mAb 1 (5C4; BMS-936558)), **PD-1 mAb 2** (MK-3475; Merck, lambrolizumab). Responder (R) cells are pan T cells; stimulator (S)cells are mature dendritic cells (mDCs).

[0028] **Figure 8** shows that soluble human LAG-3 (shLAG-3), which binds to human HLA-class II molecules expressed on both APCs and CD4 T-cells, induced a robust proliferative response compared to IgG isotype or responder (R) (pan T cells) plus stimulator (S) (mature dendritic cells (mDCs) control wells.

[0029] **Figures 9A-9B** show that the PD-1 x LAG-3 bi-specific diabodies of the present invention induced potent T-cell proliferative responses when compared against anti-PD-1 mAb (5C4) or anti-LAG-3 mAb (25F7). **Figure 9A** shows the T-cell proliferative responses obtained using the preferred PD-1 x LAG-3 bi-specific, tetra-valent diabodies of the present invention (**PD-1 x LAG-Fc-DART®-1** and **PD-1 x LAG-Fc-DART®-2**), **PD-1 mAb 1** (5C4; BMS-936558)), **LAG-3 mAb 1** (25F7; BMS-986016, Medarex/BMS), soluble human LAG-3 (ShLAG-3), a control IgG, responder+stimulator cells (pan T cells and mature dendritic cells; R+S) and stimulator cells (mature dendritic cells; S). **Figure 9B** shows the same data for **PD-1 x LAG-Fc-DART®-1** and **PD-1 x LAG-Fc-DART®-2**, **PD-1 mAb 1** (5C4; BMS-936558)), **LAG-3 mAb 1** (25F7; BMS-986016, Medarex/BMS), and **PD-1 mAb 1** (5C4; BMS-936558)) + **LAG-3 mAb 1** (25F7; BMS-986016, Medarex/BMS), using a different y-axis scale.

Summary:

[0030] The present disclosure is directed to bi-specific diabodies that comprise two or more polypeptide chains and which possess at least one Epitope-Binding Site that is immunospecific for an epitope of PD-1 and at least one Epitope-Binding Site that is immunospecific for an epitope of LAG-3 (*i.e.*, a "PD-1 x LAG-3 bi-specific diabody"). More preferably, the present disclosure is directed to bi-specific diabodies that comprise four polypeptide chains and which possess two Epitope-Binding Sites that are immunospecific for one (or two) epitope(s) of PD-1 and two Epitope-Binding Site that are immunospecific for one (or two) epitope(s) of LAG-3 (*i.e.*, a "PD-1 x LAG-3 bi-specific, tetra-valent diabody"). The present disclosure also is directed to such diabodies that additionally comprise an immunoglobulin Fc Domain ("bi-specific Fc diabodies" and "bi-specific, tetra-valent, Fc diabodies"). The diabodies of the present disclosure are capable of simultaneously binding to PD-1 and to LAG-3, particularly as such molecules are arrayed on the surfaces of human cells. The disclosure is directed to

pharmaceutical compositions that contain such diabodies, and to methods involving the use of such diabodies in the treatment of cancer and other diseases and conditions.

[0031] In detail, in one aspect, the invention provides a bi-specific Fc diabody capable of immunospecific binding to an epitope of PD-1 and to an epitope of LAG-3, wherein the diabody comprises four polypeptide chains, each having an amino terminus and a carboxy terminus, and wherein:

(A) the first and second polypeptide chains are covalently bonded to one another, the first and third polypeptide chains are covalently bonded to one another, and the third and fourth polypeptide chains are covalently bonded to one another;

(B) the first and third polypeptide chains of the diabody each comprise, in the N-terminal to C-terminal direction, a Light Chain Variable Domain of an antibody that is immunospecific for PD-1 or LAG-3, a Heavy Chain Variable Domain of an antibody that is immunospecific for LAG-3 or PD-1, a Heterodimer-Promoting Domain and a CH2-CH3 Domain, wherein the Light Chain Variable Domains and the Heavy Chain Variable Domains are incapable of associating to form an Epitope-Binding Site capable of binding an epitope of PD-1 or an epitope of LAG-3; and

(C) the second and the fourth polypeptide chains of the diabody each comprise, in the N-terminal to C-terminal direction, a Light Chain Variable Domain of an antibody that is immunospecific for PD-1 or LAG-3, a Heavy Chain Variable Domain of an antibody that is immunospecific for LAG-3 or PD-1, and a Heterodimer-Promoting Domain, wherein the Light Chain Variable Domains and the Heavy Chain Variable Domains are incapable of associating to form an Epitope-Binding Site capable of binding an epitope of PD-1 or an epitope of LAG-3;

and wherein:

(I) (1) the Light Chain Variable Domain of the first polypeptide chain and the Heavy Chain Variable Domain of the second polypeptide chain associate to form a first Epitope-Binding Site and the Heavy Chain Variable Domain of the first polypeptide chain and the Light Chain Variable Domain of the second polypeptide chain associate to form a second Epitope-Binding Site; and

(2) the Light Chain Variable Domain of the third polypeptide chain and the Heavy Chain Variable Domain of the fourth polypeptide chain associate to form a third Epitope-Binding Site and the Heavy Chain Variable Domain of the third

polypeptide chain and the Light Chain Variable Domain of the fourth polypeptide chain associate to form a fourth Epitope-Binding Site;

wherein two of the formed Epitope-Binding Sites are capable of immunospecifically binding to an epitope of PD-1 and two of the formed Epitope-Binding Sites are capable of immunospecifically binding to an epitope of LAG-3;

II. the Heterodimer-Promoting Domains of the first and second polypeptide chains differ and have an amino acid sequence selected from the group consisting of: **SEQ ID NO:16** and **SEQ ID NO:17**; and

III. the CH2-CH3 Domains of the first and third polypeptide chains associate to form an Fc Domain.

[0032] The disclosure also concerns the embodiment of such a bi-specific Fc diabody wherein the CH2-CH3 Domains of the first and third polypeptide chains each have the amino acid sequence of **SEQ ID NO:24**.

[0033] The disclosure also concerns the embodiment of such bi-specific Fc diabodies wherein the Heavy Chain Variable Domain of an antibody that is immunospecific for LAG-3 has the amino acid sequence of **SEQ ID NO:11,** and wherein the Light Chain Variable Domain of an antibody that is immunospecific for LAG-3 has the amino acid sequence of **SEQ ID NO:12**.

[0034] The disclosure also concerns the embodiment of such bi-specific Fc diabodies wherein the Heavy Chain Variable Domain of an antibody that is immunospecific for PD-1 has the amino acid sequence of **SEQ ID NO:2,** and wherein the Light Chain Variable Domain of an antibody that is immunospecific for PD-1 has the amino acid sequence of **SEQ ID NO:3**.

[0035] In one aspect, the invention also concerns a pharmaceutical composition that comprises an effective amount of any of the above-indicated bi-specific Fc diabodies, and a pharmaceutically acceptable carrier.

[0036] The disclosure also concerns the embodiment of such a pharmaceutical composition wherein the effective amount of the bi-specific Fc diabody is an amount effective to treat cancer in a recipient individual in need of such treatment.

[0037] The disclosure also concerns the embodiment of such pharmaceutical compositions wherein the cancer is an adrenal gland cancer, 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 rhabdomysarcoma, 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, or a uterine cancer.

[0038] The disclosure also concerns the embodiment of such pharmaceutical compositions wherein the effective amount of the bi-specific Fc diabody is an amount effective to treat a disease associated with the presence of a pathogen in a recipient individual in need of such treatment.

[0039] The disclosure also concerns the embodiment of such a pharmaceutical composition wherein the pathogen is a bacterium a fungus or a virus.

[0040] The disclosure also concerns a method of treating cancer which comprises administering an effective amount of such pharmaceutical compositions to an individual in need thereof.

[0041] The disclosure also concerns a method of treating a disease associated with the presence of a pathogen which comprises administering an effective amount of any of the above-indicated pharmaceutical compositions to an individual in need thereof.

[0041a] In one aspect, the invention provides use of any of the above-indicated bispecific Fc diabodies, or any of the above-indicated pharmaceutical compositions, in the manufacture of a medicament for treating cancer.

[0041b] In one aspect, the invention provides use of any of the above-indicated bispecific Fc diabodies, or any of the above-indicated pharmaceutical compositions, in the manufacture of a medicament for treating a disease associated with the presence of a pathogen.

Detailed Description of the Invention:

[0042] The present invention is directed to bi-specific diabodies that comprise two or more polypeptide chains and which possess at least one Epitope-Binding Site that is immunospecific for an epitope of PD-1 and at least one Epitope-Binding Site that is immunospecific for an epitope of LAG-3 (*i.e.*, a "PD-1 x LAG-3 bi-specific diabody"). More preferably, the present invention is directed to bi-specific diabodies that comprise four polypeptide chains and which possess two Epitope-Binding Sites that are immunospecific for one (or two) epitope(s) of PD-1 and two Epitope-Binding Site that are immunospecific for one (or two) epitope(s) of LAG-3 (*i.e.*, a "PD-1 x LAG-3 bi-specific, tetra-valent diabody"). The present invention also is directed to such diabodies that additionally comprise an immunoglobulin Fc Domain ("bi-specific Fc diabodies" and "bi-specific, tetra-valent, Fc diabodies"). The diabodies of the present invention are capable of simultaneously binding to PD-1 and to LAG-3, particularly as such molecules are arrayed on the surfaces of human cells. The invention is directed to pharmaceutical compositions that contain such diabodies, and to methods involving the use of such diabodies in the treatment of cancer and other diseases and conditions.

[0043] The bi-specific diabodies of the present invention are capable of simultaneously binding to PD-1 and to LAG-3, particularly as such molecules are arrayed on the surfaces of human cells. The invention is directed to pharmaceutical compositions that contain such diabodies, and to methods involving the use of such diabodies in the treatment of cancer and other diseases and conditions. In particular, the PD-1 x LAG-3 bi-specific diabodies of the present invention comprise polypeptide chains that are covalently complexed together.

[0044] As discussed above, T-cell activation requires two distinct signals. The first signal is provided by the T-Cell Receptor (TCR) expressed on the surface of a T-cell that has recognized a peptide antigen within the context of human leukocyte antigens (HLA) expressed on an Antigen-Presenting Cell (APCs). The second signal is provided by the interaction of cognate pairs of co-stimulatory ligands: B7-1 and B7-2 expressed on APCs and their corresponding receptors: CD28 and CTLA-4 expressed on T-cells.

Enhancement By Fusion Of Human Fc Domain," Abstract 3P-683, J. Biochem. 76(8):992; Takemura, S. et al. (2000) "*Construction Of A Diabody (Small Recombinant Bispecific Antibody) Using A Refolding System*," Protein Eng. 13(8):583-588; Lu, D. et al. (2005) "*A Fully Human Recombinant IgG-Like Bispecific Antibody To Both The Epidermal Growth Factor Receptor And The Insulin-Like Growth Factor Receptor For Enhanced Antitumor Activity*," J. Biol. Chem. 280(20):19665-19672). However, the art has recognized that bi-specific diabodies composed of non-covalently associated polypeptides are unstable and readily dissociate into non-functional monomers (see, e.g., Lu, D. et al. (2005) "*A Fully Human Recombinant IgG-Like Bispecific Antibody To Both The Epidermal Growth Factor Receptor And The Insulin-Like Growth Factor Receptor For Enhanced Antitumor Activity*," J. Biol. Chem. 280(20):19665-19672).

[0064] In the face of this challenge, the art has succeeded in developing stable, covalently bonded heterodimeric non-mono-specific diabodies, termed **DART®s** (see, e.g., United States Patent Publications No. 2013-0295121; 2010-0174053 and 2009-0060910; European Patent Publication No. EP 2714079; EP 2601216; EP 2376109; EP 2158221 and PCT Publications No. WO 2012/162068; WO 2012/018687; WO 2010/080538; and Moore, P.A. et al. (2011) "*Application Of Dual Affinity Retargeting Molecules To Achieve Optimal Redirected T-Cell Killing Of B-Cell Lymphoma*," Blood 117(17):4542-4551; Veri, M.C. et al. (2010) "*Therapeutic Control Of B Cell Activation Via Recruitment Of Fcgamma Receptor IIb (CD32B) Inhibitory Function With A Novel Bispecific Antibody Scaffold*," Arthritis Rheum. 62(7):1933-1943; Johnson, S. et al. (2010) "*Effector Cell Recruitment With Novel Fv-Based Dual-Affinity Re-Targeting Protein Leads To Potent Tumor Cytolysis And in vivo B-Cell Depletion*," J. Mol. Biol. 399(3):436-449). Such diabodies comprise two or more covalently complexed polypeptides and involve engineering one or more cysteine residues into each of the employed polypeptide species. For example, the addition of a cysteine residue to the c-terminus of such constructs has been shown to allow disulfide bonding between the polypeptide chains, stabilizing the resulting heterodimer without interfering with the binding characteristics of the bi-valent molecule.

[0065] Each of the two polypeptides of the simplest DART® comprises three Domains (**Figure 1**). The first polypeptide comprises: (i) a Domain that comprises a binding region of a Light Chain Variable Domain of a first immunoglobulin (VL1), (ii) a second Domain that comprises a binding region of a Heavy Chain Variable Domain of a second immunoglobulin

What Is Claimed Is:

Claim 1. A bi-specific Fc diabody capable of immunospecific binding to an epitope of PD-1 and to an epitope of LAG-3, wherein said diabody comprises four polypeptide chains, each having an amino terminus and a carboxy terminus, and wherein:

(A) said first and second polypeptide chains are covalently bonded to one another, said first and third polypeptide chains are covalently bonded to one another, and said third and fourth polypeptide chains are covalently bonded to one another;

(B) said first and third polypeptide chains of said diabody each comprise, in the N-terminal to C-terminal direction, a Light Chain Variable Domain of an antibody that is immunospecific for PD-1 or LAG-3, a Heavy Chain Variable Domain of an antibody that is immunospecific for LAG-3 or PD-1, a Heterodimer-Promoting Domain and a CH2-CH3 Domain, wherein said Light Chain Variable Domains and said Heavy Chain Variable Domains are incapable of associating to form an Epitope-Binding Site capable of binding an epitope of PD-1 or an epitope of LAG-3; and

(C) said second and said fourth polypeptide chains of said diabody each comprise, in the N-terminal to C-terminal direction, a Light Chain Variable Domain of an antibody that is immunospecific for PD-1 or LAG-3, a Heavy Chain Variable Domain of an antibody that is immunospecific for LAG-3 or PD-1, and a Heterodimer-Promoting Domain, wherein said Light Chain Variable Domains and said Heavy Chain Variable Domains are incapable of associating to form an Epitope-Binding Site capable of binding an epitope of PD-1 or an epitope of LAG-3;

and wherein:

(I) (1) said Light Chain Variable Domain of said first polypeptide chain and said Heavy Chain Variable Domain of said second polypeptide chain associate to form a first Epitope-Binding Site and said Heavy Chain Variable Domain of said first polypeptide chain and said Light Chain Variable Domain of said second

polypeptide chain associate to form a second Epitope-Binding Site; and

(2) said Light Chain Variable Domain of said third polypeptide chain and said Heavy Chain Variable Domain of said fourth polypeptide chain associate to form a third Epitope-Binding Site and said Heavy Chain Variable Domain of said third polypeptide chain and said Light Chain Variable Domain of said fourth polypeptide chain associate to form a fourth Epitope-Binding Site;

wherein two of said formed Epitope-Binding Sites are capable of immunospecifically binding to an epitope of PD-1 and two of said formed Epitope-Binding Sites are capable of immunospecifically binding to an epitope of LAG-3;

II. said Heterodimer-Promoting Domains of said first and second polypeptide chains differ and have an amino acid sequence selected from the group consisting of: **SEQ ID NO:16** and **SEQ ID NO:17**; and

III. said CH2-CH3 Domains of said first and third polypeptide chains associate to form an Fc Domain.

Claim 2. The bi-specific Fc diabody of claim 1, wherein said CH2-CH3 Domains of said first and third polypeptide chains each have the amino acid sequence of **SEQ ID NO:24**.

Claim 3. The bi-specific Fc diabody of any of claims 1 or 2, wherein said Heavy Chain Variable Domain of an antibody that is immunospecific for LAG-3 has the amino acid sequence of **SEQ ID NO:11,** and wherein said Light Chain Variable Domain of an antibody that is immunospecific for LAG-3 has the amino acid sequence of **SEQ ID NO:12**.

Claim 4. The bi-specific Fc diabody of any of claims 1-3, wherein said Heavy Chain Variable Domain of an antibody that is immunospecific for PD-1 has the amino acid sequence of **SEQ ID NO:2,** and wherein said Light Chain Variable Domain of an antibody that is immunospecific for PD-1 has the amino acid sequence of **SEQ ID NO:3**.

Claim 8. The pharmaceutical composition of claim 5, wherein said effective amount of said bi-specific Fc diabody is an amount effective to treat a disease associated with the presence of a pathogen in a recipient individual in need of such treatment.

Claim 9. The pharmaceutical composition of claim 8, wherein said pathogen is a bacterium a fungus or a virus.

Claim 10. Use of the bispecific Fc diabody of any one of claims 1-4, or of the pharmaceutical composition of any one of claims 6-7, in the manufacture of a medicament for treating cancer.

Claim 11. Use of the bispecific Fc diabody of any one of claims 1-4, or of the pharmaceutical composition of any one of claims 8-9, in the manufacture of a medicament for treating a disease associated with the presence of a pathogen.