

amino acid sequences: **SEQ ID NO:139**, **SEQ ID NO:140**, and **SEQ ID NO:141**; and

- (2) the CDR_{L1} Domain, CDR_{L2} Domain, and CDR_{L3} Domain are the Light Chain CDRs of hPD-1 mAb 7(1.3), and, respectively have the amino acid sequences: **SEQ ID NO:157**, **SEQ ID NO:158**, and **SEQ ID NO:145**;

or

- (R) (1) the CDR_{H1} Domain, CDR_{H2} Domain, and CDR_{H3} Domain are the Heavy Chain CDRs of hPD-1 mAb 9(2.2), and respectively have the amino acid sequences: **SEQ ID NO:183**, **SEQ ID NO:172**, and **SEQ ID NO:173**; and
- (2) the CDR_{L1} Domain, CDR_{L2} Domain, and CDR_{L3} Domain are the Light Chain CDRs of hPD-1 mAb 9(2.2), and, respectively have the amino acid sequences: **SEQ ID NO:188**, **SEQ ID NO:189**, and **SEQ ID NO:178**.

[0017a] In a first aspect, there is provided an anti-human PD-1-binding monospecific monoclonal antibody that comprises a Variable Heavy Chain Domain and a Variable Light Chain Domain, wherein: said Variable Heavy Chain Domain comprises the amino acid sequence of **SEQ ID NO:147**, and said Variable Light Chain Domain comprises the amino acid sequence of **SEQ ID NO:153**.

[0018] The disclosure further concerns the examples of all such anti-human PD-1-binding molecules wherein the molecule is an antibody, and especially wherein the molecule is a chimeric antibody or a humanized antibody.

[0019] The disclosure further concerns the examples of such anti-human PD-1-binding molecules wherein the Heavy Chain Variable Domain has the amino acid sequence

(5) M252Y, S254T and T256E; or

(6) K288D and H435K;

wherein the numbering is that of the EU index as in Kabat.

[0024a] In a second aspect, there is provided a composition comprising the anti-human PD-1-binding antibody of the first aspect and a pharmaceutically acceptable carrier.

[0025] The disclosure further concerns the examples in which any of the above-described PD-1-binding molecules is used to stimulate a T-cell mediate immune response. The disclosure additionally concerns the examples in which any of the above-described PD-1-binding molecules is used in the treatment of a disease or condition associated with a suppressed immune system, especially cancer or an infection.

[0025a] In a third aspect, there is provided use of the anti-human PD-1-binding monospecific monoclonal antibody of the first aspect, or the composition of the second aspect, in the manufacture of a medicament for stimulating a T-cell mediated immune response of a subject having cancer or an infection.

[0025b] In a fourth aspect, there is provided use of the anti-human PD-1-binding monospecific monoclonal antibody of the first aspect, or the composition of the second aspect, in the manufacture of a medicament for treating a disease or condition associated with a suppressed immune system, wherein the disease or condition is cancer or an infection.

[0025c] In a fifth aspect, there is provided use of the anti-human PD-1-binding monospecific monoclonal antibody of the first aspect, or the composition of the second aspect, in the manufacture of a medicament for the treatment of cancer or an infection.

[0026] The disclosure particularly concerns such use in the treatment or diagnosis or prognosis of cancer, wherein the cancer is characterized by the presence of a cancer cell selected from the group consisting of a cell of: an adrenal gland tumor, an AIDS-associated cancer, an alveolar soft part sarcoma, an astrocytic tumor, bladder cancer, bone cancer, a brain and spinal cord cancer, a metastatic brain tumor, a breast cancer, a carotid body tumors, a cervical cancer, a chondrosarcoma, a chordoma, a chromophobe renal cell carcinoma, a clear cell carcinoma, a colon cancer, a colorectal cancer, a cutaneous benign fibrous histiocytoma, a desmoplastic small round cell tumor, an ependymoma, a Ewing's tumor, an extraskeletal myxoid chondrosarcoma, a fibrogenesis imperfecta ossium, a fibrous dysplasia of the bone, a gallbladder or bile duct cancer, gastric cancer, a gestational trophoblastic disease, a germ cell tumor, a head and neck cancer, hepatocellular carcinoma,

Hodgkin's lymphoma, non-small cell lung cancer, ovarian cancer, pancreatic cancer, a rectal cancer, acute myeloid leukemia (AML), chronic myelogenous leukemia (CML), acute B lymphoblastic leukemia (B-ALL), chronic lymphocytic leukemia (CLL), hairy cell leukemia (HCL), blastic plasmacytoid dendritic cell neoplasm (BPDCN), non-Hodgkin's lymphomas (NHL), including mantle cell leukemia (MCL), and small lymphocytic lymphoma (SLL), Hodgkin's lymphoma, systemic mastocytosis, or Burkitt's lymphoma.

[0028] The disclosure further concerns the examples in which any of the above-described PD-1-binding molecules is detectably labeled and is used in the detection of PD-1.

[0028a] In a sixth aspect, there is provided use of the anti-human PD-1-binding monospecific monoclonal antibody of the first aspect, or the composition of the second aspect, in the *in vitro* detection of PD-1, wherein said antibody is detectably labeled.

[0028b] In an example, there is provided a multispecific anti-human PD-1-binding molecule that comprises a Variable Heavy Chain Domain and a Variable Light Chain Domain, wherein: said Variable Heavy Chain Domain comprises a CDR_{H1} Domain, a CDR_{H2} Domain and a CDR_{H3} Domain, and said Variable Light Chain Domain comprises a CDR_{L1} Domain, a CDR_{L2} Domain, and a CDR_{L3} Domain, wherein:

- (1) the CDR_{H1} Domain, CDR_{H2} Domain, and CDR_{H3} Domain are the Heavy Chain CDRs of PD-1 mAb 7, and respectively comprise the amino acid sequences: **SEQ ID NO:139**, **SEQ ID NO:140**, and **SEQ ID NO:141**; and
- (2) the CDR_{L1} Domain, CDR_{L2} Domain, and CDR_{L3} Domain are the Light Chain CDRs of PD-1 mAb 7, and, respectively comprise the amino acid sequences: **SEQ ID NO:144**, **SEQ ID NO:145**, and **SEQ ID NO:146**;
wherein said multispecific anti-human PD-1-binding molecule is capable of simultaneously binding to human PD-1 and to a second epitope.

[0028c] In an example, there is provided a composition comprising the multispecific anti-human PD-1-binding molecule as disclosed herein and a pharmaceutically acceptable carrier.

[0028d] In an example, there is provided use of the multispecific anti-human PD-1-binding molecule as disclosed herein or the composition as disclosed herein in the manufacture of a medicament for stimulating a T-cell mediate immune response of a subject in need thereof.

[0028e] In an example, there is provided use of the multispecific anti-human PD-1-binding molecule as disclosed herein or the composition as disclosed herein in the manufacture of a medicament for treating a disease or condition associated with a suppressed immune system.

Brief Description of the Drawings

[0029] **Figure 1** provides a schematic of a representative covalently bonded diabody having two epitope-binding sites composed of two polypeptide chains, each having an E-coil or K-coil Heterodimer-Promoting Domain. A cysteine residue may be present in a linker and/or in the Heterodimer-Promoting Domain as shown in **Figure 3B**. VL and VH Domains that recognize the same epitope are shown using the same shading or fill pattern.

[0030] **Figure 2** provides a schematic of a representative covalently bonded diabody molecule having two epitope-binding sites composed of two polypeptide chains, each having a CH2 and CH3 Domain, such that the associated chains form all or part of an Fc Region. VL and VH Domains that recognize the same epitope are shown using the same shading or fill pattern.

[0031] **Figures 3A-3C** provide schematics showing representative tetravalent diabodies having four epitope-binding sites composed of two pairs of polypeptide chains (*i.e.*, four polypeptide chains in all). One polypeptide of each pair possesses a CH2 and CH3 Domain, such that the associated chains form all or part of an Fc Region. VL and VH Domains that recognize the same epitope are shown using the same shading or fill pattern. The two pairs of polypeptide chains may be same. In such embodiments wherein the VL and VH Domains recognize different epitopes (as shown in **Figures 3A-3C**), the resulting molecule possesses four epitope-binding sites and is bispecific and bivalent with respect to each bound epitope. In such embodiments wherein the VL and VH Domains recognize the same epitope (*e.g.*, the same VL Domain CDRs and the same VH Domain CDRs are used on both chains), the resulting molecule possesses four epitope-binding sites and is monospecific and tetravalent with respect to a single epitope. Alternatively, the two pairs of polypeptides may be different. In such

What Is Claimed Is:

- Claim 1. An anti-human PD-1-binding monospecific monoclonal antibody that comprises a Variable Heavy Chain Domain and a Variable Light Chain Domain, wherein:
said Variable Heavy Chain Domain comprises the amino acid sequence of **SEQ ID NO:147**, and said Variable Light Chain Domain comprises the amino acid sequence of **SEQ ID NO:153**.
- Claim 2. The anti-human PD-1-binding monospecific monoclonal antibody of claim 1, wherein said antibody comprises an Fc Region, and wherein:
(a) said Fc Region, is of the IgG1, IgG2, IgG3, or IgG4 isotype;
(b) said molecule additionally comprises a Hinge Domain; or
(c) said Fc Region is an IgG4 Fc Domain and said antibody additionally comprises an IgG4 Hinge Domain that comprises a stabilizing mutation.
- Claim 3. The anti-human PD-1-antibody of claim 2, wherein said antibody comprises: **SEQ ID NOs:264 and 266**.
- Claim 4. The anti-human PD-1-binding monospecific monoclonal antibody of claim 3, wherein said Fc Region is a variant Fc Region that comprises:
(a) one or more amino acid modifications that reduces the affinity of the variant Fc Region for an FcγR; and/or
(b) one or more amino acid modifications that enhances the serum half-life of the variant Fc Region.
- Claim 5. The anti-human PD-1-binding monospecific monoclonal antibody of claim 4, wherein:
(A) said modifications that reduces the affinity of the variant Fc Region for an FcγR comprise the substitution of L234A; L235A; or L234A and L235A, wherein said numbering is that of the EU index as in Kabat; and
(B) said modifications that enhances the serum half-life of the variant Fc Region comprise the substitution of M252Y; M252Y and S254T; M252Y

and T256E; M252Y, S254T and T256E; or K288D and H435K, wherein said numbering is that of the EU index as in Kabat.

- Claim 6. A composition comprising the anti-human PD-1-binding antibody of any one of claims 1-5 and a pharmaceutically acceptable carrier.
- Claim 7. Use of the anti-human PD-1-binding monospecific monoclonal antibody of any one of claims 1-5, or the composition of claim 6, in the manufacture of a medicament for stimulating a T-cell mediated immune response of a subject having cancer or an infection.
- Claim 8. Use of the anti-human PD-1-binding monospecific monoclonal antibody of any one of claims 1-5, or the composition of claim 6, in the manufacture of a medicament for treating a disease or condition associated with a suppressed immune system, wherein the disease or condition is cancer or an infection.
- Claim 9. Use of the anti-human PD-1-binding monospecific monoclonal antibody of any one of claims 1-5, or the composition of claim 6, in the manufacture of a medicament for the treatment of cancer or an infection.
- Claim 10. The use of any one of claims 7-9, wherein said cancer is selected from the group consisting of: an adrenal gland tumor, an AIDS-associated cancer, an alveolar soft part sarcoma, an astrocytic tumor, bladder cancer, bone cancer, a brain and spinal cord cancer, a metastatic brain tumor, a breast cancer, a carotid body tumors, a cervical cancer, a chondrosarcoma, a chordoma, a chromophobe renal cell carcinoma, a clear cell carcinoma, a colon cancer, a colorectal cancer, a cutaneous benign fibrous histiocytoma, a desmoplastic small round cell tumor, an ependymoma, a Ewing's tumor, an extraskeletal myxoid chondrosarcoma, a fibrogenesis imperfecta ossium, a fibrous dysplasia of the bone, a gallbladder or bile duct cancer, gastric cancer, a gestational trophoblastic disease, a germ cell tumor, a head and neck cancer, hepatocellular carcinoma, an islet cell tumor, a Kaposi's Sarcoma, a kidney cancer, a leukemia, a lipoma/benign lipomatous tumor, a liposarcoma/malignant lipomatous tumor, a liver cancer, a lymphoma, a lung cancer, a medulloblastoma, a melanoma, a meningioma, a multiple endocrine neoplasia, a multiple myeloma, a myelodysplastic syndrome, a neuroblastoma,

a neuroendocrine tumors, an ovarian cancer, a pancreatic cancer, a papillary thyroid carcinoma, a parathyroid tumor, a pediatric cancer, a peripheral nerve sheath tumor, a pheochromocytoma, a pituitary tumor, a prostate cancer, a posterior uveal melanoma, a rare hematologic disorder, a renal metastatic cancer, a rhabdoid tumor, a rhabdomyosarcoma, a sarcoma, a skin cancer, a soft-tissue sarcoma, a squamous cell cancer, a stomach cancer, a synovial sarcoma, a testicular cancer, a thymic carcinoma, a thymoma, a thyroid metastatic cancer, and a uterine cancer.

- Claim 11. The use of any one of claims 7-9, wherein said cancer is colorectal cancer, hepatocellular carcinoma, glioma, kidney cancer, breast cancer, multiple myeloma, bladder cancer, neuroblastoma; sarcoma, non-Hodgkin's lymphoma, non-small cell lung cancer, ovarian cancer, pancreatic cancer, rectal cancer, acute myeloid leukemia (AML), chronic myelogenous leukemia (CML), acute B lymphoblastic leukemia (B-ALL), chronic lymphocytic leukemia (CLL), hairy cell leukemia (HCL), blastic plasmacytoid dendritic cell neoplasm (BPDCN), non-Hodgkin's lymphomas (NHL), including mantle cell leukemia (MCL), and small lymphocytic lymphoma (SLL), Hodgkin's lymphoma, systemic mastocytosis, or Burkitt's lymphoma.
- Claim 12. Use of the anti-human PD-1-binding monospecific monoclonal antibody of any one of claims 1-5, or the composition of claim 6, in the *in vitro* detection of PD-1, wherein said antibody is detectably labeled.
- Claim 13. The use of claim 10, wherein the cancer is a uterine cancer.
- Claim 14. The use of claim 10, wherein the cancer is a squamous cell cancer.
- Claim 15. The use of claim 10, wherein the cancer is glioma.
- Claim 16. The use of claim 10, wherein the cancer is a cervical cancer.
- Claim 17. The use of claim 10, wherein the cancer is kidney cancer.
- Claim 18. The use of claim 10, wherein the cancer is a lung cancer.
- Claim 19. The use of claim 10, wherein the cancer is non-small cell lung cancer.
- Claim 20. The use of claim 10, wherein the cancer is a head and neck cancer.
- Claim 21. The use of claim 10, wherein the cancer is a renal metastatic cancer.

- Claim 22. The use of claim 10, wherein the cancer is a carcinoma.
- Claim 23. The use of claim 10, wherein the cancer is a skin cancer.
- Claim 24. The use of claim 22, wherein the carcinoma is a chromophobe renal cell carcinoma.
- Claim 25. The use of claim 10, wherein the cancer is a gastric cancer.
- Claim 26. The use of claim 10, wherein the cancer is a pancreatic cancer.
- Claim 27. The use of claim 10, wherein the cancer is a breast cancer.