

- (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**.

[0014a] In one aspect, the present disclosure refers to 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 of an antibody that specifically binds human LAG-3 with an equilibrium binding constant (KD) of about 0.05 nM or less, 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**.

[0014b] In another aspect, the present disclosure refers to 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:

- (A) said Variable Heavy Chain Domain comprises the amino acid sequence of **SEQ ID NO:16** or **SEQ ID NO:18**; and
- (B) said Variable Light Chain Domain comprises the amino acid sequence of **SEQ ID NO:20**, **SEQ ID NO:22**, **SEQ ID NO:24** or **SEQ ID NO:26**.

[0015] The disclosure further concerns the examples 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 examples 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 examples 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 examples 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 examples 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 example 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 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, OX40, PD-1, TIGIT or TIM-3).

[0021] The disclosure further concerns the example 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 example 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 example of such LAG-3-binding molecules in which the molecule comprises an Fc Region. The disclosure additionally concerns the example 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 examples 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, the present disclosure refers to 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 examples in which any of the above-described LAG-3-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 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, the present disclosure refers to 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, the present disclosure refers to 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 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.

[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 examples 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.

What Is Claimed Is:

Claim 1. 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 of an antibody that specifically binds human LAG-3 with an equilibrium binding constant (KD) of about 0.05 nM or less, 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**.

Claim 2. The LAG-3-binding molecule of claim 1, wherein:

- (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**.

Claim 3. The LAG-3-binding molecule of claim 1, wherein:

- (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**.

Claim 4. 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:

(A) said Variable Heavy Chain Domain comprises the amino acid sequence of **SEQ ID NO:16** or **SEQ ID NO:18**; and

(B) said Variable Light Chain Domain comprises the amino acid sequence of **SEQ ID NO:20**, **SEQ ID NO:22**, **SEQ ID NO:24** or **SEQ ID NO:26**.

Claim 5. The LAG-3-binding molecule of any one of claims 1-4, wherein said molecule comprises a Heavy Chain Variable Domain having the amino acid sequence of **SEQ ID NO:16** or **SEQ ID NO:18**.

Claim 6. The LAG-3-binding molecule of any one of claims 1-5, wherein said molecule comprises a Light Chain Variable Domain having the amino acid sequence of **SEQ ID NO:20**, **SEQ ID NO:22**, **SEQ ID NO:24** or **SEQ ID NO:26**.

Claim 7. The LAG-3-binding molecule of any one of claims 5-6, wherein said molecule comprises a Heavy Chain Variable Domain having the amino acid sequence of

SEQ ID NO:16, and a Light Chain Variable Domain having the amino acid sequence of **SEQ ID NO:24**,

- Claim 8. The LAG-3-binding molecule of any one of claims 5-6, wherein said molecule comprises a Heavy Chain Variable Domain having the amino acid sequence of **SEQ ID NO:16**, and a Light Chain Variable Domain having the amino acid sequence of **SEQ ID NO:26**,
- Claim 9. The LAG-3-binding molecule of any one of claims 1-8, wherein said molecule is an antibody.
- Claim 10. The LAG-3-binding molecule of claim 9, wherein said molecule is a chimeric antibody or a humanized antibody.
- Claim 11. The LAG-3-binding molecule of any one of claims 1-8, wherein said molecule is a bispecific binding molecule, capable of simultaneously binding to human LAG-3 and to a second epitope.
- Claim 12. The LAG-3-binding molecule of claim 11, 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 13. The LAG-3-binding molecule of claim 11, 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 14. The LAG-3-binding molecule of claim 13, wherein said second epitope is an epitope of PD-1.
- Claim 15. The LAG-3-binding molecule of any one of claims 11-14, 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 16. The LAG-3-binding molecule of any one of claims 1-15, wherein said molecule comprises an Fc Region.
- Claim 17. The LAG-3-binding molecule of any one of claims 1-16, wherein said molecule comprises an Albumin-Binding Domain (ABD).
- Claim 18. The LAG-3-binding molecule of claim 17, wherein said ABD is a deimmunized ABD.
- Claim 19. The LAG-3-binding molecule of claim 16, 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 20. The LAG-3-binding molecule of claim 19, 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 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 21. A pharmaceutical composition comprising an effective amount of the LAG-3-binding molecule of any one of claims 1-20, and a pharmaceutically acceptable carrier.
- Claim 22. The LAG-3-binding molecule of any one of claims 1-20, or the pharmaceutical composition of claim 21, for use in stimulating a T-cell-mediated immune response of a subject in need thereof.

- Claim 23. The LAG-3-binding molecule of any one of claims 1-20, or the pharmaceutical composition of claim 21, for use in treating a disease or condition associated with a suppressed immune system.
- Claim 24. The LAG-3-binding molecule of claim 23, wherein the disease or condition is cancer or an infection.
- Claim 25. The LAG-3-binding molecule of claim 24, wherein disease or condition is a cancer selected from the group consisting 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 desmoplastic small round cell tumor, an ependymoma, a Ewing's tumor, an extraskeletal myxoid chondrosarcoma, 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 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 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 26. The LAG-3-binding molecule of claim 24, wherein said disease or condition is selected from the group consisting of: 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 lymphoma (NHL), including mantle cell leukemia (MCL), and small lymphocytic lymphoma (SLL), Hodgkin's lymphoma, systemic mastocytosis, and Burkitt's lymphoma.

Claim 27. The LAG-3-binding molecule of any one of claims 1-20, or the pharmaceutical composition of claim 21, wherein said molecule is detectably labeled for use in the detection of LAG-3.