA stimulated human PBMCs

IL-2 (pg/ml)

Figure 6

Figure 7A

CD4+ T cells

CFSE fluorescence

AGEN2034w

Isotype Control

Figure 7B

CD8+ T cells

CFSE fluorescence

AGEN2034w

Isotype Control

Figure 7C

Ascites fluid suppression assay
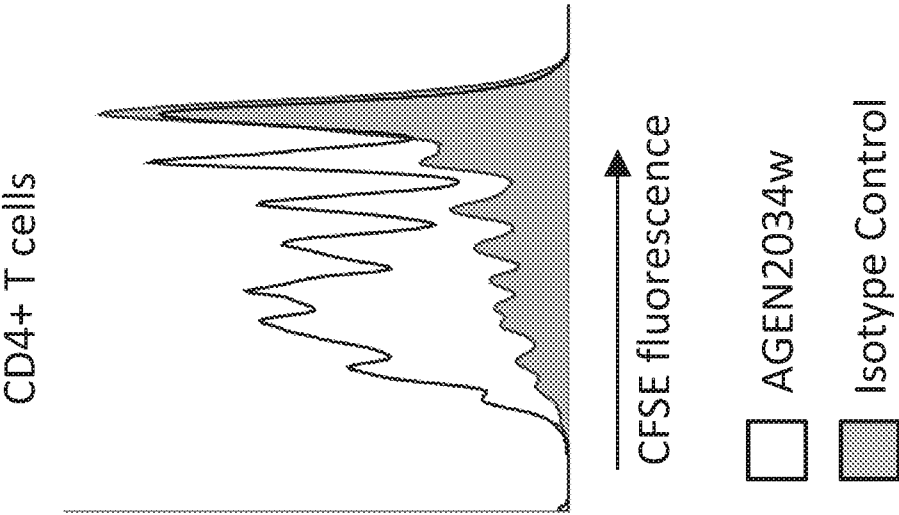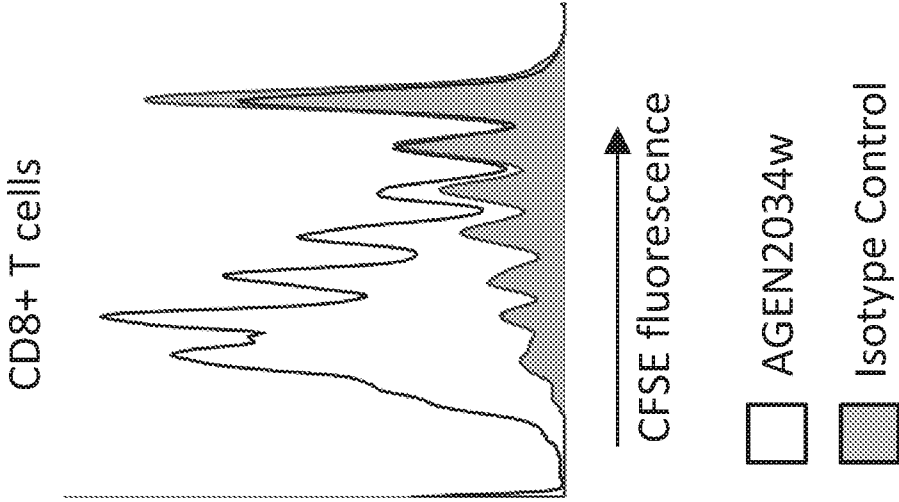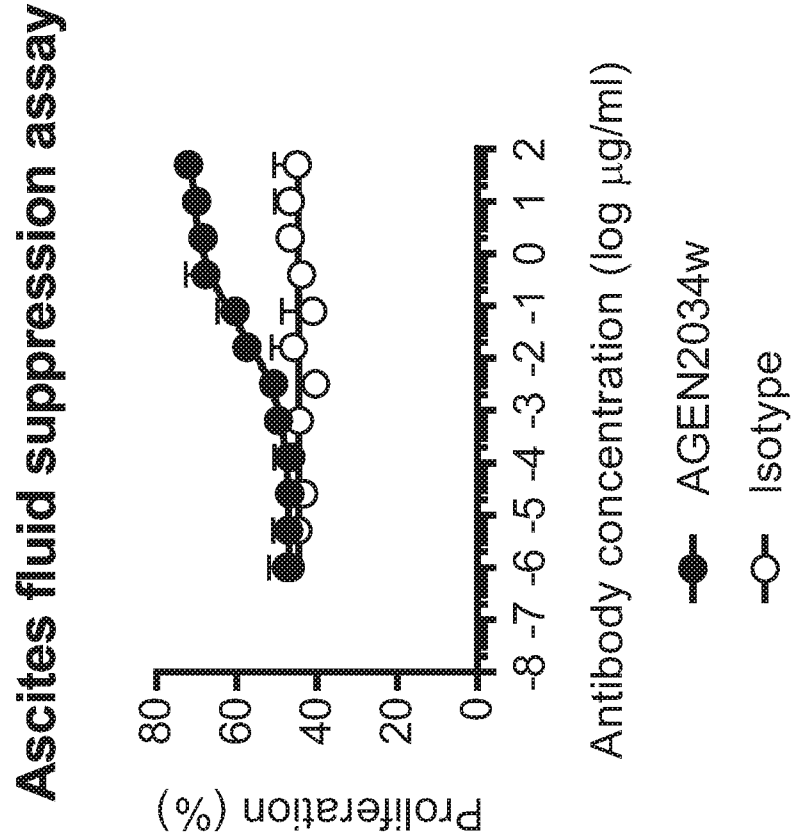
Figure 8
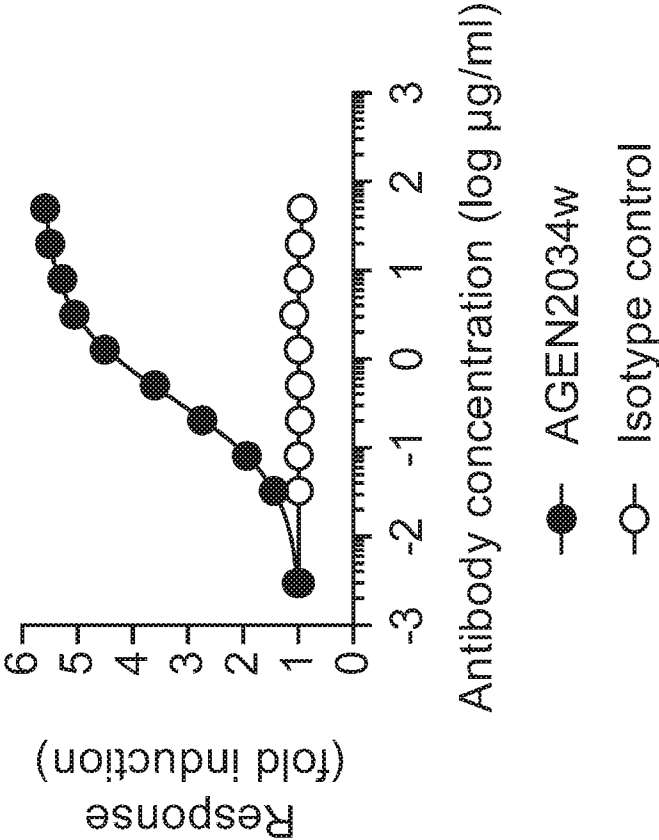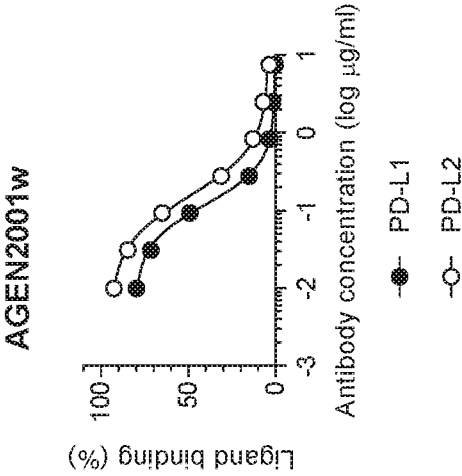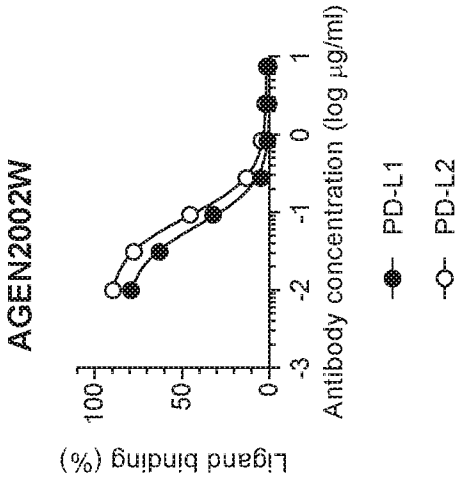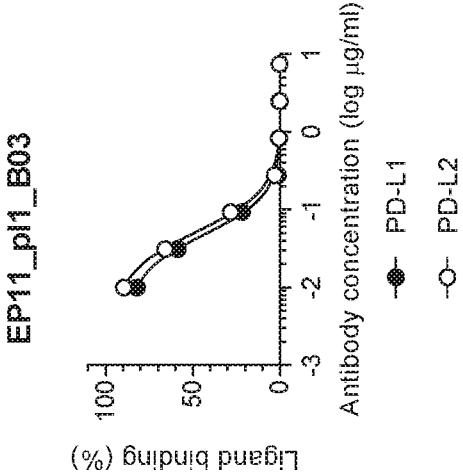
Figure 9A

Figure 9B

Figure 9C

Figure 9D
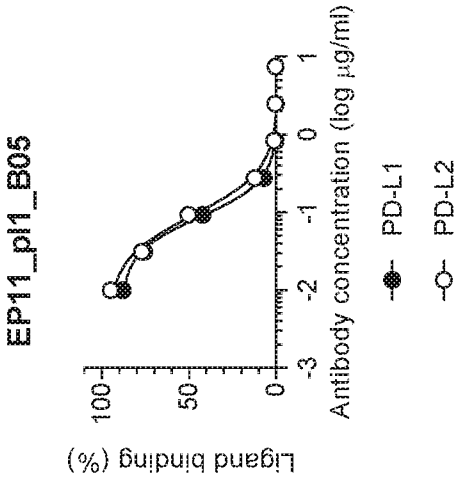
Figure 9F
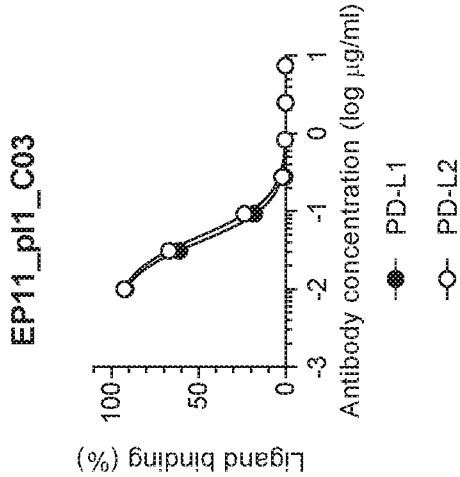
Figure 9E
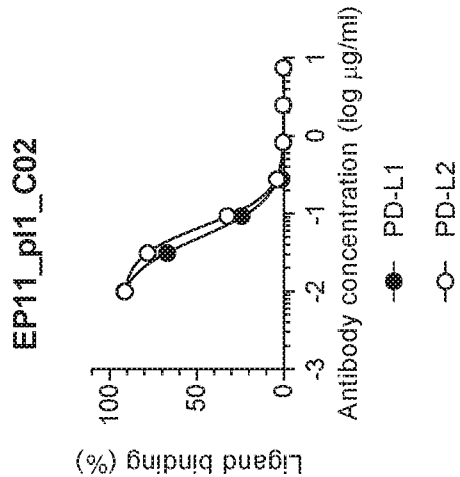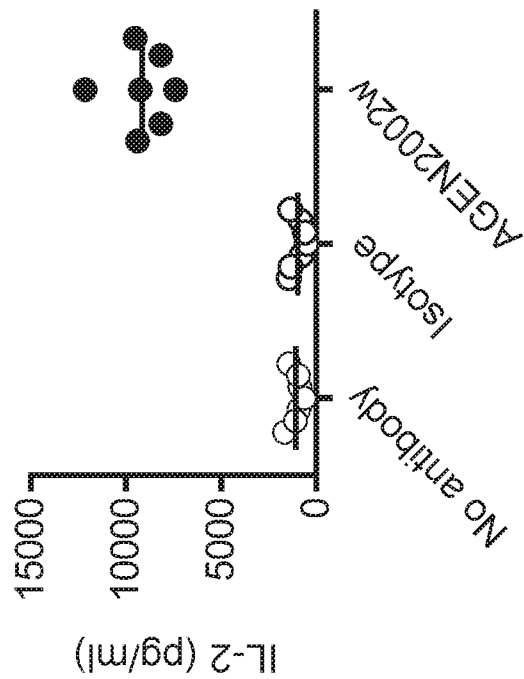
Figure 10

INTERNATIONAL SEARCH REPORT

A. CLASSIFICATION OF SUBJECT MATTER

INV. C07K16/28
ADD. A61K39/00

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 A61K

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)

EPO-Internal, BIOSIS, EMBASE, WPI Data

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	US 2015/203579 A1 (PAPADOPOULOS NICHOLAS J [US] ET AL) 23 July 2015 (2015-07-23) examples 3-5,8-9 page 3, paragraph 0034 page 19, paragraph 0178 ----- -/--	1-5, 7-10,12, 13, 17-23, 25-27, 29,32-86

X	Further documents are listed in the continuation of Box C.		X	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
7 November 2016	20/01/2017

Name and mailing address of the ISA/ European Patent Office, P.B. 5818 Patentlaan 2 NL - 2280 HV Rijswijk Tel. (+31-70) 340-2040, Fax: (+31-70) 340-3016	Authorized officer Malamoussi, A

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Form PCT/ISA/210 (second sheet) (April 2005)

	C(Continuation). DOCUMENTS CONSIDERED TO BE RELEVANT	
Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	US 2014/356363 A1 (ZHOU HEYUE [US] ET AL) 4 December 2014 (2014-12-04) abstract page 20, paragraph 0173 page 20, paragraph 0174 - paragraph 0175 page 20; example 8 -----	1-5, 7-10,12, 13, 17-23, 25-27, 29,32-86
X	WO 2014/179664 A2 (ANAPTYSBIO INC [US]) 6 November 2014 (2014-11-06) examples 1-3 -----	1-5, 7-10,12, 13, 17-23, 25-27, 29,32-86
X	WO 2008/156712 A1 (ORGANON NV [NL]; CARVEN GREGORY JOHN [US]; VAN EENENNAAM HANS [NL]; DU) 24 December 2008 (2008-12-24) table II page 47, paragraph 3 page 48, paragraph 3 page 49, paragraph 2 table III page 54 - page 57 -----	1-5, 7-10,12, 13, 17-23, 25-27, 29,32-86
X	US 2015/210769 A1 (FREEMAN GORDON JAMES [US] ET AL) 30 July 2015 (2015-07-30) example 1 page 152, paragraph 0764 - paragraph 0769 -----	1-5, 7-10,12, 13, 17-23, 25-27, 29,32-86
X	US 8 735 553 B1 (LI KANG [CN] ET AL) 27 May 2014 (2014-05-27) examples 1, 2, 3, 4, 8, 12 -----	1-5, 7-10,12, 13, 17-23, 25-27, 29,32-86

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C(Continuation).   DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	WO 2006/121168 A1 (ONO PHARMACEUTICAL CO [JP]; MEDAREX INC [US]; KORMAN ALAN J; SRINIVASA) 16 November 2006 (2006-11-16) example 1 page 81, paragraph 1 examples 5-6, 11, 13-20, 23-24 -----	1-5, 7-10,12, 13, 17-23, 25-27, 29,32-86
X	R. MOREIRA DA SILVA: "Nivolumab: Anti-PD-1 monoclonal antibody cancer immunotherapy", DRUGS OF THE FUTURE, vol. 39, no. 1, 1 January 2014 (2014-01-01), pages 15-24, XP055199597, ES ISSN: 0377-8282, DOI: 10.1358/dof.2014.039.01.2103754 page 17, left-hand column, paragraph 2 page 17, left-hand column, paragraph 4 page 20, right-hand column, paragraph 4 -----	1-5, 7-10,12, 13, 17-23, 25-27, 29,32-86
X	J. MCDERMOTT ET AL: "Pembrolizumab: PD-1 inhibition as a therapeutic strategy in cancer", DRUGS OF TODAY, vol. 51, no. 1, 1 January 2015 (2015-01-01), page 7, XP055316850, ES ISSN: 1699-3993, DOI: 10.1358/dot.2015.51.1.2250387 the whole document -----	1-5, 7-10,12, 13, 17-23, 25-27, 29,32-86
X	BENSON D M ET AL: "The PD-1/PD-L1 axis modulates the natural killer cell versus multiple myeloma effect: a therapeutic target for CT-011, a novel monoclonal anti-PD-1 antibody", BLOOD, AMERICAN SOCIETY OF HEMATOLOGY, US, vol. 116, no. 13, 30 September 2010 (2010-09-30), pages 2286-2294, XP002685329, ISSN: 0006-4971, DOI: 10.1182/BLOOD-2010-02-271874 [retrieved on 2010-05-11] page 2286, left-hand column, paragraph 3 - right-hand column, paragraph 1 page 2290, left-hand column, paragraph 2 - page 2291, right-hand column, paragraph 1 -----	1-5, 7-10,12, 13, 17-23, 25-27, 29,32-86

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INTERNATIONAL SEARCH REPORT

C(Continuation).   DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	M. SZNOL ET AL: "Antagonist Antibodies to PD-1 and B7-H1 (PD-L1) in the Treatment of Advanced Human Cancer", CLINICAL CANCER RESEARCH, vol. 19, no. 5, 1 March 2013 (2013-03-01), pages 1021-1034, XP055123957, ISSN: 1078-0432, DOI: 10.1158/1078-0432.CCR-12-2063 the whole document -----	1-5, 7-10,12, 13, 17-23, 25-27, 29,32-86
A	Anonymous: "International Nonproprietary Names for Pharmaceutical Substances (INN)", WHO Drug Information Rec. Wld Health Org.Resolution EB15.R7), 1 January 2014 (2014-01-01), pages 379-422, XP055316833, Retrieved from the Internet: URL:http://www.who.int/entity/medicines/publications/druginformation/innlists/RL72.pdf?ua=1 [retrieved on 2016-11-07] page 407 -----	1-5, 7-10,12, 13, 17-23, 25-27, 29,32-86
A	KENT SHIH ET AL: "Clinical Impact of Checkpoint Inhibitors as Novel Cancer Therapies", DRUGS, vol. 74, no. 17, 25 October 2014 (2014-10-25), pages 1993-2013, XP055316870, NZ ISSN: 0012-6667, DOI: 10.1007/s40265-014-0305-6 the whole document -----	1-5, 7-10,12, 13, 17-23, 25-27, 29,32-86

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INTERNATIONAL SEARCH REPORT

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.:
   because they relate to subject matter not required to be searched by this Authority, namely:

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).

Box No. III   Observations where unity of invention is lacking (Continuation of item 3 of first sheet)

This International Searching Authority found multiple inventions in this international application, as follows:

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see additional sheet
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1. ☐ As all required additional search fees were timely paid by the applicant, this international search report covers all searchable
   claims.

2. ☐ As all searchable claims could be searched without effort justifying an additional fees, this Authority did not invite payment of
   additional fees.

3. ☐ As only some of the required additional search fees were timely paid by the applicant, this international search report covers
   only those claims for which fees were paid, specifically claims Nos.:

4. ☒ No required additional search fees were timely paid by the applicant. Consequently, this international search report is
   restricted to the invention first mentioned in the claims; it is covered by claims Nos.:

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   1, 5, 10, 12, 13, 23, 27, 29(completely); 2-4, 7-9, 17-22, 25, 26
   32-86(partially)
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Remark on Protest   ☐ The additional search fees were accompanied by the applicant's protest and, where applicable, the
                              payment of a protest fee.

                           ☐ The additional search fees were accompanied by the applicant's protest but the applicable protest
                              fee was not paid within the time limit specified in the invitation.

                           ☐ No protest accompanied the payment of additional search fees.

FURTHER INFORMATION CONTINUED FROM   PCT/ISA/ 210

This International Searching Authority found multiple (groups of) inventions in this international application, as follows:

1. claims: 1, 5, 10, 12, 13, 23, 27, 29(completely); 2-4, 7-9, 17-22, 25, 26, 32-86(partially)

   relating to an anti-PD-1 antibody having the heavy and light chain CDR sequences with SEQ ID No 1-3 and 4-6, respectively, and heavy and light chain variable region with SEQ ID No 15 and 16, clones AGEN2033w and AGEN2046w.
   ---

2. claims: 6, 11, 14-16, 24, 28, 30, 31(completely); 2-4, 7-9, 17-22, 25, 26, 32-86(partially)

   relating to an anti-PD-1 antibody having the heavy and light chain CDR sequences with SEQ ID No 1, 2, 7 and 4-6, respectively, and heavy and light chain variable region with SEQ ID No 17 and 16, clones AGEN2034w and AGEN2047w.
   ---

3. claims: 2-4, 7-9, 17-22, 25, 26, 32-86(all partially)

   relating to an anti-PD-1 antibody having heavy and light chain variable regions with SEQ ID No 26 and 16, clone AGEN2001w.
   ---

4. claims: 2-4, 7-9, 17-22, 25, 26, 32-86(all partially)

   relating to an anti-PD-1 antibody having heavy and light chain variable regions with SEQ ID No 27 and 16, clone BADD426-2615.
   ---

5. claims: 2-4, 7-9, 17-22, 25, 26, 32-86(all partially)

   relating to an anti-PD-1 antibody having heavy and light chain variable regions with SEQ ID No 28 and 16, clone EP11_p11B03.
   ---

6. claims: 2-4, 7-9, 17-22, 25, 26, 32-86(all partially)

   relating to an anti-PD-1 antibody having heavy and light chain variable regions with SEQ ID No 29 and 16, clone EP11_p11B05.
   ---

7. claims: 2-4, 7-9, 17-22, 25, 26, 32-86(all partially)

   relating to an anti-PD-1 antibody having heavy and light chain variable regions with SEQ ID No 30 and 16, clone EP11_p11C02.

FURTHER INFORMATION CONTINUED FROM   PCT/ISA/ 210

8. claims: 2-4, 7-9, 17-22, 25, 26, 32-86(all partially)

relating to an anti-PD-1 antibody having heavy and light
chain variable regions with SEQ ID No 31 and 16, clone
EP11_p11C03.

International application No
PCT/US2016/049913

Patent document cited in search report		Publication date	Patent family member(s)		Publication date
US 2015203579	A1	23-07-2015	AU	2015209233 A1	04-08-2016
			CA	2936075 A1	30-07-2015
			CN	106068275 A	02-11-2016
			EA	201691482 A1	30-11-2016
			EP	3097119 A1	30-11-2016
			KR	20160132010 A	16-11-2016
			SG	11201605482S A	30-08-2016
			TW	201540726 A	01-11-2015
			US	2015203579 A1	23-07-2015
			UY	35964 A	31-08-2015
			WO	2015112800 A1	30-07-2015
US 2014356363	A1	04-12-2014	CA	2913977 A1	04-12-2014
			CN	105683217 A	15-06-2016
			EP	3004169 A2	13-04-2016
			JP	2016521692 A	25-07-2016
			US	2014356363 A1	04-12-2014
			WO	2014194302 A2	04-12-2014
WO 2014179664	A2	06-11-2014	AU	2014259719 A1	17-12-2015
			CA	2910278 A1	06-11-2014
			CN	105339389 A	17-02-2016
			EP	2992017 A2	09-03-2016
			JP	2016523516 A	12-08-2016
			KR	20160034247 A	29-03-2016
			SG	11201508528T A	27-11-2015
			US	2016075783 A1	17-03-2016
			WO	2014179664 A2	06-11-2014
WO 2008156712	A1	24-12-2008	AU	2008266951 A1	24-12-2008
			BR	PI0812913 A2	09-12-2014
			CA	2691357 A1	24-12-2008
			CA	2855098 A1	24-12-2008
			CN	102131828 A	20-07-2011
			CN	104945508 A	30-09-2015
			DK	2170959 T3	13-01-2014
			EP	2170959 A1	07-04-2010
			EP	2535354 A1	19-12-2012
			ES	2437327 T3	10-01-2014
			HK	1140497 A1	07-03-2014
			HR	P20131167 T1	03-01-2014
			IL	202813 A	31-03-2015
			JP	5191537 B2	08-05-2013
			JP	5640052 B2	10-12-2014
			JP	2010530753 A	16-09-2010
			JP	2012254092 A	27-12-2012
			KR	20100054780 A	25-05-2010
			KR	20140133954 A	20-11-2014
			KR	20150055114 A	20-05-2015
			LU	92936 I2	29-02-2016
			NO	2015028 I1	11-01-2016
			NZ	582150 A	31-08-2012
			NZ	600758 A	27-09-2013
			PH	12015501524 A1	22-02-2016
			PT	2170959 E	07-01-2014
			RS	53072 B	30-04-2014
			SI	2170959 T1	30-04-2014
			US	2010266617 A1	21-10-2010

Patent document cited in search report	Publication date	Patent family member(s)	Publication date
		US 2013108651 A1	02-05-2013
		US 2013109843 A1	02-05-2013
		US 2015232555 A1	20-08-2015
		WO 2008156712 A1	24-12-2008
US 2015210769 A1	30-07-2015	AU 2015209145 A1	07-07-2016
		CA 2935423 A1	30-07-2015
		EP 3097121 A1	30-11-2016
		KR 20160110995 A	23-09-2016
		SG 11201604939S A	30-08-2016
		TW 201536808 A	01-10-2015
		US 2015210769 A1	30-07-2015
		UY 35967 A	31-08-2015
		WO 2015112900 A1	30-07-2015
US 8735553 B1	27-05-2014	AU 2013400609 A1	05-05-2016
		CA 2924172 A1	19-03-2015
		CN 105531288 A	27-04-2016
		EA 201690567 A1	31-08-2016
		EP 3044234 A1	20-07-2016
		JP 2016533763 A	04-11-2016
		KR 20160044063 A	22-04-2016
		SG 11201601844T A	28-04-2016
		TW 201538525 A	16-10-2015
		US 8735553 B1	27-05-2014
		US 2015079109 A1	19-03-2015
		US 2015315274 A1	05-11-2015
		WO 2015035606 A1	19-03-2015
WO 2006121168 A1	16-11-2006	AU 2006244885 A1	16-11-2006
		BR PI0610235 A2	08-06-2010
		CA 2607147 A1	16-11-2006
		CN 103059138 A	24-04-2013
		CN 105315373 A	10-02-2016
		DK 2161336 T3	28-10-2013
		EP 1896582 A1	12-03-2008
		EP 2161336 A1	10-03-2010
		EP 2418278 A2	15-02-2012
		EP 2439272 A2	11-04-2012
		EP 2439273 A2	11-04-2012
		ES 2427646 T3	31-10-2013
		HK 1140793 A1	27-06-2014
		IL 187108 A	30-06-2011
		IL 208642 A	30-08-2012
		JP 4361545 B2	11-11-2009
		JP 5028700 B2	19-09-2012
		JP 5872377 B2	01-03-2016
		JP 2006340714 A	21-12-2006
		JP 2009155338 A	16-07-2009
		JP 2012158605 A	23-08-2012
		JP 2014077015 A	01-05-2014
		JP 2016033135 A	10-03-2016
		KR 20080011428 A	04-02-2008
		KR 20130032908 A	02-04-2013
		KR 20130114226 A	16-10-2013
		LU 92904 I2	10-02-2016
		NZ 563193 A	28-05-2010
		PT 2161336 E	03-10-2013

Patent document cited in search report	Publication date	Patent family member(s)		Publication date
		RU	2010135087 A	27-02-2012
		RU	2013133714 A	27-01-2015
		TW	I379898 B	21-12-2012
		US	2009217401 A1	27-08-2009
		US	2013133091 A1	23-05-2013
		US	2014212422 A1	31-07-2014
		US	2014294852 A1	02-10-2014
		US	2014328833 A1	06-11-2014
		US	2014348743 A1	27-11-2014
		US	2015165025 A1	18-06-2015
		WO	2006121168 A1	16-11-2006

Form PCT/ISA/210 (patent family annex) (April 2005)