

- (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, the present disclosure refers to a composition comprising:

- (A) the multispecific anti-human PD-1-binding molecule of the first aspect; and
(B) 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 mediated 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, the present disclosure refers to the multispecific anti-human PD-1-binding molecule of the first aspect, or the composition of the second aspect, for use in:

- (A) a method of stimulating a T-cell mediated immune response of a subject in need thereof; or
(B) the treatment of a disease or condition associated with a suppressed immune system.

[0025b] In a fourth aspect, the present disclosure refers to use of the multispecific anti-human PD-1-binding molecule of the first aspect or the composition of the second aspect in the manufacture of a medicament for treatment of a cancer or an infection;

~~(A) stimulating a T-cell mediated immune response of a subject in need thereof; or~~

~~(B) the treatment of a disease or condition associated with a suppressed immune system.~~

[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, 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.

[0027] The disclosure particularly concerns such use in the treatment or diagnosis or prognosis of cancer, wherein the 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, 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 fifth aspect, the present disclosure refers to use of the multispecific anti-human PD-1-binding molecule of the first aspect in the *in vitro* detection of PD-1, wherein said molecule ~~or said antibody~~ is detectably labeled.

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. 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 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 multispecific anti-human PD-1-binding molecule of claim 1, wherein said molecule is a bispecific binding molecule, capable of simultaneously binding to human PD-1 and to a second epitope.
- Claim 3. The multispecific anti-human PD-1-binding molecule of claim 2, wherein said second epitope is an epitope of a molecule involved in regulating an immune check point present on the surface of an immune cell, and wherein said second epitope is an epitope of B7-H3, B7-H4, BTLA, CD40, CD40L, CD47, CD70, CD80, CD86, CD94, CD137, CD137L, CD226, CTLA-4, Galectin-9, GITR, GITRL, HHLA2, ICOS, ICOSL, KIR, LAG-3, LIGHT, MHC class I or II, NKG2a, NKG2d, OX40, OX40L, PD1H, PD-1, PD-L1, PD-L2, PVR, SIRPa, TCR, TIGIT, TIM-3 or VISTA.
- Claim 4. The multispecific anti-human PD-1-binding molecule of claim 3, wherein said second epitope is an epitope of LAG-3.
- Claim 5. The multispecific anti-human PD-1-binding molecule of claim 4, wherein said LAG-3 epitope-binding site comprises:
- (A) (1) the CDR_{H1} Domain, CDR_{H2} Domain, and CDR_{H3} Domain of the Variable Heavy Chain of LAG-3 mAb 1, and respectively comprise the amino acid sequences: **SEQ ID NO:42**, **SEQ ID NO:43**, and **SEQ ID NO:44**; and
 - (2) the CDR_{L1} Domain, CDR_{L2} Domain, and CDR_{L3} Domain of the Variable Light Chain of LAG-3 mAb 1, and respectively

comprise the amino acid sequences: **SEQ ID NO:46**, **SEQ ID NO:47**, and **SEQ ID NO:48**;

or

- (B) (1) the CDR_H1 Domain, CDR_H2 Domain, and CDR_H3 Domain of the Variable Heavy Chain of hLAG-3 mAb 1 VH1, and respectively comprise the amino acid sequences: **SEQ ID NO:42**, **SEQ ID NO:43**, and **SEQ ID NO:44**; and
- (2) the CDR_L1 Domain, CDR_L2 Domain, and CDR_L3 Domain of the Variable Light Chain of LAG-3 mAb 1 VL4, and respectively comprise the amino acid sequences: **SEQ ID NO:55**, **SEQ ID NO:47**, and **SEQ ID NO:48**;

or

- (C) (1) the CDR_H1 Domain, CDR_H2 Domain, and CDR_H3 Domain of the Variable Heavy Chain of LAG-3 mAb 6, and respectively comprise the amino acid sequences: **SEQ ID NO:57**, **SEQ ID NO:58**, and **SEQ ID NO:59**; and
- (2) the CDR_L1 Domain, CDR_L2 Domain, and CDR_L3 Domain of the Variable Light Chain of LAG-3 mAb 6, and respectively comprise the amino acid sequences: **SEQ ID NO:61**, **SEQ ID NO:62**, and **SEQ ID NO:63**;

or

- (D) (1) the CDR_H1 Domain, CDR_H2 Domain, and CDR_H3 Domain of the Variable Heavy Chain of hLAG-3 mAb 6 VH1, and respectively comprise the amino acid sequences: **SEQ ID NO:57**, **SEQ ID NO:58**, and **SEQ ID NO:59**; and
- (2) the CDR_L1 Domain, CDR_L2 Domain, and CDR_L3 Domain of the Variable Light Chain of LAG-3 mAb 6 and respectively comprise the amino acid sequences: **SEQ ID NO:298**, **SEQ ID NO:62**, and **SEQ ID NO:63**.

Claim 6. The multispecific anti-human PD-1-binding molecule of claim 5, wherein said LAG-3 epitope-binding site comprises:

- (1) the CDR_H1 Domain, CDR_H2 Domain, and CDR_H3 Domain of the Variable Heavy Chain of hLAG-3 mAb 6 VH1, and

respectively comprise the amino acid sequences: **SEQ ID NO:57, SEQ ID NO:58, and SEQ ID NO:59**; and

- (2) the CDR_L1 Domain, CDR_L2 Domain, and CDR_L3 Domain of the Variable Light Chain of hLAG-3 mAb 6 VL1/VL2 and respectively comprise the amino acid sequences: **SEQ ID NO:298, SEQ ID NO:62, and SEQ ID NO:63**.

Claim 7. The multispecific anti-human PD-1-binding molecule of claim 6, wherein said LAG-3 epitope-binding site comprises:

- (1) the Variable Heavy Chain of hLAG-3 mAb 6 VH1 (**SEQ ID NO:294**) and
(2) the Variable Light Chain of hLAG-3 mAb 6 VL1 (**SEQ ID NO:296**).

Claim 8. The multispecific anti-human PD-1-binding molecule of any one of claims 1-7, wherein said molecule is:

- (A) a diabody, said diabody being a covalently bonded complex that comprises two, three, four or five polypeptide chains; or
(B) a trivalent binding molecule, said trivalent binding molecule being a covalently bonded complex that comprises three, four or five polypeptide chains, or
(C) a bispecific antibody.

Claim 9. The multispecific anti-human PD-1-binding molecule of claim 8, wherein said molecule is a diabody and comprises an Albumin-Binding Domain (ABD).

Claim 10. The multispecific anti-human PD-1-binding molecule of any one of claims 1-9, wherein said molecule 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;
(c) said Fc Region is an IgG4 Fc Domain and said ~~antibody~~molecule additionally comprises an IgG4 Hinge Domain that comprises a stabilizing mutation;
(d) said Fc region comprises one or more amino acid modifications that reduces the affinity of the variant Fc Region for an FcγR; and/or

(e) said Fc region comprises one or more amino acid modifications that enhances the serum half-life of the variant Fc Region.

Claim 11. The multispecific anti-human PD-1-binding molecule of claim 10, wherein:

(A) said one or more modifications that reduces the affinity of the variant Fc Region for an Fc γ R, and said modifications 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 one or more modifications that enhances the serum half-life of the variant Fc Region, and said modifications that ~~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 12. The multispecific anti-human PD-1-binding molecule of any one of claims 8, 10 or 11, wherein said molecule is a diabody comprising:

(a) **SEQ ID NO:267**, wherein X₁ is Ala; X₂ is Tyr; X₃ is Thr; X₄ is Glu, and **SEQ ID NO:268**; or

(b) **SEQ ID NO:267**, wherein X₁ is Gly; X₂ is Tyr; X₃ is Thr; X₄ is Glu, and **SEQ ID NO:268**; or

(c) **SEQ ID NO:267**, wherein X₁ is Gly; X₂ is Met; X₃ is Ser; X₄ is Thr, and **SEQ ID NO:268**; or

(d) **SEQ ID NOs:269 and 270**; or

(e) **SEQ ID NOs:271 and 272**; or

(f) **SEQ ID NOs:273, 274, 275, and 276**; or

(g) **SEQ ID NOs:277, 278, 279, and 280**; or

(h) **SEQ ID NOs:281, 282, and 283**; or

(i) **SEQ ID NOs:290 and 291**; or

(j) **SEQ ID NOs:292 and 293**.

Claim 13. The multispecific anti-human PD-1-binding molecule of claim 12, wherein said molecule is a diabody comprising **SEQ ID NOs:290 and 291**.

Claim 14. A composition comprising:

- (A) the multispecific anti-human PD-1-binding molecule of any one of claims 1-13; and
- (B) a pharmaceutically acceptable carrier.

Claim 15. The multispecific anti-human PD-1-binding molecule of any one of claims 1-13, or the composition of claim 14, for use in:

- (A) a method of stimulating a T-cell mediated immune response of a subject in need thereof; or
- (B) the treatment of a disease or condition associated with a suppressed immune system.

Claim 16. The multispecific anti-human PD-1-binding molecule or composition of claim 15, for use according to claim 15, wherein the disease or condition is cancer or an infection, optionally 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, optionally 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 mantel cell leukemia (MCL), and small lymphocytic lymphoma (SLL), Hodgkin's lymphoma, systemic mastocytosis, or Burkitt's lymphoma.

Claim 17. The multispecific anti-human PD-1-binding molecule of any one of claims 1-13, wherein said molecule is for use in an *in vitro* method of detecting PD-1, and wherein said molecule is detectably labeled.

Claim 18. Use of the multispecific anti-human PD-1-binding molecule of any one of claims 1-13 in the *in vitro* detection of PD-1, wherein said molecule ~~or said antibody~~ is detectably labeled.

Claim 19. Use of the multispecific anti-human PD-1-binding molecule of any one of claims 1-13, or the composition of claim 14, in the manufacture of a medicament for treatment of a cancer or an infection.
~~(A) stimulating a T-cell mediated immune response of a subject in need thereof;~~
~~or~~
~~(B) the treatment of a disease or condition associated with a suppressed immune system.~~

Claim 20. The use of claim 19, wherein the ~~disease or condition is cancer or an infection,~~ optionally 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, optionally 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.