

WHAT IS CLAIMED IS:

- Claim 1. A bispecific molecule possessing both one or more epitope-binding sites capable of immunospecific binding to (an) epitope(s) of PD-1 and one or more epitope-binding sites capable of immunospecific binding to (an) epitope(s) of CTLA-4, wherein said molecule comprises:
- (A) a Heavy Chain Variable Domain and a Light Chain Variable Domain of an antibody that binds PD-1; and
 - (B) a Heavy Chain Variable Domain and a Light Chain Variable Domain of an antibody that binds CTLA-4;
- wherein said molecule is:
- (i) a diabody, said diabody being a covalently bonded complex that comprises two, three, four or five polypeptide chains; or
 - (ii) a trivalent binding molecule, said trivalent binding molecule being a covalently bonded complex that comprises three, four, five, or more polypeptide chains.
- Claim 2. The bispecific molecule of claim 1, wherein said molecule exhibits an activity that is enhanced relative to such activity exhibited by two monospecific molecules one of which possesses said Heavy Chain Variable Domain and said Light Chain Variable Domain of said antibody that binds PD-1 and the other of which possesses said Heavy Chain Variable Domain and said Light Chain Variable Domain of said antibody that binds CTLA-4.
- Claim 3. The bispecific molecule of claim 1 or 2, wherein said molecule elicits fewer immune-related adverse events (irAEs) when administered to a subject in need thereof relative to such iREs elicited by the administration of a monospecific antibody that binds CTLA-4.
- Claim 4. The bispecific molecule of any one of claims 1-3, wherein said molecule comprises an Fc Region.
- Claim 5. The bispecific molecule of claim 4, 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 Fc γ R; and/or

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

- Claim 6. The bispecific molecule of claim 5, wherein said modifications that reduces the affinity of the variant Fc Region for an Fc γ R comprise the substitution of L234A; L235A; or L234A and L235A, wherein said numbering is that of the EU index as in Kabat.
- Claim 7. The bispecific molecule of claim 5 or 6, 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 8. The bispecific molecule of any one of claims 1-7, wherein said molecule is said diabody and comprises two epitope-binding sites capable of immunospecific binding to an epitope of PD-1 and two epitope-binding sites capable of immunospecific binding to an epitope of CTLA-4.
- Claim 9. The bispecific molecule of any one of claims 1-7, wherein said molecule is said trivalent binding molecule and comprises two epitope-binding sites capable of immunospecific binding to an epitope of PD-1 and one epitope-binding site capable of immunospecific binding to an epitope of CTLA-4.
- Claim 10. The bispecific molecule of any one of claims 1-9, wherein said molecule is capable of binding to PD-1 and CTLA-4 molecules present on the cell surface.
- Claim 11. The bispecific molecule of any one of claims 1-10, wherein said molecule is capable of simultaneously binding to PD-1 and CTLA-4.
- Claim 12. The bispecific molecule of any one of claims 1-11, wherein said molecule promotes the stimulation of immune cells.
- Claim 13. The bispecific molecule of claim 12, wherein said stimulation of immune cells results in:
- (A) immune cell proliferation; and/or
- (B) immune cell production and/or release of at least one cytokine; and/or

- (C) immune cell production and/or release of at least one lytic molecule;
and/or
 - (D) immune cell expression of at least one activation marker.
- Claim 14. The bispecific molecule of claim 12 or 13, wherein said immune cell is a T-lymphocyte or an NK-cell.
- Claim 15. The bispecific molecule of any one of claims 1-14, wherein said epitope-binding sites capable of immunospecific binding to an epitope of PD-1 comprise:
- (A) the VH Domain of PD-1 mAb 1 (SEQ ID NO:47) and the VL Domain of PD-1 mAb 1 (SEQ ID NO:48); or
 - (B) the VH Domain of PD-1 mAb 2 (SEQ ID NO:49) and the VL Domain of PD-1 mAb 2 (SEQ ID NO:50); or
 - (C) the VH Domain of PD-1 mAb 3 (SEQ ID NO:51) and the VL Domain of PD-1 mAb 3 (SEQ ID NO:52); or
 - (D) the VH Domain of PD-1 mAb 4 (SEQ ID NO:53) and the VL Domain of PD-1 mAb 4 (SEQ ID NO:54); or
 - (E) the VH Domain of PD-1 mAb 5 (SEQ ID NO:55) and the VL Domain of PD-1 mAb 5 (SEQ ID NO:56); or
 - (F) the VH Domain of PD-1 mAb 6 (SEQ ID NO:57) and the VL Domain of PD-1 mAb 6 (SEQ ID NO:58); or
 - (G) the VH Domain of PD-1 mAb 6-I VH (SEQ ID NO:86) and the VL Domain of PD-1 mAb 6-SQ VL (SEQ ID NO:87); or
 - (H) the VH Domain of PD-1 mAb 7 (SEQ ID NO:59) and the VL Domain of PD-1 mAb 7 (SEQ ID NO:60); or
 - (I) the VH Domain of PD-1 mAb 8 (SEQ ID NO:61) and the VL Domain of PD-1 mAb 8 (SEQ ID NO:62).
- Claim 16. The bispecific molecule of any one of claims 1-15, wherein said epitope-binding site(s) capable of immunospecific binding to an epitope of CTLA-4 comprise:
- (A) the VH Domain of CTLA-4 mAb 1 (SEQ ID NO:76) and the VL Domain of CTLA-4 mAb 1 (SEQ ID NO:77); or
 - (B) the VH Domain of CTLA-4 mAb 2 (SEQ ID NO:78) and the VL Domain of CTLA-4 mAb 2 (SEQ ID NO:79); or

- (C) the VH Domain of CTLA-4 mAb 3 (SEQ ID NO:90) and the VL Domain of CTLA-4 mAb 3 (SEQ ID NO:91).

Claim 17. The bispecific molecule of claim 16, wherein:

- (A) said epitope-binding sites capable of immunospecific binding to an epitope of PD-1 comprise the VH Domain of PD-1 mAb 6-I VH (SEQ ID NO:86) and the VL Domain of PD-1 mAb 6-SQ (SEQ ID NO:87); and
- (B) said epitope-binding site(s) capable of immunospecific binding to an epitope of CTLA-4 comprise(s) the VH Domain of CTLA-4 mAb 3 (SEQ ID NO:90) and the VL Domain of CTLA-4 mAb 3 (SEQ ID NO:91).

Claim 18. The bispecific molecule of any one of claims 1-17, wherein said molecule comprises:

- (A) two polypeptide chains having SEQ ID NO:95, and two polypeptide chain having SEQ ID NO:96; or
- (B) two polypeptide chains having SEQ ID NO:97, and two polypeptide chain having SEQ ID NO:98; or
- (C) two polypeptide chains having SEQ ID NO:99, and two polypeptide chain having SEQ ID NO:100; or
- (D) two polypeptide chains having SEQ ID NO:102, and two polypeptide chain having SEQ ID NO:103; or
- (E) two polypeptide chains having SEQ ID NO:101, and two polypeptide chain having SEQ ID NO:100; or
- (F) one polypeptide chains having SEQ ID NO:104, one polypeptide chain having SEQ ID NO:105, one polypeptide chain having SEQ ID NO:106, and one polypeptide chain having SEQ ID NO:107; or
- (G) one polypeptide chains having SEQ ID NO:108, one polypeptide chain having SEQ ID NO:105, one polypeptide chain having SEQ ID NO:109, and one polypeptide chain having SEQ ID NO:107.

Claim 19. A pharmaceutical composition that comprises an effective amount of the bispecific molecule of any of claims 1-18 and a pharmaceutically acceptable carrier.

- Claim 20. The bispecific molecule of any one of claims 1-18, wherein said molecule is used to promote stimulation of an immune-mediated response of a subject in need thereof.
- Claim 21. The bispecific molecule of any one of claims 1-18, wherein said molecule is used in the treatment of a disease or condition associated with a suppressed immune system.
- Claim 22. The bispecific molecule of claim 21, wherein the disease or condition is cancer or an infection.
- Claim 23. The bispecific molecule 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 bispecific molecule of claim 22, wherein said infection is characterized by the presence of a bacterial, fungal, viral or protozoan pathogen.