[0041] The disclosure additionally concerns the use of such pharmaceutical composition or the use of any of the above-described bispecific molecules to promote stimulation of an immune-mediated response of a subject in need thereof, and in particular, wherein such molecule promotes the stimulation of immune cells, and in particular, stimulation of NK-cells and/or T-lymphocytes. In one example, the present disclosure refers to any one of the bispecific molecules described herein, or a pharmaceutical composition described herein, for use in promoting stimulation of an immune-mediated response. In another example, the present disclosure refers to use of any one of the bispecific molecules described herein, or a pharmaceutical composition described herein, in the manufacture of a medicament for stimulating an immune-mediated response. The disclosure particularly concerns the examples wherein such stimulation results in immune cell proliferation, immune cell production and/or release of cytokines (*e.g.*, IFNγ, IL-2, TNFα, *etc*.), immune cell production and/or release of lytic molecules (*e.g.*, granzyme, perforin, *etc*.), and/or immune cell expression of activation markers (*e.g.*, CD69, CD25, CD107a, *etc*.). The disclosure further concerns methods of treating cancer or other diseases that involve the use or administration of any of the above-described PD-1 x CTLA-4 bispecific molecules to stimulate an immune mediated response. The disclosure particularly concerns the examples in which the immune stimulatory activity of any of the above-described PD-1 x CTLA-4 bispecific molecules is more potent than the joint or combined administration of a separate anti-PD-1 antibody and a separate anti-CTLA-4 antibody (especially, wherein such antibodies are monospecific for such molecules). The disclosure also concerns examples in which immune cells, particularly NK-cells and/or T-lymphocytes, stimulated by the above-described PD-1 x CTLA-4 bispecific molecules exhibit enhanced proliferation, altered production and/or release of cytokines (*e.g.*, IFNγ, IL-2, TNFα, *etc*.), altered production and/or release of lytic molecules, and/or altered expression of activation markers relative to that exhibited by such cells stimulated by the joint or combined administration of a separate anti-PD-1 antibody and a separate anti-CTLA-4 antibody. The disclosure also concerns examples in which the above-described PD-1 x CTLA-4 bispecific molecules have a reduced incidence of irAEs. The disclosure additionally concerns the examples in which any of the above-described PD-1 x CTLA-4 bispecific molecules are used in the treatment of a disease or condition associated with a suppressed immune system, especially cancer or an infection.

[0042] The disclosure additionally concerns such a use to treat a disease or condition associated with a suppressed immune system, or in the treatment of such a disease or condition. In one aspect, the present disclosure refers to any one of the bispecific molecules described herein, or a pharmaceutical composition described herein, for use in the treatment of a cancer or an infection. In another aspect, the present disclosure refers to use of any one of the bispecific molecules described herein, or a pharmaceutical composition described herein, in the manufacture of a medicament for treating a cancer or an infection. The disclosure particularly concerns such a use in in the treatment of a disease or condition associated with a suppressed immune system, or wherein the disease or condition is cancer or an infection (particularly, an infection characterized by the presence of a bacterial, fungal, viral or protozoan pathogen).

WHAT IS CLAIMED IS:

1. A bispecific molecule, comprising:

(A) a Heavy Chain Variable Domain and a Light Chain Variable Domain of an antibody that binds PD-1 comprising the VH Domain of SEQ ID NO:86 and the VL Domain of SEQ ID NO:87; and

(B) a Heavy Chain Variable Domain and a Light Chain Variable Domain of an antibody that binds CTLA-4 comprising the six CDRs of the VH Domain of SEQ ID NO:90 and the VL Domain of SEQ ID NO:91;

wherein the bispecific molecule is:

(i) a diabody, the diabody being a covalently bonded complex that comprises two, three, four or five polypeptide chains; or

(ii) a trivalent binding molecule, the trivalent binding molecule being a covalently bonded complex that comprises three, four, five, or more polypeptide chains.

2. The bispecific molecule of claim 1, wherein the Heavy Chain Variable Domain and the Light Chain Variable Domain of the antibody that binds CTLA-4 comprise the VH Domain of SEQ ID NO:90 and the VL Domain of SEQ ID NO:91.

3. The bispecific molecule of claim 1 or claim 2, wherein the molecule comprises an Fc Region.

4. The bispecific molecule of claim 3, wherein the Fc Region is a variant Fc Region that comprises:

(A) one or more amino acid modifications that reduce the affinity of the variant Fc Region for an FcγR; and/or

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

5. The bispecific molecule of claim 4, wherein the one or more modifications that reduce the affinity of the variant Fc Region for an FcγR comprise the substitution of L234A or L235A, or L234A and L235A, wherein the numbering is that of the EU index as in Kabat.

6. The bispecific molecule of claim 4, wherein the one or more modifications that enhance the serum half-life of the variant Fc Region comprise the substitution of M252Y; or M252Y

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

7. The bispecific molecule of any one of claims 1-6, which is a diabody comprising two epitope-binding sites that immunospecifically bind to an epitope of PD-1 and two epitope-binding sites that immunospecifically bind to an epitope of CTLA-4.

8. The bispecific molecule of any one of claims 1-6, which is a trivalent binding molecule comprising two epitope-binding sites that immunospecifically bind to an epitope of PD-1 and one epitope-binding site that immunospecifically binds to an epitope of CTLA-4.

9. The bispecific molecule of any one of claims 1-8, which elicits fewer immune-related adverse events (irAEs) if administered to a monkey relative to such irAEs elicited by the administration of a monospecific antibody that binds CTLA-4.

10. The bispecific molecule of any one of claims 1-8, which elicits fewer immune-related adverse events (irAEs) if administered to a subject in need thereof relative to such irAEs elicited by the administration of a monospecific antibody that binds CTLA-4.

11. A bispecific molecule comprising one polypeptide chain comprising SEQ ID NO:104, one polypeptide chain comprising SEQ ID NO:105, one polypeptide chain comprising SEQ ID NO:106, and one polypeptide chain comprising SEQ ID NO:107.

12. A bispecific molecule comprising two polypeptide chains each comprising SEQ ID NO:101 and two polypeptide chains each comprising SEQ ID NO:100.

13. A bispecific molecule comprising one polypeptide chain comprising SEQ ID NO:108, one polypeptide chain comprising SEQ ID NO:105, one polypeptide chain comprising SEQ ID NO:109, and one polypeptide chain comprising SEQ ID NO:107.

14. A pharmaceutical composition comprising the bispecific molecule of any one of claims 1-13 and a pharmaceutically acceptable carrier.

15. A bispecific molecule of any one of claims 1-13, or a pharmaceutical composition of claim 14, for use in the treatment of a cancer or an infection.

16. Use of a bispecific molecule of any one of claims 1-13, or a pharmaceutical composition of claim 14, in the manufacture of a medicament for treating a cancer or an infection.

17. A bispecific molecule for use of claim 15, a pharmaceutical composition for use of claim 15, or a use of claim 16, 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 phaeochromocytoma, 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.

18. A bispecific molecule for use of claim 15, a pharmaceutical composition for use of claim 15, or a use of claim 16, wherein the cancer is characterized by the presence of a cancer cell selected from the group consisting of a cell of: a colorectal cancer, a lung cancer, a cervical cancer, a head and neck cancer, a prostate cancer, a sarcoma, and a thymoma.

19. A bispecific molecule for use of claim 15, a pharmaceutical composition for use of claim 15, or a use of claim 16, wherein the cancer is characterized by the presence of a cancer cell selected from the group consisting of a cell of: a colorectal cancer, a hepatocellular carcinoma, a glioma, a kidney cancer, a breast cancer, a multiple myeloma, a bladder cancer, a neuroblastoma, a sarcoma, a non-Hodgkin's lymphoma, a non-small cell lung cancer, an ovarian cancer, a pancreatic cancer and a rectal cancer.

20. A bispecific molecule for use of claim 15, a pharmaceutical composition for use of claim 15, or a use of claim 16, wherein the cancer is characterized by the presence of a cancer cell selected from the group consisting of a cell of: a colorectal cancer, a gastric cancer, a melanoma, a prostate cancer, a pancreatic cancer, a renal cancer, a bladder cancer, a mammary cancer, a lung cancer, a fibrosarcoma, a human mantle cell lymphoma, a Raji Burkitt's lymphoma.

21. A bispecific molecule for use of claim 15, a pharmaceutical composition for use of claim 15, or a use of claim 16, wherein the infection is characterized by the presence of a bacterial, fungal, viral or protozoan pathogen.

22. A bispecific molecule comprising two polypeptide chains each comprising the polypeptide of SEQ ID NO:99 and two polypeptide chains each comprising the polypeptide of SEQ ID NO:100.

23. A pharmaceutical composition comprising the bispecific molecule of claim 24 and a pharmaceutically acceptable carrier.

24. A bispecific molecule of claim 22, or a pharmaceutical composition of claim 23, for use in the treatment of a cancer or an infection.

25. Use of a bispecific molecule of claim 22, or a pharmaceutical composition of claim 23, in the manufacture of a medicament for treating a cancer or an infection.

26. A bispecific molecule for use of claim 24, a pharmaceutical composition for use of claim 24, or a use of claim 25, 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 phaeochromocytoma, 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.

27. A bispecific molecule for use of claim 24, a pharmaceutical composition for use of claim 24, or a use of claim 25, wherein the cancer is characterized by the presence of a cancer cell selected from the group consisting of a cell of: a colorectal cancer, a lung cancer, a cervical cancer, a head and neck cancer, a prostate cancer, a sarcoma, and a thymoma.

28. A bispecific molecule for use of claim 24, a pharmaceutical composition for use of claim 24, or a use of claim 25, wherein the cancer is characterized by the presence of a cancer cell selected from the group consisting of a cell of: a colorectal cancer, a hepatocellular carcinoma, a glioma, a kidney cancer, a breast cancer, a multiple myeloma, a bladder cancer, a neuroblastoma, a sarcoma, a non-Hodgkin's lymphoma, a non-small cell lung cancer, an ovarian cancer, a pancreatic cancer and a rectal cancer.

29. A bispecific molecule for use of claim 24, a pharmaceutical composition for use of claim 24, or a use of claim 25, wherein the cancer is characterized by the presence of a cancer cell selected from the group consisting of a cell of: a colorectal cancer, a gastric cancer, a melanoma, a prostate cancer, a pancreatic cancer, a renal cancer, a bladder cancer, a mammary cancer, a lung cancer, a fibrosarcoma, a human mantle cell lymphoma, a Raji Burkitt's lymphoma.

30. A bispecific molecule for use of claim 24, a pharmaceutical composition for use of claim 24, or a use of claim 25, wherein the infection is characterized by the presence of a bacterial, fungal, viral or protozoan pathogen.