

via inhibition of TCR-induced calcium fluxes, and controls the size of the memory T-cell pool (Matsuzaki, J. *et al.* (2010) “*Tumor-Infiltrating NY-ESO-1-Specific CD8+ T-Cells Are Negatively Regulated By LAG-3 and PD-1 In Human Ovarian Cancer*,” *Proc. Natl. Acad. Sci. (U.S.A.)* 107(17):7875-7880; Workman C.J., *et al.* (2004) “*Lymphocyte Activation Gene-3 (CD223) Regulates The Size Of The Expanding T-Cell Population Following Antigen Activation in vivo*,” *J. Immunol.* 172:5450-5455).

[0012] 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 LAG-3. 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 LAG-3-binding molecules. In particular, a need exists for LAG-3-binding molecules that a desirable binding kinetic profile, bind different LAG-3 epitopes and/or exhibit single agent activity that 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:

[0013] The present disclosure is directed to LAG-3 binding molecules that comprise the LAG-3-binding domain of selected anti-LAG-3 antibodies: LAG-3 mAb 1, LAG-3 mAb 2, LAG-3 mAb 3, LAG-3 mAb 4, LAG-3 mAb 5, or LAG-3 mAb 6 that are capable of binding to both cynomolgus monkey LAG-3 and to human LAG-3. The disclosure particularly concerns LAG-3 binding molecules that are humanized or chimeric versions of such antibodies, or that comprise LAG-3 binding-fragments of such anti-LAG-3 antibodies (especially immunoconjugates, diabodies, BiTEs, bispecific antibodies, *etc.*). The disclosure particularly concerns such LAG-3-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 LAG-3-binding molecules to

detect LAG-3 or to stimulate an immune response. The present disclosure also pertains to methods of combination therapy in which a LAG-3-binding molecule that comprises one or more LAG-3-binding domain(s) of such selected anti-LAG-3 antibodies is administered in combination with one or more additional molecules that are effective in stimulating an immune response to thereby further enhance, stimulate or upregulate such immune response in a subject.

[0014] In detail, one aspect provides a LAG-3-binding molecule that is capable of binding both to human LAG-3 and to cynomolgus monkey LAG-3, wherein said LAG-3-binding molecule 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:

- (A) (1) the CDR_{H1} Domain, CDR_{H2} Domain, and CDR_{H3} Domain are the Heavy Chain CDRs of LAG-3 mAb 1, and respectively have the amino acid sequences: **SEQ ID NO:8**, **SEQ ID NO:9**, and **SEQ ID NO:10**; and
- (2) the CDR_{L1} Domain, CDR_{L2} Domain, and CDR_{L3} Domain are the Light Chain CDRs of LAG-3 mAb 1, and respectively have the amino acid sequences: **SEQ ID NO:13**, **SEQ ID NO:14**, and **SEQ ID NO:15**;

or

- (B) (1) the CDR_{H1} Domain, CDR_{H2} Domain, and CDR_{H3} Domain are the Heavy Chain CDRs of hLAG-3 mAb 1, and respectively have the amino acid sequences: **SEQ ID NO:8**, **SEQ ID NO:9**, and **SEQ ID NO:10**; and
- (2) the CDR_{L1} Domain, CDR_{L2} Domain, and CDR_{L3} Domain are the Light Chain CDRs of hLAG-3 mAb 1, and respectively have the amino acid sequences: **SEQ ID NO:28**, **SEQ ID NO:14**, and **SEQ ID NO:15**;

or

- (C) (1) the CDR_{H1} Domain, CDR_{H2} Domain, and CDR_{H3} Domain are the Heavy Chain CDRs of LAG-3 mAb 2, and respectively have the amino acid sequences: **SEQ ID NO:31**, **SEQ ID NO:32**, and **SEQ ID NO:33**; and

- (2) the CDR_L1 Domain, CDR_L2 Domain, and CDR_L3 Domain are the Light Chain CDRs of LAG-3 mAb 6, and, respectively have the amino acid sequences: **SEQ ID NO:76**, **SEQ ID NO:77**, and **SEQ ID NO:78**;

or

- (H) (1) the CDR_H1 Domain, CDR_H2 Domain, and CDR_H3 Domain are the Heavy Chain CDRs of LAG-3 hAb 6, 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 hLAG-3 hAb 6, and respectively have the amino acid sequences: **SEQ ID NO:87**, **SEQ ID NO:77**, and **SEQ ID NO:78**.

[0015] The disclosure further concerns the embodiments of such LAG-3-binding molecules wherein the Heavy Chain Variable Domain has the amino acid sequence of **SEQ ID NO:6**, **SEQ ID NO:29**, **SEQ ID NO:39**, **SEQ ID NO:49**, or **SEQ ID NO:59**.

[0016] The disclosure further concerns the embodiments of such LAG-3-binding molecules wherein the Light Chain Variable Domain has the amino acid sequence of **SEQ ID NO:11**, **SEQ ID NO:34**, **SEQ ID NO:44**, **SEQ ID NO:54**, or **SEQ ID NO:64**.

[0017] The disclosure further concerns the embodiments of such LAG-3-binding molecules wherein the Heavy Chain Variable Domain has the amino acid sequence of **SEQ ID NO:16**, **SEQ ID NO:18**, **SEQ ID NO:79**, or **SEQ ID NO:80**.

[0018] The disclosure further concerns the embodiments of such LAG-3-binding molecules wherein the Light Chain Variable Domain has the amino acid sequence of **SEQ ID NO:20**, **SEQ ID NO:22**, **SEQ ID NO:24**, **SEQ ID NO:26**, **SEQ ID NO:83**, or **SEQ ID NO:85**.

[0019] The disclosure further concerns the embodiments of all such LAG-3-binding molecules wherein the molecule is an antibody, and especially wherein the molecule is a chimeric antibody or a humanized antibody.

[0020] The disclosure further concerns the embodiment wherein the LAG-3-binding molecule is a bispecific binding molecule, capable of simultaneously binding to human LAG-3 and to a second epitope, and particularly concerns the embodiment 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, OX40, PD-1, TIGIT or TIM-3).

[0021] The disclosure further concerns the embodiment of such LAG-3-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, or more than five polypeptide chains. The disclosure further concerns the embodiment of such anti-LAG-3-binding molecules wherein the molecule is a trivalent binding molecule, the trivalent binding molecule being a covalently bonded complex that comprises three, four, five or more polypeptide chains. The disclosure additionally concerns the embodiment of such LAG-3-binding molecules in which the molecule comprises an Fc Region. The disclosure additionally concerns the embodiment of such LAG-3-binding molecules in which the molecule comprises an Albumin-Binding Domain, and especially a deimmunized Albumin-Binding Domain.

[0022] The disclosure further concerns the embodiments of all such LAG-3-binding molecules wherein the molecule comprises an Fc (Fraction Crystalizable) 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 Fc γ R and/or enhances serum half-life, and more particularly, wherein the modifications comprise at least one amino acid substitution selected from the group consisting of:

- (1) L234A;
- (2) L235A;
- (3) L234A and L235A;

- (4) M252Y; M252Y and S254T;
- (5) M252Y and T256E;
- (6) M252Y, S254T and T256E; or
- (7) K288D and H435K;

wherein the numbering is that of the EU index according to Kabat.

[0022a] In one aspect, there is provided a pharmaceutical composition comprising an effective amount of any of the above-described LAG-3-binding molecules, and a pharmaceutically acceptable carrier.

[0023] The disclosure further concerns the embodiments in which any of the above-described LAG-3-binding molecules is used to stimulate a T-cell mediated immune response. The disclosure additionally concerns the embodiments in which any of the above-described LAG-3-binding molecules is used in the treatment of a disease or condition associated with a suppressed immune system, especially cancer or an infection.

[0023a] In one aspect, there is provided any of the above-described LAG-3-binding molecules, or the above-described pharmaceutical composition, for use in stimulating a T-cell-mediated immune response of a subject in need thereof. In another aspect, there is provided any of the above-described LAG-3-binding molecules, or the above-described pharmaceutical composition, for use in treating a disease or condition associated with a suppressed immune system.

[0024] 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.

[0025] 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.

[0026] The disclosure further concerns the embodiments in which any of the above-described LAG-3-binding molecules is detectably labeled and is used in the detection of LAG-3.

Brief Description of the Drawings:

[0027] **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.

[0028] **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.

- Claim 6. The LAG-3-binding molecule of any one of claims 1-5, wherein said molecule is a bispecific binding molecule, capable of simultaneously binding to human LAG-3 and to a second epitope.
- Claim 7. The LAG-3-binding molecule of claim 6, 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 8. The LAG-3-binding molecule of claim 6, 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 9. The LAG-3-binding molecule of any one of claims 6-8, wherein said molecule is:
- (a) a diabody, said diabody being a covalently bonded complex that comprises two, or three or four different 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.
- Claim 10. The LAG-3-binding molecule of any one of claims 1-9, wherein said molecule comprises an Fc Region.
- Claim 11. The LAG-3-binding molecule of claim 9, wherein said molecule comprises an Albumin-Binding Domain (ABD).
- Claim 12. The LAG-3-binding molecule of claim 11, wherein said ABD is a deimmunized ABD.

- Claim 13. The LAG-3-binding molecule of claim 10, wherein said Fc Region is a variant Fc Region that comprises:
- (a) one or more amino acid modifications that reduce(s) the affinity of said variant Fc Region for an FcγR; and/or
 - (b) one or more amino acid modifications that enhance(s) the serum half-life of said variant Fc Region.
- Claim 14. The LAG-3-binding molecule of claim 13, wherein:
- (a) said modifications that reduce(s) the affinity of the variant Fc Region for an FcγR comprise the substitutions:
 - (1) L234A; L235A; or
 - (2) L234A and L235A;
- and
- (b) said modifications that enhance(s) the serum half-life of the variant Fc Region comprise the substitutions:
 - (1) M252Y; M252Y and S254T;
 - (2) M252Y and T256E;
 - (3) M252Y, S254T and T256E; or
 - (4) K288D and H435K,
- wherein said numbering is that of the EU index according to Kabat.
- Claim 15. A pharmaceutical composition comprising an effective amount of the LAG-3-binding molecule of any one of claims 1-14, and a pharmaceutically acceptable carrier.
- Claim 16. The LAG-3-binding molecule of any one of claims 1-14, or the pharmaceutical composition of claim 15, for use in stimulating a T-cell-mediated immune response of a subject in need thereof.
- Claim 17. The LAG-3-binding molecule of any one of claims 1-14, or the pharmaceutical composition of claim 15, for use in treating a disease or condition associated with a suppressed immune system.
- Claim 18. The LAG-3-binding molecule of claim 17, wherein the disease or condition is cancer or an infection.

Claim 19. The LAG-3-binding molecule of claim 18, wherein disease or condition is a cancer 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 20. The LAG-3-binding molecule of claim 18, wherein said disease or condition 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 21. The LAG-3-binding molecule of any one of claims 1-14, or the pharmaceutical composition of claim 15, wherein said molecule is detectably labeled and is used in the detection of LAG-3.