

[0015] However, despite all such prior advances, a need remains for improved compositions capable of more vigorously directing the body's immune system to attack cancer cells or pathogen-infected cells, especially at lower therapeutic concentrations. For although the adaptive immune system can be a potent defense mechanism against cancer and disease, it is often hampered by immune suppressive mechanisms in the tumor microenvironment, such as the expression of PD-1. Furthermore, co-inhibitory molecules expressed by tumor cells, immune cells, and stromal cells in the tumor milieu can dominantly attenuate T-cell responses against cancer cells. Thus, a need remains for potent PD-1-binding molecules. In particular, a need exists for potent PD-1-binding molecules having a desirable binding kinetic profile and that antagonize the PD-1/PD-L1 axis by blocking the PD-1/PD-L1 interaction, which could provide improved therapeutic value to patients suffering from cancer or other diseases and conditions. The present invention is directed to these and other goals.

Summary

[0016] The present disclosure is directed to PD-1 binding molecules that comprise the PD-1-binding domain of selected anti-PD-1 antibodies capable of binding to both cynomolgus monkey PD-1 and to human PD-1: PD-1 mAb 1, PD-1 mAb 2, PD-1 mAb 3, PD-1 mAb 4, PD-1 mAb 5, PD-1 mAb 6, PD-1 mAb 7, PD-1 mAb 8, PD-1 mAb 9, PD-1 mAb 10, PD-1 mAb 11, PD-1 mAb 12, PD-1 mAb 13, PD-1 mAb 14, or PD-1 mAb 15. The disclosure particularly concerns PD-1 binding molecules that are humanized or chimeric versions of such antibodies, or that comprise PD-1 binding-fragments of such anti-PD-1 antibodies (especially immunoconjugates, diabodies, BiTEs, bispecific antibodies, *etc.*). The disclosure particularly concerns such PD-1-binding molecules that are additionally capable of binding an epitope of a molecule involved in regulating an immune check point that is present on the surface of an immune cell. The present disclosure also pertains to methods of using such PD-1-binding molecules to detect PD-1 or to stimulate an immune response. The present disclosure also pertains to methods of combination therapy in which a PD-1-binding molecule that comprises one or more PD-1-binding domain(s) of such selected anti-PD-1 antibodies is administered in combination with one or more additional molecules that are effective in stimulating an immune response and/or in combination with one or more additional molecules that specifically bind a cancer antigen.

[0017] In detail, the disclosure provides an anti-human PD-1-binding molecule that comprises the three Heavy Chain CDR Domains, CDR_{H1}, CDR_{H2} and CDR_{H3} and the three Light Chain CDR Domains, CDR_{L1}, CDR_{L2}, and CDR_{L3}, wherein:

- (A) (1) the CDR_{H1} Domain, CDR_{H2} Domain, and CDR_{H3} Domain are the Heavy Chain CDRs of PD-1 mAb 1, and respectively have the amino acid sequences: **SEQ ID NO:71, SEQ ID NO:72, and SEQ ID NO:73**; and
- (2) the CDR_{L1} Domain, CDR_{L2} Domain, and CDR_{L3} Domain are the Light Chain CDRs of PD-1 mAb 1, and respectively have the amino acid sequences: **SEQ ID NO:76, SEQ ID NO:77, and SEQ ID NO:78**;

or

- (B) (1) the CDR_{H1} Domain, CDR_{H2} Domain, and CDR_{H3} Domain are the Heavy Chain CDRs of PD-1 mAb 2, and respectively have the amino acid sequences: **SEQ ID NO:85, SEQ ID NO:86, and SEQ ID NO:87**; and
- (2) the CDR_{L1} Domain, CDR_{L2} Domain, and CDR_{L3} Domain are the Light Chain CDRs of PD-1 mAb 2, and, respectively have the amino acid sequences: **SEQ ID NO:90, SEQ ID NO:91, and SEQ ID NO:92**;

or

- (C) (1) the CDR_{H1} Domain, CDR_{H2} Domain, and CDR_{H3} Domain are the Heavy Chain CDRs of PD-1 mAb 3, and respectively have the amino acid sequences: **SEQ ID NO:99, SEQ ID NO:100, and SEQ ID NO:101**; and
- (2) the CDR_{L1} Domain, CDR_{L2} Domain, and CDR_{L3} Domain are the Light Chain CDRs of PD-1 mAb 3, and, respectively have the amino acid sequences: **SEQ ID NO:104, SEQ ID NO:105, and SEQ ID NO:106**;

or

- (D) (1) the CDR_{H1} Domain, CDR_{H2} Domain, and CDR_{H3} Domain are the Heavy Chain CDRs of PD-1 mAb 4, and respectively have the amino acid sequences: **SEQ ID NO:109, SEQ ID NO:110, and SEQ ID NO:111**; and
- (2) the CDR_{L1} Domain, CDR_{L2} Domain, and CDR_{L3} Domain are the Light Chain CDRs of PD-1 mAb 4, and, respectively have the amino acid sequences: **SEQ ID NO:114, SEQ ID NO:115, and SEQ ID NO:116**;

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, the present disclosure refers to 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.

[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 of **SEQ ID NO:79**, **SEQ ID NO:93**, **SEQ ID NO:147**, **SEQ ID NO:149**, **SEQ ID NO:179**, **SEQ ID NO:181**, or **SEQ ID NO:250**.

[0020] The disclosure further concerns the examples of such anti-human PD-1-binding molecules wherein the Light Chain Variable Domain has the amino acid sequence of **SEQ ID NO:81**, **SEQ ID NO:95**, **SEQ ID NO:151**, **SEQ ID NO:153**, **SEQ ID NO:155**, **SEQ ID NO:184**, **SEQ ID NO:186**, or **SEQ ID NO:251**.

[0021] The disclosure further concerns the example wherein the anti-human PD-1-binding molecule is a bispecific binding molecule, capable of simultaneously binding to human PD-1 and to a second epitope, and particularly concerns the example wherein the second epitope is an epitope of a molecule involved in regulating an immune check point present on the surface of an immune cell (especially wherein the 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, and most particularly wherein the second epitope is an epitope of CD137, CTLA-4, LAG-3, OX40, TIGIT, or TIM-3).

[0022] The disclosure further concerns the examples wherein the anti-human PD-1-binding molecule is a bispecific molecule comprising a LAG-3 epitope-binding site, particularly wherein the 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, having the amino acid sequences: **SEQ ID NO:42**, **SEQ ID NO:43**, and **SEQ ID NO:44**, respectively; and
- (2) the CDR_{L1} Domain, CDR_{L2} Domain, and CDR_{L3} Domain of the Variable Light Chain of LAG-3 mAb 1, having the amino acid sequences: **SEQ ID NO:46**, **SEQ ID NO:47**, and **SEQ ID NO:48**, respectively;

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, having the amino acid sequences: **SEQ ID NO:42**, **SEQ ID NO:43**, and **SEQ ID NO:44**, respectively; and
- (2) the CDR_{L1} Domain, CDR_{L2} Domain, and CDR_{L3} Domain of the Variable Light Chain of hLAG-3 mAb 1 VL4, having the amino acid sequences: **SEQ ID NO:55**, **SEQ ID NO:47**, and **SEQ ID NO:48**, respectively;

or

- (C) (1) the CDR_{H1} Domain, CDR_{H2} Domain, and CDR_{H3} Domain of the Variable Heavy Chain of LAG-3 mAb 6, having the amino acid sequences: **SEQ ID NO:57**, **SEQ ID NO:58**, and **SEQ ID NO:59**, respectively; and
- (2) the CDR_{L1} Domain, CDR_{L2} Domain, and CDR_{L3} Domain of the Variable Light Chain of LAG-3 mAb 6, having the amino acid

sequences: **SEQ ID NO:61**, **SEQ ID NO:62**, and **SEQ ID NO:63**, respectively;

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, having the amino acid sequences: **SEQ ID NO:57**, **SEQ ID NO:58**, and **SEQ ID NO:59**, respectively; and
- (2) the CDR_{L1} Domain, CDR_{L2} Domain, and CDR_{L3} Domain of the Variable Light Chain of LAG-3 mAb 6, having the amino acid sequences: **SEQ ID NO:298**, **SEQ ID NO:62**, and **SEQ ID NO:63**, respectively.

[0023] The disclosure further concerns the example of such anti-human PD-1-binding molecules wherein the molecule is a diabody, and especially, wherein the diabody is a covalently bonded complex that comprises two, or three, or four, or five polypeptide chains. The disclosure further concerns the example of such anti-human PD-1-binding molecules wherein the molecule is a trivalent binding molecule, and especially wherein the trivalent binding molecule is a covalently bonded complex that comprises three, four, five or more than five polypeptide chains. The disclosure additionally concerns the example of such anti-human PD-1-binding molecules in which the molecule comprises an Fc Region. The disclosure additionally concerns the example of such anti-human PD-1-binding molecules in which the molecule comprises an Albumin-Binding Domain, and especially a deimmunized Albumin-Binding Domain.

[0024] The disclosure further concerns the examples of all such anti-human PD-1-binding molecules wherein the molecule comprises an Fc Region, and wherein the Fc Region is a variant Fc Region that comprises one or more amino acid modifications that reduces the affinity of the variant Fc Region for an FcγR and/or enhances the serum half-life, and more particularly, wherein the modifications comprise at least one amino acid substitution selected from the group consisting of:

- (1) L234A; L235A;
- (2) L234A and L235A;
- (3) M252Y; M252Y and S254T;
- (4) M252Y and T256E;

- (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 the multispecific anti-human PD-1-binding molecule 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, 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 stimulating a T-cell mediate immune response of a subject in need thereof.

[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 treating 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 a nucleic acid expression vector encoding the multispecific molecule for binding human PD-1 of the first aspect.

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 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.
- Claim 2. The multispecific anti-human PD-1-binding molecule of claim 1, wherein said molecule comprises a Heavy Chain Variable Domain that comprises the amino acid sequence of **SEQ ID NO:147**, or **SEQ ID NO:149**.
- Claim 3. The multispecific anti-human PD-1-binding molecule of any one of claims 1-2, wherein said molecule comprises a Light Chain Variable Domain that comprises the amino acid sequence of **SEQ ID NO:153**, or **SEQ ID NO:155**.
- Claim 4. The multispecific anti-human PD-1-binding molecule of any one of claims 1-3, 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.
- Claim 5. The multispecific anti-human PD-1-binding molecule of claim 1, 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 6. The multispecific anti-human PD-1-binding molecule of claim 5, wherein said second epitope is an epitope of CD137, CTLA-4, LAG-3, OX40, TIGIT, or TIM-3.

Claim 7. The multispecific anti-human PD-1-binding molecule of claim 6, comprising a LAG-3 epitope-binding site, 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 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 1-7, wherein said molecule comprises an Fc Region.

Claim 11. The multispecific anti-human PD-1-binding molecule of claim 10, wherein said Fc Region, is of the IgG1, IgG2, IgG3, or IgG4 isotype.

Claim 12. The multispecific anti-human PD-1-binding molecule of claim 11, wherein said molecule further comprises a Hinge Domain.

- Claim 13. The multispecific anti-human PD-1-binding molecule of claim 12, wherein said Fc Region and said Hinge Domain are of the IgG4 isotype, and wherein said Hinge Domain comprises a stabilizing mutation.
- Claim 14. The multispecific anti-human PD-1-binding molecule of any one of claims 10-13, 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 15. The multispecific anti-human PD-1-binding molecule of claim 14, wherein 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.
- Claim 16. The multispecific anti-human PD-1-binding molecule of claim 14 or 15, wherein 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 17. The multispecific anti-human PD-1-binding molecule of any one of claims 7 or 9-15, 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 18. The multispecific anti-human PD-1-binding molecule of any one of claims 1-17, wherein said molecule is detectably labeled and is used in the detection of PD-1.
- Claim 19. A composition comprising the multispecific anti-human PD-1-binding molecule of any one of claims 1-17 and a pharmaceutically acceptable carrier.
- Claim 20. Use of the multispecific anti-human PD-1-binding molecule of any one of claims 1-17 or the composition of claim 19 in the manufacture of a medicament for stimulating a T-cell mediate immune response of a subject in need thereof.
- Claim 21. Use of the multispecific anti-human PD-1-binding molecule of any one of claims 1-17 or the composition of claim 19 in the manufacture of a medicament for treating a disease or condition associated with a suppressed immune system.
- Claim 22. The use of claim 21, wherein the disease or condition is cancer or an infection.
- Claim 23. The use of claim 22, wherein said 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.

Claim 24. The use of claim 23, 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 25. A nucleic acid expression vector encoding the multispecific molecule for binding human PD-1 of any one of claims 1-17.