

CLAIMS

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 VIWX1DGSNX2YYADSVX3G (SEQ ID NO: 32), wherein
X1 is Y or F;
X2 is K or E; and
X3 is K or M;
- (c) CDRH3 comprises the amino acid sequence of NX1DX2 (SEQ ID NO: 33), wherein
X1 is G or V; and
X2 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:

- (a) CDRH2 comprises an amino acid sequence selected from the group consisting of SEQ ID NOs: 2 and 34-36; and/or
- (b) CDRH3 comprises an amino acid sequence selected from the group consisting of SEQ ID NOs: 3, 7, and 37.

3. The isolated antibody of claim 1 or claim 2, 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.

4. The isolated antibody of any one of claims 1 to 3, 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.

5. The isolated antibody of any one of claims 1 to 3, 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.
6. The isolated antibody of any one of claims 1 to 5, wherein the heavy chain variable region comprises an amino acid sequence derived from a human IGHV3-33 germline sequence.
7. The isolated antibody of any one of claims 1 to 3, 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 to 3, 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 1, wherein the antibody comprises a heavy chain comprising the amino acid sequence of SEQ ID NO: 52, 53, 54, 55, or 56.
10. The isolated antibody of any one of claims 1 to 9, wherein the light chain variable region comprises an amino acid sequence derived from a human IGKV3-15 germline sequence.
11. The isolated antibody of any one of claims 1 to 10, wherein the light chain variable region comprises 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.
12. 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; 26 and 16; 27 and 16; 28 and 16; 29 and 16; 30 and 16; or 31 and 16.

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

14. The isolated antibody of claim 1, comprising a heavy chain and a light chain, wherein the heavy chain and the light chain, respectively, comprise the amino acid sequences set forth in SEQ ID NOs: 52 and 19; 54 and 19; 55 and 19; or 56 and 19.

15. The isolated antibody of claim 1, comprising a heavy chain and a light chain, wherein the amino acid sequences of the heavy chain and the light chain, respectively, consist of the amino acid sequences set forth in SEQ ID NOs: 52 and 19; 54 and 19; 55 and 19; or 56 and 19.

16. 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 CDRH1, CDRH2, and CDRH3 comprise the amino acid sequences of SEQ ID NOs: 1, 2, and 7, respectively; and a light chain variable region comprising the amino acid sequence of SEQ ID NO: 16.

17. An isolated antibody that specifically binds to human PD-1, comprising a heavy chain comprising the amino acid sequence of SEQ ID NO: 20; and a light chain variable region comprising complementarity determining regions CDRL1, CDRL2, and CDRL3, wherein CDRL1, CDRL2, and CDRL3 comprise the amino acid sequences of SEQ ID NOs: 4, 5, and 6, respectively.

18. 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: 17; and a light chain variable region comprising the amino acid sequence of SEQ ID NO: 16.

19. The isolated antibody of claim 18, wherein the amino acid sequence of the heavy chain variable region consists of the amino acid sequence of SEQ ID NO: 17; and the amino acid sequence of the light chain variable region consists of the amino acid sequence of SEQ ID NO: 16.

20. An isolated antibody that specifically binds to human PD-1, comprising a heavy chain and a light chain, wherein the heavy chain comprises the amino acid sequence of SEQ ID NO: 53; and the light chain comprises the amino acid sequence of SEQ ID NO: 19.
21. The isolated antibody of claim 20, wherein the amino acid sequence of the heavy chain consists of the amino acid sequence of SEQ ID NO: 53, and the amino acid sequence of the light chain consists of the amino acid sequence of SEQ ID NO: 19.
22. The isolated antibody of claim 20, wherein the heavy chain comprises the amino acid sequence of SEQ ID NO: 20, and the amino acid sequence of the light chain consists of the amino acid sequence of SEQ ID NO: 19.
23. The isolated antibody of claim 22, wherein the amino acid sequence of the heavy chain consists of the amino acid sequence of SEQ ID NO: 20, and the amino acid sequence of the light chain consists of the amino acid sequence of SEQ ID NO: 19.
24. The isolated antibody of any one of claims 1 to 5, 8, or 16, wherein the heavy chain variable region comprises the amino acid sequence of SEQ ID NO: 17.
25. The isolated antibody of claim 18 or claim 24, wherein the amino acid sequence of the heavy chain variable region consists of the amino acid sequence of SEQ ID NO: 17.
26. The isolated antibody of any one of claims 1 to 6, 8, 9, 16, 18, or 19, wherein the heavy chain comprises the amino acid sequence of SEQ ID NO: 53.
27. The isolated antibody of claim 20 or claim 26, wherein the amino acid sequence of the heavy chain consists of the amino acid sequence of SEQ ID NO: 53.
28. The isolated antibody of any one of claims 1, 2, 18, or 20, wherein the heavy chain comprises the amino acid sequence of SEQ ID NO: 20.

29. The isolated antibody of claim 22 or claim 28, wherein the amino acid sequence of the heavy chain consists of the amino acid sequence of SEQ ID NO: 20.
30. The isolated antibody of any one of claims 1, 2, or 18, wherein the heavy chain comprises the amino acid sequence of SEQ ID NO: 23.
31. The isolated antibody of claim 30, wherein the amino acid sequence of the heavy chain consists of the amino acid sequence of SEQ ID NO: 23.
32. The isolated antibody of any one of claims 1 to 11, or 17, wherein the light chain variable region comprises the amino acid sequence of SEQ ID NO: 16.
33. The isolated antibody of any one of claims 16, 18, or 32, wherein the amino acid sequence of the light chain variable region consists of the amino acid sequence of SEQ ID NO: 16.
34. The isolated antibody of any one of claims 1 to 11, 16, or 18, wherein the light chain comprises the amino acid sequence of SEQ ID NO: 19.
35. The isolated antibody of any one of claims 20 or 34, wherein the amino acid sequence of the light chain consists of the amino acid sequence of SEQ ID NO: 19.
36. The isolated antibody of any one of claims 1 to 8 or 16 to 19, wherein the antibody comprises a heavy chain constant region selected from the group consisting of human IgG1, IgG2, IgG3, IgG4, IgA1, and IgA2, and/or a light chain constant region selected from the group consisting of human IgGκ and IgGλ.
37. The isolated antibody of any one of claims 1 to 8 or 16 to 19, 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 gamma receptor with lower affinity than the wild type human IgG heavy chain constant region binds to the human Fc gamma receptor.

38. The isolated antibody of claim 37, wherein the variant human IgG heavy chain constant region is a variant human IgG1, a variant human IgG2, or a variant human IgG4 heavy chain constant region.

39. The isolated antibody of claim 37, wherein the Fc gamma receptor is Fc γ RIIB.

40. The isolated antibody of claim 1, comprising:

- (a) a human IgG1 heavy chain constant region that lacks a glycan moiety at position N297;
 - (b) a human IgG1 heavy chain constant region comprising an N297A mutation;
 - (c) a human IgG1 heavy chain constant region comprising an N297Q mutation;
 - (d) a human IgG1 heavy chain constant region comprising a D265A mutation; and/or
 - (e) a human IgG4 heavy chain constant region comprising an S228P mutation,
- wherein the amino acid positions are according to the EU numbering system.

41. The isolated antibody of any one of claims 1 to 40, wherein the antibody is a human antibody.

42. The isolated antibody of any one of claims 1 to 41, wherein the antibody is antagonistic to human PD-1.

43. The isolated antibody of any one of claims 1 to 42, wherein the antibody deactivates, reduces, or inhibits an activity of human PD-1.

44. The isolated antibody of any one of claims 1 to 43, wherein the antibody inhibits binding of human PD-1 to human PD-L1 or to human PD-L2.

45. The isolated antibody of any one of claims 1 to 44, wherein the antibody increases IL-2 production by peripheral blood mononuclear cells (PBMCs) stimulated with staphylococcal enterotoxin A (SEA).

46. The isolated antibody of any one of claims 1 to 44, wherein the antibody increases IFN γ production of a co-culture of human T cells and allogenic dendritic cells.

47. The isolated antibody of any one of claims 1 to 44, wherein the antibody increases proliferation of anti-CD3-antibody-stimulated CD4+ or CD8+ T cells co-cultured with ovarian cancer ascites fluid.
48. The isolated antibody of any one of claims 1 to 44, wherein the antibody increases NFAT signaling in PD-1-expressing NFAT-luciferase reporter cells co-cultured with PD-L1-expressing target cells.
49. The isolated antibody of any one of claims 1 to 48 conjugated to a cytotoxic agent, cytostatic agent, toxin, radionuclide, or detectable label.
50. An isolated polynucleotide encoding a heavy and/or light chain variable region of the antibody of any one of claims 1 to 49.
51. A vector comprising the polynucleotide of claim 50.
52. A recombinant host cell comprising the polynucleotide of claim 50.
53. A recombinant host cell comprising the vector of claim 51.
54. A method of producing an antibody that binds to human PD-1, the method comprising culturing the host cell of claim 52 or claim 53 so that the polynucleotide is expressed and the antibody is produced.
55. A pharmaceutical composition comprising the antibody of any one of claims 1 to 49 and a pharmaceutically acceptable carrier or excipient.
56. Use of the pharmaceutical composition of claim 55 in the manufacture of a medicament for the treatment of cancer in a subject.
57. The use of claim 56, wherein the cancer is selected from the group consisting of acute leukemia, acute lymphoblastic leukemia, acute myeloid leukemia, bladder cancer, bone cancer, brain stem

glioma, breast cancer, cancer of the adrenal gland, cancer of the endocrine system, cancer of the kidney or ureter, cancer of the parathyroid gland, cancer of the small intestine, cancer of the thyroid gland, cancer of the urethra, cancers of the anus, cancers of the oropharynx, cancers of the penis, cancers of the rectum, cancers of the vagina, cancers of the vulva, cancers that express PD-L1, carcinoma of the cervix, carcinoma of the endometrium, carcinoma of the fallopian tubes, carcinoma of the renal pelvis, cervical cancer, chronic leukemia, chronic lymphocytic leukemia, chronic myeloid leukemia, colorectal cancer, endometrial cancer, environmentally induced cancers including those induced by asbestos, epidermoid cancer, esophageal cancer, glioblastoma multiforme, glioma, head and neck cancer, hepatocellular cancer, Hodgkin's Disease, HPV-associated cancers, Kaposi's sarcoma, leukemia, liver cancer, lung cancer, lymphocytic lymphoma, lymphoma, melanoma, metastatic cancers, multiple myeloma, neoplasm of the central nervous system (CNS), non-Hodgkin's lymphoma, ovarian cancer, pancreatic cancer, pituitary adenoma, primary CNS lymphoma, prostate cancer, refractory or recurrent malignancies, renal cell carcinoma, sarcoma, sarcoma of soft tissue, skin cancer, solid tumors of childhood, spinal axis tumor, squamous cell cancer, stomach cancer, T-cell lymphoma, testicular cancer, and uterine cancer.

58. The use of claim 56 or claim 57, wherein the medicament further comprises an additional therapeutic agent.

59. The use of claim 58, wherein the additional therapeutic agent is a chemotherapeutic, radiotherapeutic, or checkpoint targeting agent.

60. The use of claim 59, 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.

61. The use of claim 58, wherein the additional therapeutic agent is an inhibitor of indoleamine-2,3-dioxygenase (IDO).

62. The use of claim 61, wherein the inhibitor is selected from the group consisting of epacadostat, F001287, indoximod, and NLG919.

63. The use of claim 58, wherein the additional therapeutic agent is a vaccine.

64. The use of claim 63, wherein the vaccine comprises a heat shock protein peptide complex (HSPPC) comprising a heat shock protein complexed with an antigenic peptide.

65. The use of claim 64, wherein the heat shock protein is hsc70 and is complexed with a tumor-associated antigenic peptide.

66. The use of claim 64, 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.