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(54) Title: ANTI-LAG-3 ANTIBODIES AND USES THEREOF

(57) Abstract: Provided are antibodies that bind Lymphocyte Activation Gene-3 (LAG-3). Also provided are methods of stimulating an immune response, inhibiting growth of tumor cells, and treating an autoimmune, inflammatory, or viral disease.

ANTI-LAG-3 ANTIBODIES AND USES THEREOF

FIELD OF THE DISCLOSURE

[0001] The present disclosure relates generally to the field of molecular biology and protein biochemistry. More specifically, the disclosure relates to antibodies that bind to Lymphocyte Activation Gene-3 (LAG-3) and methods of use thereof.

BACKGROUND

[0002] Lymphocyte Activation Gene-3 (LAG-3) (also known as CD223) is a member of the immunoglobulin (Ig) superfamily, is closely related to CD4, and variously impacts T cell function. LAG-3 is expressed on activated T cells, exhausted T cells, tumor infiltrating T cells, and regulatory T cells (T_{regs}). Upon binding with major histocompatibility complex 2 (MHC class II), the LAG-3/MHC class II interaction results in the negative regulation of T cell proliferation, activation, and homeostasis.

[0003] LAG-3 represents an important immune checkpoint in cancer, similarly to cytotoxic T lymphocyte antigen-4 (CTLA-4), programmed cell death ligand-1 (PD-L1), and programmed cell death-1 (PD-1). LAG-3 not only expresses on the activated/exhausted effector T cells but also on regulatory T cells. LAG3 antagonism can not only promote the activation of effector T cells, but also block the suppressive function of regulatory T cells. Therefore, LAG-3 represents a promising target for cancer immunotherapy and preclinical evidence suggests that an anti-LAG-3 antibody can promote an anti-tumor response.

[0004] In view of the above, a need exists for developing novel agents that modulate the activity of LAG-3 in a manner that stimulates an immune response that inhibits the growth of various cancers and tumor cells, as well as being useful in the treatment of autoimmune, inflammatory, or viral diseases.

SUMMARY

[0005] The present disclosure provides antibodies and fragments thereof capable of binding to human Lymphocyte Activation Gene-3 (LAG-3) protein, as well as their uses in therapeutic, diagnostic and analytical settings. As demonstrated in the experimental examples, some of the anti-LAG-3 antibodies disclosure herein exhibited activities not shown with known anti-LAG-3

antibodies. For instance, the presently disclosed antibodies may inhibit the binding of the LAG-3 protein to Galectin-3 (LGALS3) and C-type lectin domain family 4 member G (LSECtin) protein, in addition to the binding to MHC class II molecules. Known anti-LAG-3 antibodies, by contrast, have only shown inhibitory effect to the binding to MHC class II molecules. In some embodiments, the antibodies and fragment thereof of the present disclosure are capable of reversing the inhibitory effect of regulatory T cells (T_{regs}) on effector T cells (T_{effs}).

[0006] In one embodiment, the present disclosure provides an isolated antibody or fragment thereof, wherein the antibody or fragment thereof has specificity to a human Lymphocyte Activation Gene-3 (LAG-3) protein, wherein the antibody or fragment thereof comprises a heavy chain variable region comprising heavy chain complementarity determining regions CDRH1, CDRH2, and CDRH3, and a light chain variable region comprising light chain complementarity determining regions CDRL1, CDRL2, and CDRL3, wherein the CDRH1 comprises the amino acid sequence of SEQ ID NO:240 or an amino acid sequence derived from SEQ ID NO:240 with one or two amino acid substitution; the CDRH2 comprises the amino acid sequence of SEQ ID NO:241 or an amino acid sequence derived from SEQ ID NO:241 with one or two amino acid substitution; the CDRH3 comprises the amino acid sequence of SEQ ID NO:242 or an amino acid sequence derived from SEQ ID NO:242 with one or two amino acid substitution; the CDRL1 comprises the amino acid sequence of SEQ ID NO:243 or an amino acid sequence derived from SEQ ID NO:243 with one or two amino acid substitution; the CDRL2 comprises the amino acid sequence of SEQ ID NO:244 or an amino acid sequence derived from SEQ ID NO:244 with one or two amino acid substitution; and the CDRL3 comprises the amino acid sequence of SEQ ID NO:245 or an amino acid sequence derived from SEQ ID NO:245 with one or two amino acid substitution.

[0007] In some embodiments, the amino acid substitution from SEQ ID NO:240 is at amino acid residue Y27, T28, T30, G35, or the combinations thereof, according to Kabat numbering. In some embodiments, the amino acid substitution is selected from: Y27: F; T28: M, or L; T30: E, D, or G; or G35: W, or S. In some embodiments, the CDRH1 comprises an amino acid sequence selected from the group consisting of SEQ ID NO:240 and 339-346.

[0008] In some embodiments, the amino acid substitution from SEQ ID NO:241 is at amino acid residue D50, Y52, Y56, N58, or the combinations thereof, according to Kabat numbering. In some embodiments, the amino acid substitution is selected from: D50: E; Y52: F; Y56: I, V, L, or H; or N58: V, or T. In some embodiments, the amino acid substitution comprises N58V. In some embodiments, the CDRH2 comprises an amino acid sequence selected from the group consisting of SEQ ID NO:241 and 347-353.

[0009] In some embodiments, the amino acid substitution from SEQ ID NO:242 is at amino acid residue N96, G99, Y102, or the combinations thereof, according to Kabat numbering. In some embodiments, the amino acid substitution is selected from: N96: D, or G; G99: K, R, or Q; or Y102: H. In some embodiments, the amino acid substitution comprises G99K or Y102H. In some embodiments, the CDRH3 comprises an amino acid sequence selected from the group consisting of SEQ ID NO:242 and 354-361.

[0010] In some embodiments, the amino acid substitution from SEQ ID NO:243 is at amino acid residue N28, according to Kabat numbering. In some embodiments, the amino acid substitution comprises N28Q. In some embodiments, the CDRL2 comprises an amino acid sequence of SEQ ID NO:376.

[0011] In some embodiments, the amino acid substitution from SEQ ID NO:244 is at amino acid residue Q50, V51, S52, L54, S56, or the combinations thereof, according to Kabat numbering. In some embodiments, the amino acid substitution is selected from: Q50: H; V51: K; S52: D; L54: R; or S56: R, V, L, or T. In some embodiments, the CDRL2 comprises an amino acid sequence selected from the group consisting of SEQ ID NO:244 and 362-369.

[0012] In some embodiments, the amino acid substitution from SEQ ID NO:245 is at amino acid residue A89, N91, L94, or the combinations thereof, according to Kabat numbering. In some embodiments, the amino acid substitution is selected from: A89: G; N91: Y; or L94: M, or E. In some embodiments, the amino acid substitution comprises N91Y. In some embodiments, the CDRL3 comprises an amino acid sequence selected from the group consisting of SEQ ID NO:245 and 370-375.

[0013] In some embodiments, the antibody or fragment comprises a heavy chain variable region comprising an amino acid sequence selected from the group consisting of SEQ ID NO:238, 246-259, 261, 263, 265, 267, 269, 271, 273, 275, 277, 279, 281, 283, 285, 287, 289, 291, 293, 295, 297, 299, 301, 303, 305, 307, 309, 311, 313, 315, 317, 319, 321, 323, 325, 327, 329, 331, 333, 335, and 337, or a peptide having at least 90% sequence identity to an amino acid sequence selected from the group consisting of SEQ ID NO:238, 246-259, 261, 263, 265, 267, 269, 271, 273, 275, 277, 279, 281, 283, 285, 287, 289, 291, 293, 295, 297, 299, 301, 303, 305, 307, 309, 311, 313, 315, 317, 319, 321, 323, 325, 327, 329, 331, 333, 335, and 337.

[0014] In some embodiments, the antibody or fragment comprises a light chain variable region comprising an amino acid sequence selected from the group consisting of SEQ ID NO:239, 260, 262, 264, 266, 268, 270, 272, 274, 276, 278, 280, 282, 284, 286, 288, 290, 292, 294, 296, 298, 300, 302, 304, 306, 308, 310, 312, 314, 316, 318, 320, 322, 324, 326, 328, 330, 332, 334, 336, and 338, or a peptide having at least 90% sequence identity to an amino acid sequence selected from the group consisting of SEQ ID NO:239, 260, 262, 264, 266, 268, 270, 272, 274, 276, 278, 280, 282, 284, 286, 288, 290, 292, 294, 296, 298, 300, 302, 304, 306, 308, 310, 312, 314, 316, 318, 320, 322, 324, 326, 328, 330, 332, 334, 336, and 338.

[0015] Back mutations can be incorporated to the humanized antibodies or fragments. In some embodiments, the heavy chain variable region comprises one or more amino acid residues selected from the group consisting of: (a) Ala (A) at position 71, (b) Leu (L) at position 69, (c) Lys (K) at position 66, (d) Ala (A) at position 67, (e) Ile (I) at position 48, (f) Ile (I) at position 37, (g) Lys (K) at position 38, (h) Phe (F) at position 91, and (i) Glu (E) at position 1, according to Kabat numbering, and combinations thereof.

[0016] In another embodiment, provided is an isolated antibody or fragment thereof, wherein the antibody or fragment thereof has specificity to a human Lymphocyte Activation Gene-3 (LAG-3) protein, wherein the antibody or fragment thereof comprises a heavy chain variable region comprising heavy chain complementarity determining regions CDRH1, CDRH2, and CDRH3, and a light chain variable region comprising light chain complementarity determining regions CDRL1, CDRL2, and CDRL3, wherein: the CDRH1 comprises the amino acid sequence of SEQ ID NO:1 or 2 or an amino acid sequence derived from SEQ ID NO:1 or 2 with one or two amino

acid substitution; the CDRH2 comprises the amino acid sequence of SEQ ID NO:3 or 4 or an amino acid sequence derived from SEQ ID NO:3 or 4 with one or two amino acid substitution; the CDRH3 comprises an amino acid sequence selected from the group consisting of SEQ ID NO:5-45 or an amino acid sequence derived from any one of SEQ ID NO:5-45 with one or two amino acid substitution; the CDRL1 comprises an amino acid sequence selected from the group consisting of SEQ ID NO:46-80 or an amino acid sequence derived from any one of SEQ ID NO:46-80 with one or two amino acid substitution; the CDRL2 comprises an amino acid sequence selected from the group consisting of SEQ ID NO:81-103 or an amino acid sequence derived from any one of SEQ ID NO:81-103 with one or two amino acid substitution; and the CDRL3 comprises an amino acid sequence selected from the group consisting of SEQ ID NO:104-139 or an amino acid sequence derived from any one of SEQ ID NO:104-139 with one or two amino acid substitution.

[0017] In some embodiments, the antibody or fragment comprises a heavy chain variable region comprising an amino acid sequence selected from the group consisting of SEQ ID NO:140-188 or a peptide having at least 90% sequence identity to an amino acid sequence selected from the group consisting of SEQ ID NO:140-188. In some embodiments, the antibody or fragment comprises a light chain variable region comprising an amino acid sequence selected from the group consisting of SEQ ID NO:189-237 or a peptide having at least 90% sequence identity to an amino acid sequence selected from the group consisting of SEQ ID NO:189-237.

[0018] Bispecific antibodies are also provided which further comprises a second specificity to an immune checkpoint protein or a tumor antigen. In some embodiments, the bispecificity comprises a second specificity to a protein target selected from the group consisting of PD-L1, PD-1, CTLA-4, CD28, CD122, 4-1BB, TIM3, OX-40, OX40L, CD40, CD40L, LIGHT, ICOS, ICOSL, GITR, GITRL, TIGIT, CD27, VISTA, B7H3, B7H4, HEVM, BTLA, KIR, CD47, CD73, EGFR, Her2, CD33, CD133, CEA and VEGF.

[0019] Methods of treatments are provided as well, including the treatment of autoimmune or inflammatory disease, cancer, and infections.

BRIEF DESCRIPTION OF THE DRAWINGS

[0025] FIG. 1. The D1-D2 domains are important for LAG-3 function. Wildtype (WT) LAG3 extracellular domain (ECD) fusion protein (LAG-3-ECD-huFc) fragments can bind to Daudi cells while D1-D2 truncated LAG-3-ECD-huFc fragments fail to bind Daudi cells.

[0026] FIG. 2. The binding of human anti-LAG3 antibodies to LAG3 protein derived from various species. Anti-LAG-3 antibodies were evaluated for their binding properties to human, rat, and mouse LAG3 through enzyme-linked immunosorbent assay (ELISA).

[0027] FIG. 3. The binding of human anti-LAG3 antibodies to cell surface LAG-3 antigen on activated human primary $CD4^+$ T cells. Anti-LAG-3 antibodies were assessed for binding to cell surface LAG-3 antigen on activated human primary $CD4^+$ T cells at various concentrations (10 μg/ml, 3.333 μg/ml, 1.111 μg/ml, 0.370 μg/ml, 0.123 μg/ml, 0.041 μg/ml, 0.014 μg/ml and 0.005 μg/ml).

[0028] FIG. 4. Inhibition of soluble LAG-3 (sLAG) binding to MHC class II receptor by anti-LAG-3 antibody. Anti-LAG-3 antibodies were evaluated for their ability to block the binding of sLAG-3 to MHC class II receptor in an *in vitro* binding assay using biotin-labeled LAG-3-ECD-huFcLAG-3-Fc fusion proteins and Raji cells expressing MHC class II receptor.

[0029] FIG. 5. Stimulation of IL-2 production in peripheral blood mononuclear cells (PBMCs) by anti-LAG-3 antibodies. Anti-LAG-3 antibodies were administrated into Staphylococcal Enterotoxin B (SEB) stimulated PBMCs at various concentrations starting from 20μg/ml at 1:3 serial dilution for 6 doses. Three days later, IL-2 concentration in the culture supernatant was evaluated by enzyme-linked immunosorbent assay (ELISA).

[0030] FIG. 6. Reversing the suppressive function of regulatory T cells (T_{regs}) on effector T cells (T_{effs}) using anti- LAG-3 antibodies. To evaluate the ability of anti-LAG-3 antibodies to reverse the suppressive effect of T_{regs} on T_{effs}, the antibodies of Example 1 were used in an *in vitro* T_{regs} suppression assay.

[0031] FIG. 7. Synergistic effect of anti-LAG3 and PD-1 antibody combo treatment. The anti-LAG3 antibodies were tested in combination with PD-1 antibody on SEB-stimulated PBMCs assay.

[0032] FIG. 8. Anti-LAG-3 antibodies enhance human T cell response in the presence of PD-L1 antibody. The anti-LAG3 antibodies were evaluated in combination with PD-L1 antibody on human mixed lymphocyte reaction (MLR) assay.

[0033] FIG. 9. ELISA results showing EC50 of the antibody for binding to full extracellular domain of LAG3 (D1-D4 huFc) but not D1-D2 deleted LAG3 (△D1-D2 huFc), demonstrating that 122H, 147H and 170H are potent and selective binder for D1 and D2 domain of human LAG3.

[0034] FIG. 10. 122H, 147H and 170H antibodies dose dependently inhibited the binding of LAG3 to its receptor MHC class II molecules.

[0035] FIG. 11. 122H, 147H and 170H mouse monoclonal antibodies dose dependently promoted IL2 production by Jurkat T cells.

[0036] FIG. 12. Humanized monoclonal antibody 147H-13 dose dependently promoted the IL2 production by Jurkat T cells.

[0037] FIG. 13. Binding curves of anti-LAG3 antibodies on Jurkat-LAG3 cells and activated CD4 T cell.

[0038] FIG. 14. The effect of affinity maturated anti-LAG3 antibodies on stimulating IL2 release by Jurkat T cells.

[0039] FIG. 15. The effect of anti-LAG3 antibodies on blocking the binding of Galectin-3 or LSECtin to LAG3.

[0040] FIG. 16. Anti LAG3 antibodies in combination with anti PD-L1 antibody significantly produced more IL-2 than each alone.

[0041] FIG. 17. Combination of anti-PD-L1 antibody and 147H-13 demonstrated robust inhibition of MC38 tumor growth.

DETAILED DESCRIPTION

[0042] The present disclosure relates to isolated antibodies, particularly human and humanized antibodies, which bind to human LAG-3 and that have desirable functional properties. In some embodiments, the LAG-3 antibodies can bind to LAG-3 and inhibit its binding to other molecules. In some embodiments, the other molecules include, without limitation, Galectin-3 (LGALS3), C-type lectin domain family 4 member G (LSECtin) protein, and MHC class II molecules.

[0043] In certain embodiments, the antibodies of the disclosure include certain CDR regions as disclosed herein. This disclosure provides isolated antibodies, methods of making such antibodies, immunoconjugates and bispecific molecules comprising such antibodies and pharmaceutical compositions containing the antibodies, immunoconjugates or bispecific molecules of the disclosure. This disclosure also relates to methods of using the antibodies, such as to detect LAG-3 protein, as well as to methods of using the anti-LAG-3 antibodies of the disclosure to stimulate immune responses, alone or in combination with other therapeutic agents. Accordingly, this disclosure also provides methods of using the anti-LAG-3 antibodies of the disclosure to, for example, inhibit tumor growth or treat viral infection.

[0044] In order that the present disclosure may be more readily understood, certain terms are first defined. Additional definitions are set forth throughout the detailed description.

[0045] The term "LAG-3" or "LAG3" refers to Lymphocyte Activation Gene-3. The LAG3 protein, which belongs to immunoglobulin (Ig) superfamily, comprises a 503-amino acid type I transmembrane protein with four extracellular Ig-like domains, designated D1 to D4. As described herein, the term "LAG-3" includes variants, isoforms, homologs, orthologs, and paralogs. For example, antibodies specific for a human LAG-3 protein may, in certain cases, cross-react with a LAG-3 protein from a species other than human. In other embodiments, the antibodies specific for a human LAG-3 protein may be completely specific for the human LAG-3 protein and may not exhibit species or other types of cross-reactivity, or may cross-react with LAG-3 from certain other species but not all other species (e.g., cross-react with monkey LAG-3,

but not mouse LAG-3). The term "human LAG-3" refers to human sequence LAG-3, such as the complete amino acid sequence of human LAG-3 having GenBank Accession No. NP 002277. The term "mouse LAG-3" refers to mouse sequence LAG-3, such as the complete amino acid sequence of mouse LAG-3 having GenBank Accession No. NP 032505. LAG-3 is also known in the art as, for example, CD223. The human LAG-3 sequence may differ from human LAG-3 of GenBank Accession No. NP 002277 by having, e.g., conserved mutations or mutations in non-conserved regions and the LAG-3 has substantially the same biological function as the human LAG-3 of GenBank Accession No. NP 002277. For example, a biological function of human LAG-3 is having an epitope in the extracellular domain of LAG-3 that is specifically bound by an antibody of the instant disclosure or a biological function of human LAG-3 is binding to MHC Class II molecules.

[0046] A particular human LAG-3 sequence will generally be at least 90% identical in amino acids sequence to human LAG-3 of GenBank Accession No. NP 002277 and contains amino acid residues that identify the amino acid sequence as being human when compared to LAG-3 amino acid sequences of other species (e.g., murine). In certain cases, a human LAG-3 can be at least 95%, or even at least 96%, 97%, 98%, or 99% identical in amino acid sequence to LAG-3 of GenBank Accession No. NP 002277. In certain embodiments, a human LAG-3 sequence will display no more than 10 amino acid differences from the LAG-3 sequence of GenBank Accession No. NP 002277. In certain embodiments, the human LAG-3 can display no more than 5, or even no more than 4, 3, 2, or 1 amino acid difference from the LAG-3 sequence of GenBank Accession No. NP 002277. Percent identity can be determined as described herein.

[0047] The term "immune response" refers to the action of, for example, lymphocytes, antigen presenting cells, phagocytic cells, granulocytes, and soluble macromolecules produced by the above cells or the liver (including antibodies, cytokines, and complement) that results in selective damage to, destruction of, or elimination from the human body of invading pathogens, cells or tissues infected with pathogens, cancerous cells, or, in cases of autoimmunity or pathological inflammation, normal human cells or tissues.

[0048] An "antigen-specific T cell response" refers to responses by a T cell that result from stimulation of the T cell with the antigen for which the T cell is specific. Non-limiting examples

of responses by a T cell upon antigen-specific stimulation include proliferation and cytokine production (e.g., IL-2 production).

[0049] The term "antibody" as referred to herein includes whole antibodies and any antigen binding fragment (i.e., "antigen-binding portion") or single chains thereof. Whole antibodies are glycoproteins comprising at least two heavy (H) chains and two light (L) chains inter-connected by disulfide bonds. Each heavy chain is comprised of a heavy chain variable region (abbreviated herein as V_H) and a heavy chain constant region. The heavy chain constant region is comprised of three domains, C_H1, C_H2, and C_H3. Each light chain is comprised of a light chain variable region (abbreviated herein as V_L) and a light chain constant region. The light chain constant region is comprised of one domain, C_L. The V_H and V_L regions can be further subdivided into regions of hypervariability, termed complementarity determining regions (CDRs), interspersed with regions that are more conserved, termed framework regions (FR). Each V_H and V_L is composed of three CDRs and four FRs, arranged from amino-terminus to carboxy terminus in the following order: FRl, CDRl, FR2, CDR2, FR3, CDR3, and FR4. The variable regions of the heavy and light chains contain a binding domain that interacts with an antigen. The constant regions of the antibodies can mediate the binding of the immunoglobulin to host tissues or factors, including various cells of the immune system (e.g., effector cells) and the first component (Clq) of the classical complement system.

[0050] The term "antigen-binding portion" of an antibody (or simply "antibody portion" or "fragment"), as used herein, refers to one or more fragments of an antibody that retain the ability to specifically bind to an antigen (e.g., a LAG-3 protein). It has been shown that the antigen-binding function of an antibody can be performed by fragments of a full-length antibody. Examples of binding fragments encompassed within the term "antigen binding portion" of an antibody include (i) a Fab fragment, a monovalent fragment consisting of the V_L, V_H, C_L and C_H1 domains; (ii) a $F(ab')_2$ fragment, a bivalent fragment comprising two Fab fragments linked by a disulfide bridge at the hinge region; (iii) a Fab' fragment, which is essentially a Fab with part of the hinge region (see, FUNDAMENTAL IMMUNOLOGY (Paul ed., 3.sup.rd ed. I993); (iv) a Fd fragment consisting of the V_H and C_H1 domains; (v) a F_v fragment consisting of the V_L and V_H domains of a single arm of an antibody, (vi) a dAb fragment (Ward *et al.*, (1989) *Nature* 341:544-546), which consists of a VH domain; (vii) an isolated complementarity determining

region (CDR); and (viii) a nanobody, a heavy chain variable region containing a single variable domain and two constant domains. Furthermore, although the two domains of the F_v fragment, V_L and V_H, are coded for by separate genes, they can be joined, using recombinant methods, by a synthetic linker that enables them to be made as a single protein chain in which the V_L and V_H regions pair to form monovalent molecules (known as single chain F_v (scFv); see e.g., Bird et *al.* (1988) *Science* 242:423-426; and Huston et al. (I988) *Proc. Natl. Acad. Sci. USA* 85:5879-5883). Such single chain antibodies are also intended to be encompassed within the term "antigen binding portion" of an antibody. These antibody fragments are obtained using conventional techniques known to those with skill in the art, and the fragments are screened for utility in the same manner as are intact antibodies.

[0051] An "isolated antibody", as used herein, is intended to refer to an antibody that is substantially free of other antibodies having different antigenic specificities (e.g., an isolated antibody that specifically binds a LAG-3 protein is substantially free of antibodies that specifically bind antigens other than LAG-3 proteins). An isolated antibody that specifically binds a human LAG-3 protein may, however, have cross reactivity to other antigens, such as LAG-3 proteins from other species. Moreover, an isolated antibody can be substantially free of other cellular material and/or chemicals.

[0052] The terms "monoclonal antibody" or "monoclonal antibody composition" as used herein refer to a preparation of antibody molecules of single molecular composition. A monoclonal antibody composition displays a single binding specificity and affinity for a particular epitope.

[0053] Humanized antibodies are antibodies from non-human species whose protein sequences have been modified to increase their similarity to antibody variants produced naturally in humans. The process of "humanization" is usually applied to monoclonal antibodies developed for administration to humans.

[0054] The term "human antibody", as used herein, is intended to include antibodies having variable regions in which both the framework and CDR regions are derived from human germline immunoglobulin sequences. Furthermore, if the antibody contains a constant region, the constant region also is derived from human germline immunoglobulin sequences. The human antibodies of the disclosure can include amino acid residues not encoded by human

germline immunoglobulin sequences (e.g., mutations introduced by random or site-specific mutagenesis *in vitro* or by somatic mutation *in vivo).* However, the term "human antibody", as used herein, is not intended to include antibodies in which CDR sequences derived from the germline of another mammalian species, such as a mouse, have been grafted onto human framework sequences.

[0055] The term "isotype" refers to the antibody class (e.g., IgM or IgG1) that is encoded by the heavy chain constant region genes.

[0056] The phrases "an antibody recognizing an antigen" and "an antibody specific for an antigen" are used interchangeably herein with the term "an antibody which binds specifically to an antigen."

[0057] The term "human antibody derivatives" refers to any modified form of the human antibody, e.g., a conjugate of the antibody and another agent or antibody. The term "humanized antibody" is intended to refer to antibodies in which CDR sequences derived from the germline of another mammalian species, such as a mouse, have been grafted onto human framework sequences. Additional framework region modifications can be made within the human framework sequences.

[0058] The term "chimeric antibody" is intended to refer to antibodies in which the variable region sequences are derived from one species and the constant region sequences are derived from another species, such as an antibody in which the variable region sequences are derived from a mouse antibody and the constant region sequences are derived from a human antibody.

[0059] As used herein, an antibody that "specifically binds human LAG-3" or "has specificity to human LAG-3" is intended to refer to an antibody that binds to human LAG-3 protein (and possibly a LAG-3 protein from one or more non-human species) but does not substantially bind to non-LAG-3 proteins. Preferably, the antibody binds to a human LAG-3 protein with "high affinity", namely with a K_D of 1×10^{-7} M or less, more preferably 5×10^{-8} M or less, more preferably 3×10^{-8} M or less, more preferably 1×10^{-8} M or less, more preferably 25×10^{-9} M or less or even more preferably 1×10^{-9} M or less.

[0060] The term "does not substantially bind" to a protein or cells, as used herein, means does not bind or does not bind with a high affinity to the protein or cells, i.e. binds to the protein or cells with a K_D of 1×10^{-6} M or more, more preferably 1×10^{-5} M or more, more preferably 1×10^{-4} M or more, more preferably 1×10^{-3} M or more, even more preferably 1×10^{-2} M or more. The term "K_{assoc}" or "K_a", as used herein, is intended to refer to the association rate of a particular antibody-antigen interaction, whereas the term "K_{dis}" or "K_d," as used herein, is intended to refer to the dissociation rate of a particular antibody-antigen interaction. The term "K_D ," as used herein, is intended to refer to the dissociation constant, which is obtained from the ratio of K_d to K_a (i.e., K_d/K_a) and is expressed as a molar concentration (M). K_D values for antibodies can be determined using methods well established in the art. A preferred method for determining the K_D of an antibody is by using surface plasmon resonance, preferably using a biosensor system such as a Biacore® system.

[0061] The term "high affinity" for an IgG antibody refers to an antibody having a K_D of 1×10^{-7} M or less, more preferably 5×10^{-8} M or less, even more preferably 1×10^{-8} M or less, even more preferably 5×10^{-9} M or less, and even more preferably 1×10^{-9} M or less for a target antigen. However, "high affinity" binding can vary for other antibody isotypes. For example, "high affinity" binding for an IgM isotype refers to an antibody having a K_D of 10^{-6} M or less, more preferably 10^{-7} M or less, even more preferably 10^{-8} M or less.

[0062] The term "subject" includes any human or nonhuman animal. The term "nonhuman animal" includes all vertebrates, e.g., mammals and non-mammals, such as non-human primates, sheep, dogs, cats, cows, horses, chickens, amphibians, and reptiles, although mammals are preferred, such as non-human primates, sheep, dogs, cats, cows and horses.

[0063] Various aspects of the disclosure are described in further detail in the following subsections.

Anti-LAG-3 Antibodies and Fragments

[0064] The present disclosure provides antibodies and fragments having specificity to human Lymphocyte Activation Gene-3 (LAG-3) protein. Demonstrated are human antibody as well as mouse and humanized antibodies that have high affinity to LAG-3 as well as other desired

activities associated with the binding. The antibodies of the disclosure are characterized by particular functional features or properties of the antibodies.

[0065] An example group of anti-LAG-3 antibodies and fragments was derived from mouse antibody 147H (see **Table 5**). An example humanized chimeric antibody, along with a number of humanized antibody fragments with back mutations are shown in **Table 6**. Further, based on affinity maturation, additional antibodies and fragments were prepared that had improved properties (**Table 7 and 8**). In some embodiments, provided is an isolated antibody or fragment thereof, wherein the antibody or fragment thereof has specificity to a human Lymphocyte Activation Gene-3 (LAG-3) protein and comprises a VH CDR1 of SEQ ID NO:240, a VH CDR2 of SEQ ID NO:241, a VH CDR3 of SEQ ID NO:242, a VL CDR1 of SEQ ID NO:243, a VL CDR2 of SEQ ID NO:244, and a VL CDR3 of SEQ ID NO:245.

[0066] In one embodiment, the antibody or fragment thereof comprises a heavy chain variable region comprising heavy chain complementarity determining regions CDRH1, CDRH2, and CDRH3, and a light chain variable region comprising light chain complementarity determining regions CDRL1, CDRL2, and CDRL3, wherein the CDRH1 comprises the amino acid sequence of SEQ ID NO:240 or an amino acid sequence derived from SEQ ID NO:240 with one or two amino acid substitution; the CDRH2 comprises the amino acid sequence of SEQ ID NO:241 or an amino acid sequence derived from SEQ ID NO:241 with one or two amino acid substitution; the CDRH3 comprises the amino acid sequence of SEQ ID NO:242 or an amino acid sequence derived from SEQ ID NO:242 with one or two amino acid substitution; the CDRL1 comprises the amino acid sequence of SEQ ID NO:243 or an amino acid sequence derived from SEQ ID NO:243 with one or two amino acid substitution; the CDRL2 comprises the amino acid sequence of SEQ ID NO:244 or an amino acid sequence derived from SEQ ID NO:244 with one or two amino acid substitution; and the CDRL3 comprises the amino acid sequence of SEQ ID NO:245 or an amino acid sequence derived from SEQ ID NO:245 with one or two amino acid substitution.

[0067] Non-limiting examples of amino acid residues on which substitutions can be made are shown in **Table 8**. For instance, in CDRH2, such residues include D50, Y52, Y56 and N58. In a preferred embodiment, the CDRH2 includes the N58V substitution, optionally with other

substitutions (e.g., SEQ ID NO: 347). In another example, a CDRH3 substitution occurs at N96, G99 or Y102. In a preferred embodiment, the CDRH3 includes substitution G99K, Y102, or the combination (e.g., SEQ ID NO: 354). In yet another example, a CDRL3 substitution occurs at A89, N91, or L94. In a preferred embodiment, the CHRL3 includes substitution N91Y (e.g., SEQ ID NO: 374). In one embodiment, the antibody or fragment includes all of N58V, G99K, Y102, and N91Y, optionally with other substitutions.

[0068] In some embodiments, the antibody or fragment comprises a heavy chain variable region comprising an amino acid sequence selected from the group consisting of SEQ ID NO:238, 246-259, 261, 263, 265, 267, 269, 271, 273, 275, 277, 279, 281, 283, 285, 287, 289, 291, 293, 295, 297, 299, 301, 303, 305, 307, 309, 311, 313, 315, 317, 319, 321, 323, 325, 327, 329, 331, 333, 335, and 337, or a peptide having at least 90% sequence identity to an amino acid sequence selected from the group consisting of SEQ ID NO:238, 246-259, 261, 263, 265, 267, 269, 271, 273, 275, 277, 279, 281, 283, 285, 287, 289, 291, 293, 295, 297, 299, 301, 303, 305, 307, 309, 311, 313, 315, 317, 319, 321, 323, 325, 327, 329, 331, 333, 335, and 337.

[0069] In some embodiments, the antibody or fragment comprises a light chain variable region comprising an amino acid sequence selected from the group consisting of SEQ ID NO:239, 260, 262, 264, 266, 268, 270, 272, 274, 276, 278, 280, 282, 284, 286, 288, 290, 292, 294, 296, 298, 300, 302, 304, 306, 308, 310, 312, 314, 316, 318, 320, 322, 324, 326, 328, 330, 332, 334, 336, and 338, or a peptide having at least 90% sequence identity to an amino acid sequence selected from the group consisting of SEQ ID NO:239, 260, 262, 264, 266, 268, 270, 272, 274, 276, 278, 280, 282, 284, 286, 288, 290, 292, 294, 296, 298, 300, 302, 304, 306, 308, 310, 312, 314, 316, 318, 320, 322, 324, 326, 328, 330, 332, 334, 336, and 338.

[0070] In some embodiments, the antibody or fragment thereof further comprises a heavy chain constant region, a light chain constant region, an Fc region, or the combination thereof. In some embodiments, the light chain constant region is a kappa or lambda chain constant region.

[0071] Without limitation, the antibody or fragment thereof is a chimeric antibody, a humanized antibody, or a fully human antibody. In one aspect, antibody or fragment thereof is a humanized antibody.

[0072] For a humanized antibody or fragment, certain back mutations can be incorporated. In some embodiments, the heavy chain variable region comprises one or more amino acid residues selected from the group consisting of:

(a) Ala (A) at position 71,

(b) Leu (L) at position 69,

(c) Lys (K) at position 66,

(d) Ala (A) at position 67,

(e) Ile (I) at position 48,

(f) Ile (I) at position 37,

(g) Lys (K) at position 38,

(h) Phe (F) at position 91, and

(i) Glu (E) at position 1, according to Kabat numbering, and combinations thereof.

[0073] In some embodiments, the heavy chain variable region comprises Ala (A) at position 71. In some embodiments, the heavy chain variable region comprises Leu (L) at position 69. In some embodiments, the heavy chain variable region comprises Lys (K) at position 66. In some embodiments, the heavy chain variable region comprises Ala (A) at position 67. In some embodiments, the heavy chain variable region comprises Ile (I) at position 48. In some embodiments, the heavy chain variable region comprises Ile (I) at position 37. In some embodiments, the heavy chain variable region comprises Lys (K) at position 38. In some embodiments, the heavy chain variable region comprises Phe (F) at position 91. In some embodiments, the heavy chain variable region comprises Glu (E) at position 1.

[0074] In some embodiments, the heavy chain variable region comprises one or more amino acid residues selected from the group consisting of

(a) Ala (A) at position 71,

(b) Leu (L) at position 69,

(c) Lys (K) at position 66,

(d) Ala (A) at position 67,

(e) Ile (I) at position 48,

(f) Ile (I) at position 37, and

(g) Lys (K) at position 38, according to Kabat numbering, and combinations thereof. In some embodiments, the heavy chain variable region comprises all of the above recited residues.

[0075] In some embodiments, the antibody or fragment thereof comprises a heavy chain variable region comprising an amino acid sequence of SEQ ID NO:238, or a peptide having at least 90% sequence identity to SEQ ID NO:238. In some embodiments, the antibody or fragment thereof comprises a light chain variable region comprising an amino acid sequence of SEQ ID NO:239, or a peptide having at least 90% sequence identity to SEQ ID NO:239.

[0076] In some embodiments, the antibody or fragment thereof comprises a heavy chain variable region comprising an amino acid sequence selected from the group consisting of SEQ ID NO:246-259, or a peptide having at least 90% sequence identity to an amino acid sequence selected from the group consisting of SEQ ID NO:246-259. In some embodiments, the antibody or fragment thereof comprises a light chain variable region comprising an amino acid sequence of SEQ ID NO:239, or a peptide having at least 90% sequence identity to SEQ ID NO:260.

[0077] In some embodiments, the antibody or fragment thereof comprises a heavy chain variable region comprising an amino acid sequence of SEQ ID NO:246, or a peptide having at least 90% sequence identity to SEQ ID NO:246. In some embodiments, the antibody or fragment thereof comprises a light chain variable region comprising an amino acid sequence of SEQ ID NO:260, or a peptide having at least 90% sequence identity to SEQ ID NO:260.

[0078] In some embodiments, the antibody or fragment thereof comprises a heavy chain variable region comprising an amino acid sequence of SEQ ID NO:247, or a peptide having at least 90% sequence identity to SEQ ID NO:247. In some embodiments, the antibody or fragment thereof comprises a light chain variable region comprising an amino acid sequence of SEQ ID NO:260, or a peptide having at least 90% sequence identity to SEQ ID NO:260.

[0079] In some embodiments, the antibody or fragment thereof comprises a heavy chain variable region comprising an amino acid sequence of SEQ ID NO:248, or a peptide having at least 90% sequence identity to SEQ ID NO:248. In some embodiments, the antibody or fragment thereof comprises a light chain variable region comprising an amino acid sequence of SEQ ID NO:260, or a peptide having at least 90% sequence identity to SEQ ID NO:260.

[0080] In some embodiments, the antibody or fragment thereof comprises a heavy chain variable region comprising an amino acid sequence of SEQ ID NO:249, or a peptide having at least 90% sequence identity to SEQ ID NO:249. In some embodiments, the antibody or fragment thereof comprises a light chain variable region comprising an amino acid sequence of SEQ ID NO:260, or a peptide having at least 90% sequence identity to SEQ ID NO:260.

[0081] In some embodiments, the antibody or fragment thereof comprises a heavy chain variable region comprising an amino acid sequence of SEQ ID NO:250, or a peptide having at least 90% sequence identity to SEQ ID NO:250. In some embodiments, the antibody or fragment thereof comprises a light chain variable region comprising an amino acid sequence of SEQ ID NO:260, or a peptide having at least 90% sequence identity to SEQ ID NO:260.

[0082] In some embodiments, the antibody or fragment thereof comprises a heavy chain variable region comprising an amino acid sequence of SEQ ID NO:251, or a peptide having at least 90% sequence identity to SEQ ID NO:251. In some embodiments, the antibody or fragment thereof comprises a light chain variable region comprising an amino acid sequence of SEQ ID NO:260, or a peptide having at least 90% sequence identity to SEQ ID NO:260.

[0083] In some embodiments, the antibody or fragment thereof comprises a heavy chain variable region comprising an amino acid sequence of SEQ ID NO:252, or a peptide having at least 90% sequence identity to SEQ ID NO:252. In some embodiments, the antibody or fragment thereof comprises a light chain variable region comprising an amino acid sequence of SEQ ID NO:260, or a peptide having at least 90% sequence identity to SEQ ID NO:260.

[0084] In some embodiments, the antibody or fragment thereof comprises a heavy chain variable region comprising an amino acid sequence of SEQ ID NO:253, or a peptide having at least 90% sequence identity to SEQ ID NO:253. In some embodiments, the antibody or fragment thereof comprises a light chain variable region comprising an amino acid sequence of SEQ ID NO:260, or a peptide having at least 90% sequence identity to SEQ ID NO:260.

[0085] In some embodiments, the antibody or fragment thereof comprises a heavy chain variable region comprising an amino acid sequence of SEQ ID NO:254, or a peptide having at least 90% sequence identity to SEQ ID NO:254. In some embodiments, the antibody or fragment thereof

comprises a light chain variable region comprising an amino acid sequence of SEQ ID NO:260, or a peptide having at least 90% sequence identity to SEQ ID NO:260.

[0086] In some embodiments, the antibody or fragment thereof comprises a heavy chain variable region comprising an amino acid sequence of SEQ ID NO:255, or a peptide having at least 90% sequence identity to SEQ ID NO:255. In some embodiments, the antibody or fragment thereof comprises a light chain variable region comprising an amino acid sequence of SEQ ID NO:260, or a peptide having at least 90% sequence identity to SEQ ID NO:260.

[0087] In some embodiments, the antibody or fragment thereof comprises a heavy chain variable region comprising an amino acid sequence of SEQ ID NO:256, or a peptide having at least 90% sequence identity to SEQ ID NO:256. In some embodiments, the antibody or fragment thereof comprises a light chain variable region comprising an amino acid sequence of SEQ ID NO:260, or a peptide having at least 90% sequence identity to SEQ ID NO:260.

[0088] In some embodiments, the antibody or fragment thereof comprises a heavy chain variable region comprising an amino acid sequence of SEQ ID NO:257, or a peptide having at least 90% sequence identity to SEQ ID NO:257. In some embodiments, the antibody or fragment thereof comprises a light chain variable region comprising an amino acid sequence of SEQ ID NO:260, or a peptide having at least 90% sequence identity to SEQ ID NO:260.

[0089] In some embodiments, the antibody or fragment thereof comprises a heavy chain variable region comprising an amino acid sequence of SEQ ID NO:258, or a peptide having at least 90% sequence identity to SEQ ID NO:258. In some embodiments, the antibody or fragment thereof comprises a light chain variable region comprising an amino acid sequence of SEQ ID NO:260, or a peptide having at least 90% sequence identity to SEQ ID NO:260.

[0090] In some embodiments, the antibody or fragment thereof comprises a heavy chain variable region comprising an amino acid sequence of SEQ ID NO:259, or a peptide having at least 90% sequence identity to SEQ ID NO:259. In some embodiments, the antibody or fragment thereof comprises a light chain variable region comprising an amino acid sequence of SEQ ID NO:260, or a peptide having at least 90% sequence identity to SEQ ID NO:260.

[0091] In any of these example heavy chain variable or light chain variable regions, the CDRs can be modified as illustrated in **Table 8** or replaced by the example modified CDRs as shown in **Table 8**.

[0092] In various embodiments, an antibody of the disclosure comprises heavy and light chain variable regions comprising amino acid sequences that are homologous to the amino acid sequences of the preferred antibodies described herein, and wherein the antibodies retain the desired functional properties of the anti-LAG-3 antibodies of the disclosure. For example, the antibody specifically binds to human LAG-3; blocks LAG-3 binding to major histocompatibility complex (MHC) class II molecules, Galectin-3 and LSECtin; stimulates an immune response; and reverses the inhibitory effect of regulatory T cells on effector cells.

[0093] Additionally, or alternatively, the antibody can possess one or more of the following functional properties discussed above, such as high affinity binding to human LAG-3, binding to monkey LAG-3, lack of binding to mouse LAG-3, the ability to inhibit binding of LAG-3 to MHC Class II molecules and/or the ability to stimulate antigen-specific T cell responses.

[0094] In various embodiments, the antibody can be, for example, a human antibody, a humanized antibody or a chimeric antibody. In other embodiments, the V_H and/or V_L amino acid sequences can be 85%, 90%, 95%, 96%, 97%, 98%, or 99% homologous to the sequences set forth above. An antibody having V_H and V_L regions having high (i.e., 80% or greater) homology to the V_H and V_L regions of the sequences set forth above, can be obtained by mutagenesis (e.g., site-directed or PCR-mediated mutagenesis) of nucleic acids of V_H and/or V_L amino acid sequences, followed by testing of the encoded altered antibody for retained function (i.e., the functions set forth above) using the functional assays described herein.

[0095] As used herein, the percent homology between two amino acid sequences is equivalent to the percent identity between the two sequences. The percent identity between the two sequences is a function of the number of identical positions shared by the sequences (i.e., %homology = # of identical positions/total # of positions x 100), taking into account the number of gaps, and the length of each gap, which need to be introduced for optimal alignment of the two sequences. The comparison of sequences and determination of percent identity between two sequences can be accomplished using a mathematical algorithm, as described in the non-limiting examples below.

[0096] The percent identity between two amino acid sequences can be determined using the algorithm of E. Meyers and W. Miller (Comput. Appl. Biosci. 4:11-7, 1988) which has been incorporated into the ALIGN program (version 2.0), using a PAM120 weight residue table, a gap length penalty of 12 and a gap penalty of 4. In addition, the percent identity between two amino acid sequences can be determined using the Needleman and Wunsch (J. Mol. Biol. 48:444-53, 1970) algorithm which has been incorporated into the GAP program in the GCG software package, using either a Blossum 62 matrix or a PAM250 matrix, and a gap weight of 16, 14, 12, 10, 8, 6, or 4 and a length weight of 1, 2, 3, 4, 5, or 6.

[0097] Additionally, or alternatively, the protein sequences of the present disclosure can further be used as a "query sequence" to perform a search against public databases to, e.g., to identify related sequences. Such searches can be performed using the XBLAST program (version 2.0) of Altschul et al. (J. Mol. Biol. 215:403-10, 1990). BLAST protein searches can be performed with the XBLAST program, score = 50, word length =3 to obtain amino acid sequences homologous to the antibody molecules of the disclosure. To obtain gapped alignments for comparison purposes, Gapped BLAST can be utilized as described in Altschul et al. (Nucl. Acid Res. 25(17):3389-402, 1997). When utilizing BLAST and Gapped BLAST programs, the default parameters of the respective programs (e.g., XBLAST and NBLAST) are useful.

[0098] In some embodiments, the sequence identity is at least 95%, 96%, 97%, 98%, 99% or 99.5%. In some embodiments, the sequence identity encompasses amino acid substitution, deletion or addition of one, two, three, four, five, six, seven, eight, nine of ten residues. Such substitutions, in some embodiments, are conservative substitutions.

[0099] A "conservative amino acid substitution" is one in which the amino acid residue is replaced with an amino acid residue having a similar side chain. Families of amino acid residues having similar side chains have been defined in the art, including basic side chains (*e.g.*, lysine, arginine, histidine), acidic side chains (*e.g.*, aspartic acid, glutamic acid), uncharged polar side chains (*e.g.*, glycine, asparagine, glutamine, serine, threonine, tyrosine, cysteine), nonpolar side chains (*e.g.*, alanine, valine, leucine, isoleucine, proline, phenylalanine, methionine, tryptophan), beta-branched side chains (*e.g.*, threonine, valine, isoleucine) and aromatic side chains (*e.g.*, tyrosine, phenylalanine, tryptophan, histidine). Thus, a nonessential amino acid residue in an

immunoglobulin polypeptide is preferably replaced with another amino acid residue from the same side chain family. In another embodiment, a string of amino acids can be replaced with a structurally similar string that differs in order and/or composition of side chain family members.

[0100] Non-limiting examples of conservative amino acid substitutions are provided in the tables below, where a similarity score of 0 or higher indicates conservative substitution between the two amino acids.

Amino Acid Similarity Matrix

	C	G	P	S	A	T	D	E	N	Q	H	K	R	V	M	I	L	F	Y	W
W	-8	-7	-6	-2	-6	-5	-7	-7	-4	-5	-3	-3	2	-6	-4	-5	-2	0	0	17
Y	0	-5	-5	-3	-3	-3	-4	-4	-2	-4	0	-4	-5	-2	-2	-1	-1	7	10	
F	-4	-5	-5	-3	-4	-3	-6	-5	-4	-5	-2	-5	-4	-1	0	1	2	9		
L	-6	-4	-3	-3	-2	-2	-4	-3	-3	-2	-2	-3	-3	2	4	2	6			
I	-2	-3	-2	-1	-1	0	-2	-2	-2	-2	-2	-2	-2	4	2	5				
M	-5	-3	-2	-2	-1	-1	-3	-2	0	-1	-2	0	0	2	6					
V	-2	-1	-1	-1	0	0	-2	-2	-2	-2	-2	-2	-2	4						
R	-4	-3	0	0	-2	-1	-1	-1	0	1	2	3	6							
K	-5	-2	-1	0	-1	0	0	0	1	1	0	5								
H	-3	-2	0	-1	-1	-1	1	1	2	3	6									
Q	-5	-1	0	-1	0	-1	2	2	1	4										
N	-4	0	-1	1	0	0	2	1	2											
E	-5	0	-1	0	0	0	3	4												
D	-5	1	-1	0	0	0	4													
T	-2	0	0	1	1	3														
A	-2	1	1	1	2															
S	0	1	1	1																
P	-3	-1	6																	
G	-3	5																		
C	12																			

Conservative Amino Acid Substitutions

For Amino Acid	**Substitution With**
Alanine	D-Ala, Gly, Aib, β-Ala, L-Cys, D-Cys
Arginine	D-Arg, Lys, D-Lys, Orn D-Orn
Asparagine	D-Asn, Asp, D-Asp, Glu, D-Glu Gln, D-Gln
Aspartic Acid	D-Asp, D-Asn, Asn, Glu, D-Glu, Gln, D-Gln
Cysteine	D-Cys, S-Me-Cys, Met, D-Met, Thr, D-Thr, L-Ser, D-Ser
Glutamine	D-Gln, Asn, D-Asn, Glu, D-Glu, Asp, D-Asp

Glutamic Acid	D-Glu, D-Asp, Asp, Asn, D-Asn, Gln, D-Gln
Glycine	Ala, D-Ala, Pro, D-Pro, Aib, β-Ala
Isoleucine	D-Ile, Val, D-Val, Leu, D-Leu, Met, D-Met
Leucine	Val, D-Val, Met, D-Met, D-Ile, D-Leu, Ile
Lysine	D-Lys, Arg, D-Arg, Orn, D-Orn
Methionine	D-Met, S-Me-Cys, Ile, D-Ile, Leu, D-Leu, Val, D-Val
Phenylalanine	D-Phe, Tyr, D-Tyr, His, D-His, Trp, D-Trp
Proline	D-Pro
Serine	D-Ser, Thr, D-Thr, allo-Thr, L-Cys, D-Cys
Threonine	D-Thr, Ser, D-Ser, allo-Thr, Met, D-Met, Val, D-Val
Tyrosine	D-Tyr, Phe, D-Phe, His, D-His, Trp, D-Trp
Valine	D-Val, Leu, D-Leu, Ile, D-Ile, Met, D-Met

[0101] Human antibodies were also prepared herein. The human antibodies or fragments may include heavy chain CDRs as shown in **Table 2**. Examples of heavy chain variable regions are shown in **Table 1**. The human antibodies or fragments may include light chain CDRs as shown in **Table 4**. Examples of heavy chain variable regions are shown in **Table 3**.

[0102] In one embodiment, accordingly, provided is an isolated antibody or fragment thereof, wherein the antibody or fragment thereof has specificity to a human Lymphocyte Activation Gene-3 (LAG-3) protein, wherein the antibody or fragment thereof comprises a heavy chain variable region comprising heavy chain complementarity determining regions CDRH1, CDRH2, and CDRH3, and a light chain variable region comprising light chain complementarity determining regions CDRL1, CDRL2, and CDRL3, wherein: the CDRH1 comprises the amino acid sequence of SEQ ID NO:1 or 2 or an amino acid sequence derived from SEQ ID NO:1 or 2 with one or two amino acid substitution; the CDRH2 comprises the amino acid sequence of SEQ ID NO:3 or 4 or an amino acid sequence derived from SEQ ID NO:3 or 4 with one or two amino acid substitution; the CDRH3 comprises an amino acid sequence selected from the group consisting of SEQ ID NO:5-45 or an amino acid sequence derived from any one of SEQ ID NO:5-45 with one or two amino acid substitution; the CDRL1 comprises an amino acid sequence selected from the group consisting of SEQ ID NO:46-80 or an amino acid sequence derived from any one of SEQ ID NO:46-80 with one or two amino acid substitution; the CDRL2 comprises an amino acid sequence selected from the group consisting of SEQ ID NO:81-103 or an amino acid

sequence derived from any one of SEQ ID NO:81-103 with one or two amino acid substitution; and the CDRL3 comprises an amino acid sequence selected from the group consisting of SEQ ID NO:104-139 or an amino acid sequence derived from any one of SEQ ID NO:104-139 with one or two amino acid substitution.

[0103] In some embodiments, the antibody or fragment includes the same three heavy chain CDRs as one of the combination as shown in **Table. 2**. For instance, the heavy chain may include CDRH1 of SEQ ID NO: 1, CDRH2 of SEQ ID NO:3 and CDRH3 of SEQ ID NO:5, as shown in the first row. In some embodiments, the antibody or fragment includes the same three light chain CDRs as one of the combination as shown in **Table. 4**. For instance, the light chain may include CDRL1 of SEQ ID NO: 46, CDRL2 of SEQ ID NO:81 and CDRL3 of SEQ ID NO:104, as shown in the first row.

[0104] In some embodiments, the antibody or fragment comprises a heavy chain variable region comprising an amino acid sequence selected from the group consisting of SEQ ID NO:140-188 or a peptide having at least 90% sequence identity to an amino acid sequence selected from the group consisting of SEQ ID NO:140-188. In some embodiments, the antibody or fragment comprises a light chain variable region comprising an amino acid sequence selected from the group consisting of SEQ ID NO:189-237 or a peptide having at least 90% sequence identity to an amino acid sequence selected from the group consisting of SEQ ID NO:189-237.

[0105] In some embodiments, the sequence identity is at least 95%, 96%, 97%, 98%, 99% or 99.5%. In some embodiments, the sequence identity encompasses amino acid substitution, deletion or addition of one, two, three, four, five, six, seven, eight, nine of ten residues. Such substitutions, in some embodiments, are conservative substitutions.

[0106] The antibodies of the disclosure are characterized by particular functional features or properties of the antibodies. For example, the antibodies specifically bind to human LAG-3 and may bind to LAG-3 from certain other species, e.g., monkey LAG-3, e.g., cynomolgus monkey, rhesus monkey, but may not substantially bind to LAG-3 from certain other species, e.g., mouse LAG-3. Preferably, an antibody of the disclosure binds to human LAG-3 with high affinity.

[0107] The ability of the antibody to stimulate an immune response, such as an antigen- specific T cell response, can be indicated by, for example, the ability of the antibody to stimulate interleukin-2 (IL-2) or interferon gamma (IFN-γ) production in an antigen-specific T cell response. In certain embodiments, an antibody of the disclosure binds to human LAG-3 and exhibits an ability to stimulate an antigen-specific T cell response. In other embodiments, an antibody of the disclosure binds to human LAG-3 but does not exhibit an ability to stimulate an antigen-specific T cell response. Other means by which to evaluate the ability of the antibody to stimulate an immune response include the ability of the antibody to inhibit tumor growth, such as in an *in vivo* tumor graft model or the ability of the antibody to stimulate an autoimmune response, such as the ability to promote the development of an autoimmune disease in an autoimmune model, such as the ability to promote the development of diabetes in the NOD mouse model.

[0108] The binding of an antibody of the disclosure to LAG-3 can be assessed using one or more techniques well established in the art. For example, in a preferred embodiment, an antibody can be tested by a flow cytometry assay in which the antibody is reacted with a cell line that expresses human LAG-3, such as CHO cells that have been transfected to express LAG-3, e.g., human LAG-3, or monkey LAG-3, e.g., rhesus or cynomolgus monkey or mouse LAG-3 on their cell surface. Other suitable cells for use in flow cytometry assays include anti-CD3-stimulated $CD4^+$ activated T cells, which express native LAG-3. Additionally, or alternatively, the binding of the antibody, including the binding kinetics (e.g., K_D value) can be tested in BIAcore binding assays. Still other suitable binding assays include ELISA assays, for example using a recombinant LAG-3 protein. Preferably, an antibody of the disclosure binds to a LAG-3 protein with a K_D of 5×10^{-8} M or less, binds to a LAG-3 protein with a K_D of 2×10^{-8} M or less, binds to a LAG-3 protein with a K_D of 5×10^{-9} M or less, binds to a LAG-3 protein with a K_D of 4×10^{-9} M or less, binds to a LAG-3 protein with a K_D of 3×10^{-9} M or less, binds to a LAG-3 protein with a K_D of 2×10^{-9} M or less, binds to a LAG-3 protein with a K_D of 125×10^{-9} M or less, binds to a LAG-3 protein with a K_D of 5×10^{-10} M or less, or binds to a LAG-3 protein with a K_D of 1×10^{-10} M or less.

[0109] Preferred antibodies of the disclosure are the human monoclonal antibodies S27, S31, T99, and S119 isolated and structurally characterized as described [Examples 2-8]. The V_H

amino acid sequences of S27, S31, T99 and S119 are shown in SEQ ID NO:149, SEQ NO:150, SEQ ID NO:158, and SEQ ID NO:162, respectively. The V_L amino acid sequences of S27, S31, T99, and S119 are shown in SEQ ID NO:198, SEQ NO:199, SEQ ID NO:207, and SEQ ID NO:211, respectively.

[0110] Given that each of these antibodies can bind to human LAG-3, the V_H and V_L sequences can be "mixed and matched" to create other anti-LAG-3 binding molecules of the disclosure. Preferably, when V_H and V_L chains are mixed and matched, a V_H sequence from a particular V_H/V_L pairing is replaced with a structurally similar V_H sequence. Likewise, preferably a V_L sequence from a particular V_H/V_L pairing is replaced with a structurally similar V_L sequence.

[0111] Accordingly, in one aspect, this disclosure provides an isolated monoclonal antibody, or antigen binding portion thereof comprising:

(a) a heavy chain variable region comprising an amino acid sequence selected from the group consisting of SEQ ID NO: 140-SEQ ID NO:188 and

(b) a light chain variable region comprising an amino acid sequence selected from the group consisting of SEQ ID NO:189-SEQ ID NO:237

wherein the antibody specifically binds human LAG-3.

Preferred variable heavy and variable light chain combinations include:

(a) a heavy chain variable region comprising the amino acid sequence of SEQ ID NO:149 and a light chain variable region comprising the amino acid sequence of SEQ ID NO:198;

(b) a heavy chain variable region comprising the amino acid sequence of SEQ ID NO:150 and a light chain variable region comprising the amino acid sequence of SEQ ID NO:199;

(c) a heavy chain variable region comprising the amino acid sequence of SEQ ID NO:158 and a light chain variable region comprising the amino acid sequence of SEQ ID NO:207;

(d) a heavy chain variable region comprising the amino acid sequence of SEQ ID NO:162 and a light chain variable region comprising the amino acid sequence of SEQ ID NO:211.

[0112] It is well known in the art that the CDR3 domain, independently from the CDR1and/or CDR2 domain(s), alone can determine the binding specificity of an antibody for a cognate antigen and that multiple antibodies can predictably be generated having the same binding specificity based on a common CDR3 sequence. *See,* e.g., Klimka et al., *Brit. J. of Can.* 83(2):252-60, 2000; Beiboer et al., *J. Mol. Biol.* 296:833-49, 2000; Rader et al., *PNAS* 95:8910-15, 1998; Barbas et al., *JACS* 116:2161-2, 29914; Barbas et al., *PNAS* 92:2529-33, 1995; Ditzel

et al., *J. Immunol.* 157:739-49, 1996; Berezov et al., *BIAJournal* 8(1): Scientific Review, 2001; Igarashi et al., *J. Biochem* 117:452-7, 1995; Bourgeois et al., *J. Virol.* 72:807-10, 1998; Levi et al., *PNAS* 90:4374-8, 1993; Polymenis and Stoller, *J. Immunol.* 152:5218-329, 1994; and Xu and Davis, *Immunity* 13:37-45, 2000. *See also,* U.S. Patent Nos. 6,951,646; 6,914,128; 6,090,382; 6,818,216; 6,156,313; 6,827,925; 5,833,943; 5,762,905 and 5,760,185. Each of these references is hereby incorporated by reference in its entirety.

[0113] Accordingly, the present disclosure provides monoclonal antibodies comprising one or more heavy and/or light chain CDR3 domains from an antibody derived from a human or non-human animal, wherein the monoclonal antibody is capable of specifically binding to human LAG-3. Within certain aspects, the present disclosure provides monoclonal antibodies comprising one or more heavy and/or light chain CDR3 domain from a non-human antibody, such as a mouse or rat antibody, wherein the monoclonal antibody is capable of specifically binding to LAG-3. Within some embodiments, such inventive antibodies comprising one or more heavy and/or light chain CDR3 domain from a non-human antibody (a) are capable of competing for binding with; (b) retain the functional characteristics; (c) bind to the same epitope; and/or (d) have a similar binding affinity as the corresponding parental non-human antibody. Within other aspects, the present disclosure provides monoclonal antibodies comprising one or more heavy and/or light chain CDR3 domain from a human antibody, such as, e.g., a human antibody obtained from a non-human animal, wherein the human antibody is capable of specifically binding to human LAG-3. Within other aspects, the present disclosure provides monoclonal antibodies comprising one or more heavy and/or light chain CDR3 domain from a first human antibody, such as, for example, a human antibody obtained from a non-human animal, wherein the first human antibody is capable of specifically binding to human LAG-3 and wherein the CDR3 domain from the first human antibody replaces a CDR3 domain in a human antibody that is lacking binding specificity for LAG-3 to generate a second human antibody that is capable of specifically binding to human LAG-3. Within some embodiments, such inventive antibodies comprising one or more heavy and/or light chain CDR3 domain from the first human antibody (a) are capable of competing for binding with; (b) retain the functional characteristics; (c) bind to the same epitope; and/or (d) have a similar binding affinity as the corresponding parental first human antibody.

Engineered and Modified Antibodies

[0114] As used herein, the terms "humanized", "humanization", and the like, refer to grafting of the murine monoclonal antibody CDRs disclosed herein to human FRs and constant regions. Also encompassed by these terms are possible further modifications to the murine CDRs, and human FRs, by the methods disclosed in, for example, Kashmiri et al. (*Methods*, 36(1):25-34, 2005) and Hou et al. (*J. Biochem.* 144(1):115-20, 2008), respectively, to improve various antibody properties, as discussed below.

[0115] As used herein, the term "FR" or "framework sequence" refers to any one of FRs 1 to 4. Humanized antibodies and antigen binding fragments encompassed by the present disclosure include molecules wherein any one or more of FRs 1 to 4 is substantially or fully human, i.e., wherein any of the possible combinations of individual substantially or fully human FRs 1 to 4, is present. For example, this includes molecules in which FR1 and FR2, FR1 and FR3, FR1, FR2, and FR3, etc., are substantially or fully human. Substantially human frameworks are those that have at least 80% sequence identity to a known human germline framework sequence. Preferably, the substantially human frameworks have at least 85%, at least 86%, at least 87%, at least 88%, at least 89%, at least 90%, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98%, or at least 99% sequence identity, to a framework sequence disclosed herein, or to a known human germline framework sequence.

[0116] Fully human frameworks are those that are identical to a known human germline framework sequence. Human FR germline sequences can be obtained from the international ImMunoGeneTics (IMGT) database and from *The Immunoglobulin FactsBook* by Marie-Paule Lefranc and Gerard Lefranc, Academic Press, 2001, the contents of which are herein incorporated by reference in their entirety.

[0117] CDRs encompassed by the present disclosure include not only those specifically disclosed herein, but also CDR sequences having sequence identities of at least 80%, at least 85%, at least 90%, at least 95%, at least 96%, at least 97%, at least 98%, or at least 99% sequence identity to a CDR sequence disclosed herein. Alternatively, CDRs encompassed by the present disclosure include not only those specifically disclosed herein, but also CDR sequences having 1, 2, 3, 4, or 5 amino acid changes at corresponding positions compared to CDR

sequences disclosed herein. Such sequence identical, or amino acid modified, CDRs preferably bind to the antigen recognized by the intact antibody.

[0118] Humanized antibodies in addition to those disclosed herein exhibiting similar functional properties according to the present disclosure can be generated using several different methods Almagro et al. (Front. Biosci., Humanization of antibodies Jan 1(13):1619-33, 2008). In one approach, the parent antibody compound CDRs are grafted into a human framework that has a high sequence identity with the parent antibody compound framework. The sequence identity of the new framework will generally be at least 80%, at least 85%, at least 86%, at least 87%, at least 88%, at least 89%, at least 90%, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98%, or at least 99% sequence identical to the sequence of the corresponding framework in the parent antibody compound. In the case of frameworks having fewer than 100 amino acid residues, one, two, three, four, five, six, seven, eight, nine, or ten amino acid residues can be changed. This grafting may result in a reduction in binding affinity compared to that of the parent antibody. If this is the case, the framework can be back-mutated to the parent framework at certain positions based on specific criteria disclosed by Queen et al. (*PNAS* 88:2869, 1991). Additional references describing methods useful to generate humanized variants based on homology and back mutations include as described in Olimpieri et al. (*Bioinformatics* Feb 1;31(3):434-5, 2015) and U.S. Patent Nos. 4,816,397, 5,225,539, and 5,693,761; and the method of Winter and co-workers (Jones et al., *Nature* 321:522-5, 1996; Riechmann et al., *Nature* 332:323-7, 1988; and Verhoeyen et al., *Science* 239:1534-6, 1988).

[0119] Antibodies of the disclosure can be tested for binding to human LAG-3 by, for example, standard ELISA. Anti-LAG-3 human IgG antibodies can be further tested for reactivity with a LAG-3 antigen by Western blotting. The binding specificity of an antibody of the disclosure can also be determined by monitoring binding of the antibody to cells expressing a LAG-3 protein, e.g., flow cytometry. These methods are known in the art. *See,* e.g., Harlow and Lane (1988), cited *supra.*

Bi-functional Molecules

[0120] Antibodies of this disclosure can be conjugated to a therapeutic agent to form an immunoconjugate such as an antibody-drug conjugate (ADC). Suitable therapeutic agents

include antimetabolites, alkylating agents, DNA minor groove binders, DNA intercalators, DNA cross linkers, histone deacetylase inhibitors, nuclear export inhibitors, proteasome inhibitors, topoisomerase I or II inhibitors, heat shock protein inhibitors, tyrosine kinase inhibitors, antibiotics, and anti-mitotic agents. In the ADC, the antibody and therapeutic agent preferably are conjugated via a linker cleavable such as a peptidyl, disulfide, or hydrazone linker. More preferably, the linker is a peptidyl linker such as Val-Cit, Ala-Val, Val-Ala-Val, Lys-Lys, Pro-Val-Gly-Val-Val, Ala-Asn-Val, Val-Leu-Lys, Ala-Ala-Asn, Cit-Cit, Val-Lys, Lys, Cit, Ser, or Glu. The ADC scan be prepared as described in U.S. Patent Nos. 7,087,600; 6,989,452; and 7,129,261; PCT Publications WO 02/096910; WO 07/038658; WO 07/051081; WO 07/059404; WO 08/083312; and WO 08/103693; U.S. Patent Publications 20060024317;20060004081; and 20060247295; the disclosures of which are incorporated herein by reference.

[0121] In another aspect, the present disclosure features bispecific molecules comprising an anti-LAG-3 antibody linked to at least one other functional molecule, e.g., another peptide or protein (e.g., another antibody or ligand for a receptor) to generate a bispecific molecule that binds to at least two different binding sites or target molecules. Thus, as used herein, "bispecific molecule" includes molecules that have three or more specificities. In a preferred embodiment, the bispecific molecule comprises a first binding specificity for LAG-3 and a second binding specificity for a triggering molecule that recruits cytotoxic effector cells that can kill a LAG-3 expressing target cell. Examples of suitable triggering molecules are CD64, CD89, CD16, and CD3. *See, e.g.,* Kufer et al., Trends in Biotech. 22(5):238-44, 2004.

[0122] In an embodiment, a bispecific molecule has, in addition to an anti-Fc binding specificity and an anti-LAG-3 binding specificity, a third specificity. The third specificity can be for an anti-enhancement factor (EF), e.g., a molecule that binds to a surface protein involved in cytotoxic activity and thereby increases the immune response against the target cell. For example, the anti-enhancement factor can bind a cytotoxic T cell (e.g. via CD2, CD3, CDS, CD28, CD4, CD40, or ICAM-1), other immune regulatory molecules (e.g. via PD-1, PD-L1, CTLA-4, CD122, 4-1BB, TIM3, OX-40, OX40L, CD40L, LIGHT, ICOS, ICOSL, GITR, GITRL, TIGIT, CD27, VISTA, B7H3, B7H4, HEVM, BTLA, KIR, CD47 or CD73) or other immune cell, resulting in an increased immune response against the target cell.

[0123] As an immune receptor modulator, an antibody or antigen-binding fragment specific to LAG-3 can be combined with a second antigen-binding fragment specific to a tumor antigen to generate a bispecific antibody. A "tumor antigen" is an antigenic substance produced in tumor cells, *i.e.*, it triggers an immune response in the host. Tumor antigens are useful in identifying tumor cells and are potential candidates for use in cancer therapy. Normal proteins in the body are not antigenic. Certain proteins, however, are produced or overexpressed during tumorigenesis and thus appear "foreign" to the body. This may include normal proteins that are well sequestered from the immune system, proteins that are normally produced in extremely small quantities, proteins that are normally produced only in certain stages of development, or proteins whose structure is modified due to mutation.

[0124] An abundance of tumor antigens are known in the art and new tumor antigens can be readily identified by screening. Non-limiting examples of tumor antigens include EGFR, Her2, EpCAM, CD20, CD30, CD33, CD47, CD52, CD133, CD73, CEA, gpA33, Mucins, TAG-72, CIX, PSMA, folate-binding protein, GD2, GD3, GM2, VEGF, VEGFR, Integrin, $\alpha V\beta 3$, $\alpha 5\beta 1$, ERBB2, ERBB3, MET, IGF1R, EPHA3, TRAILR1, TRAILR2, RANKL, FAP and Tenascin.

[0125] In some aspects, the monovalent unit has specificity to a protein that is overexpressed on a tumor cell as compared to a corresponding non-tumor cell. A "corresponding non-tumor cell" as used here, refers to a non-tumor cell that is of the same cell type as the origin of the tumor cell. It is noted that such proteins are not necessarily different from tumor antigens. Non-limiting examples include carcinoembryonic antigen (CEA), which is overexpressed in most colon, rectum, breast, lung, pancreas and gastrointestinal tract carcinomas; heregulin receptors (HER-2, *neu* or c-*erbB*-2), which is frequently overexpressed in breast, ovarian, colon, lung, prostate and cervical cancers; epidermal growth factor receptor (EGFR), which is highly expressed in a range of solid tumors including those of the breast, head and neck, non-small cell lung and prostate; asialoglycoprotein receptor; transferrin receptor; serpin enzyme complex receptor, which is expressed on hepatocytes; fibroblast growth factor receptor (FGFR), which is overexpressed on pancreatic ductal adenocarcinoma cells; vascular endothelial growth factor receptor (VEGFR), for anti-angiogenesis gene therapy; folate receptor, which is selectively overexpressed in 90% of nonmucinous ovarian carcinomas; cell surface glycocalyx; carbohydrate receptors; and polymeric immunoglobulin receptor, which is useful for gene delivery to respiratory epithelial

cells and attractive for treatment of lung diseases such as Cystic Fibrosis. Non-limiting examples of bispecificity in this respect include LAG-3/EGFR, LAG-3/Her2, LAG-3/CD33, LAG-3/CD133, LAG-3/CEA and LAG-3/VEGF.

[0126] Different format of bispecific antibodies are also provided. In some embodiments, each of the anti-LAG-3 fragment and the second fragment each is independently selected from a Fab fragment, a single-chain variable fragment (scFv), or a single-domain antibody. In some embodiments, the bispecific antibody further includes a Fc fragment.

[0127] Bifunctional molecules that include not just antibody or antigen binding fragment are also provided. As a tumor antigen targeting molecule, an antibody or antigen-binding fragment specific to LAG-3, such as those described here, can be combined with an immune cytokine or ligand optionally through a peptide linker. The linked immune cytokines or ligands include, but not limited to, IL-2, IL-3, IL-4, IL-5, IL-6, IL-7, IL-10, IL-12, IL-13, IL-15, GM-CSF, TNF-α, CD40L, OX40L, CD27L, CD30L, 4-1BBL, LIGHT and GITRL. Such bi-functional molecules can combine the immune checkpoint blocking effect with tumor site local immune modulation.

[0128] Bispecific molecules can come in many different formats and sizes. At one end of the size spectrum, a bispecific molecule retains the traditional antibody format, except that, instead of having two binding arms of identical specificity, it has two binding arms each having a different specificity. At the other extreme are bispecific molecules consisting of two single-chain antibody fragments (scFv's) linked by a peptide chain, a so-called $Bs(scFv)_2$ construct. Intermediate-sized bispecific molecules include two different F(ab) fragments linked by a peptidyl linker. Bispecific molecules of these and other formats can be prepared by genetic engineering, somatic hybridization, or chemical methods. *See, e.g.,* Kufer et al., *supra*; Cao and Suresh, Bioconjugate Chem. 9(6):635-44, 1988; and van Spriel et al., Immunol. Today 21(8):391-7, 2000; and the references cited therein.

Pharmaceutical Compositions

[0129] In another aspect, the present disclosure provides a pharmaceutical composition comprising an antibody of the present disclosure formulated together with a pharmaceutically acceptable earlier. It may optionally contain one or more additional pharmaceutically active

ingredients, such as another antibody or a drug. The pharmaceutical compositions of the disclosure also can be administered in a combination therapy with, for example, another immunostimulatory agent, anti-cancer agent, an anti-viral agent, or a vaccine, such that the anti-LAG-3 antibody enhances the immune response against the vaccine.

[0130] The pharmaceutical composition can comprise any number of excipients. Excipients that can be used include carriers, surface active agents, thickening or emulsifying agents, solid binders, dispersion or suspension aids, solubilizers, colorants, flavoring agents, coatings, disintegrating agents, lubricants, sweeteners, preservatives, isotonic agents, and combinations thereof. The selection and use of suitable excipients is taught in Gennaro, ed., Remington: *The Science and Practice of Pharmacy,* 20th Ed. (Lippincott Williams & Wilkins 2003), the disclosure of which is incorporated herein by reference. Preferably, a pharmaceutical composition is suitable for intravenous, intramuscular, subcutaneous, parenteral, spinal or epidermal administration (e.g., by injection or infusion). Depending on the route of administration, the active compound can be coated in a material to protect it from the action of acids and other natural conditions that may inactivate it. The phrase "parenteral administration" as used herein means modes of administration other than enteral and topical administration, usually by injection, and includes, without limitation, intravenous, intramuscular, intraarterial, intrathecal, intracapsular, intraorbital, intracardiac, intradermal, intraperitoneal, transtracheal, subcutaneous, subcuticular, intraarticular, subcapsular, subarachnoid, intraspinal, epidural and intrastemal injection and infusion. Alternatively, an antibody of the disclosure can be administered via a non-parenteral route, such as a topical, epidermal or mucosal route of administration, e.g., intranasally, orally, vaginally, rectally, sublingually or topically.

[0131] The pharmaceutical compounds of the disclosure can be in the form of pharmaceutically acceptable salts. A "pharmaceutically acceptable salt" refers to a salt that retains the desired biological activity of the parent compound and does not impart any undesired toxicological effects. Examples of such salts include acid addition salts and base addition salts. Acid addition salts include those derived from nontoxic inorganic acids, such as hydrochloric, nitric, phosphoric, sulfuric, hydrobromic, hydroiodic, phosphorous and the like, as well as from nontoxic organic acids such as aliphatic mono- and dicarboxylic acids, phenyl-substituted alkanoic acids, hydroxyl alkanoic acids, aromatic acids, aliphatic and aromatic sulfonic acids and

the like. Base addition salts include those derived from alkaline earth metals, such as sodium, potassium, magnesium, calcium and the like, as well as from nontoxic organic amines, such as N,N'-dibenzylethylenediamine, N-methylglucamine, chloroprocaine, choline, diethanolamine, ethylenediamine, procaine and the like.

[0132] Pharmaceutical compositions can be in the form of sterile aqueous solutions or dispersions. They can also be formulated in a microemulsion, liposome, or other ordered structure suitable to high drug concentration.

[0133] The amount of active ingredient which can be combined with a carrier material to produce a single dosage form will vary depending upon the subject being treated and the particular mode of administration and will generally be that amount of the composition which produces a therapeutic effect. Generally, out of one hundred percent, this amount will range from about 0.01% to about ninety-nine percent of active ingredient, preferably from about 0.1% to about 70%, most preferably from about 1% to about 30% of active ingredient in combination with a pharmaceutically acceptable carrier.

[0134] Dosage regimens are adjusted to provide the optimum desired response (e.g., a therapeutic response). For example, a single bolus can be administered, several divided doses can be administered over time or the dose can be proportionally reduced or increased as indicated by the exigencies of the therapeutic situation. It is especially advantageous to formulate parenteral compositions in dosage unit form for ease of administration and uniformity of dosage. Dosage unit form as used herein refers to physically discrete units suited as unitary dosages for the subjects to be treated; each unit contains a predetermined quantity of active compound calculated to produce the desired therapeutic effect in association with the required pharmaceutical carrier. Alternatively, antibody can be administered as a sustained release formulation, in which case less frequent administration is required.

[0135] For administration of the antibody, the dosage ranges from about 0.0001 to 100mg/kg, and more usually 0.01 to 5 mg/kg, of the host body weight. For example dosages can be 0.3 mg/kg body weight, 1 mg/kg body weight, 3 mg/kg body weight, 5mg/kg body weight or 10 mg/kg body weight or within the range of 1-10 mg/kg. An exemplary treatment regime entails administration once per week, once every two weeks, once every three weeks, once every four

weeks, once a month, once every 3 months or once every 3 to 6 months. Preferred dosage regimens for an anti-LAG-3antibody of the disclosure include 1 mg/kg body weight or 3 mg/kg body weight via intravenous administration, with the antibody being given using one of the following dosing schedules: (i) every four weeks for six dosages, then every three months; (ii) every three weeks; (iii) 3 mg/kg body weight once followed by 1 mg/kg body weight every three weeks. In some methods, dosage is adjusted to achieve a plasma antibody concentration of about 1-1000 μg/mL and in some methods about 25-300 μg/mL.

[0136] A "therapeutically effective dosage" of an anti-LAG-3 antibody of the disclosure preferably results in a decrease in severity of disease symptoms, an increase infrequency and duration of disease symptom-free periods, or a prevention of impairment or disability due to the disease affliction. For example, for the treatment of tumor bearing subjects, a "therapeutically effective dosage" preferably inhibits tumor growth by at least about 20%, more preferably by at least about 40%, even more preferably by at least about 60%, and still more preferably by at least about 80% relative to untreated subjects. A therapeutically effective amount of a therapeutic compound can decrease tumor size, or otherwise ameliorate symptoms in a subject, which is typically a human or can be another mammal.

[0137] The pharmaceutical composition can be a controlled release formulation, including implants, transdermal patches, and microencapsulated delivery systems. Biodegradable, biocompatible polymers can be used, such as ethylene vinyl acetate, polyanhydrides, polyglycolic acid, collagen, polyorthoesters, and polylactic acid. *See, e.g.,* Sustained and Controlled Release Drug Delivery Systems, J.R. Robinson, ed., Marcel Dekker, Inc., New York, 1978.

[0138] Therapeutic compositions can be administered via medical devices such as (1)needleless hypodermic injection devices (e.g., U.S. Patent Nos. 5,399,163; 5,383,851; 5,312,335;5,064,413; 4,941,880; 4,790,824; and 4,596,556); (2) micro-infusion pumps (U.S. Patent No. 4,487,603); (3) transdermal devices (U.S. Patent No. 4,486,194); (4) infusion apparati (U.S. Patent Nos. 4,447,233 and 4,447,224); and (5) osmotic devices (U.S. Patent Nos. 4,439,196 and 4,475,196); the disclosures of which are incorporated herein by reference.

[0139] In certain embodiments, the human monoclonal antibodies of the disclosure can be formulated to ensure proper distribution *in vivo*. For example, to ensure that the therapeutic compounds of the disclosure cross the blood-brain barrier, they can be formulated in liposomes, which may additionally comprise targeting moieties to enhance selective transport to specific cells or organs. *See, e.g.,* U.S. Patent Nos. 4,522,811; 5,374,548; 5,416,016; and 5,399,331; V.V. Ranade, J. Clin. Pharmacol. 29:685, 1989; Umezawa *et al.,* (1988) *Biochem. Biophys. Res. Commun.* 153:1038; Bloeman *et al.* (1995) *FEBSLett.* 357:140; M. Owais *et al.* (1995) *Antimicrob. Agents Chemother.* 39:180; Briscoe *etal.* (1995) *Am. J. Physiol.* 1233:134; Schreier *et al.* (1994) *J. Biol. Chern.* 269:9090;Keinanen and Laukkanen (1994) *FEBS Lett.* 346:123; and Killion and Fidler (1994) *Immunomethods* 4:273.

Uses and Methods

[0140] The antibodies, antibody compositions and methods of the present disclosure have numerous *in vitro* and *in vivo* utilities involving, for example, detection of LAG-3 or enhancement of immune response by blockade of LAG-3. In a preferred embodiment, the antibodies of the present disclosure are human antibodies. For example, these molecules can be administered to cells in culture, *in vitro* or *ex vivo,* or to human subjects, e.g., *in vivo,* to enhance immunity in a variety of situations. Accordingly, in one aspect, the disclosure provides a method of modifying an immune response in a subject comprising administering to the subject the antibody, or antigen-binding portion thereof, of the disclosure such that the immune response in the subject is modified. Preferably, the response is enhanced, stimulated or up-regulated.

[0141] Preferred subjects include human patients in need of enhancement of an immune response. The methods are particularly suitable for treating human patients having a disorder that can be treated by augmenting an immune response (e.g., the T-cell mediated immune response). In a particular embodiment, the methods are particularly suitable for treatment of cancer *in vivo.* To achieve antigen-specific enhancement of immunity, the anti-LAG-3 antibodies can be administered together with an antigen of interest or the antigen may already be present in the subject to be treated (e.g., a tumor bearing or virus-bearing subject). When antibodies to LAG-3 are administered together with another agent, the two can be administered in either order or simultaneously.

[0142] The disclosure further provides methods for detecting the presence of humanLAG-3 antigen in a sample, or measuring the amount of human LAG-3 antigen, comprising contacting the sample, and a control sample, with a human monoclonal antibody, or an antigen binding portion thereof, which specifically binds to human LAG-3, under conditions that allow for formation of a complex between the antibody or portion thereof and human LAG-3. The formation of a complex is then detected, wherein a difference complex formation between the sample compared to the control sample is indicative the presence of human LAG-3 antigen in the sample. Moreover, the anti-LAG-3 antibodies of the disclosure can be used to purify human LAG-3 via immunoaffinity purification.

[0143] Given the ability of anti-LAG-3 antibodies of the disclosure to inhibit the binding of LAG-3 to MHC Class II molecules and to stimulate antigen-specific T cell responses, the disclosure also provides *in vitro* and *in vivo* methods of using the antibodies of the disclosure to stimulate, enhance or upregulate antigen-specific T cell responses. For example, the disclosure provides a method of stimulating an antigen-specific T cell response comprising contacting said T cell with the antibody of the disclosure such that an antigen-specific T cell response is stimulated. Any suitable indicator of an antigen-specific T cell response can be used to measure the antigen-specific T cell response. Non-limiting examples of such suitable indicators include increased T cell proliferation in the presence of the antibody and/or increase cytokine production in the presence of the antibody. In a preferred embodiment, interleukin-2 production by the antigen specific T cell is stimulated.

[0144] The disclosure also provides a method of stimulating an immune response (e.g., an antigen-specific T cell response) in a subject comprising administering an antibody of the disclosure to the subject such that an immune response (e.g., an antigen-specific T cell response) in the subject is stimulated. In a preferred embodiment, the subject is a tumor-bearing subject and an immune response against the tumor is stimulated. In another preferred embodiment, the subject is a virus-bearing subject and an immune response against the virus is stimulated.

[0145] In another aspect, the disclosure provides a method for inhibiting growth of tumor cells in a subject comprising administering to the subject an antibody of the disclosure such that growth of the tumor is inhibited in the subject. In yet another aspect, the disclosure provides a method of

treating viral infection in a subject comprising administering to the subject an antibody of the disclosure such that the viral infection is treated in the subject.

[0146] These and other methods of the disclosure are discussed in further detail below.

Cancer

[0147] Blockade of LAG-3 by antibodies can enhance the immune response to cancerous cells in the patient. In one aspect, the present disclosure relates to treatment of a subject *in vivo* using an anti-LAG-3 antibody such that growth of cancerous tumors is inhibited. An anti-LAG-3 antibody can be used alone to inhibit the growth of cancerous tumors. Alternatively, an anti-LAG-3 antibody can be used in conjunction with other immunogenic agents, standard cancer treatments, or other antibodies, as described below.

[0148] Accordingly, in one embodiment, the disclosure provides a method of inhibiting growth of tumor cells in a subject, comprising administering to the subject a therapeutically effective amount of an anti-LAG-3 antibody, or antigen-binding portion thereof. Preferably, the antibody is a human anti-LAG-3 antibody (such as any of the human anti-human LAG-3 antibodies described herein). Additionally or alternatively, the antibody can be a chimeric or humanized anti-LAG-3 antibody.

[0149] Preferred cancers whose growth may be inhibited using the antibodies of the disclosure include cancers typically responsive to immunotherapy. Non-limiting examples of preferred cancers for treatment include melanoma (e.g., metastatic malignant melanoma), renal cancer (e.g. clear cell carcinoma), prostate cancer (e.g., hormone refractory prostate adenocarcinoma), breast cancer, colon cancer and lung cancer (e.g., non-small cell lung cancer). Additionally, the disclosure includes refractory or recurrent malignancies whose growth may be inhibited using the antibodies of the disclosure. Examples of other cancers that can be treated using the methods of the disclosure include bone cancer, pancreatic cancer, skin cancer, cancer of the head or neck, cutaneous or intraocular malignant melanoma, uterine cancer, ovarian cancer, rectal cancer, cancer of the anal region, stomach cancer, testicular cancer, carcinoma of the fallopian tubes, carcinoma of the endometrium, carcinoma of the cervix, carcinoma of the vagina, carcinoma of the vulva, Hodgkin's Disease, non-Hodgkin's lymphoma, cancer of the esophagus, cancer of the

small intestine, cancer of the endocrine system, cancer of the thyroid gland, cancer of the parathyroid gland, cancer of the adrenal gland, sarcoma of soft tissue, cancer of the urethra, cancer of the penis, chronic or acute leukemias including acute myeloid leukemia, chronic myeloid leukemia, acute lymphoblastic leukemia, chronic lymphocytic leukemia, solid tumors of childhood, lymphocytic lymphoma, cancer of the bladder, cancer of the kidney or ureter, carcinoma of the renal pelvis, neoplasm of the central nervous system (CNS), primary CNS lymphoma, tumor angiogenesis, spinal axis tumor, brain stem glioma, pituitary adenoma, Kaposi's sarcoma, epidermoid cancer, squamous cell cancer, T-cell lymphoma, environmentally induced cancers including those induced by asbestos, and combinations of said cancers. The present disclosure is also useful for treatment of metastatic cancers, especially metastatic cancers that express PD-L1 (Iwai *et al.* (2005) *Int. Immunol.* 17:133-144).

[0150] Optionally, antibodies to LAG-3 can be combined with an immunogenic agent, such as cancerous cells, purified tumor antigens (including recombinant proteins, peptides, and carbohydrate molecules), cells, and cells transfected with genes encoding immune stimulating cytokines (He *et al* (2004) *J. Immunol.* 173:4919-28). Non-limiting examples of tumor vaccines that can be used include peptides of melanoma antigens, such as peptides of gp100, MAGE antigens, Trp-2, MARTI and/or tyrosinase, or tumor cells transfected to express the cytokine GM-CSF (discussed further below).

[0151] In humans, some tumors have been shown to be immunogenic such as melanomas. By raising the threshold of T cell activation by LAG-3 blockade, the tumor responses in the host can be activated.

[0152] LAG-3 blockade is likely to be more effective when combined with a vaccination protocol. Many experimental strategies for vaccination against tumors have been devised *(see* Rosenberg, S., 2000, Development of Cancer Vaccines, ASCO Educational Book Spring: 60-62; Logothetis, C., 2000, ASCO Educational Book Spring:300-302; Khayat, D. 2000, ASCO Educational Book Spring: 414-428; Foon, K. 2000,ASCO Educational Book Spring: 730-738; *see* also Restifo, N. and Sznol, M., Cancer Vaccines, Ch. 61, pp. 3023-3043 in DeVita *et al.* (eds.), 1997, Cancer: Principles and Practice of Oncology, Fifth Edition). In one of these strategies, a vaccine is prepare dusing autologous or allogeneic tumor cells. These cellular

vaccines have been shown to be most effective when the tumor cells are transduced to express GM-CSF. GM-CSF has been shown to be a potent activator of antigen presentation for tumor vaccination (Dranoff *et al.* (1993) *Proc. Natl. Acad. Sci U.S.A.* 90: 3539-43).

[0153] The study of gene expression and large scale gene expression patterns in various tumors has led to the definition of so called tumor specific antigens (Rosenberg, SA (1999) *Immunity* 10: 281-7). In many cases, these tumor specific antigens are differentiation antigens expressed in the tumors and in the cell from which the tumor arose, for example melanocyte antigens gp100, MAGE antigens, and Trp-2. More importantly, many of these antigens can be shown to be the targets of tumor specific T cells found in the host. LAG-3 blockade can be used in conjunction with a collection of recombinant proteins and/or peptides expressed in a tumor in order to generate an immune response to these proteins. These proteins are normally viewed by the immune system as self antigens and are therefore tolerant to them. The tumor antigen can include the protein telomerase, which is required for the synthesis of telomeres of chromosomes and which is expressed in more than 85% of human cancers and in only a limited number of somatic tissues (Kim *et al.* (1994) *Science* 266: 2011-2013). (These somatic tissues may be protected from immune attack by various means). Tumor antigen can also be "neo-antigens" expressed in cancer cells because of somatic mutations that alter protein sequence or create fusion proteins between two unrelated sequences (i.e., bcr-abl in the Philadelphia chromosome), or idiotype from B cell tumors.

[0154] Other tumor vaccines can include the proteins from viruses implicated in human cancers such a Human Papilloma Viruses (HPV), Hepatitis Viruses (HBV and HCV) and Kaposi's Herpes Sarcoma Virus (KHSV). Another form of tumor specific antigen which can be used in conjunction with LAG-3 blockade is purified heat shock proteins (HSP) isolated from the tumor tissue itself. These heat shock proteins contain fragments of proteins from the tumor cells and these HSPs are highly efficient at delivery to antigen presenting cells for eliciting tumor immunity (Suot & Srivastava (1995) *Science*269:1585-1588; Tamura *et al.* (1997) *Science* 278:117-120).

[0155] Dendritic cells (DC) are potent antigen presenting cells that can be used to prime antigen-specific responses. DC's can be produced *ex vivo* and loaded with various protein and peptide

antigens as well as tumor cell extracts (Nestle *et al.* (1998) *Nature Medicine* 4: 328-332). DCs can also be transduced by genetic means to express these tumor antigens as well. DCs have also been fused directly to tumor cells for the purposes of immunization (Kugler *et al.* (2000) *Nature Medicine* 6: 332-336). As a method of vaccination, DC immunization can be effectively combined with LAG-3 blockade to activate more potent anti-tumor responses.

[0156] LAG-3 blockade can also be combined with standard cancer treatments. LAG-3 blockade can be effectively combined with chemotherapeutic regimes. In these instances, it may be possible to reduce the dose of chemotherapeutic reagent administered (Mokyr *et al.* (1998) *Cancer Research* 58: 5301-5304). An example of such a combination is an anti-LAG-3 antibody in combination with decarbazine for the treatment of melanoma. Another example of such a combination is an anti-LAG-3antibody in combination with interleukin-2 (IL-2) for the treatment of melanoma. The scientific rationale behind the combined use of LAG-3 blockade and chemotherapy is that cell death, that is a consequence of the cytotoxic action of most chemotherapeutic compounds, should result in increased levels of tumor antigen in the antigen presentation pathway. Other combination therapies that may result in synergy with LAG-3 blockade through cell death are radiation, surgery, and hormone deprivation. Each of these protocols creates a source of tumor antigen in the host. Angiogenesis inhibitors can also be combined with LAG-3 blockade. Inhibition of angiogenesis leads to tumor cell death which may feed tumor antigen into host antigen presentation pathways.

[0157] LAG-3 blocking antibodies can also be used in combination with bispecific antibodies that target Fca or Fey receptor-expressing effectors cells to tumor cells (see, e.g., U.S. Pat. Nos. 5,922,845 and 5,837,243). Bispecific antibodies can be used totarget two separate antigens. For example anti-Fc receptor/anti-tumor antigen (e.g., Her-2/neu) bispecific antibodies have been used to target macrophages to sites of tumor.This targeting may more effectively activate tumor specific responses. The T cell arm of these responses would be augmented by the use of LAG-3 blockade. Alternatively, antigen may be delivered directly to DCs by the use of bispecific antibodies which bind to tumor antigen and a dendritic cell specific cell surface marker.

[0158] Tumors evade host immune surveillance by a large variety of mechanisms. Many of these mechanisms may be overcome by the inactivation of proteins which are expressed by the

tumors and which are immunosuppressive. These include among others TGF-β (Kehrl *etal.* (1986) *J. Exp. Med.* 163: 1037-1050), IL-10 (Howard &O'Garra (1992) *Immunology Today* 13: 198-200), and Fas ligand (Hahne *et al.* (1996) *Science* 4: 1363-1365). Antibodies to each of these entities can be used in combination with anti-LAG-3 to counteract the effects of the immunosuppressive agent and favor tumor immune responses by the host.

[0159] Other antibodies which activate host immune responsiveness can be used in combination with anti-LAG-3. These include molecules on the surface of dendritic cells which activate DC function and antigen presentation. Anti-CD40 antibodies are able to substitute effectively for T cell helper activity (Ridge *et al.* (1998) Nature 393: 474-478) and can be used in conjunction with LAG-3 antibodies (Ito *et al.* (2000) *Immunobiology*201 (5) 527-40). Activating antibodies toT cell costimulatory molecules such as CTLA-4 (e.g., US Patent No. 5,811,097), OX-40 (Weinberg *et al.* (2000) *Immunol.* 164:2160-2169), 4-1BB (Melero *et al.* (1997) *Nature Medicine* 3: 682-685 (1997), and ICOS (Hutloff *et al.* (1999) *Nature* 397: 262-266) may also provide for increased levels of T cell activation.

[0160] Bone marrow transplantation is currently being used to treat a variety of tumors of hematopoietic origin. While graft versus host disease is a consequence of this treatment, therapeutic benefit may be obtained from graft vs. tumor responses. LAG-3 blockade can be used to increase the effectiveness of the donor engrafted tumor specific T cells.

[0161] There are also several experimental treatment protocols that involve *ex vivo* activation and expansion of antigen specific T cells and adoptive transfer of these cells into recipients in order to stimulate antigen-specific T cells against tumor (Greenberg &Riddell (1999) *Science* 285: 546-51). These methods can also be used to activate T cell responses to infectious agents such as CMV. *Ex vivo* activation in the presence of anti-LAG-3 antibodies can increase the frequency and activity of the adoptively transferred T cells.

[0162] Cellular therapies, and more specifically chimeric antigen receptor (CAR) T-cell therapies, are also provided in the present disclosure. A suitable T cell can be used, that is put in contact with an anti-LAG-3 antibody of the present disclosure (or alternatively engineered to express an anti-LAG-3 antibody of the present disclosure). Upon such contact or engineering, the T cell can then be introduced to a cancer patient in need of a treatment. The cancer patient may

have a cancer of any of the types as disclosed herein. The T cell can be, for instance, a tumor-infiltrating T lymphocyte, a CD4+ T cell, a CD8+ T cell, or the combination thereof, without limitation.

[0163] In some embodiments, the T cell was isolated from the cancer patient him- or her-self. In some embodiments, the T cell was provided by a donor or from a cell bank. When the T cell is isolated from the cancer patient, undesired immune reactions can be minimized.

Infectious Diseases

[0164] Other methods of the disclosure are used to treat patients that have been exposed to particular toxins or pathogens. Accordingly, another aspect of the disclosure provides a method of treating an infectious disease in a subject comprising administering to the subject an anti-LAG-3 antibody, or antigen-binding portion thereof, such that the subject is treated for the infectious disease. Preferably, the antibody is a human anti-human LAG-3 antibody (such as any of the human anti-LAG-3 antibodies described herein). Additionally or alternatively, the antibody can be a chimeric or humanized antibody.

[0165] Similar to its application to tumors as discussed above, antibody mediated LAG-3 blockade can be used alone, or as an adjuvant, in combination with vaccines, to stimulate the immune response to pathogens, toxins, and self-antigens. Examples of pathogens for which this therapeutic approach can be particularly useful, include pathogens for which there is currently no effective vaccine, or pathogens for which conventional vaccines are less than completely effective. These include, but are not limited to HIV, Hepatitis (A, B, & C), Influenza, Herpes, Giardia, Malaria, Leishmania, Staphylococcus aureus, Pseudomonas aeruginosa. LAG-3 blockade is particularly useful against established infections by agents such as HIV that present altered antigens over the course of the infections. These novel epitopes are recognized as foreign at the time of anti-human LAG-3 administration, thus provoking a strong T cell response that is not dampened by negative signals through LAG-3.

[0166] Some examples of pathogenic viruses causing infections treatable by methods ofthe disclosure include HIV, hepatitis (A, B, or C), herpes virus (e.g., VZV, HSV-1,HAV-6, HSV-11, and CMV, Epstein Barr virus), adenovirus, influenza virus, flaviviruses, echovirus, rhinovirus,

coxsackie virus, coronavirus, respiratory syncytial virus, mumps virus, rotavirus, measles virus, rubella virus, parvovirus, vaccinia virus, HTL-V virus, dengue virus, papilloma virus, molluscum virus, poliovirus, rabies virus, JCvirus and arboviral encephalitis virus.

[0167] Some examples of pathogenic bacteria causing infections treatable by methods ofthe disclosure include chlamydia, rickettsial bacteria, mycobacteria, staphylococci, streptococci, pneumonococci, meningococci and gonococci, klebsiella, proteus, serratia, pseudomonas, legionella, diphtheria, salmonella, bacilli, cholera, tetanus, botulism, anthrax, plague, leptospirosis, and Lymes disease bacteria.

[0168] Some examples of pathogenic fungi causing infections treatable by methods of the disclosure include *Candida albicans, krusei, glabrata, tropicalis, etc.), Cryptococcus neoformans, Aspergillus (fumigatus, niger*, etc.), Genus *Mucorales (mucor, absidia,rhizopus*), *Sporothrix schenkii, Blastomyces dermatitidis, Paracoccidioides brasiliensis, Coccidioides immitis* and *Histoplasma capsulatum.*

[0169] Some examples of pathogenic parasites causing infections treatable by methods of the disclosure include *Entamoeba histolytica, Balantidium coli, Naegleriafowleri, Acanthamoeba sp., Giardia lambia, Cryptosporidium sp., Pneumocystis carinii, Plasmodium vivax, Babesia microti, Trypanosoma brucei, Trypanosoma cruzi, Leishmania donovani, Toxoplasma gondii, Nippostrongylus brasiliensis.*

[0170] In all of the above methods, LAG-3 blockade can be combined with other forms of immunotherapy such as cytokine treatment (e.g., interferons, GM-CSF, G-CSF, IL-2), or bispecific antibody therapy, which provides for enhanced presentation of tumor antigens *(see,* e.g., Bolliger (1993) *Proc. Natl. Acad. Sci.* USA 90:6444-6448; Poljak (1994) *Structure* 2:1121-1123).

Autoimmune Reactions

[0171] Anti-LAG-3 antibodies may provoke and amplify autoimmune responses. Indeed, induction of anti-tumor responses using tumor cell and peptide vaccines revealsthat many anti-tumor responses involve anti-self reactivities (van Elsas *et al.* (2001) *J.*112 *Exp. Med.* 194:481-489; Overwijk, *et al.* (1999) *Proc. Natl. Acad. Sci. U.S.A.* 96: 2982-2987; Hurwitz, (2000) *supra;*

Rosenberg & White (1996) *J. Immunother Emphasis Tumor Immunol.19* (1): 81-4). Therefore, it is possible to consider using anti-LAG-3 blockade in conjunction with various self-proteins in order to devise vaccination protocols to efficiently generate immune responses against these self-proteins for disease treatment. For example, Alzheimer's disease involves inappropriate accumulation of Aβ peptide in amyloid deposits in the brain; antibody responses against amyloid are able to clear these amyloid deposits (Schenk *et al.,* (1999) *Nature* 400: 173-177).

[0172] Other self-proteins can also be used as targets such as IgE for the treatment of allergy and asthma, and TNFα for rheumatoid arthritis. Finally, antibody responses to various hormones may be induced by the use of anti-LAG-3 antibody. Neutralizing antibody responses to reproductive hormones can be used for contraception. Neutralizing antibody response to hormones and other soluble factors that are required for the growth of particular tumors can also be considered as possible vaccination targets.

[0173] Analogous methods as described above for the use of anti-LAG-3 antibody can be used for induction of therapeutic autoimmune responses to treat patients having an inappropriate accumulation of other self-antigens, such as amyloid deposits, including Aβ in Alzheimer's disease, cytokines such as TNFα, and IgE.

Vaccines

[0174] Anti-LAG-3 antibodies can be used to stimulate antigen-specific immune responses by co-administration of an anti-LAG-3 antibody with an antigen of interest (e.g., a vaccine). Accordingly, in another aspect the disclosure provides a method of enhancing an immune response to an antigen in a subject, comprising administering to the subject: (i) the antigen; and (ii) an anti-LAG-3 antibody, or antigen-binding portion thereof, such that an immune response to the antigen in the subject is enhanced. Preferably, the antibody is a human anti-human LAG-3 antibody (such as any of the human anti-LAG-3 antibodies described herein). Additionally or alternatively, the antibody can be a chimeric or humanized antibody. The antigen can be, for example, a tumor antigen, a viral antigen, a bacterial antigen or an antigen from a pathogen. Non-limiting examples of such antigens include those discussed in the sections above, such as the tumor antigens (or tumor vaccines) discussed above, or antigens from the viruses, bacteria or other pathogens described above.

[0175] Suitable routes of administering the antibody compositions (e.g., human monoclonal antibodies, multi-specific and bispecific molecules and immunoconjugates) of the disclosure *in vivo* and *in vitro* are well known in the art and can be selected by those of ordinary skill. For example, the antibody compositions can be administered by injection (e.g., intravenous or subcutaneous). Suitable dosages of the molecules used will depend on the age and weight of the subject and the concentration and/or formulation of the antibody composition.

[0176] As previously described, human anti-LAG-3 antibodies of the disclosure can be co-administered with one or other more therapeutic agents, e.g., a cytotoxic agent, a radiotoxic agent or an immunosuppressive agent. The antibody can be linked to the agent (as an immuno-complex) or can be administered separate from the agent. In the latter case (separate administration), the antibody can be administered before, after or concurrently with the agent or can be co-administered with other known therapies, e.g., an anti-cancer therapy, e.g., radiation. Such therapeutic agents include, among others, anti-neoplastic agents such as doxorubicin (adriamycin), cisplatin bleomycin sulfate, carmustine, chlorambucil, dacarbazine and cyclophosphamide hydroxyurea which, by themselves, are only effective at levels which are toxic or subtoxic to a patient. Cisplatin is intravenously administered as a 100 mg/mL dose once every four weeks and adriamycin is intravenously administered as a 60-75 mg/mL dose once every 21 days. Co-administration of the human anti-LAG-3 antibodies, or antigen binding fragments thereof, of the present disclosure with chemotherapeutic agents provides two anti-cancer agents which operate via different mechanisms which yield a cytotoxic effect to human tumor cells. Such co-administration can solve problems due to development of resistance to drugs or a change in the antigenicity of the tumor cells which would render them unreactive with the antibody.

[0177] Also within the scope of the present disclosure are kits comprising the antibody compositions of the disclosure (e.g., human antibodies, bispecific or multi-specific molecules, or immunoconjugates) and instructions for use. The kit can further contain at least one additional reagent, or one or more additional human antibodies of the disclosure (e.g., a human antibody having a complementary activity which binds to an epitope inLAG-3 antigen distinct from the first human antibody). Kits typically include a label indicating the intended use of the contents

of the kit. The term label includes any writing, or recorded material supplied on or with the kit, or which otherwise accompanies the kit.

Nervous System Disorders

[0178] Other methods of the disclosure are used to treat patients that have a progressive disorder of the nervous system that affects movement. In one embodiment, the progressive disorder of the nervous system that affects movement is Parkinson's disease. Accordingly, another aspect of the disclosure provides a method of treating Parkinson's disease in a subject comprising administering to the subject an anti-LAG-3 antibody, or antigen-binding portion thereof, such that the subject is treated for Parkinson's disease. Preferably, the antibody is a human anti-human LAG-3 antibody (such as any of the human anti-LAG-3 antibodies described herein). Additionally or alternatively, the antibody can be a chimeric or humanized antibody.

[0179] In addition to immune system organ e.g. thymus and spleen, LAG3 is enriched in the brain as well (C. J. Workman (2002)， Eur. J. Immunol. 32, 2255–2263). Immunoblot analysis indicates that LAG3 is expressed predominantly in neurons. According to the Allen Brain Atlas, LAG3 is localized to neurons throughout the central nervous system (CNS), including DA neurons. X. Mao et al., (Science. 2016 Sep 30;353(6307)) reported that LAG3 preferentially binds α-synuclein (α-syn) misfolded preformed fibrils (PFF) with high affinity mainly through its D1 domain (29-167AA). In addition, deletion of the D2 (168-252AA), D3 (265-343AA), or intracellular domain (ICD, 472-525AA) substantially weakens binding of LAG3 to α-syn PFF, X. Mao et al have shown that α-syn PFF binding to LAG3 initiated a-syn PFF endocytosis, transmission, and toxicity. Emerging evidence indicates that the pathogenesis of Parkinson's disease (PD) may be due to cell-to-cell transmission of misfolded α-syn PFF. Parkinson's disease (PD) is the second most common neurodegenerative disorder and leads to slowness of movement, tremor, rigidity, and, in the later stages of PD, cognitive impairment. Pathologically, PD is characterized by the accumulation of a-synuclein in Lewy bodies and neurites. There is degeneration of neurons throughout the nervous system, with the degeneration of dopamine neurons in the substantia nigra pars compacta leading to the major symptoms of PD. Anti-LAG3 antibody specifically bind to D1 or D2 domain can reduce a-syn PFF toxicity and cell-to-cell transmission, suggesting its potential for PD therapy. As shown in the Example 1, our antibody

can specifically bind to D1 or D2 domain of LAG3 protein. Therefore, there antibody can be used for the PD therapy.

Combination Therapy

[0180] In another aspect, the disclosure provides methods of combination therapy in which an anti-LAG-3 antibody is co-administered with one or more additional antibodies that are effective in stimulating immune responses to thereby further enhance, stimulate or upregulate immune responses in a subject. For example, the disclosure provides a method for stimulating an immune response in a subject comprising administering to the subject an anti-LAG-3 antibody and one or more additional immunostimulatory antibodies, such as an anti-PD-1 antibody, an anti-PD-L1 antibody and/or an anti-CTLA-4 antibody, such that an immune response is stimulated in the subject, for example to inhibit tumor growth or to stimulate an anti-viral response. In one embodiment, the subject is administered an anti-LAG-3 antibody and an anti-PD-1 antibody. In another embodiment, the subject is administered an anti-LAG-3 antibody and an anti-PD-L1 antibody. In yet another embodiment, the subject is administered ananti-LAG-3 antibody and an anti-CTLA-4 antibody. In one embodiment, the anti-LAG-3 antibody is a human antibody, such as an antibody of the disclosure. Alternatively, theanti-LAG-3 antibody can be, for example, a chimeric or humanized antibody (e.g., prepared from a mouse anti-LAG-3 mAb). In another embodiment, the at least oneadditional immunostimulatory antibody (e.g., anti-PD-1, anti-PD-L1 and/or anti-CTLA-4 antibody) is a human antibody. Alternatively, the at least one additional immunostimulatory antibody can be, for example, a chimeric or humanized antibody (e.g., prepared from a mouse anti-PD-1, anti-PD-L1 and/or anti-CTLA-4 antibody).

[0181] In one embodiment, the present disclosure provides a method for treating a hyperproliferative disease (e.g., cancer), comprising administering a LAG-3 antibody and a CTLA-4 antibody to a subject. In further embodiments, the anti-LAG-3 antibody is administered at a subtherapeutic dose, the anti-CTLA-4 antibody is administered at a subtherapeutic dose, or both are administered at a subtherapeutic dose. In another embodiment, the present disclosure provides a method for altering an adverse event associated with treatment of a hyperproliferative disease with an immunostimulatory agent, comprising administering an anti-LAG-3 antibody and a subtherapeutic dose ofanti-CTLA-4 antibody to a subject. In certain embodiments, the

subject is human. In certain embodiments, the anti-CTLA-4 antibody is human sequence monoclonal antibody 10D1 (described in PCT Publication WO 01114424) and the anti-LAG-3 antibody is human sequence monoclonal antibody, such as S27, S31, T99, or S119 as described herein. Other anti-CTLA-4 antibodies encompassed by the methods of the present disclosure include, for example, those disclosed in: WO98/42752; WO 00/37504; U.S. Patent No. 6,207,156; Hurwitz *et al.* (1998) *Proc. Natl.Acad. Sci. USA* 95(17):10067-10071; Camacho *et al.* (2004) *J. Clin. Oncology* 22(145): Abstract No. 2505 (antibody CP-675206); and Mokyr *et al.* (1998) *Cancer Res.*58:5301-5304. In certain embodiments, the anti-CTLA-4 antibody binds to human CTLA-4 with a K_D of 5 x 10^{-8} M or less, binds to human CTLA-4 with a K_D of 1 x 10^{-8} M or less, binds to human CTLA-4 with a K_D of 5 x 10^{-9} M or less, or binds to human CTLA-4 with a K_D of between 1 x 10^{-8} M and 1 x 10^{-10} M or less.

[0182] In one embodiment, the present disclosure provides a method for treating a hyperproliferative disease (e.g., cancer), comprising administering a LAG-3 antibody and a PD-1 antibody to a subject. In further embodiments, the anti-LAG-3 antibody is administered at a subtherapeutic dose, the anti-PD-1 antibody is administered at a subtherapeutic dose, or both are administered at a subtherapeutic dose. In another embodiment, the present disclosure provides a method for altering an adverse event associated with treatment of a hyperproliferative disease with an immunostimulatory agent, comprising administering an anti-LAG-3 antibody and a subtherapeutic dose ofanti-PD-1 antibody to a subject. In certain embodiments, the subject is human. In certain embodiments, the anti-PD-1 antibody is a human sequence monoclonal antibody nd the anti-LAG-3 antibody is human sequence monoclonal antibody, such as S27, S31, T99, or S119 as described herein. Examples of human sequence antiPD-1 antibodies include 17D8, 2D3, 4H1, 5C4 and 4A11, which are described in PCT Publication WO 061121168. In certain embodiments, the anti-PD-1 antibody binds to human PD-1 with a K_D of 5 x 10^{-8} M or less, binds to human PD-1 with a K_D of 1 x 10^{-8} M or less, binds to human PD-1 with a K_D of 5 x 10^{-9} M or less, or binds to human PD-1with a K_D of between 1 X 10^{-8} M and 1 X 10^{-10} M or less.

[0183] In one embodiment, the present disclosure provides a method for treating a hyperproliferative disease (e.g., cancer), comprising administering a LAG-3 antibody and a PD-L1 antibody to a subject. In further embodiments, the anti-LAG-3 antibody is administered at a subtherapeutic dose, the anti-PD-L1 antibody is administered at a subtherapeutic dose, or both

are administered at a subtherapeutic dose. In another embodiment, the present disclosure provides a method for altering an adverse event associated with treatment of a hyperproliferative disease with an immunostimulatory agent, comprising administering an anti-LAG-3 antibody and a subtherapeutic dose of anti-PD-L1 antibody to a subject. In certain embodiments, the subject is human. In certain embodiments, the anti-PD-L1 antibody is a human sequence monoclonal antibody and the anti-LAG-3 antibody is human sequence monoclonal antibody, such as S27, S31, T99, or S119 as described herein. Examples of human sequence anti-PD-L1 antibodies include 3G10, 12A4, 10A5, 5F8, 10H10, 1B12, 7H1, 11E6, 12B7and 13G4, which are described in PCT Publication WO 07/005874. In certain embodiments, the anti-PD-L1 antibody binds to human PD-L1 with a K_D of 5 x 10^{-8} M or less, binds to human PD-L1 with a K_D of 1 x 10^{-8} M or less, binds to human PD-L1with a K_D of 5 x 10^{-9} M or less, or binds to human PD-L1 with a K_D of between 1 x 10^{-8} M and 1 x 10^{-10} M or less.

[0184] Blockade of LAG-3 and one or more second target antigens such as CTLA-4 and/or PD-1 and/or PD-L1 by antibodies can enhance the immune response to cancerous cells in the patient. Cancers whose growth may be inhibited using the antibodies of the instant disclosure include cancers typically responsive to immunotherapy. Representative examples of cancers for treatment with the combination therapy of the instant disclosure include those cancers specifically listed above in the discussion of monotherapy with anti-LAG-3 antibodies.

[0185] In certain embodiments, the combination of therapeutic antibodies discussed herein can be administered concurrently as a single composition in a pharmaceutically acceptable carrier, or concurrently as separate compositions with each antibody in a pharmaceutically acceptable carrier. In another embodiment, the combination of therapeutic antibodies can be administered sequentially. For example, an anti-CTLA-4antibody and an anti-LAG-3 antibody can be administered sequentially, such as anti-CTLA-4 antibody being administered first and anti-LAG-3 antibody second, or anti-LAG-3 antibody being administered first and anti-CTLA-4 antibody second. Additionally or alternatively, an anti-PD-1 antibody and an anti-LAG-3 antibody can be administered sequentially, such as anti-PD-1 antibody being administered first and anti-LAG-3 antibody second, or anti-LAG-3 antibody being administered first and anti-PD-1 antibody second. Additionally or alternatively, an anti-PD-L1 antibody and an anti-LAG-3 antibody can be administered sequentially, such as anti-PD-L1 antibody being administered first and anti-LAG-3

antibody second, or anti-LAG-3 antibody being administered first and anti-PD-Ll antibody second.

[0186] Furthermore, if more than one dose of the combination therapy is administered sequentially, the order of the sequential administration can be reversed or kept in the same order at each time point of administration, sequential administrations can be combined with concurrent administrations, or any combination thereof. For example, the first administration of a combination anti-CTLA-4 antibody and anti-LAG-3antibody can be concurrent, the second administration can be sequential with anti-CTLA-4 first and anti-LAG-3 second, and the third administration can be sequential with anti-LAG-3 first and anti-CTLA-4 second, *etc.* Additionally or alternatively, the first administration of a combination anti-PD-1 antibody and anti-LAG-3 antibody can be concurrent, the second administration can be sequential with anti-PD-1 first and anti-LAG-3 second, and the third administration can be sequential with anti-LAG-3 first andanti-PD-1 second, etc. Additionally or alternatively, the first administration of a combination anti-PD-Ll antibody and anti-LAG-3 antibody can be concurrent, the second administration can be sequential with anti-PD-L1 first and anti-LAG-3 second, and the third administration can be sequential with anti-LAG-3 first and anti-PD-L1 second, *etc.* Another representative dosing scheme can involve a first administration that is sequential with anti-LAG-3 first and anti-CTLA-4 (and/or anti-PD-1 and/or anti-PD-L1) second, and subsequent administrations may be concurrent.

[0187] Optionally, the combination of anti-LAG-3 and one or more additional antibodies (e.g., anti-CTLA-4 and/or anti-PD-1 and/or anti-PD-L1 antibodies) can be further combined with an immunogenic agent, such as cancerous cells, purified tumor antigens (including recombinant proteins, peptides, and carbohydrate molecules), cells, and cells transfected with genes encoding immune stimulating cytokines (He *et al.*(2004) *J. Immunol.* 173:4919-28). Non-limiting examples of tumor vaccines that can beused include peptides of melanoma antigens, such as peptides of gpl00, MAGE antigens, Trp-2, MARTI and/or tyrosinase, or tumor cells transfected to express the cytokine GM-CSF. A combined LAG-3 and CTLA-4 and/or PD-1 and/or PD-Ll blockade can be further combined with a vaccination protocol, such as any of the vaccination protocols discussed in detail above with respect to monotherapy with anti-LAG-3 antibodies.

[0188] A combined LAG-3 and CTLA-4 and/or PD-1 and/or PD-L1 blockade can also be further combined with standard cancer treatments. For example, a combined LAG-3and CTLA-4 and/or PD-1 and/or PD-L1 blockade can be effectively combined with chemotherapeutic regimes. In these instances, it is possible to reduce the dose of other chemotherapeutic reagent administered with the combination of the instant disclosure (Mokyr *et al.* (1998) *Cancer Research* 58: 5301-5304). An example of such a combination is a combination of anti-LAG-3 and anti-CTLA-4 antibodies and/or anti-PD-1 antibodies and/or anti-PD-L1 antibodies further in combination with decarbazine for the treatment of melanoma. Another example is a combination of anti-LAG-3 and anti-CTLA-4 antibodies and/or anti-PD-1 antibodies and/or anti-PD-L1 antibodies further in combination with interleukin-2 (IL-2) for the treatment of melanoma. The scientific rationale behind the combined use of LAG-3 and CTLA-4 and/or PD-1 and/or PD-L1 blockade with chemotherapy is that cell death, which is a consequence of the cytotoxic action of most chemotherapeutic compounds, should result in increased levels of tumor antigen in the antigen presentation pathway. Other combination therapies that may result in synergy with a combined LAG-3 and CTLA-4 and/or PD-1 and/or PD-L1 blockade through cell death include radiation, surgery, or hormone deprivation. Each of these protocols creates a source of tumor antigen in the host. Angiogenesis inhibitors can also be combined with a combined LAG-3 and CTLA-4 and/or PD-1 and/or PD-L1 blockade. Inhibition of angiogenesis leads to tumor cell death, which can be a source of tumor antigen fed into host antigen presentation pathways.

[0189] A combination of LAG-3 and CTLA-4 and/or PD-1 and/or PD-L1 blocking antibodies can also be used in combination with bispecific antibodies that target Fcα or Fcγ receptor-expressing effector cells to tumor cells (see, e.g., U.S. Pat. Nos. 5,922,845 and 5,837,243). Bispecific antibodies can be used to target two separate antigens. The T cell arm of these responses would be augmented by the use of a combined LAG-3 and CTLA-4 and/or PD-1 and/or PD-L1 blockade. In another example, a combination of anti-LAG-3 and anti-CTLA-4 and/or antiPD-1 antibodies and/or anti-PD-L1 antibodies can be used in conjunction with anti-neoplastic antibodies, such as Rituxan® (rituximab), Herceptin® (trastuzumab), Bexxar®(tositumomab), Zevalin® (ibritumomab), Campath® (alemtuzumab), Lymphocide® (eprtuzumab), Avastin® (bevacizumab), and Tarceva® (erlotinib), and the like. By wayof example and not wishing to be bound by theory, treatment with an anti-cancer antibody or an

anti-cancer antibody conjugated to a toxin can lead to cancer cell death (e.g., tumor cells) which would potentiate an immune response mediated by CTLA-4, PD-1, PD-L1 or LAG-3. In an exemplary embodiment, a treatment of a hyperproliferative disease (e.g., a cancer tumor) can include an anti-cancer antibody in combination with anti-LAG-3 and anti-CTLA-4 and/or anti-PD-1 and/or anti-PD-L1antibodies, concurrently or sequentially or any combination thereof, which can potentiate an anti-tumor immune responses by the host.

[0190] Tumors evade host immune surveillance by a large variety of mechanisms. Many of these mechanisms may be overcome by the inactivation of proteins, which are expressed by the tumors and which are immunosuppressive. These include, among others, TGF-β (Kehrl *et al.* (1986) *J. Exp. Med.* 163: 1037-1050), IL-10 (Howard & O'Garra (1992) *Immunology Today* 13: 198-200), and Fas ligand (Hahne *et al.* (1996) *Science* 27 4: 1363-1365). In another example, antibodies to each of these entities can be further combined with an anti-LAG-3 and anti-CTLA-4 and/or anti-PD-1 and/or anti-PD-L1 antibody combination to counteract the effects of immunosuppressive agents and favor anti-tumor immune responses by the host.

[0191] Other antibodies that can be used to activate host immune responsiveness can be further used in combination with an anti-LAG-3 and anti-CTLA-4 and/or anti-PD-1and/or anti-PD-L1 antibody combination. These include molecules on the surface of dendritic cells that activate DC function and antigen presentation. Anti-CD40 antibodies (Ridge *et al., supra)* can be used in conjunction with an anti-LAG-3 and anti-CTLA-4and/or anti-PD-1 and/or anti-PD-L1 combination (Ito *et al., supra).* Other activating antibodies to T cell co stimulatory molecules Weinberg *et al., supra,* Melero *et al. supra,* Hutloff *et al., supra)* may also provide for increased levels of T cell activation.

[0192] As discussed above, bone marrow transplantation is currently being used to treat a variety of tumors of hematopoietic origin. A combined LAG-3 and CTLA-4 and/orPD-1 and/or PD-L1 blockade can be used to increase the effectiveness of the donor engrafted tumor specific T cells.

[0193] Several experimental treatment protocols involve *ex vivo* activation and expansion of antigen specific T cells and adoptive transfer of these cells into recipients in order to antigen-specific T cells against tumor (Greenberg & Riddell, *supra).* These methods can also be used to activate T cell responses to infectious agents such as CMV. *Ex vivo* activation in the presence of

anti-LAG-3 and anti-CTLA-4 and/or anti-PD-1and/or anti-PD-L1 antibodies can be expected to increase the frequency and activity of the adoptively transferred T cells.

[0194] In certain embodiments, the present disclosure provides a method for altering an adverse event associated with treatment of a hyperproliferative disease (e.g., cancer) with an immunostimulatory agent, comprising administering an anti-LAG-3 antibody and a subtherapeutic dose of anti-CTLA-4 and/or anti-PD-1and/or anti-PD-L1 antibody to a subject. For example, the methods of the present disclosure provide for a method of reducing the incidence of immunostimulatory therapeutic antibody-induced colitis or diarrhea by administering a non-absorbable steroid to the patient. Because any patient who will receive an immunostimulatory therapeutic antibody is at risk for developing colitis or diarrhea induced by such an antibody, this entire patient population is suitable for therapy according to the methods of the present disclosure. Although steroids have been administered to treat inflammatory bowel disease (IBD) and prevent exacerbations of IBD, they have not been used to prevent (decrease the incidence of) IBD in patients who have not been diagnosed with IBD. The significant side effects associated with steroids, even non-absorbable steroids, have discouraged prophylactic use.

[0195] In further embodiments, a combination LAG-3 and CTLA-4 and/or PD-1 and/orPD-L1 blockade *(*i.e., immunostimulatory therapeutic antibodies anti-LAG-3 and antiCTLA-4 and/or anti-PD-1 antibodies and/or anti-PD-L1 antibodies) can be further combined with the use of any non-absorbable steroid. As used herein, a "nonabsorbable steroid" is a glucocorticoid that exhibits extensive first pass metabolism such that, following metabolism in the liver, the bioavailability of the steroid is low, i.e., less than about 20%. In one embodiment of the disclosure, the non-absorbable steroid isbudesonide. Budesonide is a locally-acting glucocorticosteroid, which is extensivelymetabolized, primarily by the liver, following oral administration. ENTOCORT EC® (Astra-Zeneca) is a pH- and time-dependent oral formulation of budesonide developed to optimize drug delivery to the ileum and throughout the colon. ENTOCORT EC® is approved in the U.S. for the treatment of mild to moderate Crohn's disease involving theileum and/or ascending colon. The usual oral dosage of ENTOCORT EC® for thetreatment of Crohn's disease is 6 to 9 mg/day. ENTOCORT EC® is released in the intestines before being absorbed and retained in the gut mucosa. Once it passes through the gut mucosa target tissue, ENTOCORT EC® is extensively metabolized by the cytochrome P450 system in

the liver to metabolites with negligible glucocorticoid activity. Therefore, the bioavailability is low (about 10%). The low bioavailability of budesonide results in an improved therapeutic ratio compared to other glucocorticoids with less extensive first-pass metabolism. Budesonide results in fewer adverse effects, including less hypothalamic-pituitary suppression, than systemically-acting corticosteroids. However, chronic administration of ENTOCORT EC® can result in systemic glucocorticoid effects such as hypercorticism and adrenal suppression. *See* PDR 58th ed. 2004; 608-610.

[0196] In still further embodiments, a combination LAG-3 and CTLA-4 and/or PD-1and/or PD-L1 blockade *(*i.e., immunostimulatory therapeutic antibodies anti-LAG-3 andanti-CTLA-4 and/or anti-PD-1 and/or anti-PD-L1 antibodies) in conjunction with a nonabsorbable steroid can be further combined with a salicylate. Salicylates include 5-ASAagents such as, for example: sulfasalazine (AZULFIDINE®, Pharmacia & UpJohn); olsalazine (DIPENTUM®, Pharmacia & UpJohn); balsalazide (COLAZAL ®, Salix Pharmaceuticals, Inc.); and mesalamine (ASACOL ®, Procter & Gamble Pharmaceuticals; PENTASA ®, Shire US; CANASA ®, Axcan Scandipharm, Inc.; ROW ASA ®' Solvay).

[0197] In accordance with the methods of the present disclosure, a salicylate administered in combination with anti-LAG-3 and anti-CTLA-4 and/or anti-PD-1 and/oranti-PD-L1 antibodies and a non-absorbable steroid can include any overlapping or sequential administration of the salicylate and the non-absorbable steroid for the purpose of decreasing the incidence of colitis induced by the immunostimulatory antibodies. Thus, for example, methods for reducing the incidence of colitis induced by the immunostimulatory antibodies according to the present disclosure encompass administering a salicylate and a non-absorbable concurrently or sequentially (e.g., a salicylate is administered 6 hours after a non-absorbable steroid), or any combination thereof. Further, according to the present disclosure, a salicylate and a non-absorbable steroid can be administered by the same route (e.g., both are administered orally) or by different routes (e.g., a salicylate is administered orally and a non-absorbable steroid is administered rectally), which may differ from the route(s) used to administer the anti-LAG-3 and anti-CTLA-4 and/or anti-PD-1 and/or anti-PD-L1 antibodies.

Diagnostic Methods

[0198] Over-expression of LAG-3 is observed in certain tumor samples, and patients having LAG-3-over-expressing cells are likely responsive to treatments with the anti-LAG-3 antibodies of the present disclosure. Accordingly, the antibodies of the present disclosure can also be used for diagnostic and prognostic purposes.

[0199] A sample that preferably includes a cell can be obtained from a patient, which can be a cancer patient or a patient desiring diagnosis. The cell be a cell of a tumor tissue or a tumor block, a blood sample, a urine sample or any sample from the patient. Upon optional pre-treatment of the sample, the sample can be incubated with an antibody of the present disclosure under conditions allowing the antibody to interact with a LAG-3 protein potentially present in the sample. Methods such as ELISA can be used, taking advantage of the anti-LAG-3 antibody, to detect the presence of the LAG-3 protein in the sample.

[0200] Presence of the LAG-3 protein in the sample (optionally with the amount or concentration) can be used for diagnosis of cancer, as an indication that the patient is suitable for a treatment with the antibody, or as an indication that the patient has (or has not) responded to a cancer treatment. For a prognostic method, the detection can be done at once, twice or more, at certain stages, upon initiation of a cancer treatment to indicate the progress of the treatment.

[0201] The present disclosure is further illustrated by the following examples, which should not be construed as further limiting. The contents of all figures and all references, GenBank sequences, patents and published patent applications cited throughout this application are expressly incorporated herein by reference. In particular, the disclosures of PCT publications WO 09/045957, WO 09/073533, WO 09/073546, and WO 09/054863 are expressly incorporated herein by reference.

EXAMPLES

[0202] The following examples are included to demonstrate preferred embodiments of the disclosure. It should be appreciated by those of skill in the art that the techniques disclosed in the examples which follow represent techniques discovered by the inventors to function well in the practice of the disclosure, and thus can be considered to constitute preferred modes for its

practice. However, those of skill in the art should, in light of the present disclosure, appreciate that many changes can be made in the specific embodiments which are disclosed and still obtain a like or similar result without departing from the concept, spirit and scope of the disclosure. More specifically, it will be apparent that certain agents which are both chemically and physiologically related may be substituted for the agents described herein while the same or similar results would be achieved. All such similar substitutes and modifications apparent to those skilled in the art are deemed to be within the spirit, scope and concept of the disclosure as defined by the appended claims.

Example 1

Screening of full human monoclonal antibodies against LAG-3.

[0203] Anti-LAG3 human monoclonal antibodies (α-LAG-3 mAbs) were generated by screening full human Fab phage-display libraries. Wildtype LAG-3-ECD-huFc fragments can bind to Daudi cells while D1-D2 truncated LAG-3-ECD-huFc fragments fail to bind Daudi cells (**FIG. 1**). Consequently, the D1-D2 domains are critical for LAG-3 function.

[0204] Antigens for phage-display library-panning. LAG-3 is a single-pass type I membrane protein which belongs to the immunoglobulin (Ig) superfamily and contains 4 extracellular Ig-like domains (ECD): domain (D)1, D2, D3 and D4. A recombinant human LAG-3-ECD-human IgG1 (LAG-3-huFc) fusion protein or a human D1-D2 truncated LAG-3-ECD-human IgG1 (ΔD1D2-LAG-3-huFc) fusion protein were expressed in a 293T cell system.

[0205] Phage library. Ig gene segments in mammals are arranged in groups of variable (V), diversity (D), joining (J), and constant (C) exons. The human Fab phage libraries were construed using the phage vectors, which consists of: 1) all human variable kappa (VK) repertoires; and 2) the VH of VH3-23 and VH1-69 germline genes, respectively, with genetically randomized CDR3 regions from healthy human subjects.

[0206] Antigen screening and generation. To select the D1-D2 domain-specific phage binders, the phage libraries were subjected to antigen-based panning.

I) Phage library solution panning against LAG-3.

[0207] 293F cells were transfected with a plasmid containing a D1-D2 deleted LAG-3 (ΔD1D2-LAG-3) sequence with a FLAG-tag at the N-terminus. At 3 days post-transfection, the ΔD1D2-

LAG-3 293F cells were used for phage library screening. The phage libraries were performed the sequential negative screenings: streptavidin beads, △D1D2-LAG-3 transfected 293F cells and biotin-labeled-human IgG1Fc protein. The resulting library was then incubated with biotinylated LAG-3-huFc LAG-3 for 2 hrs under motion, followed by incubation with 100μL of casein blocked streptavidin-magnetic beads for 15 min. Unbound phages were removed by washing with PBS 5-20 times. The bound phages were then eluted with freshly prepared 100mM triethylamine (TEA) and neutralized with the addition of Tris-HCl buffer. The resulting phages were labeled as the Output-1 phage libraries. Output-1 phage libraries were subjected to the same screening as described above to generate the Output-2 and subsequent Output-3 phage libraries. Three rounds of phage library screening were performed in total.

II) Phage library immunotube panning against LAG-3.

[0208] The phage libraries were used to perform sequential negative screenings: casein-coated immunotubes, ΔD1D2-LAG-3 transfected 293F cells and human IgG1Fc protein. The resulting library was then incubated in LAG3-huFc-coated immunotubes for 2 hrs under motion. Unbound phages were removed by washing with PBST 5-20 times. Similar with cell-based panning, three rounds of phage library screening were performed in total.

[0209] Output-3 phage libraries were diluted and plated to grow at 37°C for 8 hrs and captured by anti-kappa antibody-coated filters overnight at 22°C. Biotinylated LAG-3-huFc (50nM) and NeutrAvidin-AP conjugate were applied to the filter to detect antigen binding anti-LAG3 phages. Positive phage plaques were picked and eluted into 100 μL of phage elution buffer. About 10-15 μL of eluted phages were then used to infect 1 mL of XL1-Blue competent cells to make a high-titer (HT) phage for phage single point ELISA (SPE) (ELISA immobilized substrate coated with 50 nM of each protein tested). $1x10^{10}$ plaque forming units (pfus) of each phage hit was used for SPE confirmation. The positive clones picked from the filter lift were then tested for LAG-3 antigen binding with LAG-3-huFc and ΔD1D2-LAG-3-huFc. The D1-D2 specific binders were amplified from antigen positive phages by PCR and sequenced. Ig light chain V genes (VL) and VH sequences were analyzed to identify unique sequences and determine sequence diversity.

[0210] VL and VH gene sequences of all hits were cloned into expression vectors pFUSE2ss-CLIg-hk (light chain, InvivoGen Cat No. pfuse2ss-hclk) and pFUSEss-CHIg-hG1 (heavy chain,

InvivoGen Cat No. pfusess-hchg1). The antibodies were expressed in HEK293 cells and purified using Protein A PLUS-Agarose. Sequences of the antibodies and their CDR regions are provided in the table below.

Table 1. Antibody heavy chain variable regions

Antibody No.	VH	SEQ ID NO:
NLAG3-HDB169-T03	QVQLVQSGAEVKKPGSSVKVSCKASGGTFSSYAISWVRQAPGQGLEWMGG IIPIFGTANYAQKFQGRVTITADESTSTAYMELSSLRSEDTAVYYCARGS SWFDYWGQGTLVTVSS	140
NLAG3-HDB169-T05	QVQLVQSGAEVKKPGSSVKVSCKASGGTFSSYAISWVRQAPGQGLEWMGG IIPIFGTANYAQKFQGRVTITADESTSTAYMELSSLRSEDTAVYYCASSY HGGGYHRYWGQGTLVTVSS	141
NLAG3-HDB169-T06	QVQLVQSGAEVKKPGSSVKVSCKASGGTFSSYAISWVRQAPGQGLEWMGG IIPIFGTANYAQKFQGRVTITADESTSTAYMELSSLRSEDTAVYYCTTSK YSGSALRYWGQGTLVTVSS	142
NLAG3-HDB169-T07	QVQLVQSGAEVKKPGSSVKVSCKASGGTFSSYAISWVRQAPGQGLEWMGG IIPIFGTANYAQKFQGRVTITADESTSTAYMELSSLRSEDTAVYYCARDR TGAFDYWGQGTLVTVSS	143
NLAG3-HDB169-T08	QVQLVQSGAEVKKPGSSVKVSCKASGGTFSSYAISWVRQAPGQGLEWMGG IIPIFGTANYAQKFQGRVTITADESTSTAYMELSSLRSEDTAVYYCARHE TVAGSFDYWGQGTLVTVSS	144
NLAG3-HDB169-T10	QVQLVQSGAEVKKPGSSVKVSCKASGGTFSSYAISWVRQAPGQGLEWMGG IIPIFGTANYAQKFQGRVTITADESTSTAYMELSSLRSEDTAVYYCARTG YYGGNSGAFDIWGQGTMVTVSS	145
NLAG3-HDB169-T13	QVQLVQSGAEVKKPGSSVKVSCKASGGTFSSYAISWVRQAPGQGLEWMGG IIPIFGTANYAQKFQGRVTITADESTSTAYMELSSLRSEDTAVYYCARAG TGMDLVFNSWGQGTLVTVSS	146
NLAG3-HDB169-T23	QVQLVQSGAEVKKPGSSVKVSCKASGGTFSSYAISWVRQAPGQGLEWMGG IIPIFGTANYAQKFQGRVTITADESTSTAYMELSSLRSEDTAVYYCARGL ARGDLNFGYWGQGTLVTVSS	147
NLAG3-HDB169-S24	QVQLVQSGAEVKKPGSSVKVSCKASGGTFSSYAISWVRQAPGQGLEWMGG IIPIFGTANYAQKFQGRVTITADESTSTAYMELSSLRSEDTAVYYCTREP HFDYWGQGTLVTVSS	148
NLAG3-HDB169-S27	QVQLVQSGAEVKKPGSSVKVSCKASGGTFSSYAISWVRQAPGQGLEWMGG IIPIFGTANYAQKFQGRVTITADESTSTAYMELSSLRSEDTAVYYCTTAA PGSYYLVFHYWGQGTLVTVSS	149
NLAG3-HDB169-S31	QVQLVQSGAEVKKPGSSVKVSCKASGGTFSSYAISWVRQAPGQGLEWMGG IIPIFGTANYAQKFQGRVTITADESTSTAYMELSSLRSEDTAVYYCARDA GPVGYYGMDVWGQGTTVTVSS	150
NLAG3-HDB169-S32	QVQLVQSGAEVKKPGSSVKVSCKASGGTFSSYAISWVRQAPGQGLEWMGG IIPIFGTANYAQKFQGRVTITADESTSTAYMELSSLRSEDTAVYYCAGDG LYGSGSFGYWGQGTPVTVSS	151
NLAG3-HDB169-S61	QVQLVQSGAEVKKPGSSVKVSCKASGGTFSSYAISWVRQAPGQGLEWMGG	152

	IIPIFGTANYAQKFQGRVTITADESTSTAYMELSSLRSEDTAVYYCAKDI RWFYGMDVWGQGTTVTVSSw	
NLAG3-HDB169-S64	QVQLVQSGAEVKKPGSSVKVSCKASGGTFSSYAISWVRQAPGQGLEWMGG IIPIFGTANYAQKFQGRVTITADESTSTAYMELSSLRSEDTAVYYCARHE SGIAGGHFDYWGQGTLVTVSS	153
NLAG3-HDB169-S86	QVQLVQSGAEVKKPGSSVKVSCKASGGTFSSYAISWVRQAPGQGLEWMGG IIPIFGTANYAQKFQGRVTITADESTSTAYMELSSLRSEDTAVYYCARDA GPVGYYGMDVWGQGTTVTVS	154
NLAG3-HDB169-S87	QVQLVQSGAEVKKPGSSVKVSCKASGGTFSSYAISWVRQAPGQGLEWMGG IIPIFGTANYAQKFQGRVTITADESTSTAYMELSSLRSEDTAVYYCAKDI RWYYGMDVWGQGTTVTVSS	155
NLAG3-HDB169-T94	QVQLVQSGAEVKKPGSSVKVFCKASGGTFSSYAISWVRQAPGQGLEWMGG IIPIFGTANYAQKFQGRVTITADESTSTAYMELSSLRSEDTAVYYCAKGV RGTYQIGYYGMDVWGQGTTVTVSS	156
NLAG3-HDB169-T97	QVQLVQSGAEVKKPGSSVKVSCKASGGTFSSYAISWVRQAPGQGLEWMGG IIPIFGTANYAQKFQGRVTITADESTSTAYMELSSLRSEDTAVYYCARQG TAMALDYWGQGTLVTVSS	157
NLAG3-HDB169-T99	QVQLVQSGAEVKKPGSSVKVSCKASGGTFSSYAISWVRQAPGQGLEWMGG IIPIFGTANYAQKFQGRVTITADESTSTAYMELSSLRSEDTAVYYCVRDL QDWNYGGAAYWGQGTLVTVSS	158
NLAG3-HDB169-S103	QVQLVQSGAEVKKPGSSVKVSCKASGGTFSSYAISWVRQAPGQGLEWMGG IIPIFGTANYAQKFQGRVTITADESTSTAYMELSSLRSEDTAVYYCARDD YYYGQFDSWGQGTLVTVSS	159
NLAG3-HDB169-S107	QVQLVQSGAEVKKPGSSVKVSCKASGGTFSSYAISWVRQAPGQGLEWMGG IIPIFGTANYAQKFQGRVTITADESTSTAYMELSSLRSEDTAVYYCAREI TGTSYTALDSWGQGTLVTVSS	160
NLAG3-HDB169-S109	QVQLVQSGAEVKKPGSSVKVSCKASGGTFSSYAISWVRQAPGQGLEWMGG IIPIFGTANYAQKFQGRVTITADESTSTAYMELSSLRSEDTAVYYCARGH IDGQAAGDYWGQGTLVTVSS	161
NLAG3-HDB169-S119	QVQLVQSGAEVKKPGSSVKVSCKASGGTFSSYAISWVRQAPGQGLEWMGG IIPIFGTANYAQKFQGRVTITADESTSTAYMELSSLRSEDTAVYYCAAST LRVPNPPYWGQGTLVTVSS	162
NLAG3-HDB169-S120	QVQLVQSGAEVKKPGSSVKVSCKASGGTFSSYAISWVRQAPGQGLEWMGG IIPIFGTANYAQKFQGRVTITADESTSTAYMELSSLRSEDTAVYYCARSG DRYDFWSGYWGQGTLVTVSS	163
NLAG3-HDB169-S127	QVQLVQSGAEVKKPGSSVKVSCKASGGTFSSYAISWVRQAPGQGLEWMGG IIPIFGTANYAQKFQGRVTITADESTSTAYMELSSLRSEDTAVYYCAAST LRVPNPPYWGQGTLVTVSS	164
NLAG3-HDB169-S128	QVQLVQSGAEVKKPGSSVKVSCKASGGTFSSYAISWVRQAPGQGLEWMGG IIPIFGTANYAQKFQGRVTITADESTSTAYMELSSLRSEDTAVYYCARDA	165

	GPVGYYGMDVWGQGTMVTVSS	
NLAG3-HDB169-S136	QVQLVQSGAEVKKPGSSVKVSCKASGGTFSSYAISWVRQAPGQGLEWMGG IIPIFGTANYAQKFQGRVTITADESTSTAYMELSSLRSEDTAVYYCTRGQ DSTWYSSFDYWGQGTLVTVSS	166
NLAG3-HDB169-S139	QVQLVQSGAEVKKPGSSVKVSCKASGGTFSSYAISWVRQAPGQGLEWMGG IIPIFGTANYAQKFQGRVTITADESTSTAYMELSSLRSEDTAVYYCAAST LRLPNPPYWGQGTLVTVSS	167
NLAG3-HDB169-S150	QVQLVQSGAEVKKPGSSVKVSCKASGGTFSSYAISWVRQAPGQGLEWMGG IIPIFGTANYAQKFQGRVTITADESTSTAYMELSSLRSEDTAVYYCATTQ TSFYSHGMDVWGQGTTVTVSS	168
NLAG3-HDB169-S157	QLLESGGGLVQPGGSLRLSCAASGFTFSSYAMSWVRQAPGKGLEWVSAIS GSGGSTYYADSVKGRFTISRDNSKNTLYLQMNSLRAEDTAVYYCARVRKT PFWGALDSWGRGTLVTVSS	169
NLAG3-HDB169-S164	QVQLVQSGAEVKKPGSSVKVSCKASGGTFSSYAISWVRQAPGQGLEWMGG IIPIFGTANYAQKFQGRVTITADESTSTAYMELSSLRSEDTAVYYCARGF TYGDFIFDYWGQGTLVTVSS	170
NLAG3-HDB169-S177	QVQLVQSGAEVKKPGSSVKVSCKASGGTFSSYAISWVRQAPGQGLEWMGG IIPIFGTANYAQKFQGRVTITADESTSTAYMELSSLRSEDTAVYYCARDV RGVTYLGMDVWGQGTTVTVSS	171
NLAG3-HDB323-S20	QLLESGGGLVQPGGSLRLSCAASGFTFSSYAMSWVRQAPGKGLEWVSAIS GSGGSTYYADSVKGRFTISRDNSKNTLYLQMNSLRAEDTAVYYCARVRKT PFWGTLDSWGRGTLVTVSS	172
NLAG3-HDB323-S21	QLLESGGGLVQPGGSLRLSCAASGFTFSSYAMSWVRQAPGKGLEWVSAIS GSGGSTYYADSVKGRFTISRDNSKNTLYLQMNSLRAEDTAVYYCARVRRT PFWGALDSWGRGTLVTVSS	173
NLAG3-HDB323-S32	QLLESGGGLVQPGGSLRLSCAASGFTFSSYAMSWVRQAPGKGLEWVSAIS GSGGSTYYADSVKGRFTISRDNSKNTLYLQMNSLRAEDTAVYYCARVRKT PFWGALDSWGRGTLVTVSS	174
NLAG3-HDB323-S35	QLLESGGGLVQPGGSLRLSCAASGFTFSSYAMSWVRQAPGKGLEWVSAIS GSGGSTYYADSVKGRFTISRDNSKNTLYLQMNSLRAEDTAVYYCAKRKGL GSPTDYYYGMDVWGQGTTVTVSS	175
NLAG3-HDB323-S52	QLLESGGGLVQPGGSLRLSCAASGFTFSSYAMSWVRQAPGKGLEWVSAIS GSGGSTYYADSVKGRFTISRDNSKNTLYLQMNSLRAEDTAVYYCARVRKT PFWGALDSWGRGTLVTVSS	176
NLAG3-HDB323-S55	QLLESGGGLVQPGGSLRLSCAASGFTFSSYAMSWVRQAPGKGLEWVSAIS GSGGSTYYADSVKGRFTISRDNSKNTLYLQMNSLRAEDTAVYYCARVRKT PFWGTLDSWGRGSLVTVSS	177
NLAG3-HDB323-T89	QLLESGGGLVQPGGSLRLSCAASGFTFSSYAMSWVRQAPGKGLEWVSAIS GSGGSTYYADSVKGRFTISRDNSKNTLYLQMNSLRAEDTAVYYCVRPEYD TYYYGMDVWGQGTTVTVSS	178

NLAG3-HDB323-T92	QLLESGGGLVQPGGSLRLSCAASGFTFSSYAMSWVRQAPGKGLEWVSAIS GSGGSTYYADSVKGRFTISRDNSKNTLYLQMNSLRAEDTAVYYCAKGGGS YDYWGQGTLVTVSS	179
NLAG3-HDB323-T94	QLLESGGGLVQPGGSLRLSCAASGFTFSSYAMSWVRQAPGKGLEWVSAIS GSGGSTYYADSVKGRFTISRDNSKNTLYLQMNSLRAEDTAVYYCARALNG MDVWGQGTMVTVSS	180
NLAG3-HDB323-S102	QLLESGGGLVQPGGSLRLSCAASGFTFSSYAMSWVRQAPGKGLEWVSAIS GSGGSTYYADSVKGRFTISRDNSKNTLYLQMNSLRAEDTAVYYCTRPLQG IAAADSYYYYAMDVWGQGTTVTVSS	181
NLAG3-HDB323-S103	QLLESGGGLVQPGGSLRLSCAASGFTFSSYAMSWVRQAPGKGLEWVSAIS GSGGSTYYADSVKGRFTISRDNSKNTLYLQMNSLRAEDTAVYYCARLHSY LSEEFDPWGQGTLVTVSS	182
NLAG3-HDB323-S107	QLLESGGGLVQPGGSLRLSCAASGFTFSSYAMSWVRQAPGKGLEWVSAIS GSGGSTYYADSVKGRFTISRDNSKNTLYLQMNSLRAEDTAVYYCARVRKT PFWGALDSWGRGTLVTVSS	183
NLAG3-HDB323-S114	QLLESGGGLVQPGGSLRLSCAASGFTFSSYAMSWVRQAPGKGLEWVSAIS GSGGSTYYADSVKGRFTISRDNSKNTLYLQMNSLRAEDTAVYYCAKLSAV NTYIDDWGQGTLVTVSS	184
NLAG3-HDB323-S135	QLLESGGGLVQPGGSLRLSCAASGFTFSSYAMSWVRQAPGKGLEWVSAIS GSGGSTYYADSVKGRFTISRDNSKNTLYLQMNSLRAEDTAVYYCARVTKT PFWGTLDYWGQGTLVTVSS	185
NLAG3-HDB323-S143	QLLESGGGLVQPGGSLRLSCAASGFTFSSYAMSWVRQAPGKGLEWVSAIS GSGGSTYYADSVKGRFTISRDNSKNTLYLQMNSLRAEDTAVYYCARVRRT PFWGALDSWGRGTLVTVSS	186
NLAG3-HDB323-S146	QLLESGGGLVQPGGSLRLSCAASGFTFSSYAMSWVRQAPGKGLEWVSAIS GSGGSTYYADSVKGRFTISRDNSKNTLYLQMNSLRAEDTAVYYCARVSQS PVWGYFDYWGQGMLVTVSS	187
NLAG3-HDB323-S161	QLLESGGGLVQPGGSLRLSCAASGFTFSSYAMSWVRQAPGKGLEWVSAIS GSGGSTYYADSVKGRFTISRDNSKNTLYLQMNSLRAEDTAVYYCAKDGYY DFWSGYSDYWGQGTLVTVSS	188

Table 2. Heavy chain CDRs

Antibody No.	CDR H1	SEQ ID NO:	CDR H2	SEQ ID NO:	CDR H3	SEQ ID NO:
NLAG3-HDB169-T03	SYAIS	1	GIIPIFGTANYAQKFQG	3	ARGSSWFDY	5
NLAG3-HDB169-T05	SYAIS	1	GIIPIFGTANYAQKFQG	3	ASSYHGGGYHRY	6
NLAG3-HDB169-T06	SYAIS	1	GIIPIFGTANYAQKFQG	3	TTSKYSGSALRY	7

NLAG3-HDB169-T07	SYAIS	1	GIIPIFGTANYAQKFQG	3	ARDRTGAFDY	8
NLAG3-HDB169-T08	SYAIS	1	GIIPIFGTANYAQKFQG	3	ARHETVAGSFDY	9
NLAG3-HDB169-T10	SYAIS	1	GIIPIFGTANYAQKFQG	3	ARTGYYGGNSGAFDI	10
NLAG3-HDB169-T13	SYAIS	1	GIIPIFGTANYAQKFQG	3	ARAGTGMDLVFNS	11
NLAG3-HDB169-T23	SYAIS	1	GIIPIFGTANYAQKFQG	3	ARGLARGDLNFGY	12
NLAG3-HDB169-S24	SYAIS	1	GIIPIFGTANYAQKFQG	3	TREPHFDY	13
NLAG3-HDB169-S27	SYAIS	1	GIIPIFGTANYAQKFQG	3	TTAAPGSYYLVFHY	14
NLAG3-HDB169-S31	SYAIS	1	GIIPIFGTANYAQKFQG	3	ARDAGPVCYYGMDV	15
NLAG3-HDB169-S32	SYAIS	1	GIIPIFGTANYAQKFQG	3	AGDGLYGSGSFGY	16
NLAG3-HDB169-S61	SYAIS	1	GIIPIFGTANYAQKFQG	3	AKDIRWFYGMDV	17
NLAG3-HDB169-S64	SYAIS	1	GIIPIFGTANYAQKFQG	3	ARHESGIAGGHFDY	18
NLAG3-HDB169-S86	SYAIS	1	GIIPIFGTANYAQKFQG	3	ARDAGPVCYYGMDV	15
NLAG3-HDB169-S87	SYAIS	1	GIIPIFGTANYAQKFQG	3	AKDIRWYYGMDV	19
NLAG3-HDB169-T94	SYAIS	1	GIIPIFGTANYAQKFQG	3	AKGVRGTYQIGYYGMDV	20
NLAG3-HDB169-T97	SYAIS	1	GIIPIFGTANYAQKFQG	3	ARQGTAMALDY	21
NLAG3-HDB169-T99	SYAIS	1	GIIPIFGTANYAQKFQG	3	VRDLQDWNYGGAAY	22
NLAG3-HDB169-S103	SYAIS	1	GIIPIFGTANYAQKFQG	3	ARDDYYYCQFDS	23
NLAG3-HDB169-S107	SYAIS	1	GIIPIFGTANYAQKFQG	3	AREITGTSYTALDS	24
NLAG3-HDB169-S109	SYAIS	1	GIIPIFGTANYAQKFQG	3	ARGHIDGQAAGDY	25
NLAG3-HDB169-S119	SYAIS	1	GIIPIFGTANYAQKFQG	3	AASTLRVPNPPY	26
NLAG3-HDB169-S120	SYAIS	1	GIIPIFGTANYAQKFQG	3	ARSGDRYDFWSGY	27
NLAG3-HDB169-S127	SYAIS	1	GIIPIFGTANYAQKFQG	3	AASTLRVPNPPY	26
NLAG3-HDB169-S128	SYAIS	1	GIIPIFGTANYAQKFQG	3	ARDAGPVCYYGMDV	15
NLAG3-HDB169-S136	SYAIS	1	GIIPIFGTANYAQKFQG	3	TRGQDSTWYSSFDY	28
NLAG3-HDB169-S139	SYAIS	1	GIIPIFGTANYAQKFQG	3	AASTLRLPNPPY	29
NLAG3-HDB169-S150	SYAIS	1	GIIPIFGTANYAQKFQG	3	ATTQTSFYSHGMDV	30
NLAG3-HDB169-S157	SYAIS	1	GIIPIFGTANYAQKFQG	3	ARVRKTPFWGALDS	31
NLAG3-HDB169-S164	SYAIS	1	GIIPIFGTANYAQKFQG	3	ARGFTYGDFIFDY	32
NLAG3-HDB169-S177	SYAIS	1	GIIPIFGTANYAQKFQG	3	ARDVRGVTYLGMDV	33
NLAG3-HDB323-S20	SYAMS	2	AISGSGGSTYYADSVKG	4	ARVRKTPFWGTLDS	34
NLAG3-HDB323-S21	SYAMS	2	AISGSGGSTYYADSVKG	4	ARVRRTPFWGALDS	35

NLAG3-HDB323-S32	SYAMS	2	AISGSGGSTYYADSVKG	4	ARVRKTPFWGALDS	31
NLAG3-HDB323-S35	SYAMS	2	AISGSGGSTYYADSVKG	4	AKRKGLGSPTDYYYGMDV	36
NLAG3-HDB323-S52	SYAMS	2	AISGSGGSTYYADSVKG	4	ARVRKTPFWGALDS	31
NLAG3-HDB323-S55	SYAMS	2	AISGSGGSTYYADSVKG	4	ARVRKTPFWGTLDS	34
NLAG3-HDB323-T89	SYAMS	2	AISGSGGSTYYADSVKG	4	VRPEYDTYYYGMDV	37
NLAG3-HDB323-T92	SYAMS	2	AISGSGGSTYYADSVKG	4	AKGGGSYDY	38
NLAG3-HDB323-T94	SYAMS	2	AISGSGGSTYYADSVKG	4	ARALNGMDV	39
NLAG3-HDB323-S102	SYAMS	2	AISGSGGSTYYADSVKG	4	TRPLQGIAAADSYYYYAMDV	40
NLAG3-HDB323-S103	SYAMS	2	AISGSGGSTYYADSVKG	4	ARLHSYLSEEFDP	41
NLAG3-HDB323-S107	SYAMS	2	AISGSGGSTYYADSVKG	4	ARVRKTPFWGALDS	31
NLAG3-HDB323-S114	SYAMS	2	AISGSGGSTYYADSVKG	4	AKLSAVNTYIDD	42
NLAG3-HDB323-S135	SYAMS	2	AISGSGGSTYYADSVKG	4	ARVTKTPFWGTLDY	43
NLAG3-HDB323-S143	SYAMS	2	AISGSGGSTYYADSVKG	4	ARVRRTPFWGALDS	35
NLAG3-HDB323-S146	SYAMS	2	AISGSGGSTYYADSVKG	4	ARVSQSPVWGYFDY	44
NLAG3-HDB323-S161	SYAMS	2	AISGSGGSTYYADSVKG	4	AKDGYYDFWSGYSDY	45

Table 3. Light chain variable regions

Antibody No.	VL	SEQ ID NO:
NLAG3-HDB169-T03	DIQLTQSPSSLSAFVGDRVTITCQANQDIHHYLNWYQQKPGKAPKLLIYD ASILQSGVPSRFSGSGSGTDFTLTISSLQPEDFATYFCQQADSFPITFGQ GTRLEIKR	189
NLAG3-HDB169-T05	EIVLTQSPDSLAVSLGERATINCKSSQSVLYSSSNKNYLAWYQQKPGQPP KLLIYWASTRESGVPDRFSGSGSGTDFTLTISSLQPEDFATYYCQQSYST PWTFGPGTKLEIKR	190
NLAG3-HDB169-T06	DIQMTQSPDSLAVSLGERATINCKSSQSVLYSSNNKNYLAWYQQKPGHPP KLLVYWASTRESGVPARFSASGSGTDFTLAISNLQAEDVAVYYCQQYYST PWTFGQGTKVEIKR	191
NLAG3-HDB169-T07	EIVLTQSPLSLPVTPGEPASISCRSSQNLLHSDGYNYLNWYLQKPGQSPQ LLIYLGSNRATGVPDRFSGSGSGTDFTLTISSLQPEDFATYYCQQSYSTP WTFGQGTKVEIKR	192
NLAG3-HDB169-T08	DIVMTQSPDSLAVSLGERATINCKSSQSVLYTSNNKNYLAWYQQKPGQPP KLLIYWASTRESGVPDRFSGSGSGTDFTLTISSLQAEDVAIYYCQQYYST PWTFGQGTKLEIKR	193

NLAG3-HDB169-T10	AIQLTQSPDSLAVSLGERATINCKSSQSVLYSSNNKNYLAWYQQKPGQPP KLLIYWASTRESGVPDRFSGSGSGTDFTLTISSLQAEDSATYYCQQSFTT PWTFGQGTKVEIKR	194
NLAG3-HDB169-T13	DIQMTQSPSSLSASVGDRVTITCQASQDINRYLSWYQQKPGKAPKLLIYD ASNLETGVPSRFSGSASGTDFTFAISSLQPEDIATYYCQQYDNLPPTFGQ GTRLEIKR	195
NLAG3-HDB169-T23	EIVMTQSPSSLSASVGDRVTITCQASQDISNYLNWYQQKPGKAPKLLIYA ASSLQSGVPSRFSGSGSGTDFTLTISSLQPEDFASYYCQQSYGSPVTFGQ GTKLEIKR	196
NLAG3-HDB169-S24	EIVMTQSPSSLSASVGDRVTITCQASQDISNYLNWYQQKPGKAPKLLIYD ASNLETGVPSRFSGSGSGTEFTLTISSLRPEDFATYFCQQADSFPITFGQ GTRLEIKR	197
NLAG3-HDB169-S27	DIQLTQSPSSLSASVGDRVTITCRASQTISSHLNWYQQKPGKAPKVLIYA ASSLQSGVPSRFSGSGSGTEFTLTISSLQPDDFATYYCQQGNSFPFTFGP GTKVEIKR	198
NLAG3-HDB169-S31	AIRMTQSPSTLSASVGDRVTITCRASQGIAGWLAWYQQKPGKAPKLLIYA ASSLQSGVPSRFSGSASGTDFTLTISNLQPEDFATYYCQQAKSFPLTFGG GTKVEIKR	199
NLAG3-HDB169-S32	DIVMTQSPDSLAVSLGERATINCKSSQSVLYSSNNKNYLAWYQQKPGQPP KLLIYWASTRESGVPDRFSGTGSGTDFTLTISSLQAEDVAVYYCQQSYST PWTFGQGTKLEIK	200
NLAG3-HDB169-S61	DIVMTQSPSSVSAFVGDRVTITCRASQGVSSWLAWFQQKPGKAPKLLIYA ASTLQSGVPSRFSGRGYGTEFTLTISSLQPEDLATYYCQQVKSFPLTFGG GTKVDIKR	201
NLAG3-HDB169-S64	DIVMTQSPDSLAVSLGERATINCKSSQSLFYHSNNHNYLAWYQQKPGQPP KLLIYWASTRQSGVPDRFTGSGSGTDFTLTISSLQAEDVAVYYCQQYYNT PWTFGQGTKVEIKR	202
NLAG3-HDB169-S86	AIRMTQSPSTLSASVGDRVTITCRASQGIAGWLAWYQQKPGKAPKLLIYA ASSLQSGVPSRFSGSASGTDFTLTISNLQPEDFATYYCQQAKSFPLTFGG GTKVEIKR	203
NLAG3-HDB169-S87	DIVMTQSPSSVSAFVGDRVTITCRASQGVSSWLAWFQQKPGKAPKLLIYA ASTLQSGVPSRFSGRGYGTEFTLTISSLQPEDLATYYCQQVKSFPLTFGG GTKVDIKR	204
NLAG3-HDB169-T94	DIVMTQSPSSLSASVGDRVTITCRASQGISSSLAWYQQKPGKAPNLLIYT ASTLQNGVPSRFSGSGSGTDFTLTISGLQPEDFATYYCQQTKNFPLTFGQ GTRLEIKR	205
NLAG3-HDB169-T97	EIVLTQSPDSLAVSLGERATINCKSSQSVLYSSNNKNYLAWYQQRPGQPP KLLISWASTRESGVPDRFSGSGSGADFSLTISSLQAEDVAVYYCQQYYST PWTFGQGTKLEIKR	206
NLAG3-HDB169-T99	VIWMTQSPSSLSASVGDSVTITCQASRDISNSLSWHQQKPGKAPKLLIYA	207

	ASSLQSGVPSRFSGSGSGTDFTLTISSLQPEDFATYYCQQTKSFPLTFGG GTKVEIKR	
NLAG3-HDB169-S103	EIVMTQSPSSLSASVGDRVTISCRASQSISRYLNWYQQKPGQAPKLLIYA AFSLQSGVPSRFSGSGSGTDFTLTISSLQPEDFATYYCQQSYNTPRTFGQ GTKLEIKR	208
NLAG3-HDB169-S107	DVVMTQSPSTVSASVGDRITITCRASRSISNWLAWYQQKPGKAPKLLIYA ASSLQSGVPSRFSGSGSGTDFTLTISSLQPEDFATYYCQQAKSFPLTFGG GTKVEIK	209
NLAG3-HDB169-S109	DIQLTQSPDSLAVSLGERATINCKSSQSVFYRSNQKNYLAWYQQKPGQTP RLLIYGASSRATGIPDRFSGSGSGTDFTLTISSLQPEDFATYYCQQSYRA PWTFGQGTKVEIKR	210
NLAG3-HDB169-S119	EIVLTQSPGTLSLSPGERATLSCRASQSVSSYLAWYQQKPGQAPRLLIYG ISSRATGIPDRFSGSGSGTDFTLTISSLQPEDFATYYCQQANNFPLTFGG GTKLEIKR	211
NLAG3-HDB169-S120	EIVLTQSPSSVSASVGDRVTITCRASRGISSWLAWYQQKPGKAPKLLIYA ASTLQSGVPSRFSGSGSGTDFTLTISSLQPEDFATYYCQQAKSFPLTFGG GTKVEIKR	212
NLAG3-HDB169-S127	EIVLTQSPGTLSLSPGERATLSCRASQSVSSYLAWYQQKPGQAPRLLIYG ISSRATGIPDRFSGSGSGTDFTLTISSLQPEDFATYYCQQANNFPLTFGG GTKLEIKR	213
NLAG3-HDB169-S128	AIQMTQSPSSLSASVGDRVTITCRASQGISSWLAWYQQKPGKAPKLLIYA ASSLQSGVPSRFSGSGSGTDFTLTISRLQPEDFATYYCQQAKSFPLTFGG GTKVEIKR	214
NLAG3-HDB169-S136	AIRMTQSPSSLSASVGDRVTITCRASQSISSYLNWYQQKPGKAPNLLIYA VSTLQSGVPSRFSGSGSGTVFTLTISSLQPEDFATYFCQQGNSFPLTFGG GTKVEIKR	215
NLAG3-HDB169-S139	DIQLTQSPSTLSASVGDRVTITCRASQAISNLLAWYQQKPGKPPNLLIYD ISTLQNGVPSRFSGSGSGTDFTLTINSLQPEDFAIYYCQQSKNFPVTFGG GTKVEIKR	216
NLAG3-HDB169-S150	DIQLTQSPSSVSASVGDRVTITCRASQGISSWLAWYQQKPGKAPKLLIYG ASTLQSGVPSRFSGSGSGADYTLTISSLQPEDFATYYCQQANSFPLTFAG GTKLEIKR	217
NLAG3-HDB169-S157	DIQLTQSPSSLSASPGDRVTITCRASQGISTWLAWYQQKPGNAPKLLIYA ASSLQSGVPSRFSGSKSGTEYTLTISSLQPEDFATYYCQQLESYPLTFGG GTKVEIKR	218
NLAG3-HDB169-S164	AIRMTQSPDSLVVSLGERATINCKSSQSVLYSSNNKNYLAWYQQKPGQPP KLLIYWASTRESGVPDRFSGSGSGTDFTLSISSLQAEDVAVYYCQQYYSS PTFGGGTKVEIKR	219
NLAG3-HDB169-S177	DVVMTQSPFFLSASVGDRVTITCRASQGIASNLAWYQQKPGKAPKLLIYA ASTLQSGVPSRFTGSGSGTEFTLTVTSLQPEDFATYYCQQLKTFPLTFGG	220

	GTKVEIKR	
NLAG3-HDB323-S20	VIWMTQSPSSLSASVGDRVTITCRASQGVSSYLAWYQQKPGKAPKLLIYA ASSLQSGVPSRFSGSGSGTEFTLTISSLQPEDFATYYCQQTNWFPLTFGP GTRLEIKR	221
NLAG3-HDB323-S21	DIQMTQSPSSLSTSAGDTVTITCRASQSIYTYLNWYQQKPGKAPNLLIYG ASSLQSGVPSRFSGSGSGTDFTLTISSLQPEDFATYYCQQAQSFPITFGQ GTRLEIKR	222
NLAG3-HDB323-S32	VIWMTQSPSSVSASVGDRVTITCRASQGISSWLAWYQQKPGKAPKLLIYA ASSLQSGVPSRFSGSGSGTDFTLTISSLQPEDFATYYCQQAHSFPLTFGG GTKVEIKR	223
NLAG3-HDB323-S35	AIQLTQSPSTLSASVGDRVTITCRASQFVSDWLAWYQQKPGKAPKLLIYA ASTLQSGVPSRFSGSGSGTDFTLTISSLQPEDLATYYCLQDYHFPLTFGG GTKLEIKR	224
NLAG3-HDB323-S52	DVVMTQSPSSVSASVGDRVTITCRASQDIVNWLAWYQQKPGKAPKLLIYA ASTLESGAPSRFSASGSGTDFTLTISSLQPDDFATYYCQQGHSFPLTFGP GTKLEIKR	225
NLAG3-HDB323-S55	DIVMTQSPSSLSASVGDRVTITCRASQSIYTYLNWYQQKPGKAPKLLIYD ASSLQSGVPSRFSGSGYGTEFTLTISGLQPEDFATYYCQQSYIFPLTFGR GTKVEIKR	226
NLAG3-HDB323-T89	AIRMTQSPSFVSASVGDRVTIACRASQTISTWLAWYQQKPGKAPKVLISK ASNLQSGVPSRFSGSGSGTEFTLTISSLQPDDFATYYCQQYDTYWTFGQG TKVEIKR	227
NLAG3-HDB323-T92	AIRMTQSPSFVSASVGDRVTIACRASQTISTWLAWYQQKPGKAPKVLISK ASNLQSGVPSRFSGSGSGTEFTLTISSLQPDDFATYYCQQYDTYWTFGQG TKVEIKR	228
NLAG3-HDB323-T94	DIVMTQSPSFVSASVGDTVTITCRASQGISSYLAWYQQKPGKAPKLLIYA ASTLQSGVPSRFSGSGSGTEFTLTISSLQPEDFATYYCQQLNSYPLFTFG PGTKVEIKR	229
NLAG3-HDB323-S102	DIQMTQSPSTLSASVGDRVTITCRASQSIGYWLAWYQQKPGKAPKLLIYR ASSLQSGVPSRFSGSGSATEFTLTITSLQPDDFATYFCQQYSSYWTFGQG TKVEIKR	230
NLAG3-HDB323-S103	EIVLTQSPSSLSASVGDTVTITCRATQSISSWLAWYQQKPGKAPQRLISG ASTLQSGVPSRFSGSGSGTEFTLTISGLQPEDFATYYCLQHNTYPFTFGQ GTKVEIKR	231
NLAG3-HDB323-S107	DIVMTQSPSSVSASVGDRVTITCRASQGVRNWLAWYQQKPGKAPKLLIYA ASHLQSGVPSRFSGSGSGTDFTLTISSLQTDDFATYYCQQGHSFPLTFGG GTKVEIKR	232
NLAG3-HDB323-S114	DIVMTQSPSSVSASVGDRVTITCRASQGVRNWLAWYQQKPGKAPKLLIYA ASHLQSGVPSRFSGSGSGTDFTLTISSLQTDDFATYYCQQGHSFPLTFGG GTKVEIKR	233

NLAG3-HDB323-S135	VIWMTQSPSTLSASVGDRVTITCRASQSINNYLAWYQQKPGKAPKLLIYD ASTLQSGVPSRFSGGGSGTDFTLTINSLQPDDFASYYCQQAHSFPFTFGG GTKLEIKR	234
NLAG3-HDB323-S143	EIVMTQSPSSVSASVGDRVTITCRASQDITSWLAWYQQKPGKAPKLLIYA ASTLESGVPSRFSGSGSGTDFTLTITGLQPEDFATYYCQQANMFPLTFGG GTKVEIKR	235
NLAG3-HDB323-S146	AIRMTQSPSSLSASVGDRVTITCRASQGIYDYLAWYQQKPGKAPSLLIYA ASNLERGVPSRFSGSGSGKYFILTISSLQPEDFATYYCQQANSFPLTFGG GTKVEIKR	236
NLAG3-HDB323-S161	AIQLTQSPSSLSASVGDRVTITCRASEGISGWLAWYQQIPGKAPKLLIYA ASSLETGVPSRFSGSGYGTDFTLTISSLQPEDFATYYCQQADSFPFTFGP GTKVEIKR	237

Table 4. Light chain variable regions

Antibody No.	CDR L1	SEQ ID NO:	CDR L2	SEQ ID NO:	CDR L3	SEQ ID NO:
NLAG3-HDB169-T03	QANQDIHHYLN	46	DASILQS	81	QQADSFPIT	104
NLAG3-HDB169-T05	KSSQSVLYSSSNKNYLA	47	WASTRES	82	QQSYSTPWT	105
NLAG3-HDB169-T06	KSSQSVLYSSNNKNYLA	48	WASTRES	82	QQYYSTPWT	106
NLAG3-HDB169-T07	RSSQNLLHSDGYNYLN	49	LGSNRAT	83	QQSYSTPWT	105
NLAG3-HDB169-T08	KSSQSVLYTSNNKNYLA	50	WASTRES	82	QQYYSTPWT	106
NLAG3-HDB169-T10	KSSQSVLYSSNNKNYLA	48	WASTRES	82	QQSFTTPWT	107
NLAG3-HDB169-T13	QASQDINRYLS	51	DASNLET	84	QQYDNLPPT	108
NLAG3-HDB169-T23	QASQDISNYLN	52	AASSLQS	85	QQSYGSPVT	109
NLAG3-HDB169-S24	QASQDISNYLN	52	DASNLET	84	QQADSFPIT	104
NLAG3-HDB169-S27	RASQTISSHLN	53	AASSLQS	85	QQGNSFPFT	110
NLAG3-HDB169-S31	RASQGIAGWLA	54	AASSLQS	85	QQAKSFPLT	111
NLAG3-HDB169-S32	KSSQSVLYSSNNKNYLA	48	WASTRES	82	QQSYSTPWT	105
NLAG3-HDB169-S61	RASQGVSSWLA	55	AASTLQS	86	QQVKSFPLT	112
NLAG3-HDB169-S64	KSSQSLFYHSNNHNYLA	56	WASTRQS	87	QQYYNTPWT	113
NLAG3-HDB169-S86	RASQGIAGWLA	54	AASSLQS	85	QQAKSFPLT	111
NLAG3-HDB169-S87	RASQGVSSWLA	55	AASTLQS	86	QQVKSFPLT	112
NLAG3-HDB169-T94	RASQGISSSLA	57	TASTLQN	98	QQTKNFPLT	114

NLAG3-HDB169-T97	KSSQSVLYSSNNKNYLA	48	WASTRES	82	QQYYSTPWT	106
NLAG3-HDB169-T99	QASRDISNSLS	58	AASSLQS	85	QQTKSFPLT	116
NLAG3-HDB169-S103	RASQSISRYLN	59	AAFSLQS	88	QQSYNTPRT	117
NLAG3-HDB169-S107	RASRSISNWLA	60	AASSLQS	85	QQAKSFPLT	111
NLAG3-HDB169-S109	KSSQSVFYRSNQKNYLA	61	GASSRAT	89	QQSYRAPWT	118
NLAG3-HDB169-S119	RASQSVSSYLA	62	GISSRAT	90	QQANNFPLT	119
NLAG3-HDB169-S120	RASRGISSWLA	63	AASTLQS	86	QQAKSFPLT	111
NLAG3-HDB169-S127	RASQSVSSYLA	62	GISSRAT	90	QQANNFPLT	119
NLAG3-HDB169-S128	RASQGISSWLA	64	AASSLQS	85	QQAKSFPLT	111
NLAG3-HDB169-S136	RASQSISSYLN	65	AVSTLQS	91	QQGNSFPLT	120
NLAG3-HDB169-S139	RASQAISNLLA	66	DISTLQN	92	QQSKNFPVT	121
NLAG3-HDB169-S150	RASQGISSWLA	64	GASTLQS	93	QQANSFPLT	122
NLAG3-HDB169-S157	RASQGISTWLA	67	AASSLQS	85	QQLESYPLT	123
NLAG3-HDB169-S164	KSSQSVLYSSNNKNYLA	48	WASTRES	82	QQYYSSPT	124
NLAG3-HDB169-S177	RASQGIASNLA	68	AASTLQS	86	QQLKTFPLT	125
NLAG3-HDB323-S20	RASQGVSSYLA	69	AASSLQS	85	QQTNWFPLT	126
NLAG3-HDB323-S21	RASQSIYTYLN	70	GASSLQS	94	QQAQSFPIT	127
NLAG3-HDB323-S32	RASQGISSWLA	64	AASSLQS	85	QQAHSFPLT	128
NLAG3-HDB323-S35	RASQFVSDWLA	71	AASTLQS	86	LQDYHFPLT	129
NLAG3-HDB323-S52	RASQDIVNWLA	115	AASTLES	95	QQGHSFPLT	130
NLAG3-HDB323-S55	RASQSIYTYLN	70	DASSLQS	96	QQSYIFPLT	131
NLAG3-HDB323-T89	RASQTISTWLA	72	KASNLQS	97	QQYDTYWT	132
NLAG3-HDB323-T92	RASQTISTWLA	72	KASNLQS	97	QQYDTYWT	132
NLAG3-HDB323-T94	RASQGISSYLA	73	AASTLQS	86	QQLNSYPLFT	133
NLAG3-HDB323-S102	RASQSIGYWLA	74	RASSLQS	99	QQYSSYWT	134
NLAG3-HDB323-S103	RATQSISSWLA	75	GASTLQS	93	LQHNTYPFT	135
NLAG3-HDB323-S107	RASQGVRNWLA	76	AASHLQS	100	QQGHSFPLT	130
NLAG3-HDB323-S114	RASQGVRNWLA	76	AASHLQS	100	QQGHSFPLT	136
NLAG3-HDB323-S135	RASQSINNYLA	77	DASTLQS	101	QQAHSFPFT	137
NLAG3-HDB323-S143	RASQDITSWLA	78	AASTLES	95	QQANMFPLT	138
NLAG3-HDB323-S146	RASQGIYDYLA	79	AASNLER	102	QQANSFPLT	122

NLAG3-HDB323-S161	RASEGISGWLA	80	AASSLET	103	QQADSFPFT	139

Example 2

The binding of human anti-LAG3 antibodies to LAG3 protein derived from various species.

[0211] To evaluate the capability of the anti-LAG-3 antibodies to bind to human, rat, and mouse LAG3 the antibodies identified in Example 1 were evaluated for their binding property through ELISA. The human, rat and mouse LAG3 ECD-Fc protein were coated to ELISA plate at 1μg/ml with 100μl/well. Antibodies from Example 1 were serially diluted with ELISA diluent buffer. To assess binding, LAG-3 antibodies at various concentrations 10 μg/ml, 3.333 μg/ml, 1.111 μg/ml, 0.370 μg/ml, 0.123 μg/ml, 0.041 μg/ml, 0.014 μg/ml, 0.005 μg/ml, 0.0015 μg/ml and 0.0005 μg/ml) were then added to LAG3 antigen coated plate for 1.5hr RT. The resulting plates were washed and then labeled with anti-human IgG(Fab)-HRP antibody. The S31 can only bind to human LAG3. The S27 and T99 can bind to human LAG3 and rat/mouse LAG3 with lower potency. The S119 antibody can bind to human, rat and mouse LAG3 at high potency (**FIG. 2**).

Example 3

The binding of human anti-LAG3 antibodies to cell surface LAG-3 antigen on activated human primary $CD4^+$ T cells.

[0212] LAG-3 is expressed on activated or exhausted T cells. CD4+ T cells were isolated using CD4 magnetic beads. The purified human CD4+ T cells were stimulated with Dynabeads® Human T-Activator CD3/CD28 for 72 hrs. Antibodies from Example 1 were serially diluted with FACS buffer. To assess binding, LAG-3 antibodies at various concentrations (10 μg/ml, 3.333 μg/ml, 1.111 μg/ml, 0.370 μg/ml, 0.123 μg/ml, 0.041 μg/ml, 0.014 μg/ml and 0.005 μg/ml) were then added to the activated human CD4 T cells in the presence of mouse anti-human LAG3 PE antibody (eBioscience, clone: 3DS223H) for 30 min on ice. The labeled cells were washed with FACS buffer and subsequently labeled with APC-conjugated anti-human IgG antibodies for 30 min on ice. The resulting cells were washed once with FACS buffer. Labeled cells were evaluated for fluorescence intensity by flow cytometry in a BD FACSCalibur™. As shown in **FIG. 3**, the S27, S31, T99 and S119 antibodies can dose-dependently bind to LAG3 expressed on the activated human $CD4^+$ T cells.

Example 4

Anti-LAG-3 antibody inhibition of soluble LAG-3 (sLAG) binding to MHC class II receptor.

[0213] To evaluate the ability of anti-LAG-3 antibodies to block the binding of sLAG-3 to MHC class II receptor, an *in vitro* binding assay was designed using biotin-labeled LAG-3-ECD-huFc fusion proteins and Raji cells expressing MHC class II receptor. Antibodies from Example 1 were serially diluted from 20μg/mL with FACS buffer and pre-incubated with 6 μg/mL of biotin-LAG-3-ECD-huFcc for 30 min at room temperature. The antibody mixture was then added to FcR blocked Raji cells and incubated for 30 min on ice. Cells were then washed with FACS buffer and subsequently stained with streptavidin PE for 30 min on ice and subsequently washed once with FACS buffer. Labeled cells were evaluated for fluorescence intensity by flow cytometry in a BD FACSCalibur™. As shown in **FIG. 4**, the S27, S31, S119 and T99 antibodies can dose dependently inhibit the binding of LAG3 to its receptor MHC class II molecules.

Example 5

Stimulation of IL-2 production in peripheral blood mononuclear cells (PBMCs) by anti-LAG-3 antibodies.

[0214] Staphylococcal enterotoxin B (SEB) is a superantigen that simultaneously binds to MHC class II antigens and T cell receptors (TCRs), bringing them together in such a way as to induce T cell proliferation and cytokine production. 2×10^5 PBMCs were stimulated with SEB in the presence of the antibodies from Example 1 at various concentrations starting from 20μg/ml at 1:3 serial dilutions for 6 doses. Three days later, IL-2 concentration in the culture supernatant was evaluated by ELISA. As shown in **FIG. 5**, similar to PD-1 antibody, anti-LAG3 antibodies(S24, S27, S31, S87, S119, T99 and S20) can dose dependently enhanced IL-2 production as compared with SEB stimulation only.

Example 6

Reversing the inhibition of regulatory T cells (T_{regs}) on effector T cells (T_{effs}) using anti- LAG-3 antibodies.

[0215] LAG-3 is highly expressed on T_{regs} ($CD4^+CD25^{hi}$) and mediates their suppressive function (*Journal of Immunology* 184:6545-51, 2010). To evaluate the ability of anti-LAG-3 antibodies on reversing the suppressive effect of T_{regs} on effector T cells ($CD4^+CD25^-CD127^{hi}$), antibodies of Example 1 were used in an *in vitro* suppression assay. First, T_{regs} ($CD4^+CD25^{hi}CD127^{low}$) and T_{effs} ($CD4^+CD25^-CD127^{hi}$) were FACS-sorted by using a BD FACSAria II system. T_{effs} were then labeled with carboxyfluorescein succinimidyl ester (CFSE)

and co-cultured with T_{regs} at a 1:1 ratio in the presence of plate bound anti-CD3 antibodies and mitomycin C-treated antigen presenting cells. Anti-LAG-3 antibodies were next added to the cell culture and T_{effs} cell proliferation were tested 5 days later. The results in **FIG. 6**, indicate that when Tregs were co-cultured with effector T cells, effector T cell proliferation and cytokine production was inhibited. S119 and T99 can reverse the inhibition of Teffs by Tregs.

Example 7

Synergistic effect of anti-LAG3 and PD-1 antibody combo treatment.

[0216] Staphylococcal enterotoxin B (SEB) is a superantigen that stimulate the human immune response. PD-1 blocking antibody can enhance the SEB stimulated IL-2 production. As shown in Example 5, anti-LAG3 antibodies can also enhance SEB mediated IL-2 production. To explore the effect of anti-LAG3 antibodies in combination with PD-1 antibody, we investigated the effect of anti-LAG3 antibody on SEB stimulation in the presence of suboptimal PD-1 stimulation. In the presence of 0.1μg/ml PD-1 antibody, serial diluted anti-LAG3 antibodies were added to the SEB culture. IL-2 production was evaluated 72hr later. The results in **FIG. 7** indicate that anti-LAG-3 antibodies can enhance SEB stimulated T cell response in a dose-dependent manner in the presence of suboptimal PD-1 treatment, suggesting that anti-LAG3 and anti-PD-1 combo treatment have synergistic effect.

Example 8

Anti-LAG-3 antibodies enhance human T cell response in the presence of PD-L1 antibody.

[0217] To evaluate the effect of anti-LAG-3 antibodies in combination with PD-L1 antibody, the response of human T cells was assessed in a mixed lymphocyte reaction setting. Human DCs were differentiated from $CD14^+$ monocytes in the presence of GM-CSF and IL-4 for 7 days. $CD4^+$ T cells isolated from another donor were then co-cultured with DCs and serially dilutedanti-LAG-3 antibodies and PD-L1 blocking antibody. 2 days after mixed culture, , the culture supernatant was assayed for IL-2 production. The results in **FIG. 8** indicate that anti-LAG-3 antibodies can significantly promote IL-2 production in conjunction with a PD-L1 antibody.

Example 9

LAG-3 antibody BIACORE Analysis

[0218] The binding of the S20, S24, S27, S31, S87, S119, S120, S128, S136, S161 and T99 antibodies to recombinant his-tag human LAG3-ECD protein was examined by Biacore T200 using a capture method. Anti-LAG3 antibodies were captured using anti-human Fc antibody. The anti-human Fc antibody was coated on chip. Serial concentrations of his-tag human LAG3-ECD protein (0-4nM) were injected over capture antibodies at the flow rate of 30 μl/min. The dissociation phase was 900s or 550s. The results are shown in the table below. The Biacore results for the anti-LAG3 antibodies have shown that these anti-LAG3 antibodies are high affinity binder to human LAG3.

	K_a ($M^{-1}s^{-1}$)	k_d (s^{-1})	K_D (M)
S20	1.65E+05	7.33E-06	4.43E-11
S24	1.79E+06	1.20E-02	6.73E-09
S27	7.04E+06	1.10E-04	1.56E-11
S31	2.08E+06	6.25E-05	3.00E-11
S87	9.28E+05	2.33E-06	2.51E-12
S119	2.17E+07	1.49E-04	6.87E-12
S120	1.40E+06	2.64E-03	1.88E-09
S128	1.00E+06	8.17E-04	8.15E-10
S136	7.98E+05	8.27E-05	1.04E-10
S161	6.20E+05	5.53E-04	8.92E-10
T99	7.62E+06	1.70E-04	2.24E-11

Example 10

Generation of mouse monoclonal antibodies against human LAG3

[0219] This example shows how anti-human-LAG3 mouse monoclonal antibodies were generated using hybridoma technology.

[0220] Antigen: Recombinant human LAG-3 fusion proteins were used as the immunogen to raise anti-human LAG-3 antibodies. A fusion protein comprising the entire extracellular region (domains 1-4) of human LAG-3 fused to a mouse immunoglobulin Fc domain (D1-D4 mFc) was used as the immunogen. For the ELISA binding test, a fusion protein comprising entire extracellular region (domains 1-4) or extracellular region without D1-D2 domain of human LAG-3 fused to human immunoglobulin Fc domain (D1-D4 huFc or △D1-D2 huFc respectively). The LAG-3 fusion proteins were prepared using standard recombinant DNA techniques.

Immunizations:

[0221] The LAG-3 fusion proteins were prepared using standard recombinant DNA techniques. Mice were immunized intraperitoneally (IP) and/or subcutaneously (SC). The mice were firstly SC immunized 50mg immunogen and then IP immunized biweekly with 25μg immunogen. The immune response was monitored by retroorbital bleeds. The plasma was screened by ELISA and cell-based receptor blocking assay (as described below). Mice with sufficient titers of anti-LAG-3 D1-D2 domain immunoglobulin and functional LAG3 blocker were used for fusions. Prior to sacrifice and removal of the spleens, the mice were boosted intraperitoneally with 25 μg of antigen followed by a subsequent boost with μg of antigen. The spleens were used for fusion. The hybridoma supernatant was tested for anti-LAG-3 D1-D2 domain binding and its function to block the binding of LAG3 to its receptor by cell based receptor blocking assay.

Selection of mice producing anti-LAG3 blocking antibodies.

[0222] To select mice producing anti-LAG3 blocking antibodies, sera from immunized mice was tested for binding to D1-D2 domain by ELISA. Briefly, sera were evaluated for their binding to D1-D4 huFc and its binding to△D1-D2 huFc was served as a counter screen. In short, D1-D4 huFc or △D1-D2 huFc was coated at 0.5μg/ml overnight and then blocked by 5% BSA in PBS. The serially diluted sera were incubated with the coated antigen for 1h at room temperature. The resulting plates were washed with PBS/T and incubated with goat anti-mouse IgG-HRP for 1h at room temperature. The plates were developed with TMB substrate and analyzed by spectrophotometer at OD 450-630nm. In parallel, sera were evaluated to their function to blocking the binding of LAG3 to MHCII molecules expressed on Raji cells as described Example 4. The mice with high titers specific to LAG3 D1-D2 domain and function to block the binding of LAG3 to Raji cells were selected for fusion and further screening.

[0223] Hybridoma clones 122H, 147H and 170H were selected for further analysis and sequencing.

Example 11

Binding properties of anti-LAG3 mouse monoclonal antibodies

[0224] This example tested the binding properties of the anti-LAG3 mouse antibodies to the LAG3 proteins.

D1-D2 specific binders:

[0225] To evaluate the binding specificity, the purified 122H, 147H and 170H mouse monoclonal antibodies were subjected to ELISA binding test for D1-D4 huFc and ▵D1-D2 huFc antigens. Briefly, D1-D4 huFc or ▵D1-D2 huFc was coated at 0.5μg/ml overnight and then blocked by 5% BSA in PBS. The serially diluted antibodies (starting from 1μg/ml and 1:3 serialdilution for 10 doses) were incubated with the coated antigen for 1hr at room temperature. The resulting plates were washed with PBS/T and incubated with goat anti-mouse IgG-HRP for 1h at room temperature. the plates were developed with TMB substrate and analyzed by spectrophotometer at OD 450-630nm.

[0226] The results of the ELISA are summarized in **FIG. 9**, which show strong binding to full extracellular domain of LAG3 (D1-D4 huFc) but not D1-D2 deleted LAG3 (▵D1-D2 huFc), confirm that 122H, 147H and 170H are potent and selective binder for D1 and D2 domain of human LAG3.

Example 12

Functional properties of anti-LAG3 mouse monoclonal antibodies

Blocking the binding of LAG3 to its receptor

[0227] To evaluate the ability of anti-LAG-3 antibodies to block the binding of sLAG-3 to MHC class II receptor, an *in vitro* binding assay was designed using biotin-labeled LAG-3-ECD-huFc fusion proteins and Raji cells expressing MHC class II receptor. 122H, 147H and 170H mouse monoclonal antibodies were serially diluted (1:5 for 6 doses) from 20μg/mL with FACS buffer and pre-incubated with 6 μg/mL of biotin-LAG-3-ECD-huFc for 30 min at room temperature. The antibody mixture was then added to FcR blocked Raji cells and incubated for 30 min on ice. Cells were then washed with FACS buffer and subsequently stained with streptavidin PE for 30 min on ice and subsequently washed once with FACS buffer. Labeled cells were evaluated for

fluorescence intensity by flow cytometry in a BD FACSCalibur™. As shown in **FIG. 10**, the 122H, 147H and 170H antibodies can dose dependently inhibit the binding of LAG3 to its receptor MHC class II molecules.

Stimulation of human T cell response by anti-LAG3 antibodies

[0228] To test the ability of the anti-LAG3 antibodies to stimulated T cell response, Jurkat T cell stimulation assay was used. Jurkat is human T cell leukemia cell line that can produce IL2 upon TCR stimulation. In this assay, Jurkat cells transfected with human LAG3 gene by lentivirus were used as the responder cells. The Raji cells which expressed MHCII was used as the antigen presenting cells (APC). Staphylococcal Enterotoxins (SE) are superantigen, which can crosslink the MHCII molecules and T cell receptor beta (TCRVβ) and stimulate T cell response. SE was used as the stimulator in this assay. In this system, ectopically expressed huLAG3 can suppress SE stimulated IL-2 production by Jurkat cells, while anti-LAG3 antibodies can reverse IL-2 production. In short, APCs (2.5×10^4) were co-cultured with LAG3 expressing Jurkat T cells (1×10^5) in the presence of SE stimulation. Anti-LAG3 antibodies (starting from 20ug/ml and 1:5 serially diluted for 6 dose) were added at the beginning of the culture. 48hr later, culture supernatant was evaluated for IL2 production by ELISA. As shown in **FIG. 11**, 122H, 147H and 170H mouse monoclonal antibodies can dose dependently promote IL2 production by Jurkat T cells, suggesting they can stimulate TCR stimulation by suppressing LAG3 signal to T cells.

Example 13

147H mouse mAb humanization design

[0229] The mAb 147H variable region genes were employed to create a humanized mAb. In the first step of this process, the amino acid sequences of the VH and VK of mAb 147H were compared against the available database of human Ig gene sequences to find the overall best-matching human germline Ig gene sequences. For the light chain, the closest human match was the A19/JK4 gene, and for the heavy chain the closest human match was the VH1-f/JH6 gene. Humanized variable domain sequences were then designed where the CDR1 (SEQ ID NO:243), 2 (SEQ ID NO:244) and 3 (SEQ ID NO:245) of the 147H light chain were grafted onto framework sequences of the A19/JK4 gene, and the CDR1 (SEQ ID NO:240), 2 (SEQ ID NO:241), and 3 (SEQ ID NO:242) sequences of the 147H VH were grafted onto framework

sequences of the VH1-f/JH6 gene. A 3D model was then generated to determine if there were any framework positions where replacing the mouse amino acid to the human amino acid could affect binding and/or CDR conformation. In the case of the heavy chain, R71, M69, R66, V67, M48, V37, R38, Y91 and Q1 (Kabat numbering) in human framework were identified and subjected to back-mutation to their mouse counterpart amino acid i.e.: R71A, M69L, R66K, V67A, M48I, V37I, R38K, Y91F and Q1E.

Table 5. Mouse antibody sequences

Antibody chain or domain	Sequences (CDR residues with VH and VL are underlined)	SEQ ID NO:
147H VH	QVQLQQSGSE LVRPGTSVKI SCKASGYTFT NYWLGWIKQR PGHGLEWIGD IYPGGDYINY NEKFKGKATL SADTSSSTAY MQLSSLTSED SAVYFCARPN LPGDYWGQGT SVTVSS	238
147H VL	DIVMTQAAFS NPVTLGTSAS ISCRSSKSLL HSNGITYLYW YLQKPGQSPQ LLIYQVSNLA SGVPGRFSGS GSGTDFTLRI SRVEAEDVGV YYCAQNLELP WTFGGGTKLE IK	239
CDRH1	GYTFTNYWLG	240
CDRH2	DIYPGGDYIN YNEKFKG	241
CDRH3	PNLPGDY	242
CDRL1	RSSKSLLHSN GITYLY	243
CDRL2	QVSNLAS	244
CDRL3	AQNLELPWT	245

[0230] The amino acid sequences of the humanized antibodies are listed: 147H-1, 147H-2, 147H-3, 147H-4, 147H-5, 147H-6, 147H-7, 147H-8, 147H-9, 147H-10, 147H-11, 147H-12, 147H-13, and 147H-14, each having a different heavy chain but all share a common light chain.

Table 6. Humanized antibodies and back mutations

Antibody chain	Sequences (CDR underlined; back mutations bold and underlined)	SEQ ID NO:
147H-1 VH	QVQLVQSGAE VKKPGASVKV SCKASGYTFT NYWLGWVRQA PGQGLEWMGD IYPGGDYINY NEKFKGRVTM TRDTSISTAY MELSRLRSDD TAVYYCARPN LPGDYWGQGT TVTVSS	246

147H-2 VH	QVQLVQSGAE VKKPGASVKV SCKASGYTFT NYWLGWVRQA PGQGLEWMGD IYPGGDYINY NEKFKGRVTM TADTSISTAY MELSRLRSDD TAVYYCARPN LPGDYWGQGT TVTVSS	247
147H-3 VH	QVQLVQSGAE VKKPGASVKV SCKASGYTFT NYWLGWVRQA PGQGLEWMGD IYPGGDYINY NEKFKGRVTL TADTSISTAY MELSRLRSDD TAVYYCARPN LPGDYWGQGT TVTVSS	248
147H-4 VH	QVQLVQSGAE VKKPGASVKV SCKASGYTFT NYWLGWVRQA PGQGLEWMGD IYPGGDYINY NEKFKGKATL TADTSISTAY MELSRLRSDD TAVYYCARPN LPGDYWGQGT TVTVSS	249
147H-5 VH	QVQLVQSGAE VKKPGASVKV SCKASGYTFT NYWLGWVRQA PGQGLEWIGD IYPGGDYINY NEKFKGKATL TADTSISTAY MELSRLRSDD TAVYYCARPN LPGDYWGQGT TVTVSS	250
147H-6 VH	QVQLVQSGAE VKKPGASVKV SCKASGYTFT NYWLGWIKQA PGQGLEWIGD IYPGGDYINY NEKFKGKATL TADTSISTAY MELSRLRSDD TAVYYCARPN LPGDYWGQGT TVTVSS	251
147H-7 VH	QVQLVQSGAE VKKPGASVKV SCKASGYTFT NYWLGWIKQA PGQGLEWIGD IYPGGDYINY NEKFKGKATL TADTSISTAY MELSRLRSDD TAVYFCARPN LPGDYWGQGT TVTVSS	252
147H-8 VH	EVQLVQSGAE VKKPGASVKV SCKASGYTFT NYWLGWVRQA PGQGLEWMGD IYPGGDYINY NEKFKGRVTM TRDTSISTAY MELSRLRSDD TAVYYCARPN LPGDYWGQGT TVTVSS	253
147H-9 VH	EVQLVQSGAE VKKPGASVKV SCKASGYTFT NYWLGWVRQA PGQGLEWMGD IYPGGDYINY NEKFKGRVTM TADTSISTAY MELSRLRSDD TAVYYCARPN LPGDYWGQGT TVTVSS	254
147H-10 VH	EVQLVQSGAE VKKPGASVKV SCKASGYTFT NYWLGWVRQA PGQGLEWMGD IYPGGDYINY NEKFKGRVTL TADTSISTAY MELSRLRSDD TAVYYCARPN LPGDYWGQGT TVTVSS	255
147H-11 VH	EVQLVQSGAE VKKPGASVKV SCKASGYTFT NYWLGWVRQA PGQGLEWMGD IYPGGDYINY NEKFKGKATL TADTSISTAY MELSRLRSDD TAVYYCARPN LPGDYWGQGT TVTVSS	256
147H-12 VH	EVQLVQSGAE VKKPGASVKV SCKASGYTFT NYWLGWVRQA PGQGLEWIGD IYPGGDYINY NEKFKGKATL TADTSISTAY MELSRLRSDD TAVYYCARPN LPGDYWGQGT TVTVSS	257
147H-13	EVQLVQSGAE VKKPGASVKV SCKASGYTFT NYWLGWIKQA PGQGLEWIGD	258

VH	IYPGGDYINY NEKFKGKATL TADTSISTAY MELSRLRSDD TAVYYCARPN LPGDYWGQGT TVTVSS	
147H-14 VH	EVQLVQSGAE VKKPGASVKV SCKASGYTFT NYWLGWIKQA PGQGLEWIGD IYPGGDYINY NEKFKGKATL TADTSISTAY MELSRLRSDD TAVYFCARPN LPGDYWGQGT TVTVSS	259
147H VL	DIVMTQSPLS LPVTPGEPAS ISCRSSKSLL HSNGITYLYW YLQKPGQSPQ LLIYQVSNLA SGVPDRFSGS GSGTDFTLKI SRVEAEDVGV YYCAQNLELP WTFGGGTKVE IK	260

[0231] The humanized VH and VK genes were produced synthetically and then respectively cloned into vectors containing the human gamma 1 and human kappa constant domains. The pairing of the human VH and the human VK created 40 humanized antibodies.

Example 14

Binding properties of anti-LAG3 147H humanized monoclonal antibodies

Affinity ranking of humanized antibodies by Octet® RED96 System

[0232] To explore the binding kinetics of the humanized antibody, this example performed the affinity ranking by using Octet Red 96. As shown in the table below, 147H-6, 147H-7, 147H-13 and 147H-14 show better affinity.

Antibody	KD (M)	kon(1/Ms)	kdis(1/s)
147H-1	3.54E-08	1.09E+05	3.86E-03
147H-2	3.16E-08	9.93E+04	3.14E-03
147H-3	3.65E-08	9.25E+04	3.38E-03
147H-4	3.98E-08	8.62E+04	3.43E-03
147H-5	3.13E-08	9.58E+04	3.00E-03
147H-6	1.53E-08	1.20E+05	1.84E-03
147H-7	1.57E-08	1.52E+05	2.39E-03
147H-8	3.23E-08	1.65E+05	5.33E-03
147H-9	6.64E-08	6.74E+04	4.48E-03
147H-10	8.23E-08	4.91E+04	4.04E-03
147H-11	4.22E-08	1.07E+05	4.51E-03

147H-12	5.52E-08	6.23E+04	3.44E-03
147H-13	2.16E-08	1.08E+05	2.34E-03
147H-14	2.32E-08	1.08E+05	2.50E-03

Full kinetic affinity of humanized antibodies by Octet® RED96 System

[0233] To explore the binding kinetics of the humanized antibody, this example further performed the full kinetic affinity testing by running various dose of antigen (50 nM, 25 nM, 12.5 nM, 6.15 nM, 3.125 nM) by using Octet Red 96. The binding affinity was calculated by software in Octet® RED96 System. As shown in the table, 147H-6, 147H-7, 147H-13 and 147H-14 showed comparable affinity with 147H chimeric antibody.

Antibody	KD (M)	kon(1/Ms)	kdis(1/s)
147H chimeric	2.71E-08	8.01E+04	2.17E-03
147H-6	2.48E-08	1.05E+05	2.59E-03
147H-6	2.65E-08	1.18E+05	3.12E-03
147H-13	1.82E-08	1.04E+05	1.90E-03
147H-14	2.07E-08	9.87E+04	2.04E-03

Example 15

Functional properties of anti-LAG3 mouse monoclonal antibodies

Stimulation of human T cell response by anti-LAG3 antibodies

[0234] To test the ability of anti-LAG3 antibodies to stimulated T cell response, Jurkat T cell stimulation assay was used as described in Example 12. Anti-LAG3 antibodies (starting from 30μg/ml and 1:3 serially diluted for 6 doses) were added at the beginning of the culture. 48hr later, culture supernatant was evaluated for IL2 production by ELISA. As shown in **FIG. 12**, 147H-13 humanized monoclonal antibodies can dose dependently promote IL2 production by Jurkat T cells, suggesting they can stimulate the TCR stimulation by suppressing LAG3 signal to T cells.

Example 16

Affinity maturation of anti-LAG3 147H humanized monoclonal antibodies

[0235] To improve antigen binding affinity, this example performed affinity maturation of 147H4-13 using phage display technology. Strategy 1: The CDRH3 and CDRL3 of 147H-13 were targeted for codon-based mutagenesis. CDRH3 and CDRL3 were randomized at position H95-H102 and L89-L97 (Kabat numbering), respectively. Strategy 2: Each CDR was targeted for single codon based mutagenesis using CDR walking approach. Then CDRH1, CDRH2, CDRL1 combined to library 1. The CDRH3, CDRL2, CDRL3 combined to library 2.

[0236] In both strategies, libraries were subject to three or four rounds of affinity-based solution-phase phage display selection with decreasing concentration of antigen at each round. A relatively high antigen concentration (10 nM) was used for the first round. The antigen concentration was decreased 10-fold each of the subsequent three rounds or 100-fold each the subsequent two rounds to select for high affinity variants. Individual variants from the final round were tested for positive binding to antigen by ELISA screening. Off-rate ranking of individual variants was determined by Octet Red 96 (Fortebio, USA). Mutations with improved affinity were combined to generate new LAG3 antibodies. Affinity was further confirmed by Biacore which suggested N58V of CDR H2 significantly increased Koff, while N91Y of CDR L3 improved Kon.

Table 7. Antibody affinity maturation

No.	Sequence (CDR underlined, mutation bold)
147H3421	VH: (SEQ ID NO: 261) EVQLVQSGAE VKKPGASVKV SCKASGYTFT NYWLGWIKQA PGQGLEWIGD IYPGGDYINY NEKFKGKATL TADTSISTAY MELSRLRSDD TAVYYCARPN LP**K**D**H**WGQGT TVTVSS VL: (SEQ ID NO: 262) DIVMTQSPLS LPVTPGEPAS ISCRSSKSLL HSNAITYLYW YLQKPGQSPQ LLIYQVSNLA SGVPDRFSGS GSGTDFTLKI SRVEAEDVGV YYCAQNLELP WTFGGGTKVE IK
147H 3422	VH: (SEQ ID NO: 263) EVQLVQSGAE VKKPGASVKV SCKASGYTFT NYWLGWIKQA PGQGLEWIGD IYPGGDYINY NEKFKGKATL TADTSISTAY MELSRLRSDD TAVYYCARP**D** LPGDYWGQGT TVTVSS

	VL: (SEQ ID NO: 264) DIVMTQSPLS LPVTPGEPAS ISCRSSKSLL HSNAITYLYW YLQKPGQSPQ LLIYQVSNLA SGVPDRFSGS GSGTDFTLKI SRVEAEDVGV YYCAQNLELP WTFGGGTKVE IK
147H 3423	VH: (SEQ ID NO: 265) EVQLVQSGAE VKKPGASVKV SCKASGYTFT NYWLGWIKQA PGQGLEWIGD IYPGGDYINY NEKFKGKATL TADTSISTAY MELSRLRSDD TAVYYCARPG LPKDYWGQGT TVTVSS VL: (SEQ ID NO: 266) DIVMTQSPLS LPVTPGEPAS ISCRSSKSLL HSNAITYLYW YLQKPGQSPQ LLIYQVSNLA SGVPDRFSGS GSGTDFTLKI SRVEAEDVGV YYCAQNLELP WTFGGGTKVE IK
147H 3424	VH: (SEQ ID NO: 267) EVQLVQSGAE VKKPGASVKV SCKASGYTFT NYWLGWIKQA PGQGLEWIGD IYPGGDYINY NEKFKGKATL TADTSISTAY MELSRLRSDD TAVYYCARPN LPKDYWGQGT TVTVSS VL: (SEQ ID NO: 268) DIVMTQSPLS LPVTPGEPAS ISCRSSKSLL HSNAITYLYW YLQKPGQSPQ LLIYQVSNLA SGVPDRFSGS GSGTDFTLKI SRVEAEDVGV YYCAQNLELP WTFGGGTKVE IK
147H 3425	VH: (SEQ ID NO: 269) EVQLVQSGAE VKKPGASVKV SCKASGYTFT NYWLGWIKQA PGQGLEWIGD IYPGGDYINY NEKFKGKATL TADTSISTAY MELSRLRSDD TAVYYCARPN LPRDYWGQGT TVTVSS VL: (SEQ ID NO: 270) DIVMTQSPLS LPVTPGEPAS ISCRSSKSLL HSNAITYLYW YLQKPGQSPQ LLIYQVSNLA SGVPDRFSGS GSGTDFTLKI SRVEAEDVGV YYCAQNLELP WTFGGGTKVE IK
147H 3426	VH: (SEQ ID NO: 271) EVQLVQSGAE VKKPGASVKV SCKASGYTFT NYWLGWIKQA PGQGLEWIGD IYPGGDYINY NEKFKGKATL TADTSISTAY MELSRLRSDD TAVYYCARPG LPRDYWGQGT TVTVSS VL: (SEQ ID NO: 272) DIVMTQSPLS LPVTPGEPAS ISCRSSKSLL HSNAITYLYW YLQKPGQSPQ LLIYQVSNLA SGVPDRFSGS GSGTDFTLKI SRVEAEDVGV YYCAQNLELP WTFGGGTKVE IK

147H 3427	VH: (SEQ ID NO: 273) EVQLVQSGAE VKKPGASVKV SCKASGYTFT NYWLGWIKQA PGQGLEWIGD IYPGGDYINY NEKFKGKATL TADTSISTAY MELSRLRSDD TAVYYCARP**G** LP**Q**DYWGQGT TVTVSS VL: (SEQ ID NO: 274) DIVMTQSPLS LPVTPGEPAS ISCRSSKSLL HSNAITYLYW YLQKPGQSPQ LLIYQVSNLA SGVPDRFSGS GSGTDFTLKI SRVEAEDVGV YYCAQNLELP WTFGGGTKVE IK
147H 3428	VH: (SEQ ID NO: 275) EVQLVQSGAE VKKPGASVKV SCKASGYTFT NYWLGWIKQA PGQGLEWIGD IYPGGDYINY NEKFKGKATL TADTSISTAY MELSRLRSDD TAVYYCARP**D** LP**K**DYWGQGT TVTVSS VL: (SEQ ID NO: 276) DIVMTQSPLS LPVTPGEPAS ISCRSSKSLL HSNAITYLYW YLQKPGQSPQ LLIYQVSNLA SGVPDRFSGS GSGTDFTLKI SRVEAEDVGV YYCAQNLELP WTFGGGTKVE IK
147H 3429	VH: (SEQ ID NO: 277) EVQLVQSGAE VKKPGASVKV SCKASGYTFT NYWLGWIKQA PGQGLEWIGD IYPGGDYINY NEKFKGKATL TADTSISTAY MELSRLRSDD TAVYYCARPN LPGDYWGQGT TVTVSS VL: (SEQ ID NO: 278) DIVMTQSPLS LPVTPGEPAS ISCRSSKSLL HSNAITYLYW YLQKPGQSPQ LLIYQVSNLA SGVPDRFSGS GSGTDFTLKI SRVEAEDVGV YYC**G**QNLELP WTFGGGTKVE IK
147H 3430	VH: (SEQ ID NO: 279) EVQLVQSGAE VKKPGASVKV SCKASGYTFT NYWLGWIKQA PGQGLEWIGD IYPGGDYINY NEKFKGKATL TADTSISTAY MELSRLRSDD TAVYYCARPN LPGDYWGQGT TVTVSS VL: (SEQ ID NO: 280) DIVMTQSPLS LPVTPGEPAS ISCRSSKSLL HSNAITYLYW YLQKPGQSPQ LLIYQVSNLA SGVPDRFSGS GSGTDFTLKI SRVEAEDVGV YYCAQNLE**M**P WTFGGGTKVE IK
147H 3431	VH: (SEQ ID NO: 281) EVQLVQSGAE VKKPGASVKV SCKASGYTFT NYWLGWIKQA PGQGLEWIGD IYPGGDYINY NEKFKGKATL TADTSISTAY MELSRLRSDD TAVYYCARPN LPGDYWGQGT TVTVSS

	VL: (SEQ ID NO: 282) DIVMTQSPLS LPVTPGEPAS ISCRSSKSLL HSNAITYLYW YLQKPGQSPQ LLIYQVSNLA SGVPDRFSGS GSGTDFTLKI SRVEAEDVGV YYC**G**QNLE**M**P WTFGGGTKVE IK
147H 3432	VH: (SEQ ID NO: 283) EVQLVQSGAE VKKPGASVKV SCKASGYTFT NYWLGWIKQA PGQGLEWIGD IYPGGDYINY NEKFKGKATL TADTSISTAY MELSRLRSDD TAVYYCARPN LPGDYWGQGT TVTVSS VL: (SEQ ID NO: 284) DIVMTQSPLS LPVTPGEPAS ISCRSSKSLL HSNAITYLYW YLQKPGQSPQ LLIYQVSNLA SGVPDRFSGS GSGTDFTLKI SRVEAEDVGV YYCAQ**Y**LE**E**P WTFGGGTKVE IK
147H 3433	VH: (SEQ ID NO: 285) EVQLVQSGAE VKKPGASVKV SCKASGYTFT NYWLGWIKQA PGQGLEWIGD IYPGGDYINY NEKFKGKATL TADTSISTAY MELSRLRSDD TAVYYCARPN LPGDYWGQGT TVTVSS VL: (SEQ ID NO: 286) DIVMTQSPLS LPVTPGEPAS ISCRSSKSLL HSNAITYLYW YLQKPGQSPQ LLIYQVSNLA SGVPDRFSGS GSGTDFTLKI SRVEAEDVGV YYCAQ**Y**LELP WTFGGGTKVE IK
147H 3508	VH: (SEQ ID NO: 287) EVQLVQSGAE VKKPGASVKV SCKASGYTFT NYWLGWIKQA PGQGLEWIGD IYPGGDYINY NEKFKGKATL TADTSISTAY MELSRLRSDD TAVYYCARPN LP**K**D**H**WGQGT TVTVSS VL: (SEQ ID NO: 288) DIVMTQSPLS LPVTPGEPAS ISCRSSKSLL HSNAITYLYW YLQKPGQSPQ LLIYQVSNLA SGVPDRFSGS GSGTDFTLKI SRVEAEDVGV YYC**G**QNLELP WTFGGGTKVE IK
147H 3549	VH: (SEQ ID NO: 289) EVQLVQSGAE VKKPGASVKV SCKASGYTFT NYWLGWIKQA PGQGLEWIGD IYPGGDYINY NEKFKGKATL TADTSISTAY MELSRLRSDD TAVYYCARPN LP**K**D**H**WGQGT TVTVSS VL: (SEQ ID NO: 290) DIVMTQSPLS LPVTPGEPAS ISCRSSKSLL HSNAITYLYW YLQKPGQSPQ LLIYQVSNLA SGVPDRFSGS GSGTDFTLKI SRVEAEDVGV YYCAQ**Y**LE**E**P WTFGGGTKVE IK

147H 3550	VH: (SEQ ID NO: 291) EVQLVQSGAE VKKPGASVKV SCKASGYTFT NYWLGWIKQA PGQGLEWIGD IYPGGDYINY NEKFKGKATL TADTSISTAY MELSRLRSDD TAVYYCARPN LP**K**D**H**WGQGT TVTVSS VL: (SEQ ID NO: 292) DIVMTQSPLS LPVTPGEPAS ISCRSSKSLL HSNAITYLYW YLQKPGQSPQ LLIYQVSNLA SGVPDRFSGS GSGTDFTLKI SRVEAEDVGV YYCAQ**Y**LELP WTFGGGTKVE IK
147H 3663	VH: (SEQ ID NO: 293) EVQLVQSGAE VKKPGASVKV SCKASGYTF**E** NYWLGWIKQA PGQGLEWIGD IYPGGDYI**V**Y NEKFKGKATL TADTSISTAY MELSRLRSDD TAVYYCARPN LPGDYWGQGT TVTVSS VL: (SEQ ID NO: 294) DIVMTQSPLS LPVTPGEPAS ISCRSSKSLL HSNAITYLYW YLQKPGQSPQ LLIYQVSNLA **R**GVPDRFSGS GSGTDFTLKI SRVEAEDVGV YYCAQNLELP WTFGGGTKVE IK
147H 3664	VH: (SEQ ID NO: 295) EVQLVQSGAE VKKPGASVKV SCKASGY**M**FT NYWLGWIKQA PGQGLEWIGD IYPGGDYINY NEKFKGKATL TADTSISTAY MELSRLRSDD TAVYYCARPN LPGDYWGQGT TVTVSS VL: (SEQ ID NO: 296) DIVMTQSPLS LPVTPGEPAS ISCRSSKSLL HSNAITYLYW YLQKPGQSPQ LLIYQ**K**SNLA SGVPDRFSGS GSGTDFTLKI SRVEAEDVGV YYCAQNLELP WTFGGGTKVE IK
147H 3665	VH: (SEQ ID NO: 297) EVQLVQSGAE VKKPGASVKV SCKASGYTF**D** NYWLGWIKQA PGQGLEWIGD IYPGGD**I**INY NEKFKGKATL TADTSISTAY MELSRLRSDD TAVYYCARPN LPGDYWGQGT TVTVSS VL: (SEQ ID NO: 298) DIVMTQSPLS LPVTPGEPAS ISCRSSKSLL HSNAITYLYW YLQKPGQSPQ LLIYQVSNLA **V**GVPDRFSGS GSGTDFTLKI SRVEAEDVGV YYCAQNLELP WTFGGGTKVE IK
147H 3666	VH: (SEQ ID NO: 299) EVQLVQSGAE VKKPGASVKV SCKASGYTF**G** NYWLGWIKQA PGQGLEWIGD IYPGGD**V**INY NEKFKGKATL TADTSISTAY MELSRLRSDD TAVYYCARPN LPGDYWGQGT TVTVSS VL: (SEQ ID NO: 300)

	DIVMTQSPLS LPVTPGEPAS ISCRSSKSLL HSNAITYLYW YLQKPGQSPQ LLIYQVSNLA **L**GVPDRFSGS GSGTDFTLKI SRVEAEDVGV YYCAQNLELP WTFGGGTKVE IK
147H 3667	VH: (SEQ ID NO: 301) EVQLVQSGAE VKKPGASVKV SCKASGYTFT NYWL**W**WIKQA PGQGLEWIGD I**F**PGGDYINY NEKFKGKATL TADTSISTAY MELSRLRSDD TAVYYCARPN LPGDYWGQGT TVTVSS VL: (SEQ ID NO: 302) DIVMTQSPLS LPVTPGEPAS ISCRSSKSLL HSNAITYLYW YLQKPGQSPQ LLIYQV**D**NLA SGVPDRFSGS GSGTDFTLKI SRVEAEDVGV YYCAQNLELP WTFGGGTKVE IK
147H 3668	VH: (SEQ ID NO: 303) EVQLVQSGAE VKKPGASVKV SCKASGYTFT NYWLGWIKQA PGQGLEWIGD IYPGGDYI**V**Y NEKFKGKATL TADTSISTAY MELSRLRSDD TAVYYCARPN LPGDYWGQGT TVTVSS VL: (SEQ ID NO: 304) DIVMTQSPLS LPVTPGEPAS ISCRSSKSLL HSNAITYLYW YLQKPGQSPQ LLIYQVSNLA **T**GVPDRFSGS GSGTDFTLKI SRVEAEDVGV YYCAQNLELP WTFGGGTKVE IK
147H 3669	VH: (SEQ ID NO: 305) EVQLVQSGAE VKKPGASVKV SCKASGY**L**FT NYWLGWIKQA PGQGLEWIGD IYPGGDYI**V**Y NEKFKGKATL TADTSISTAY MELSRLRSDD TAVYYCARPN LPGDYWGQGT TVTVSS VL: (SEQ ID NO: 306) DIVMTQSPLS LPVTPGEPAS ISCRSSKSLL HSNAITYLYW YLQKPGQSPQ LLIYQVSNLA SGVPDRFSGS GSGTDFTLKI SRVEAEDVGV YYCAQNLELP WTFGGGTKVE IK
147H 3670	VH: (SEQ ID NO: 307) EVQLVQSGAE VKKPGASVKV SCKASGYTFT NYWLGWIKQA PGQGLEWIGD IYPGGDYINY NEKFKGKATL TADTSISTAY MELSRLRSDD TAVYYCARPN LPGDYWGQGT TVTVSS VL: (SEQ ID NO: 308) DIVMTQSPLS LPVTPGEPAS ISCRSSKSLL HSNAITYLYW YLQKPGQSPQ LLIY**H**VSNLA SGVPDRFSGS GSGTDFTLKI SRVEAEDVGV YYCAQNLELP WTFGGGTKVE IK
147H 3675	VH: (SEQ ID NO: 309) EVQLVQSGAE VKKPGASVKV SCKASGYTFT NYWL**W**WIKQA PGQGLEWIGD

	IYPGGD**L**INY NEKFKGKATL TADTSISTAY MELSRLRSDD TAVYYCARPN LPGDYWGQGT TVTVSS VL: (SEQ ID NO: 310) DIVMTQSPLS LPVTPGEPAS ISCRSSKSLL HSNAITYLYW YLQKPGQSPQ LLIY**H**VSNLA SGVPDRFSGS GSGTDFTLKI SRVEAEDVGV YYCAQNLELP WTFGGGTKVE IK
147H 3676	VH: (SEQ ID NO: 311) EVQLVQSGAE VKKPGASVKV SCKASGYTFT NYWL**S**WIKQA PGQGLEWIGD IYPGGD**H**INY NEKFKGKATL TADTSISTAY MELSRLRSDD TAVYYCARPN LPGDYWGQGT TVTVSS VL: (SEQ ID NO: 312) DIVMTQSPLS LPVTPGEPAS ISCRSSKSLL HSNAITYLYW YLQKPGQSPQ LLIYQVSNLA SGVPDRFSGS GSGTDFTLKI SRVEAEDVGV YYCAQNLELP WTFGGGTKVE IK
147H 3677	VH: (SEQ ID NO: 313) EVQLVQSGAE VKKPGASVKV SCKASGYTFT NYWL**W**WIKQA PGQGLEWIG**E** IYPGGDYI**T**Y NEKFKGKATL TADTSISTAY MELSRLRSDD TAVYYCARPN LPGDYWGQGT TVTVSS VL: (SEQ ID NO: 314) DIVMTQSPLS LPVTPGEPAS ISCRSSKSLL HSNAITYLYW YLQKPGQSPQ LLIYQVSN**R**A SGVPDRFSGS GSGTDFTLKI SRVEAEDVGV YYCAQNLELP WTFGGGTKVE IK
147H 3678	VH: (SEQ ID NO: 315) EVQLVQSGAE VKKPGASVKV SCKASGYTFT NYWLGWIKQA PGQGLEWIGD IYPGGDYINY NEKFKGKATL TADTSISTAY MELSRLRSDD TAVYYCARPN LPGDYWGQGT TVTVSS VL: (SEQ ID NO: 316) DIVMTQSPLS LPVTPGEPAS ISCRSSKSLL HSNAITYLYW YLQKPGQSPQ LLIYQV**D**NLA SGVPDRFSGS GSGTDFTLKI SRVEAEDVGV YYCAQNLELP WTFGGGTKVE IK
147H 3679	VH: (SEQ ID NO: 317) EVQLVQSGAE VKKPGASVKV SCKASG**F**TFT NYWLGWIKQA PGQGLEWIGD IYPGGDYI**V**Y NEKFKGKATL TADTSISTAY MELSRLRSDD TAVYYCARPN LPGDYWGQGT TVTVSS VL: (SEQ ID NO: 318) DIVMTQSPLS LPVTPGEPAS ISCRSSKSLL HSNAITYLYW YLQKPGQSPQ LLIYQVSNLA SGVPDRFSGS GSGTDFTLKI SRVEAEDVGV YYCAQNLELP

	WTFGGGTKVE IK
147H 3790	VH: (SEQ ID NO: 319) EVQLVQSGAE VKKPGASVKV SCKASGYTFT NYWLGWIKQA PGQGLEWIGD IYPGGDYINY NEKFKGKATL TADTSISTAY MELSRLRSDD TAVYYCARPN LP**K**D**H**WGQGT TVTVSS VL: (SEQ ID NO: 320) DIVMTQSPLS LPVTPGEPAS ISCRSSKSLL HSNAITYLYW YLQKPGQSPQ LLIYQVSNLA **T**GVPDRFSGS GSGTDFTLKI SRVEAEDVGV YYCAQNLELP WTFGGGTKVE IK
147H 3791	VH: (SEQ ID NO: 321) EVQLVQSGAE VKKPGASVKV SCKASGYTFT NYWLGWIKQA PGQGLEWIGD IYPGGDYI**V**Y NEKFKGKATL TADTSISTAY MELSRLRSDD TAVYYCARPN LPGDYWGQGT TVTVSS VL: (SEQ ID NO: 322) DIVMTQSPLS LPVTPGEPAS ISCRSSKSLL HSNAITYLYW YLQKPGQSPQ LLIYQVSNLA SGVPDRFSGS GSGTDFTLKI SRVEAEDVGV YYC**G**QNLELP WTFGGGTKVE IK
147H 3792	VH: (SEQ ID NO: 323) EVQLVQSGAE VKKPGASVKV SCKASGYTFT NYWLGWIKQA PGQGLEWIGD IYPGGDYI**V**Y NEKFKGKATL TADTSISTAY MELSRLRSDD TAVYYCARPN LPGDYWGQGT TVTVSS VL: (SEQ ID NO: 324) DIVMTQSPLS LPVTPGEPAS ISCRSSKSLL HSNAITYLYW YLQKPGQSPQ LLIYQVSNLA SGVPDRFSGS GSGTDFTLKI SRVEAEDVGV YYCAQ**Y**LELP WTFGGGTKVE IK
147H 3793	VH: (SEQ ID NO: 325) EVQLVQSGAE VKKPGASVKV SCKASGY**L**FT NYWLGWIKQA PGQGLEWIGD IYPGGDYI**V**Y NEKFKGKATL TADTSISTAY MELSRLRSDD TAVYYCARPN LPGDYWGQGT TVTVSS VL: (SEQ ID NO: 326) DIVMTQSPLS LPVTPGEPAS ISCRSSKSLL HSNAITYLYW YLQKPGQSPQ LLIYQVSNLA SGVPDRFSGS GSGTDFTLKI SRVEAEDVGV YYC**G**QNLELP WTFGGGTKVE IK
147H 3794	VH: (SEQ ID NO: 327) EVQLVQSGAE VKKPGASVKV SCKASGY**L**FT NYWLGWIKQA PGQGLEWIGD IYPGGDYI**V**Y NEKFKGKATL TADTSISTAY MELSRLRSDD TAVYYCARPN LPGDYWGQGT TVTVSS

	VL: (SEQ ID NO: 328) DIVMTQSPLS LPVTPGEPAS ISCRSSKSLL HSNAITYLYW YLQKPGQSPQ LLIYQVSNLA SGVPDRFSGS GSGTDFTLKI SRVEAEDVGV YYCAQ**Y**LELP WTFGGGTKVE IK
147H 3807	VH: (SEQ ID NO: 329) EVQLVQSGAE VKKPGASVKV SCKASGYTFT NYWLGWIKQA PGQGLEWIGD IYPGGDYI**V**Y NEKFKGKATL TADTSISTAY MELSRLRSDD TAVYYCARPN LP**K**D**H**WGQGT TVTVSS VL: (SEQ ID NO: 330) DIVMTQSPLS LPVTPGEPAS ISCRSSKSLL HSNAITYLYW YLQKPGQSPQ LLIYQVSNLA SGVPDRFSGS GSGTDFTLKI SRVEAEDVGV YYCAQ**Y**LELP WTFGGGTKVE IK
147H 3808	VH: (SEQ ID NO: 331) EVQLVQSGAE VKKPGASVKV SCKASGYTFT NYWLGWIKQA PGQGLEWIGD IYPGGDYI**V**Y NEKFKGKATL TADTSISTAY MELSRLRSDD TAVYYCARPN LP**K**D**H**WGQGT TVTVSS VL: (SEQ ID NO: 332) DIVMTQSPLS LPVTPGEPAS ISCRSSKSLL HSNAITYLYW YLQKPGQSPQ LLIYQVSNLA SGVPDRFSGS GSGTDFTLKI SRVEAEDVGV YYC**G**Q**Y**LELP WTFGGGTKVE IK
147H 3809	VH: (SEQ ID NO: 333) EVQLVQSGAE VKKPGASVKV SCKASGY**L**FT NYWLGWIKQA PGQGLEWIGD IYPGGDYI**V**Y NEKFKGKATL TADTSISTAY MELSRLRSDD TAVYYCARPN LP**K**D**H**WGQGT TVTVSS VL: (SEQ ID NO: 334) DIVMTQSPLS LPVTPGEPAS ISCRSSKSLL HSNAITYLYW YLQKPGQSPQ LLIYQVSNLA SGVPDRFSGS GSGTDFTLKI SRVEAEDVGV YYCAQ**Y**LELP WTFGGGTKVE IK
147H 3810	VH: (SEQ ID NO: 335) EVQLVQSGAE VKKPGASVKV SCKASGY**L**FT NYWLGWIKQA PGQGLEWIGD IYPGGDYI**V**Y NEKFKGKATL TADTSISTAY MELSRLRSDD TAVYYCARPN LP**K**D**H**WGQGT TVTVSS VL: (SEQ ID NO: 336) DIVMTQSPLS LPVTPGEPAS ISCRSSKSLL HSNAITYLYW YLQKPGQSPQ LLIYQVSNLA **T**GVPDRFSGS GSGTDFTLKI SRVEAEDVGV YYCAQ**Y**LELP WTFGGGTKVE IK
147H	VH: (SEQ ID NO: 337)

3811	EVQLVQSGAE VKKPGASVKV SCKASGYLFT NYWLGWIKQA PGQGLEWIGD IYPGGDYIVY NEKFKGKATL TADTSISTAY MELSRLRSDD TAVYYCARPN LPKDHWGQGT TVTVSS VL: (SEQ ID NO: 338) DIVMTQSPLS LPVTPGEPAS ISCRSSKSLL HSNAITYLYW YLQKPGQSPQ LLIYQVSNLA TGVPDRFSGS GSGTDFTLKI SRVEAEDVGV YYCGQYLELP WTFGGGTKVE IK

Table 8. Summary of mutations and mutated CDR regions:

	Original sequence (SEQ ID NO:__)	Example substitutions	Example mutated sequences (SEQ ID NO:__)
CDRH1	GYTFTNYWLG (240)	Y27: F T28: M, L T30: E, D, G G35: W, S	GYTFENYWLG (339) GYMFTNYWLG (340) GYTFDNYWLG (341) GYTFGNYWLG (342) GYTFTNYWLW (343) GYLFTNYWLG (344) GYTFTNYWLS (345) GFTFTNYWLG (346)
CDRH2	DIYPGGDYINYNEKFKG (241)	D50: E Y52: F Y56: I, V, L, H N58: V, T	DIYPGGDYIVYNEKFKG (347) DIYPGGDIINYNEKFKG (348) DIYPGGDVINYNEKFKG (349) DIFPGGDYINYNEKFKG (350) DIYPGGDLINYNEKFKG (351) DIYPGGDHINYNEKFKG (352) EIYPGGDYITYNEKFKG (353)
CDRH3	PNLPGDY (242)	N96: D, G G99: K, R, Q Y102: H	PNLPKDH (354) PDLPGDY (355) PGLPKDY (356) PNLPKDY (357) PNLPRDY (358) PGLPRDY (359) PGLPQDY (360) PDLPKDY (361)
CDRL1	RSSKSLLHSNGITYLY (243)	N28: Q	RSSKSLLHSQGITYLY (376)
CDRL2	QVSNLAS (244)	Q50: H V51: K S52: D L54: R S56: R, V, L, T	QVSNLAR (362) QKSNLAS (363) QVSNLAV (364) QVSNLAL (365) QVDNLAS (366) QVSNLAT (367) HVSNLAS (368) QVSNRAS (369)
CDRL3	AQNLELPWT (245)	A89: G	GQNLELPWT (370)

		N91: Y L94: M, E	AQNLE**M**PWT (371) **G**QNLE**M**PWT (372) AQ**Y**LE**E**PWT (373) AQ**Y**LELPWT (374) **G**Q**Y**LELPWT (375)

Example 17

Binding properties of affinity matured anti-LAG3 147H humanized monoclonal antibodies

[0237] The binding kinetics of affinity matured antibodies to recombinant his-tag human LAG3-ECD protein was examined by Biacore T200, as stated in Example 9. The results were shown in Table below. The Biacore results showed that these anti-LAG3 antibodies had better affinity than parent 147H-13.

	KD (M)	kon(1/Ms)	kdis(1/s)
147H-13	1.4E-08	2.2E+06	3.0E-02
147H 3421	8.1E-09	1.4E+06	1.2E-02
147H 3508	1.4E-09	2.9E+06	4.2E-03
147H 3549	9.2E-10	7.4E+06	6.8E-03
147H 3550	9.8E-10	8.7E+06	8.5E-03
147H 3663	6.8E-09	7.9E+05	5.4E-03
147H 3669	8.8E-09	7.2E+05	6.3E-03
147H 3790	5.9E-09	7.7E+05	4.5E-03
147H 3791	1.2E-09	2.1E+06	2.5E-03
147H 3792	5.9E-10	4.9E+06	2.9E-03
147H 3793	1.3E-09	1.8E+06	2.3E-03
147H 3794	7.2E-10	3.7E+06	2.7E-03
147H 3807	5.1E-10	4.0E+06	2.0E-03
147H B3808	7.5E-10	4.3E+06	3.2E-03
147H 3809	4.7E-10	4.3E+06	2.0E-03
147H 3810	4.1E-10	4.7E+06	1.9E-03
147H 3811	5.9E-10	4.9E+06	2.9E-03

[0238] To confirm the capability of affinity matured anti-LAG-3 antibodies binding to human LAG3, 2 antibodies with highest affinity (B3807 and B3810) along with parent antibody 147H-13 were evaluated using ELISA, which was described in Example 2. EC50 of B3807, B3810 along with parent antibody was showed in table below. Both 3807 and B3810 showed superior binding capability than parent antibody 147H-13.

Name	EC50 （nM）
147H-13	6.5

147H 3807	0.41
147H 3810	0.49

[0239] To further confirm affinity matured anti-LAG-3 antibodies could bind to cell-derived human LAG3， both inducible hLAG3 expressed Jurkat cells and activated PBMCs were used to test the binding capability of B3807 and B3810. In brief, Jurkat cells were resuspended in FACS buffer. Anti-LAG3 antibodies and isotype control were 4-fold serially diluted in FACS buffer with a dose ranging from 20nM to 30 pM. The serially diluted antibodies were added to the cell suspension and incubated for 30 minutes on ice. Then after removal of unbound antibodies, cells were stained with anti-human IgG conjugated with Alexa Fluor 633 (Thermo, A21091). Fluorescence measurement was acquired on FACSCelesta flow cytometer and analyzed in Flowjo to determine the mean fluorescence intensities (MFI). To test anti-LAG3 antibodies' ability of binding to native human LAG3, PBMCs from health donor were stimulated with anti-CD3 (BD, 555336) and anti-CD28 (BD, 555725) both at a concentration of 1ug/ml. Following 3 days' stimulation, cells were harvested and incubated with anti-LAG3 antibodies for 30 mins on ice. The cells were stained with anti-human CD4 and anti-human IgG. Analysis of antibodies binding to CD4+ cells were carried out on FACSCelesta flow cytometry. The results of cytometry analysis were summarized in table below which showed EC50 of antibodies binding to cell-derived human LAG3. **FIG. 13** is a graph showing the binding curve of anti-LAG3 antibodies. EC50 of tested antibodies was showed below.

Cell-based binding assay	EC50 (nM)		
	147H-13	147H 3807	147H 3810
Jurkat-LAG3	1.2	0.4	0.5
Activated CD4 T cells	0.77	0.33	0.39

Example 18

Blocking of LAG3 binding to MHC class II

[0240] To measure the ability of anti-LAG3 monoclonal antibodies to block the interaction between human LAG3 and MHCII, the LAG3 and MHC II binding assay (Cisbio, 64ICP03PEG) were performed utilizing homogeneous TR-FRET technology, following the protocol provided by the kit manufacturer. Anti-human LAG3 antibodies were 3-fold diluted ranging from 100 nM to 5pM (10 points). Fluorescence data was acquired on a PerkinElmer Envision plate reader and

a four-parameter dose-response curve was fitted to obtain IC50 of each antibody. IC50 of tested antibodies was showed in table below.

Name	IC50 (nM)
147H-13	2.2-7.6
147H 3421	1.5
147H 3508	0.55
147H 3549	0.44
147H 3550	0.39
147H 3663	2.7
147H 3668	0.9
147H 3669	1.2
147H 3792	0.73
147H 3794	0.63
147H 3807	0.31
147H 3808	0.5
147H 3809	0.96
147H 3810	0.63
147H 3811	0.59

Example 19

Stimulation of human T cell response by anti-LAG3 antibodies

[0241] To test the ability of anti-LAG3 antibodies to stimulate T cell response, hLAG3-expressed Jurkat cells were used, as described in Example 13. Similarly, in each well of 96-well plate, Jurkat cells (1×10^5) were incubated with Raji cells (1×10^4) in the presence of 0.1ng/ml SE. Anti-LAG3 antibodies were 3-fold diluted and added to the cells at a final concentration ranging from 100nM to 5pm. 48 hours later, IL2 from the culture medium was measured using a homogeneous TR-FRET assay. (PerkinElmer, TRF1221M) **FIG. 14** shows the curve of anti-LAG3 antibodies in stimulating IL2 release. Affinity matured clones showed better potency in stimulating T cell response.

Example 20

The effect of anti-LAG3 antibodies on blocking the binding of Galectin-3 or LSECtin to LAG3

[0242] It has been reported that LAG3 has other ligands including Galectin-3 (Cancer Immunol Res. 2015;3:412–423.) and LSECtin (J Biol Chem. 2004;279:18748–18758). Interactions with these two potential alternative ligands may serve to broaden LAG3's impact on T-cell function,

particularly with regard to an intrinsic role for LAG3 on CD8+ T cells in the tumor microenvironment. Recombinant Galectin-3 or LSECtin were coated on the 96 well plated overnight at 4°C. Serially diluted anti-LAG3 antibodies (starting from 10μg/ml and 1:3 dilution) and biotin-labeled LAG3-Fc protein were incubated with Glectin-3 or LSECtin coated wells at room temperature for 2 hours. After extensive wash with the wash buffer, streptavidin-HRP was added. As shown in **FIG. 15**, the 147H, S27 and S119 antibodies dose-dependently inhibited the binding of Galectin-3 or LSECtin to LAG3 protein.

Example 21

Synergistic effect of combination treatment of anti-human LAG3 and anti-human PD-L1 antibody

[0243] To evaluate the effect of anti-LAG-3 antibodies in combination with PD-L1 antibody, Jurkat T cell stimulation assay were used. Jurkat cells were overexpressed with human LAG3 and human PD-1, and Raji cells which endogenously expressed MHCII were transfected with human PD-L1. SE was used as the stimulator in this assay. In brief, PD-L1 expressing Raji (1×10^4) was co-cultured with LAG3-PD-1 expressing Jurkat T cells (1×10^5) in the presence of SE stimulation. The anti-LAG3 antibodies with or without an anti-PD-L1 antibody were serially diluted and added at the beginning of the culture. 48hr later, the culture supernatant was collected for IL2 release using TR-FRET assay(PerkinElmer, TRF1221M). As shown in **FIG. 16**, anti LAG3 antibodies in combination with the anti PD-L1 antibody can significantly produce more IL-2 than its corresponding mono-antibodies.

[0244] Double humanized mice that express the extracellular domain of human PD-1 and human LAG3 were used. Mouse colon adenocarcinoma cells (MC38) were engineered to express human PD-L1. Double humanized mice (hLAG3/hPD-1) were subcutaneously implanted with $5x10^5$ MC38-hPD-L1 cells on day 0. On day 10, mice with an average tumor volume of 137 mm^3 were selected and randomized into four treatment groups (N=7/group). Mouse were intraperitoneally administered isotype control (5mg/kg), anti-PD-L1 antibody (5mg/kg), anti-LAG3 antibody 147H-13 (5mg/kg) and anti-PD-L1 antibody (5mg/kg) + anti-LAG3 antibody (5mg/kg) every other day for 8 doses, starting from day 10. The anti-PD-L1 antibody used in this example binds with high affinity to human PD-L1 and blocks the interaction with PD-1. Tumor volumes were monitored by caliper measurement twice per week for the duration of the experiment (29 days).

Neither the PD-L1 antibody nor 147H-13 showed tumor inhibition at 5mg/kg. By contrast, combination of the PD-L1 antibody and 147H-13 demonstrated robust inhibition of MC38 tumor growth, with a TGI of 74.2% at the end of the study (**FIG. 17**). Thus, in an established MC38 colon adenocarcinoma model, a combination treatment of anti-PD-L1 and anti-LAG3 antibodies was significantly more efficacious than the corresponding monotherapies.

* * *

[0245] The present disclosure is not to be limited in scope by the specific embodiments described which are intended as single illustrations of individual aspects of the disclosure, and any compositions or methods which are functionally equivalent are within the scope of this disclosure. It will be apparent to those skilled in the art that various modifications and variations can be made in the methods and compositions of the present disclosure without departing from the spirit or scope of the disclosure. Thus, it is intended that the present disclosure cover the modifications and variations of this disclosure provided they come within the scope of the appended claims and their equivalents.

[0246] All publications and patent applications mentioned in this specification are herein incorporated by reference to the same extent as if each individual publication or patent application was specifically and individually indicated to be incorporated by reference.

CLAIMS

What is claimed is:

1. An isolated antibody or fragment thereof, wherein the antibody or fragment thereof has specificity to a human Lymphocyte Activation Gene-3 (LAG-3) protein, wherein the antibody or fragment thereof comprises a heavy chain variable region comprising heavy chain complementarity determining regions CDRH1, CDRH2, and CDRH3, and a light chain variable region comprising light chain complementarity determining regions CDRL1, CDRL2, and CDRL3, wherein

the CDRH1 comprises the amino acid sequence of SEQ ID NO:240 or an amino acid sequence derived from SEQ ID NO:240 with one or two amino acid substitution;

the CDRH2 comprises the amino acid sequence of SEQ ID NO:241 or an amino acid sequence derived from SEQ ID NO:241 with one or two amino acid substitution;

the CDRH3 comprises the amino acid sequence of SEQ ID NO:242 or an amino acid sequence derived from SEQ ID NO:242 with one or two amino acid substitution;

the CDRL1 comprises the amino acid sequence of SEQ ID NO:243 or an amino acid sequence derived from SEQ ID NO:243 with one or two amino acid substitution;

the CDRL2 comprises the amino acid sequence of SEQ ID NO:244 or an amino acid sequence derived from SEQ ID NO:244 with one or two amino acid substitution; and

the CDRL3 comprises the amino acid sequence of SEQ ID NO:245 or an amino acid sequence derived from SEQ ID NO:245 with one or two amino acid substitution.

2. The antibody or fragment thereof of claim 1, wherein the amino acid substitution from SEQ ID NO:240 is at amino acid residue Y27, T28, T30, G35, or the combinations thereof, according to Kabat numbering.

3. The antibody or fragment thereof of claim 2, wherein the amino acid substitution is selected from:

Y27: F;

T28: M, or L;

T30: E, D, or G; or

G35: W, or S.

4. The antibody or fragment thereof of claim 1, wherein the CDRH1 comprises an amino acid sequence selected from the group consisting of SEQ ID NO:240 and 339-346.

5. The antibody or fragment thereof of claim 1, wherein the amino acid substitution from SEQ ID NO:241 is at amino acid residue D50, Y52, Y56, N58, or the combinations thereof, according to Kabat numbering.

6. The antibody or fragment thereof of claim 5, wherein the amino acid substitution is selected from:

D50: E;

Y52: F;

Y56: I, V, L, or H; or

N58: V, or T.

7. The antibody or fragment thereof of claim 5, wherein the amino acid substitution comprises N58V.

8. The antibody or fragment thereof of claim 1, wherein the CDRH2 comprises an amino acid sequence selected from the group consisting of SEQ ID NO:241 and 347-353.

9. The antibody or fragment thereof of claim 1, wherein the amino acid substitution from SEQ ID NO:242 is at amino acid residue N96, G99, Y102, or the combinations thereof, according to Kabat numbering.

10. The antibody or fragment thereof of claim 9, wherein the amino acid substitution is selected from:

N96: D, or G;

G99: K, R, or Q; or

Y102: H.

11. The antibody or fragment thereof of claim 10, wherein the amino acid substitution comprises G99K or Y102H.

12. The antibody or fragment thereof of claim 1, wherein the CDRH3 comprises an amino acid sequence selected from the group consisting of SEQ ID NO:242 and 354-361.

13. The antibody or fragment thereof of claim 1, wherein the amino acid substitution from SEQ ID NO:243 is at amino acid residue N28, according to Kabat numbering.

14. The antibody or fragment thereof of claim 13, wherein the amino acid substitution comprises N28Q.

15. The antibody or fragment thereof of claim 1, wherein the CDRL2 comprises an amino acid sequence of SEQ ID NO:376.

16. The antibody or fragment thereof of claim 1, wherein the amino acid substitution from SEQ ID NO:244 is at amino acid residue Q50, V51, S52, L54, S56, or the combinations thereof, according to Kabat numbering.

17. The antibody or fragment thereof of claim 16, wherein the amino acid substitution is selected from:

Q50: H;

V51: K;

S52: D;

L54: R; or

S56: R, V, L, or T.

18. The antibody or fragment thereof of claim 1, wherein the CDRL2 comprises an amino acid sequence selected from the group consisting of SEQ ID NO:244 and 362-369.

19. The antibody or fragment thereof of claim 1, wherein the amino acid substitution from SEQ ID NO:245 is at amino acid residue A89, N91, L94, or the combinations thereof, according to Kabat numbering.

20. The antibody or fragment thereof of claim 19, wherein the amino acid substitution is selected from:

A89: G;

N91: Y; or

L94: M, or E.

21. The antibody or fragment thereof of claim 20, wherein the amino acid substitution comprises N91Y.

22. The antibody or fragment thereof of claim 1, wherein the CDRL3 comprises an amino acid sequence selected from the group consisting of SEQ ID NO:245 and 370-375.

23. The antibody or fragment thereof of claim 1, which comprises a heavy chain variable region comprising an amino acid sequence selected from the group consisting of SEQ ID NO:238, 246-259, 261, 263, 265, 267, 269, 271, 273, 275, 277, 279, 281, 283, 285, 287, 289, 291, 293, 295, 297, 299, 301, 303, 305, 307, 309, 311, 313, 315, 317, 319, 321, 323, 325, 327, 329, 331, 333, 335, and 337, or a peptide having at least 90% sequence identity to an amino acid sequence selected from the group consisting of SEQ ID NO:238, 246-259, 261, 263, 265, 267, 269, 271, 273, 275, 277, 279, 281, 283, 285, 287, 289, 291, 293, 295, 297, 299, 301, 303, 305, 307, 309, 311, 313, 315, 317, 319, 321, 323, 325, 327, 329, 331, 333, 335, and 337.

24. The antibody or fragment thereof of claim 1, which comprises a light chain variable region comprising an amino acid sequence selected from the group consisting of SEQ ID NO:239, 260, 262, 264, 266, 268, 270, 272, 274, 276, 278, 280, 282, 284, 286, 288, 290, 292, 294, 296, 298, 300, 302, 304, 306, 308, 310, 312, 314, 316, 318, 320, 322, 324, 326, 328, 330, 332, 334, 336, and 338, or a peptide having at least 90% sequence identity to an amino acid sequence selected from the group consisting of SEQ ID NO:239, 260, 262, 264, 266, 268, 270, 272, 274, 276, 278, 280, 282, 284, 286, 288, 290, 292, 294, 296, 298, 300, 302, 304, 306, 308, 310, 312, 314, 316, 318, 320, 322, 324, 326, 328, 330, 332, 334, 336, and 338.

25. The antibody or fragment thereof of any one of claims 1-24, wherein the heavy chain variable region comprises one or more amino acid residues selected from the group consisting of:

(a) Ala (A) at position 71,

(b) Leu (L) at position 69,

(c) Lys (K) at position 66,

(d) Ala (A) at position 67,

(e) Ile (I) at position 48,

(f) Ile (I) at position 37,

(g) Lys (K) at position 38,

(h) Phe (F) at position 91, and

(i) Glu (E) at position 1, according to Kabat numbering, and combinations thereof.

26. An isolated antibody or fragment thereof, wherein the antibody or fragment thereof has specificity to a human Lymphocyte Activation Gene-3 (LAG-3) protein, wherein the antibody or fragment thereof comprises a heavy chain variable region comprising heavy chain complementarity determining regions CDRH1, CDRH2, and CDRH3, and a light chain variable region comprising light chain complementarity determining regions CDRL1, CDRL2, and CDRL3, wherein:

the CDRH1 comprises the amino acid sequence of SEQ ID NO:1 or 2 or an amino acid sequence derived from SEQ ID NO:1 or 2 with one or two amino acid substitution;

the CDRH2 comprises the amino acid sequence of SEQ ID NO:3 or 4 or an amino acid sequence derived from SEQ ID NO:3 or 4 with one or two amino acid substitution;

the CDRH3 comprises an amino acid sequence selected from the group consisting of SEQ ID NO:5-45 or an amino acid sequence derived from any one of SEQ ID NO:5-45 with one or two amino acid substitution;

the CDRL1 comprises an amino acid sequence selected from the group consisting of SEQ ID NO:46-80 or an amino acid sequence derived from any one of SEQ ID NO:46-80 with one or two amino acid substitution;

the CDRL2 comprises an amino acid sequence selected from the group consisting of SEQ ID NO:81-103 or an amino acid sequence derived from any one of SEQ ID NO:81-103 with one or two amino acid substitution; and

the CDRL3 comprises an amino acid sequence selected from the group consisting of SEQ ID NO:104-139 or an amino acid sequence derived from any one of SEQ ID NO:104-139 with one or two amino acid substitution.

27. The antibody or fragment thereof of claim 26, which comprises a heavy chain variable region comprising an amino acid sequence selected from the group consisting of SEQ ID NO:140-188 or a peptide having at least 90% sequence identity to an amino acid sequence selected from the group consisting of SEQ ID NO:140-188.

28. The antibody or fragment thereof of claim 26, which comprises a light chain variable region comprising an amino acid sequence selected from the group consisting of SEQ ID

NO:189-237 or a peptide having at least 90% sequence identity to an amino acid sequence selected from the group consisting of SEQ ID NO:189-237.

29. An isolated antibody or fragment thereof, wherein the antibody or fragment thereof has specificity to a human Lymphocyte Activation Gene-3 (LAG-3) protein, and is capable of inhibiting binding of the LAG-3 protein to Galectin-3 (LGALS3) or C-type lectin domain family 4 member G (LSECtin).

30. The antibody or fragment thereof of claim 29, which is capable of inhibiting binding of the LAG-3 protein to both Galectin-3 and LSECtin.

31. The antibody or fragment thereof of claim 29 or 30, which is further capable of inhibiting binding of the LAG-3 protein to MHC class II molecule.

32. An isolated antibody or fragment thereof, which:

(i) binds human LAG-3,

(ii) blocks LAG-3 binding to major histocompatibility (MHC) class II molecules;

(iii) stimulates an immune response; and

(iv) reverses the inhibitory effect of regulatory T cells on effector T cells.

33. The antibody or fragment thereof of claim 32, which stimulates interleukin-2 (IL-2) production in an antigen-specific T cell response.

34. The antibody or fragment thereof of claim 32 or 33, which stimulates interferon gamma (IFN-γ) production in an antigen-specific T cell response.

35. The antibody or fragment thereof of any one of claims 1-34, further comprising a heavy chain constant region or a light chain constant region.

36. The antibody or fragment thereof of any one of claims 1-34, which is bispecific.

37. The antibody or fragment thereof of claim 36, wherein the bispecificity comprises a second specificity to an immune checkpoint protein or a tumor antigen.

38. The antibody or fragment thereof of claim 36, wherein the bispecificity comprises a second specificity to a protein target selected from the group consisting of PD-L1, PD-1, CTLA-4, CD28, CD122, 4-1BB, TIM3, OX-40, OX40L, CD40, CD40L, LIGHT, ICOS, ICOSL, GITR,

GITRL, TIGIT, CD27, VISTA, B7H3, B7H4, HEVM, BTLA, KIR, CD47, CD73, EGFR, Her2, CD33, CD133, CEA and VEGF.

39. The antibody or fragment thereof of claim 36, wherein the bispecificity comprises a second specificity to PD-L1.

40. A composition comprising the antibody or fragment thereof of any one of claims 1-39 and a pharmaceutically acceptable carrier.

41. An isolated cell comprising one or more polynucleotide encoding the antibody or fragment thereof of any one of claims 1-39.

42. A method of treating an autoimmune or inflammatory disease in a patient in need thereof, comprising administering to the patient the antibody or fragment thereof of any one of claims 1-39.

43. The method of claim 42, wherein said autoimmune or inflammatory disease is selected from the group consisting of a Parkinson's disease, rthritis, rheumatoid arthritis, multiple sclerosis, psoriasis, psoriatic arthritis, Crohn's disease, inflammatory bowel disease, ulcerative colitis, lupus, systemic lupus erythematous, juvenile rheumatoid arthritis, juvenile idiopathic arthritis, Grave's disease, Hashimoto's thyroiditis, Addison's disease, celiac disease, dermatomyositis, multiple sclerosis, myasthenia gravis, pernicious anemia, Sjogren syndrome, type I diabetes, vasculitis, uveitis, atherosclerosis and ankylosing spondylitis.

44. A method of treating cancer in a patient in need thereof, comprising administering to the patient the antibody or fragment thereof of any one of claims 1-39.

45. The method of claim 44, wherein the cancer is selected from the group consisting of a leukemia, a lymphoma, ovarian cancer, breast cancer, endometrial cancer, colon cancer (colorectal cancer), rectal cancer, bladder cancer, urothelial cancer, lung cancer (non-small cell lung cancer, adenocarcinoma of the lung, squamous cell carcinoma a of the lung), bronchial cancer, bone cancer, prostate cancer, pancreatic cancer, gastric cancer, hepatocellular carcinoma, gall bladder cancer, bile duct cancer, esophageal cancer, renal cell carcinoma, thyroid cancer, squamous cell carcinoma of the head and neck (head and neck cancer), testicular cancer, cancer of the endocrine gland, cancer of the adrenal gland, cancer of the pituitary gland, cancer of the skin, cancer of soft tissues, cancer of blood vessels, cancer of brain, cancer of nerves, cancer of

eyes, cancer of meninges, cancer of oropharynx, cancer of hypopharynx, cancer of cervix, and cancer of uterus, glioblastoma, meduloblastoma, astrocytoma, glioma, meningioma, gastrinoma, neuroblastoma, melanoma, myelodysplastic syndrome, and a sarcoma.

46. The method of claim 45, wherein the leukemia is selected from the group consisting of systemic mastocytosis, acute lymphocytic (lymphoblastic) leukemia (ALL), T cell – ALL, acute myeloid leukemia (AML), myelogenous leukemia, chronic lymphocytic leukemia (CLL), multiple myeloma (MM), chronic myeloid leukemia (CML), myeloproliferative disorder / neoplasm, myelodysplastic syndrome, monocytic cell leukemia, and plasma cell leukemia; wherein said lymphoma is selected from the group consisting of histiocytic lymphoma and T cell lymphoma, B cell lymphomas, including Hodgkin's lymphoma and non-Hodgkin's lymphoma, such as low grade/follicular non-Hodgkin's lymphoma (NHL), cell lymphoma (FCC), mantle cell lymphoma (MCL), diffuse large cell lymphoma (DLCL), small lymphocytic (SL) NHL, intermediate grade/follicular NHL, intermediate grade diffuse NHL, high grade immunoblastic NHL, high grade lymphoblastic NHL, high grade small non-cleaved cell NHL, bulky disease NHL, and Waldenstrom's Macroglobulinemia; and wherein said sarcoma is selected from the group consisting of osteosarcoma, Ewing's sarcoma, leiomyosarcoma, synovial sarcoma, alveolar soft part sarcoma, angiosarcoma, liposarcoma, fibrosarcoma, rhabdomyosarcoma, and chrondrosarcoma.

47. A method of treating cancer in a patient in need thereof, comprising:

(a) treating a T cell, in vitro, with the antibody or fragment thereof of any one of claims 1-39; and

(b) administering the treated T cell to the patient.

48. A method of treating or inhibiting infection in a patient in need thereof, comprising administering to the patient the antibody or fragment thereof of any one of claims 1-39.

49. A method of detecting expression of LAG3 in a sample, comprising contacting the sample with the antibody or fragment thereof of any one of claims 1-39 under conditions for the antibody or fragment thereof to bind to the LAG3, and detecting the binding which indicates expression of LAG3 in the sample.

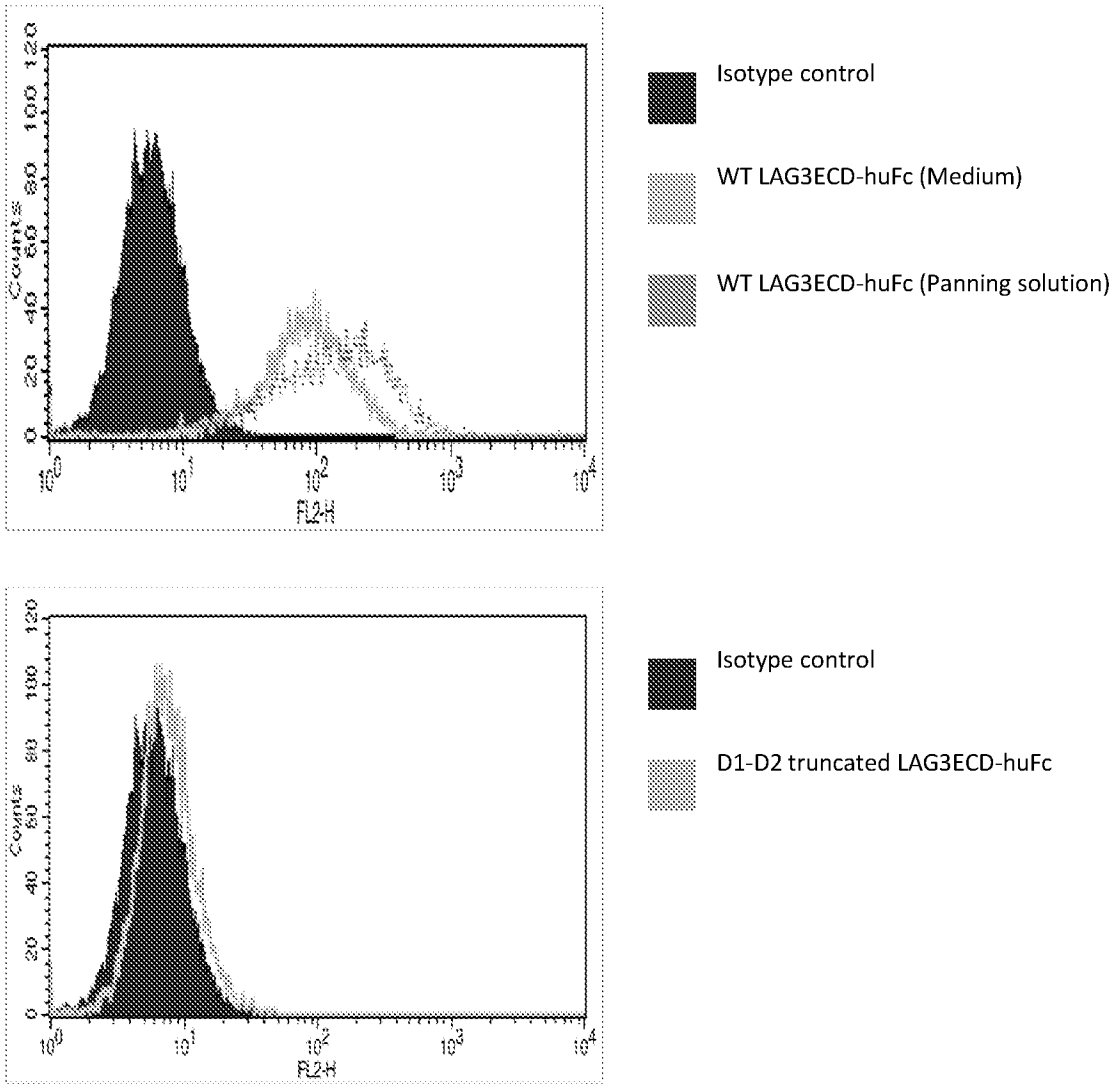
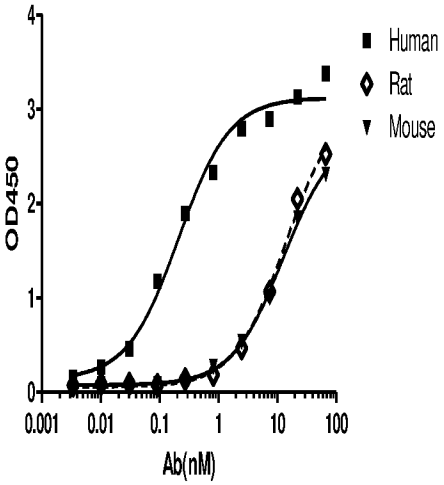

Counts
FL2-H
Isotype control
WT LAG3ECD-huFc (Medium)
WT LAG3ECD-huFc (Panning solution)
Counts
FL2-H
Isotype control
D1-D2 truncated LAG3ECD-huFc

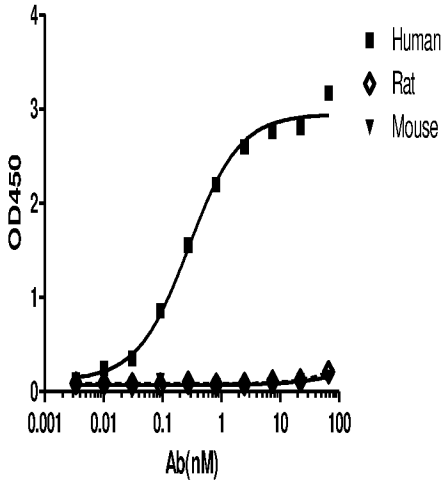
FIG. 1

S27



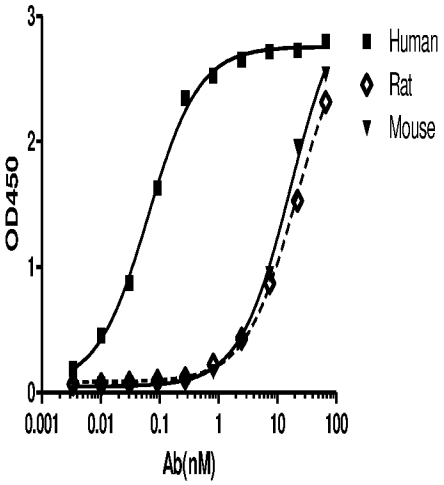
	EC50
Human	0.2096
Rat	13.95
Mouse	12.62

S31



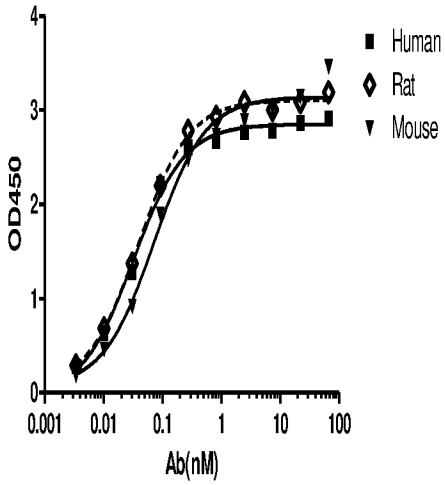
	EC50
Human	0.2789
Rat	~271270
Mouse	119.4

T99



	EC50
Human	0.06384
Rat	20.85
Mouse	16.94

S119



	EC50
Human	0.03207
Rat	0.03870
Mouse	0.07237

FIG. 2

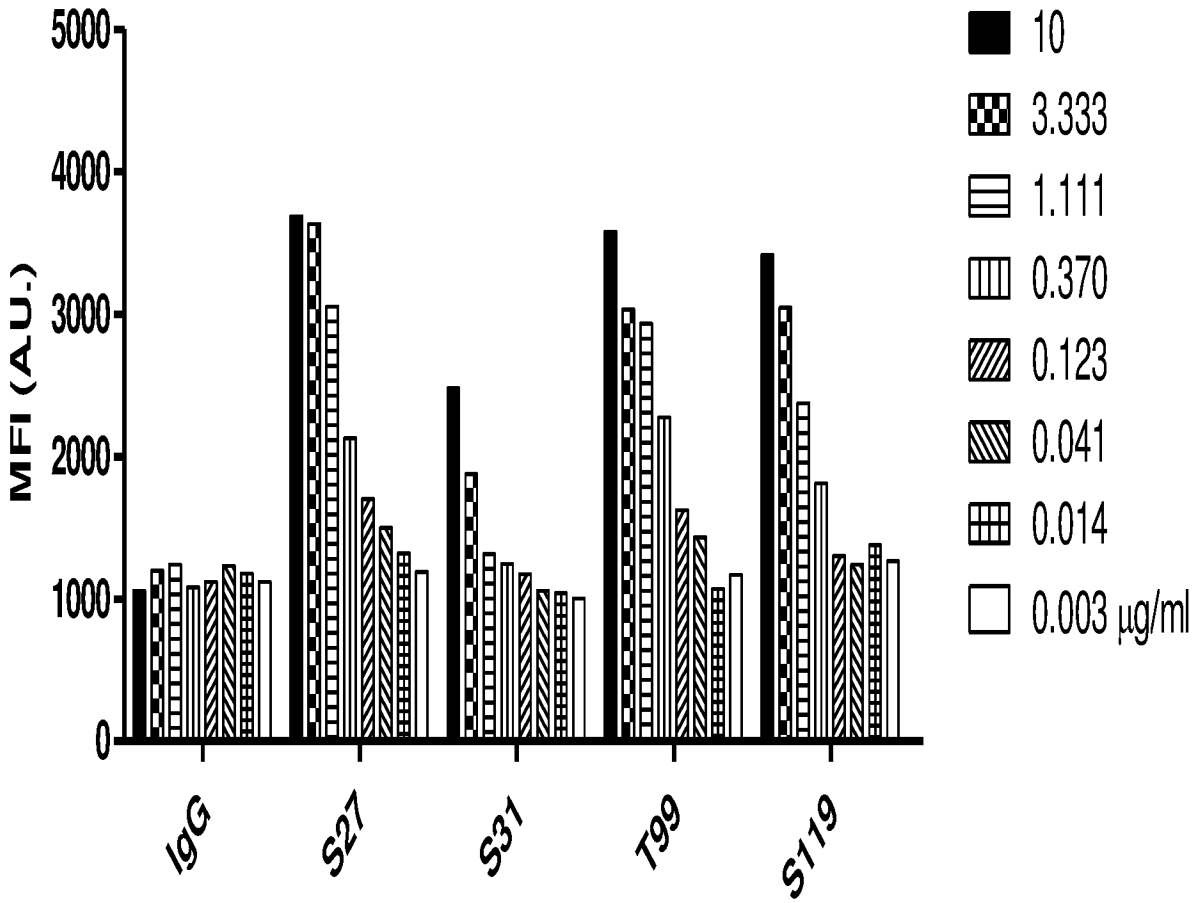

5000
4000
3000
2000
1000
0
MFI (A.U.)
IgG
S27
S31
T99
S119
10
3.333
1.111
0.370
0.123
0.041
0.014
0.003 μg/ml

FIG. 3

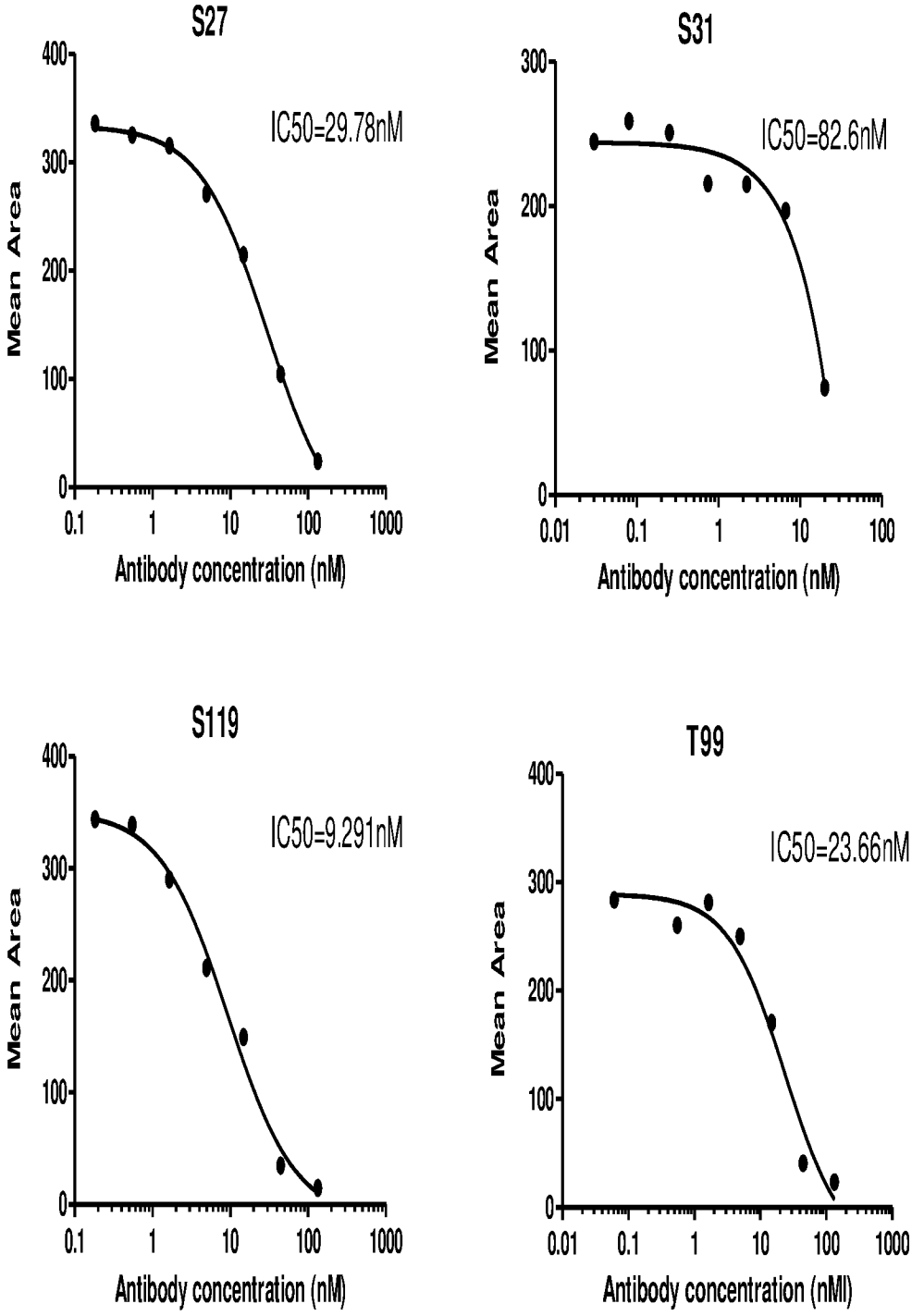

S27
IC50=29.78nM
Mean Area
Antibody concentration (nM)
S31
IC50=82.6nM
Mean Area
Antibody concentration (nM)
S119
IC50=9.291nM
Mean Area
Antibody concentration (nM)
T99
IC50=23.66nM
Mean Area
Antibody concentration (nM)

FIG. 4

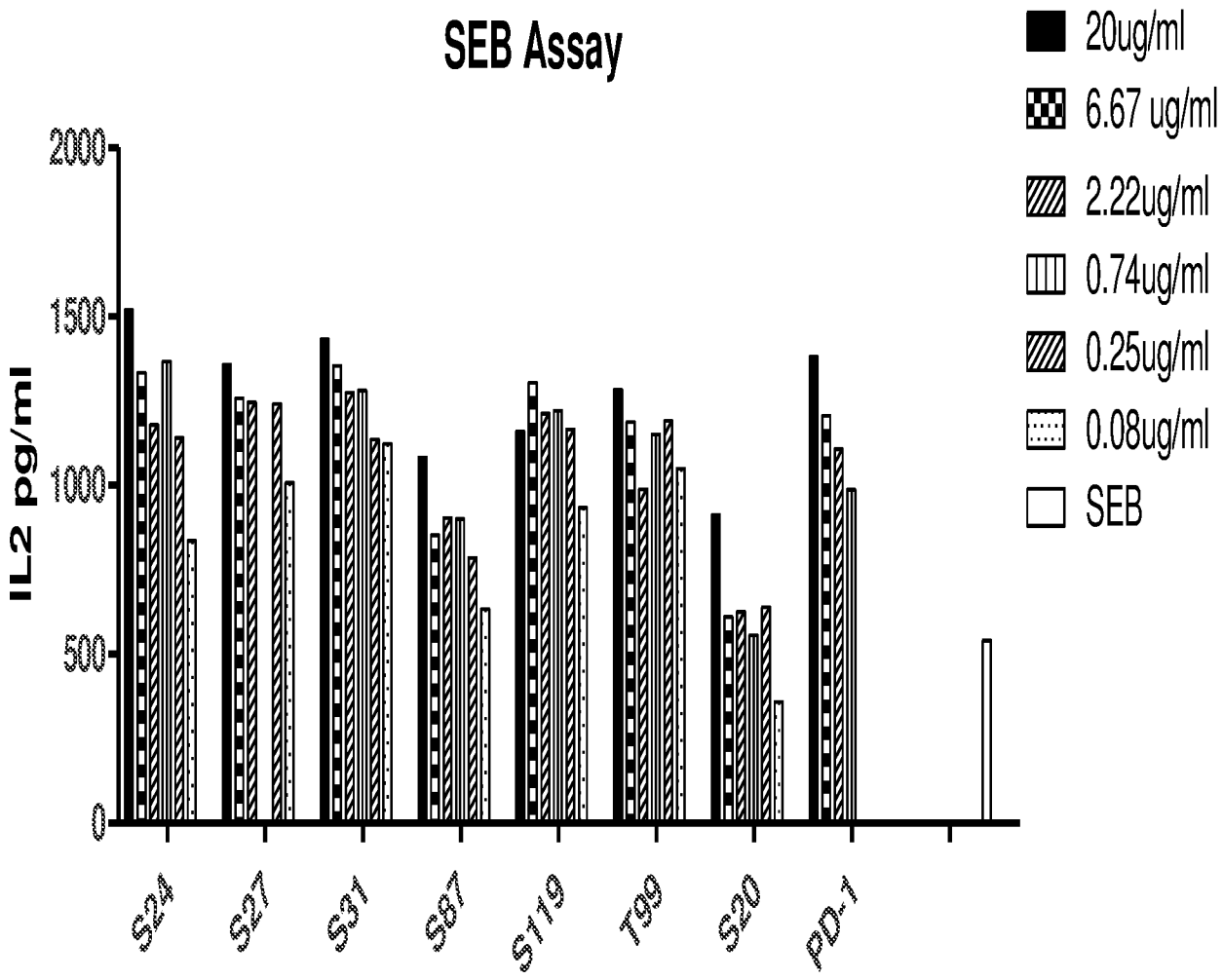

SEB Assay
IL2 pg/ml
2000
1500
1000
500
0
S24
S27
S31
S87
S119
T99
S20
PD-1
20ug/ml
6.67 ug/ml
2.22ug/ml
0.74ug/ml
0.25ug/ml
0.08ug/ml
SEB

FIG. 5

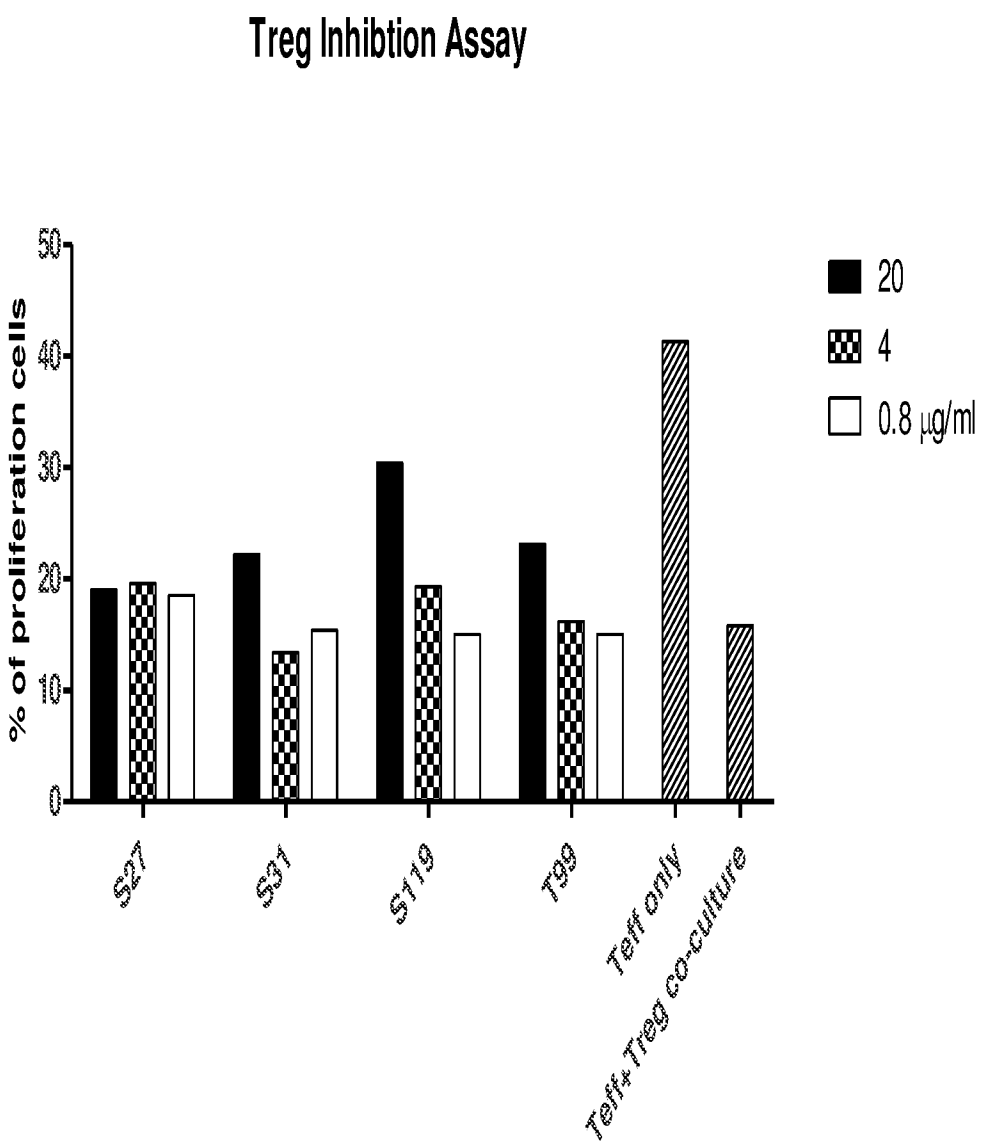

Treg Inhibtion Assay
% of proliferation cells
0
10
20
30
40
50
20
4
0.8 μg/ml
S27
S31
S119
T99
Teff only
Teff+Treg co-culture

FIG. 6

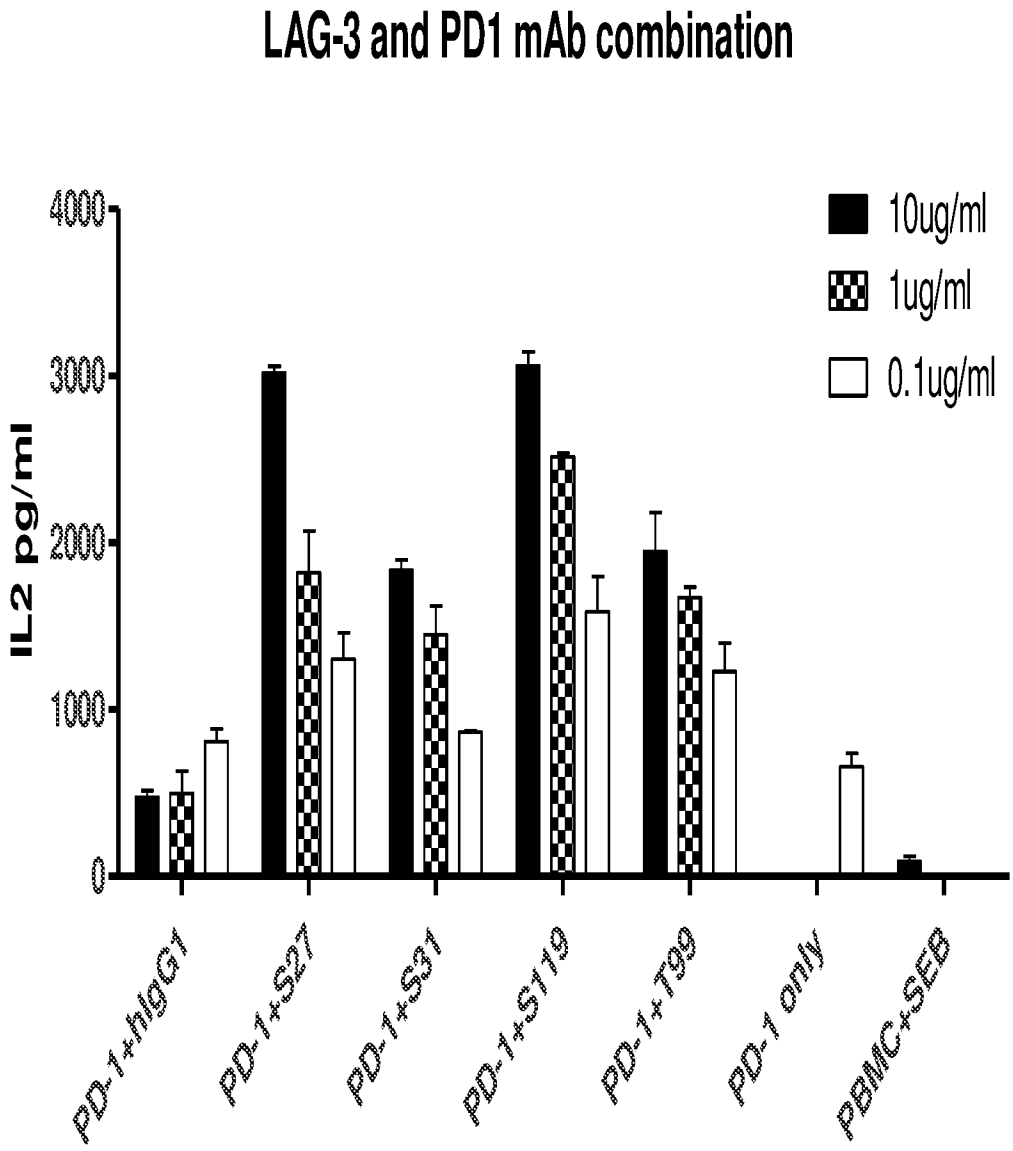

LAG-3 and PD1 mAb combination
IL2 pg/ml
4000
3000
2000
1000
0
10ug/ml
1ug/ml
0.1ug/ml
PD-1+hIgG1
PD-1+S27
PD-1+S31
PD-1+S119
PD-1+T99
PD-1 only
PBMC+SEB

FIG. 7

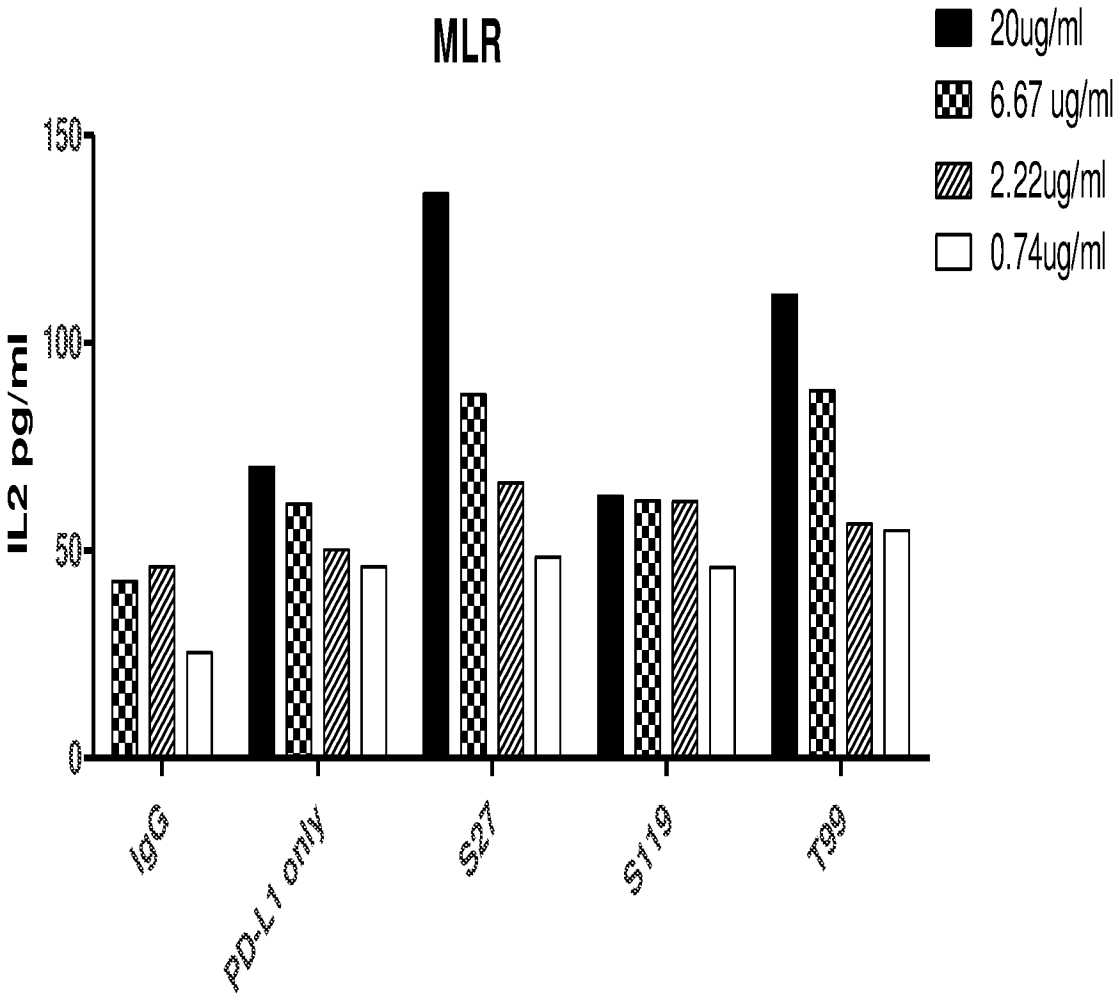
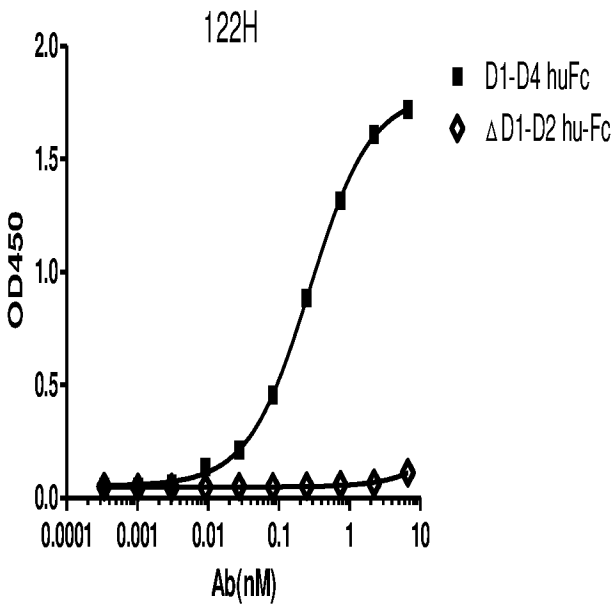
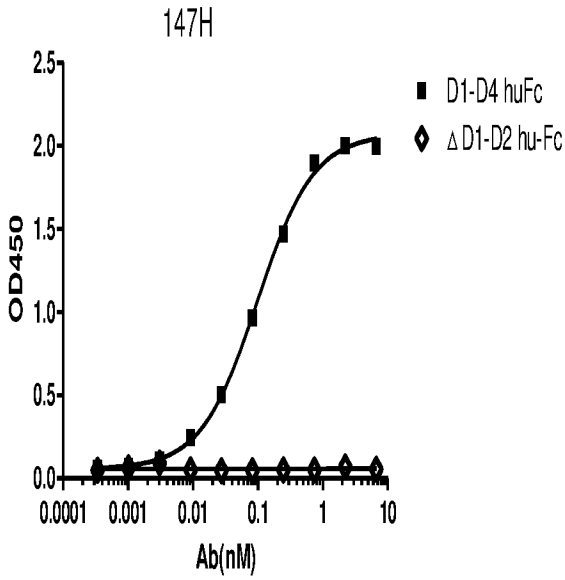

MLR
20ug/ml
6.67 ug/ml
2.22ug/ml
0.74ug/ml
IL2 pg/ml
150
100
50
0
IgG
PD-L1 only
S27
S119
T99

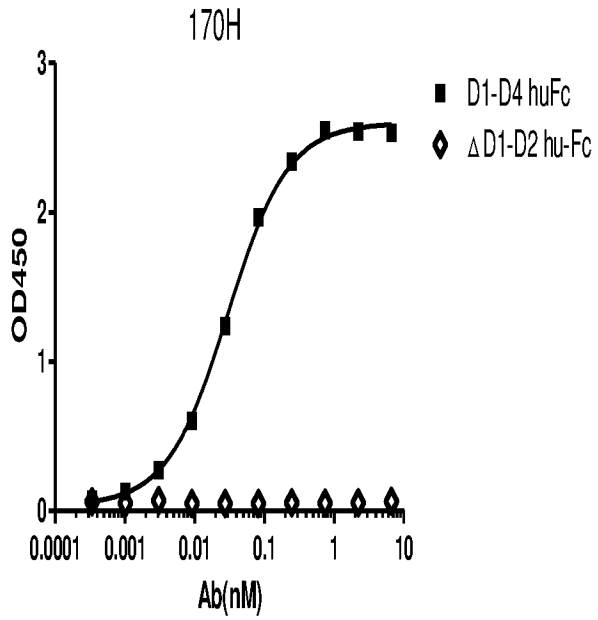
FIG. 8



	EC50
D1-D4 huFc	0.2706
ΔD1-D2 LAG3-Fc	~ 44937

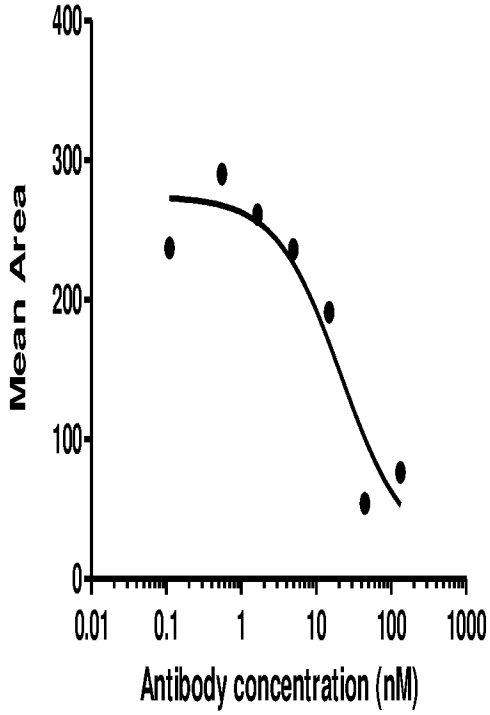


	EC50
D1-D4 huFc	0.09787
ΔD1-D2 hu-Fc	2.618

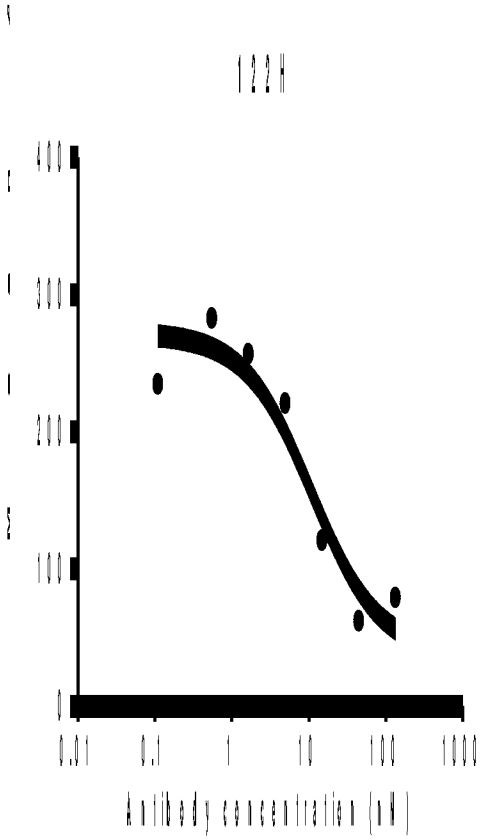


	EC50
D1-D4 huFc	0.02982

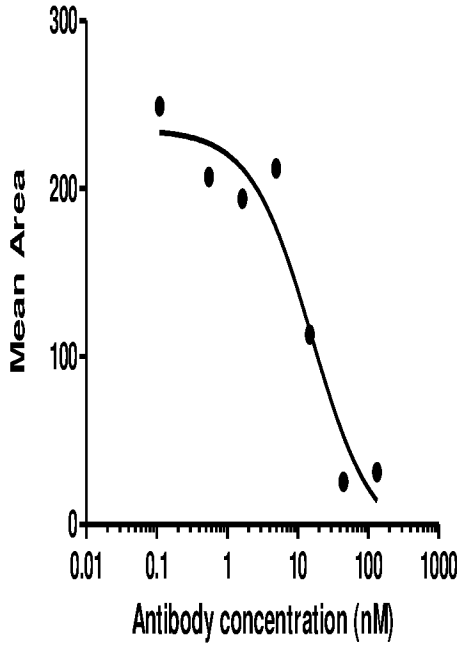
FIG. 9



IC50=21.40 nM

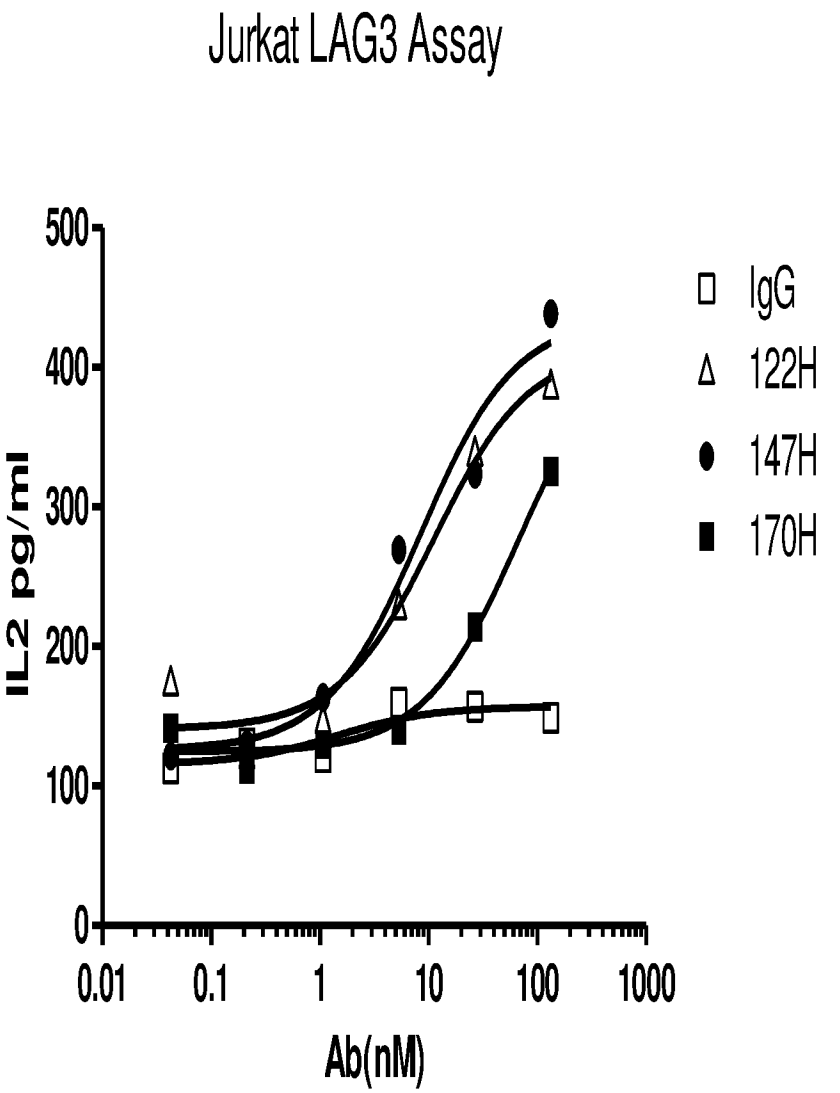


IC50=10.94 nM



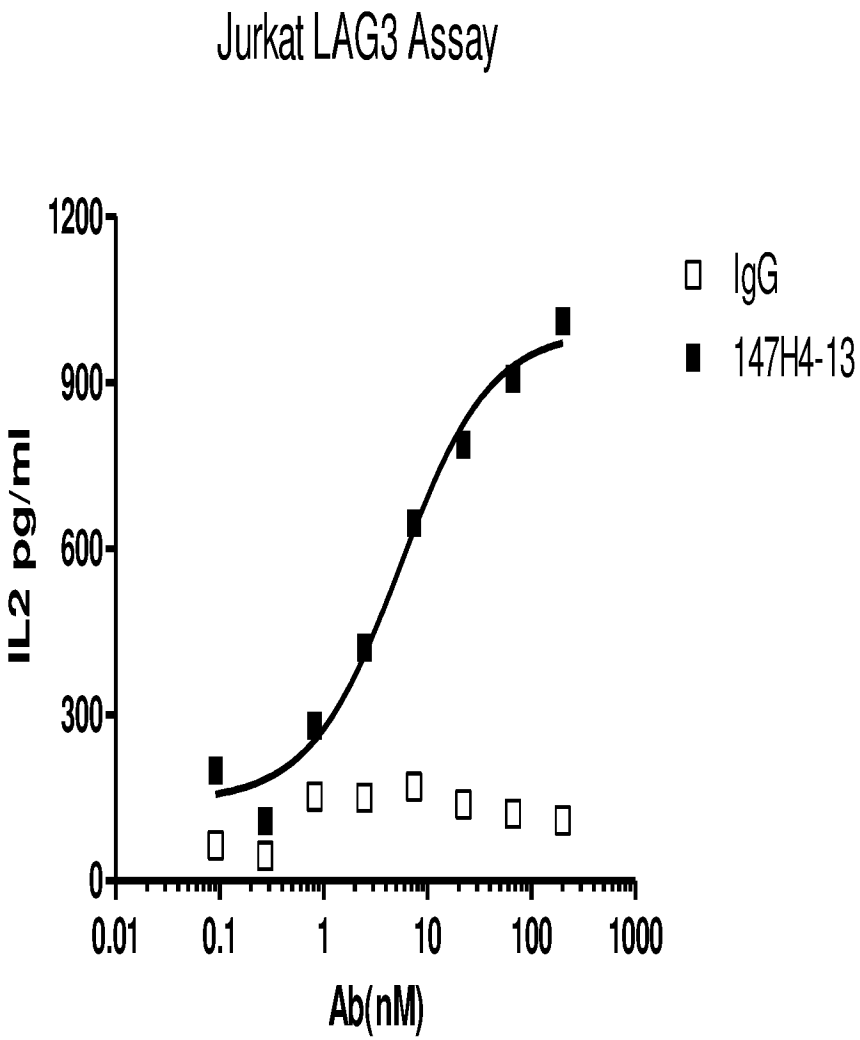
IC50=15.85 nM

FIG. 10



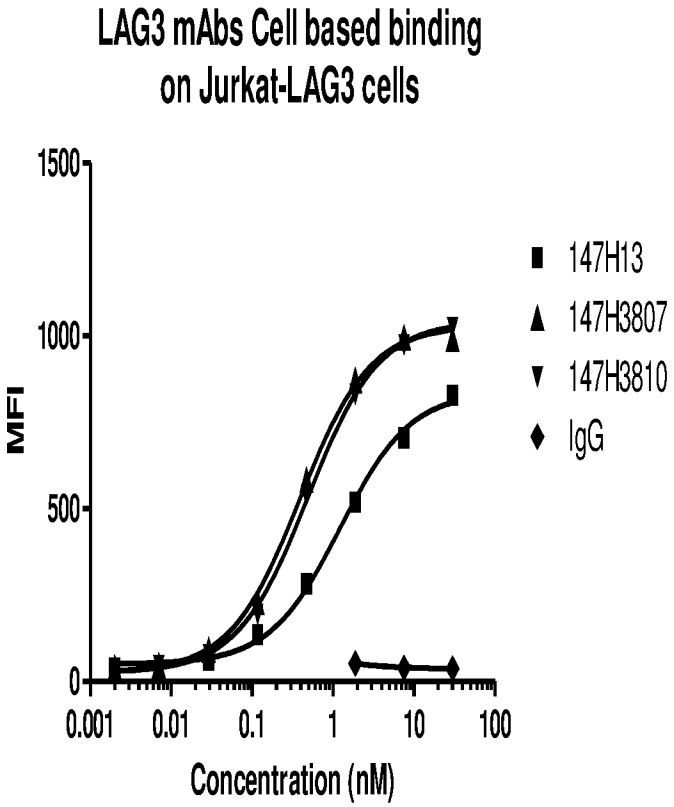
	EC50
IgG	1.471
122H	11.20
147H	8.474
170H	66.50

FIG. 11

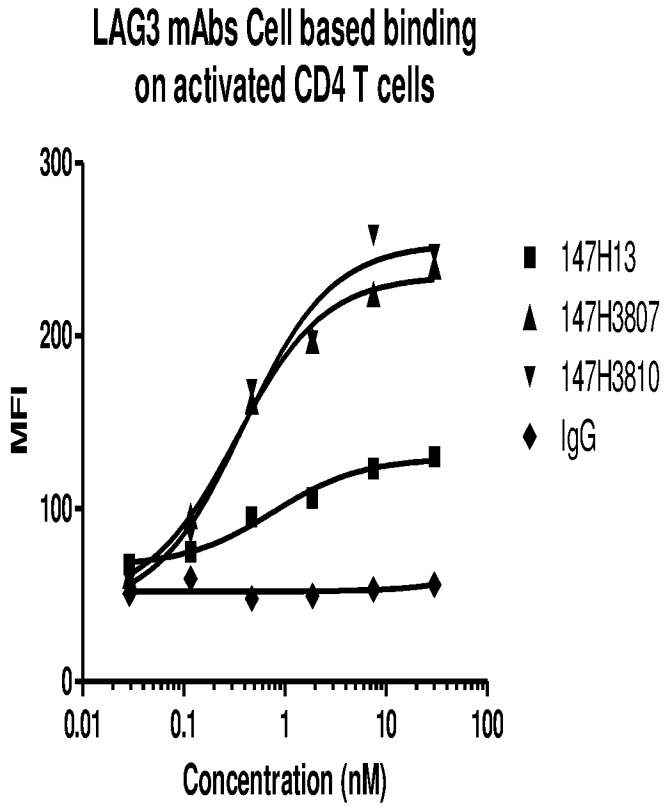


	EC50
147H-13	5.476

FIG. 12

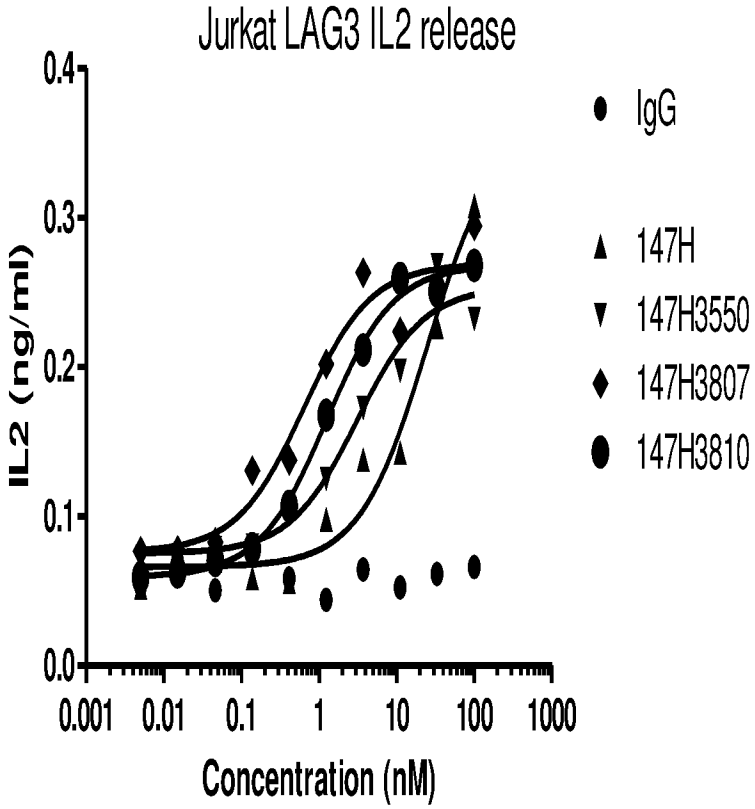


	EC50
147H13	1.225
147H3807	0.3897
147H3810	0.4925
IgG	0.4632



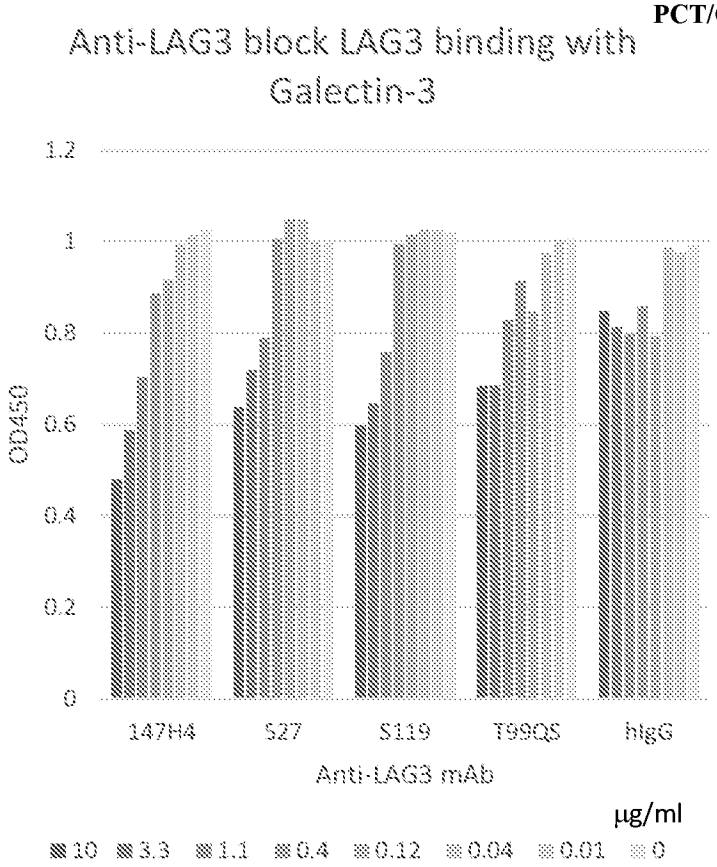
	EC50
147H13	0.7671
147H3807	0.3328
147H3810	0.3914
IgG	3.590e+006

FIG. 13



	147H	147H3550	147H3807	147H3810
EC50	24.83	3.112	0.6400	1.244

FIG. 14


Anti-LAG3 block LAG3 binding with Galectin-3
OD450
1.2
1
0.8
0.6
0.4
0.2
0
147H4
S27
S119
T99QS
hIgG
Anti-LAG3 mAb
μg/ml
10
3.3
1.1
0.4
0.12
0.04
0.01
0

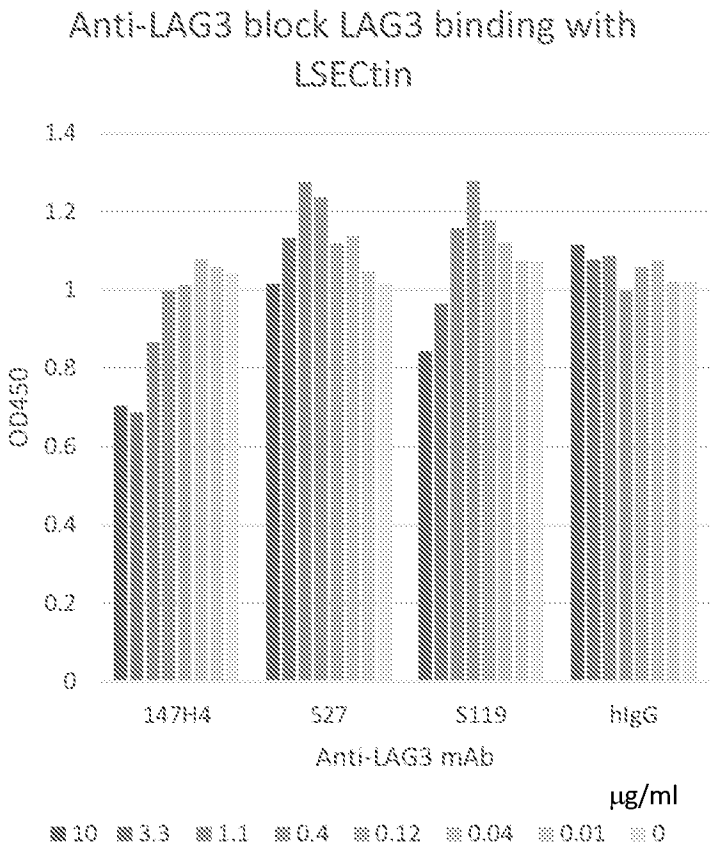

Anti-LAG3 block LAG3 binding with LSECtin
OD450
1.4
1.2
1
0.8
0.6
0.4
0.2
0
147H4
S27
S119
hIgG
Anti-LAG3 mAb
μg/ml
10
3.3
1.1
0.4
0.12
0.04
0.01
0

FIG. 15

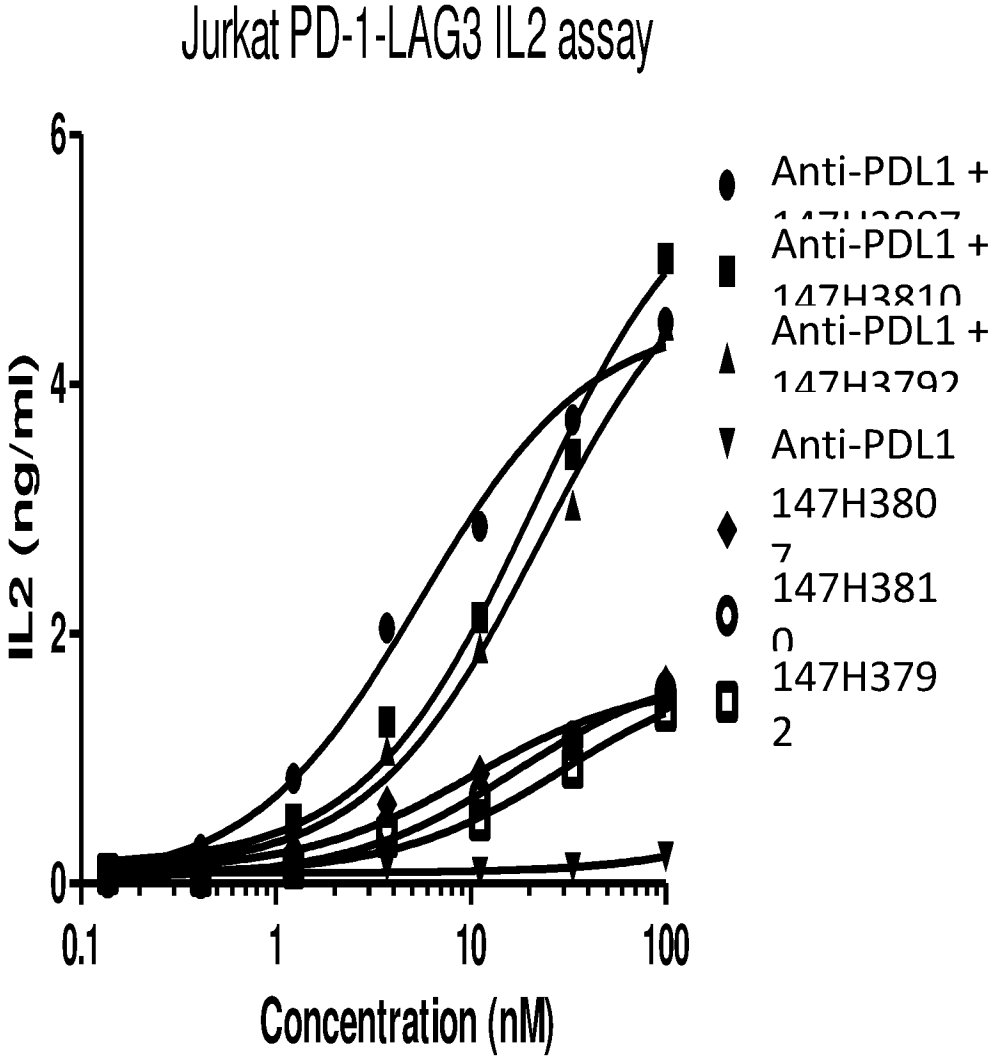

Jurkat PD-1-LAG3 IL2 assay
IL2 (ng/ml)
6
4
2
0
0.1
1
10
100
Concentration (nM)
Anti-PDL1 +
Anti-PDL1 +
147H3810
Anti-PDL1 +
Anti-PDL1
147H380
7
147H381
0
147H379
2

FIG. 16

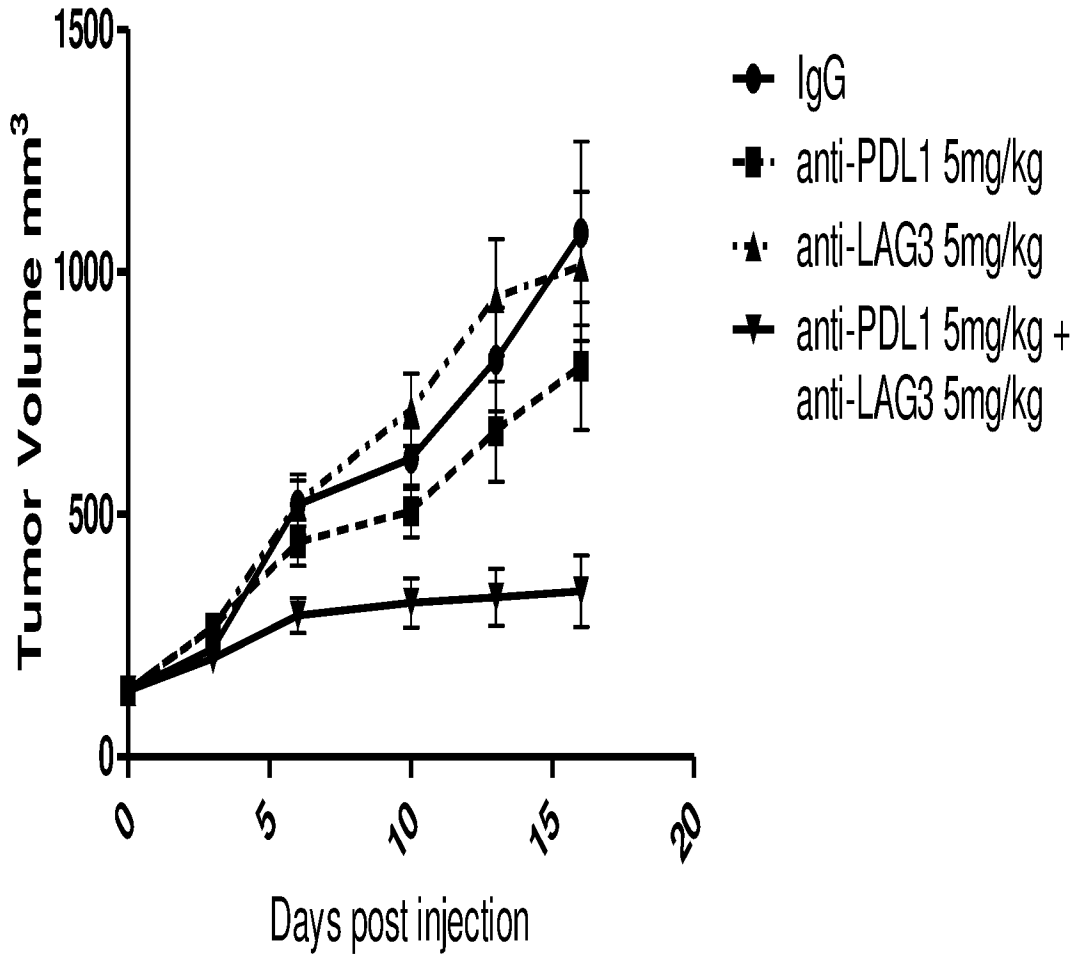

1500
1000
500
0
Tumor Volume mm3
0
5
10
15
20
Days post injection
IgG
anti-PDL1 5mg/kg
anti-LAG3 5mg/kg
anti-PDL1 5mg/kg +
anti-LAG3 5mg/kg

FIG. 17

INTERNATIONAL SEARCH REPORT

International application No.
PCT/CN2018/076940

A. CLASSIFICATION OF SUBJECT MATTER

A61K 39/00(2006.01)i; C07K 16/28(2006.01)i; C12N 15/13(2006.01)i; A61P 35/00(2006.01)i

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)

A61K; C07K; C12N; A61P

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)

DWPI, SIPOABS, CNPABS, CNKI, PubMed, ISI web of Knowledge, GenBank, EBI, STN:LAG-3, LAG3, lymphocyte activation gene,CD223,antagonist,antibody,anti-LAG3,inhibit, extracellular domain,ECD,cancer,tumor,infection, MHC-II,bispecific,I-MAB,ZANG Jingwu,FANG Lei,WANG Zhengyi,GUO Bingshi,SEQ ID NO:1-4,240-245,339-376

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	CN 106103484 A (NOVARTIS AG ET AL.) 09 November 2016 (2016-11-09) description, paragraphs 0009-0028, 0039-0041, 0114-0116, 0133-0142, 0152-0155, 0189-0191, 0632-0652	29-46, 48, 49
A	CN 106103484 A (NOVARTIS AG ET AL.) 09 November 2016 (2016-11-09) description, paragraphs 0009-0028, 0122-0124, table 1	1-31, 47
X	CN 102176921 A (MEDAREX, INC.) 07 September 2011 (2011-09-07) description, paragraphs 0004, 0007-0014, 0052, 0054-0058, 0304-0309, 0321-0333, 0346-0356, 0378	29-38,40,41,44-49
A	CN 102176921 A (MEDAREX, INC.) 07 September 2011 (2011-09-07) description, paragraphs 0015-0052, example 2	1-31
X	CN 104411723 A (BRISTOL-MYERS SQUIBB COMPANY) 11 March 2015 (2015-03-11) description, paragraphs 0014-0029, 0163-0169, 0181-0216	29-33, 35-46, 48, 49
A	CN 104411723 A (BRISTOL-MYERS SQUIBB COMPANY) 11 March 2015 (2015-03-11) description, paragraphs 0009, example 5	1-31, 34, 47

☑ Further documents are listed in the continuation of Box C. ☑ See patent family annex.

* Special categories of cited documents:

"A" document defining the general state of the art which is not considered to be of particular relevance

"E" earlier application or patent but published on or after the international filing date

"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)

"O" document referring to an oral disclosure, use, exhibition or other means

"P" document published prior to the international filing date but later than the priority date claimed

"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention

"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone

"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art

"&" document member of the same patent family

Date of the actual completion of the international search	Date of mailing of the international search report
09 May 2018	**22 May 2018**

Name and mailing address of the ISA/CN	Authorized officer
STATE INTELLECTUAL PROPERTY OFFICE OF THE P.R.CHINA **6, Xitucheng Rd., Jimen Bridge, Haidian District, Beijing 100088** **China**	**MAO,Ying**
Facsimile No. **(86-10)62019451**	Telephone No. **86-(010)-53961979**

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International application No.
PCT/CN2018/076940

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	US 2017022273 A1 (SORRENTO THERAPEUTICS INC.) 26 January 2017 (2017-01-26) description, paragraphs 0006, 0009-0036, 0077, 0133, 0157-0162, 0170-0177, example 3	29-46, 48, 49
A	US 2017022273 A1 (SORRENTO THERAPEUTICS INC.) 26 January 2017 (2017-01-26) description, paragraphs 0006, 0009-0036, 0077, table 3	1-31, 47
X	WO 2016028672 A1 (MERCK SHARP & DOHME CORP.) 25 February 2016 (2016-02-25) claims 1-13, 15, 16, 21-23, 27, description, page 68, examples 1, 7-13	29-46, 48, 49
A	WO 2016028672 A1 (MERCK SHARP & DOHME CORP.) 25 February 2016 (2016-02-25) claims 1-7, description, examples 1-6	1-31, 47
A	KOUO, T. et al. "Galectin-3 Shapes Antitumor Immune Responses by Suppressing CD8+ T Cells via LAG-3 and Inhibiting Expansion of Plasmacytoid Dendritic Cells" *CANCER IMMUNOLOGY RESEARCH*, Vol. 3, No. 4, 17 February 2015 (2015-02-17), pages 412-423	29-31
A	HE, Y.Y. et al. "Lymphocyte-activation gene-3, an important immune checkpoint in cancer" *CANCER SCIENCE*, Vol. 107, No. 9, 30 September 2016 (2016-09-30), pages 1193-1197	1-49

INTERNATIONAL SEARCH REPORT

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Box No. I Nucleotide and/or amino acid sequence(s) (Continuation of item 1.c of the first sheet)

1. With regard to any nucleotide and/or amino acid sequence disclosed in the international application, the international search was carried out on the basis of a sequence listing:
   a. ☑ forming part of the international application as filed:
      ☑ in the form of an Annex C/ST.25 text file.
      ☐ on paper or in the form of an image file.
   b. ☐ furnished together with the international application under PCT Rule 13*ter*.1(a) for the purposes of international search only in the form of an Annex C/ST.25 text file.
   c. ☐ furnished subsequent to the international filing date for the purposes of international search only:
      ☐ in the form of an Annex C/ST.25 text file (Rule 13*ter*.1(a)).
      ☐ on paper or in the form of an image file (Rule 13*ter*.1(b) and Administrative Instructions, Section 713).

2. ☐ In addition, in the case that more than one version or copy of a sequence listing has been filed or furnished, the required statements that the information in the subsequent or additional copies is identical to that forming part of the application as filed or does not go beyond the application as filed, as appropriate, were furnished.

3. Additional comments:

INTERNATIONAL SEARCH REPORT

International application No.
PCT/CN2018/076940

Box No. II Observations where certain claims were found unsearchable (Continuation of item 2 of first sheet)

This international search report has not been established in respect of certain claims under Article 17(2)(a) for the following reasons:

1. ☑ Claims Nos.: **42-48**
because they relate to subject matter not required to be searched by this Authority, namely:

[1] Claims 42-48 relate to a method of treating an autoimmune or inflammatory disease or cancer in a patient in need thereof or a method of treating or inhibiting infection in a patient in need thereof, and therefore do not warrant an international search according to the criteria set out in PCT Rule 39.1(iv). An international search is still carried out on the basis of a LAG-3 antibody or its fragment in the manufacture of a medicament for treating or inhibiting aforementioned diseases in a patient in need thereof.

2. ☐ Claims Nos.:
because they relate to parts of the international application that do not comply with the prescribed requirements to such an extent that no meaningful international search can be carried out, specifically:

3. ☐ Claims Nos.:
because they are dependent claims and are not drafted in accordance with the second and third sentences of Rule 6.4(a).

INTERNATIONAL SEARCH REPORT
Information on patent family members

International application No.
PCT/CN2018/076940

Patent document cited in search report			Publication date (day/month/year)	Patent family member(s)			Publication date (day/month/year)
CN	106103484	A	09 November 2016	JP	2017516489	A	22 June 2017
				EA	201691765	A1	30 December 2016
				US	9908936	B2	06 March 2018
				TW	201540727	A	01 November 2015
				CA	2936962	A1	17 September 2015
				WO	2015138920	A1	17 September 2015
				EP	3116909	A1	18 January 2017
				CL	2016002112	A1	09 June 2017
				CR	20160425	A	26 May 2017
				MX	2016011993	A	09 December 2016
				US	2017210804	A1	27 July 2017
				PE	00712017	A1	17 March 2017
				SG	11201605951Y	A	28 October 2016
				UY	36032	A	30 October 2015
				US	2018086830	A1	29 March 2018
				US	2015259420	A1	17 September 2015
				PH	12016501545	A1	14 September 2016
				AU	2015229103	A1	04 August 2016
				KR	20160134668	A	23 November 2016
CN	102176921	A	07 September 2011	HK	1212911	A1	24 June 2016
				TW	I491408	B	11 July 2015
				AU	2014221286	A1	02 October 2014
				KR	101700459	B1	26 January 2017
				SI	2320940	T1	31 July 2015
				AR	072999	A1	06 October 2010
				JP	2017052765	A	16 March 2017
				DK	2320940	T3	26 May 2015
				CN	103923213	A	16 July 2014
				SM	T201500125	B	09 July 2015
				JP	2015096503	A	21 May 2015
				EP	2320940	B1	04 March 2015
				TW	201019958	A	01 June 2010
				PT	2320940	E	19 June 2015
				KR	20110050507	A	13 May 2011
				SG	10201706497Q	A	28 September 2017
				JP	5647981	B2	07 January 2015
				MX	2011001250	A	29 March 2011
				PE	03062011	A1	21 May 2011
				HR	P20150453	T1	19 June 2015
				HK	1151985	A1	11 December 2015
				ES	2537203	T3	03 June 2015
				NZ	623319	A	28 August 2015
				EP	2905030	A1	12 August 2015
				CA	2734335	C	16 January 2018
				EP	2320940	A4	23 January 2013
				CL	2016002624	A1	09 June 2017
				PE	16582014	A1	08 November 2014
				CN	102176921	B	07 May 2014
				SI	EP2320940	T1	31 July 2015
				CY	1116582	T1	15 March 2017

INTERNATIONAL SEARCH REPORT
Information on patent family members

International application No.
PCT/CN2018/076940

Patent document cited in search report	Publication date (day/month/year)	Patent family member(s)	Publication date (day/month/year)
		CL 2013002062 A1	10 January 2014
		EA 023032 B1	29 April 2016
		IL 210731 D0	31 March 2011
		AU 2009282134 A1	18 February 2010
		CN 107474137 A	15 December 2017
		HU E025250 T2	29 February 2016
		WO 2010019570 A2	18 February 2010
		EP 2320940 A2	18 May 2011
		NZ 590991 A	30 November 2012
		US 2011150892 A1	23 June 2011
		WO 2010019570 A3	17 June 2010
		CO 6351751 A2	20 December 2011
		KR 20170010901 A	01 February 2017
		CA 2734335 A1	18 February 2010
		NZ 602780 A	30 April 2014
		US 2018066054 A1	08 March 2018
		EA 201590777 A1	29 January 2016
		BR PI0917470 A2	01 December 2015
CN 104411723 A	11 March 2015	CO 7170127 A2	28 January 2015
		PE 02212015 A1	19 February 2015
		SI 2867258 T1	30 October 2017
		HR P20171315 T1	20 October 2017
		SG 11201408780X A	29 January 2015
		CA 2877746 A1	09 January 2014
		EA 201590138 A1	30 July 2015
		TW 201726743 A	01 August 2017
		DK 2867258 T3	02 October 2017
		PT 2867258 T	31 August 2017
		EP 2867258 B1	28 June 2017
		HK 1207386 A1	29 January 2016
		PH 12014502854 A1	23 February 2015
		MX 2015000116 A	14 April 2015
		RS 56398 B1	29 December 2017
		UY 34887 A	31 December 2013
		EP 3275899 A1	31 January 2018
		AR 091649 A1	18 February 2015
		TW 201406784 A	16 February 2014
		AU 2013286914 B2	29 June 2017
		TW I576355 B	01 April 2017
		PL 2867258 T3	30 November 2017
		KR 20150023909 A	05 March 2015
		TN 2014000536 A1	30 March 2016
		US 2017137514 A1	18 May 2017
		US 2015307609 A1	29 October 2015
		ES 2638545 T3	23 October 2017
		SG 10201610960Y A	27 February 2017
		NZ 628528 A	29 July 2016
		WO 2014008218 A1	09 January 2014
		HU E034553 T2	28 February 2018
		CN 104411723 B	30 January 2018

INTERNATIONAL SEARCH REPORT
Information on patent family members

International application No.
PCT/CN2018/076940

Patent document cited in search report			Publication date (day/month/year)	Patent family member(s)			Publication date (day/month/year)
				AU	2017221874	A1	21 September 2017
				AU	2013286914	A1	29 January 2015
				IL	236517	A	26 February 2015
				EP	2867258	A1	06 May 2015
				LT	2867258	T	11 September 2017
				US	2014093511	A1	03 April 2014
				JP	2015527880	A	24 September 2015
				US	9505839	B2	29 November 2016
				IL	236517	D0	26 February 2015
				CL	2014003637	A1	27 March 2015
US	2017022273	A1	26 January 2017	WO	2017015560	A2	26 January 2017
				WO	2017015560	A3	02 March 2017
				US	9902772	B2	27 February 2018
				CA	2993177	A1	26 January 2017
				TW	201713692	A	16 April 2017
				AR	105444	A1	04 October 2017
WO	2016028672	A1	25 February 2016	CN	107001470	A	01 August 2017
				CL	2017000311	A1	18 August 2017
				EA	201790403	A1	30 June 2017
				PE	02882017	A1	05 April 2017
				CR	20170059	A	28 April 2017
				TW	201613979	A	16 April 2016
				AU	2015305770	A1	09 March 2017
				JP	2017532059	A	02 November 2017
				AP	201709763	A0	28 February 2016
				MX	2017002230	A	09 May 2017
				MD	20170031	A2	31 July 2017
				DO	P2017000047	A	15 May 2017
				PH	12017500297	A1	17 July 2017
				AP	201709763	D0	28 February 2016
				CA	2957275	A1	25 February 2016
				KR	20170043589	A	21 April 2017
				EP	3182999	A1	28 June 2017
				US	2017267759	A1	21 September 2017
				US	2017290914	A1	12 October 2017
				SG	11201701090V	A	30 March 2017