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(54) Title: ANTI-PD-L1/ANTI-LAG3 BISPECIFIC ANTIBODIES AND USES THEREOF

(57) Abstract: Provided is an anti-PD-L1/anti-LAG3 bispecific antibody capable to effectively block the interactions between PD-L1 and its receptor PD-1 and between LAG3 and its ligand (e. g., a MHC class II molecule and FGL1). The bispecific antibody may have high binding affinity to both of a PD-L1 protein (e. g., a human PD-L1 protein) and a LAG3 protein (e. g., a human LAG3 protein). Also provided are antibodies and fragments that have specificity to the PD-L1 or LAG3 protein alone, or antibodies and fragments having additional specificity to one or more other antigens.

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ANTI-PD-L1/ANTI-LAG3 BISPECIFIC ANTIBODIES AND USES THEREOF

The present invention claims the priority of the PCT/CN2018/101547 filed on August 21, 2018 and PCT/CN2019/087943, filed on May 22, 2019, the contents of which are incorporated herein by their entity.

Field of invention

The present invention relates to the field of antibody, specifically relates to an Anti-PD-L1/Anti-LAG3 bispecific antibodies and use thereof.

BACKGROUND

Programmed death-ligand 1 (PD-L1), also known as cluster of differentiation 274 (CD274) or B7 homolog 1 (B7-H1), is a 40kDa type 1 transmembrane protein believed to play a major role in suppressing the immune system during particular events such as pregnancy, tissue allografts, autoimmune disease and other disease states such as hepatitis. The binding of PD-L1 to PD-1 or B7.1 transmits an inhibitory signal which reduces the proliferation of CD8+ T cells at the lymph nodes and supplementary to that PD-1 is also able to control the accumulation of foreign antigen specific T cells in the lymph nodes through apoptosis which is further mediated by a lower regulation of the gene Bcl-2.

It has been shown that upregulation of PD-L1 may allow cancers to evade the host immune system. An analysis of tumor specimens from patients with renal cell carcinoma found that high tumor expression of PD-L1 was associated with increased tumor aggressiveness and an increased risk of death. Many PD-L1 inhibitors are in development as immuno-oncology therapies and are showing good results in clinical trials.

In addition to treatment of cancers, PD-L1 inhibition has also shown promises in treating infectious diseases. In a mouse model of intracellular infection, L. monocytogenes induced PD-L1 protein expression in T cells, NK cells, and macrophages. PD-L1 blockade (e.g., using blocking antibodies) resulted in increased mortality for infected mice. Blockade reduced TNFα and nitric oxide production by macrophages, reduced granzyme B production

by NK cells, and decreased proliferation of L. monocytogenes antigen-specific CD8 T cells (but not CD4 T cells). This evidence suggests that PD-L1 acts as a positive costimulatory molecule in intracellular infection.

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 (Tregs). 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.

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.

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

The present disclosure provides an anti-PD-L1/anti-LAG3 bispecific antibody capable to effectively block the interactions between PD-L1 and its receptor PD-1 and between LAG3 and its ligand (e.g., a MHC class II molecule). The bispecific antibody may have high binding affinity to both of a PD-L1 protein (e.g., a human PD-L1 protein) and a LAG3 protein (e.g., a human LAG3 protein).

The anti-PD-L1/anti-LAG3 bispecific antibody may comprise an anti-PD-L1 antibody or an antigen-binding fragment thereof as a PD-L1 targeting moiety, which is capable of specifically recognizing and/or binding to a PD-L1 protein, and an anti-LAG3 antibody or an antigen-binding fragment thereof as a LAG3 targeting moiety, which is capable of specifically recognizing and/or binding to a LAG3 protein.

The anti-PD-L1/anti-LAG3 bispecific antibody may comprise an anti-PD-L1 antibody or an antigen-binding fragment thereof as a PD-L1 targeting moiety.

In an embodiment, the anti-PD-L1 antibody or fragment thereof comprised in the bispecific antibody can specifically bind to an immunoglobulin C (IgC) domain of PD-L1 (e.g., human PD-L1) protein. In some embodiments, the IgC domain consists of amino acid residues 133-225 of a human PD-L1 protein. In some embodiments, the anti-PD-L1 antibody or fragment thereof can bind to at least one of amino acid residues Y134, K162, and N183 of a human PD-L1 protein. In some embodiments, the anti-PD-L1 antibody or fragment thereof does not bind to an immunoglobulin V (IgV) domain of the PD-L1 protein, and for example, the IgV domain consists of amino acid residues 19-127 of a human PD-L1 protein. For example, the human PD-L1 protein may be selected from the group consisting of proteins represented by GenBank Accession No. NP_001254635.1 NP_001300958.1, NP_054862.1, etc., but may not be limited thereto. These anti-PD-L1 antibodies may be useful for therapeutic purposes such as treating various types of cancer, infections (inflammations), etc., and can also be used for diagnostic and prognostic purposes. In an embodiment, the anti-PD-L1 antibody or fragment thereof is capable of specificity to a human PD-L1 protein.

The anti-PD-L1 antibody or fragment thereof may comprise (1) a VH CDR1 having an amino acid sequence selected from the group consisting of SEQ ID NO: 1 and SEQ ID NO: 61-67; (2) a VH CDR2 having an amino acid sequence selected from the group consisting of SEQ ID NO: 2, SEQ ID NO: 68-77, and 525-527; (3) a VH CDR3 having an amino acid sequence selected from the group consisting of SEQ ID NO: 3, SEQ ID NO: 78-90 and SEQ ID NO: 513-519; (4) a VL CDR1 having an amino acid sequence selected from the group consisting of SEQ ID NO: 4, SEQ ID NO: 91-92, and SEQ ID NO: 520-521; (5) a VL CDR2 having an amino acid sequence selected from the group consisting of SEQ ID NO: 5 and SEQ ID NO: 93-105; and (6) a VL CDR3 having an amino acid sequence selected from the group consisting of SEQ ID NO: 6, SEQ ID NO: 106-111, and SEQ ID NO: 522-524. For example, the anti-PD-L1 antibody or fragment thereof may comprise a VH CDR1 having an amino acid sequence of SEQ ID NO: 1; a VH CDR2 having an amino acid sequence of SEQ ID NO: 2; (3) a VH CDR3 having an amino acid sequence of SEQ ID NO: 3 or 515; a VL CDR1 having an amino acid sequence of SEQ ID NO: 4; a VL CDR2 having an amino acid sequence of SEQ ID NO: 5; and a VL CDR3 having an amino acid sequence of SEQ ID NO: 6.

The anti-PD-L1/anti-LAG3 bispecific antibody may comprise an anti-LAG3 antibody or an antigen-binding fragment thereof as a LAG3 targeting moiety. In an embodiment, the anti-LAG3 antibody or fragment thereof can specifically bind to LAG3 (e.g., human LAG3)

protein; for example, the anti-LAG3 antibody or fragment thereof may bind to an extracellular domain of LAG-3.

For instance, the anti-LAG3 antibody or fragment thereof described herein 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 inhibiting the binding to MHC class II molecules, which is a unique and considerable effect of the anti-LAG3 antibody or fragment thereof of the present disclosure, considering that existing anti-LAG-3 antibodies have only shown inhibitory effect to the binding to MHC class II molecules. In some embodiments, the antibodies and fragments thereof of the present disclosure are capable of reversing the inhibitory effect of regulatory T cells (T_{regs}) on effector T cells (T_{effs}). In some embodiments, the antibodies and fragments thereof of the present disclosure are capable of inhibiting the binding between LAG3 Fibrinogen-like Protein 1 (FGL1).

For example, the human LAG3 protein may be selected from the group consisting of proteins represented by GenBank Accession No. NP_002277.4, etc., but may not be limited thereto. These anti-LAG3 antibodies may be useful for therapeutic purposes such as treating various types of cancer, infections (inflammations), etc., and can also be used for diagnostic and prognostic purposes.

In an embodiment, the anti-LAG3 antibody or fragment thereof is capable of specificity to a human LAG3 protein. The anti-LAG3 antibody or fragment thereof may comprise (i) a VH CDR1 having an amino acid sequence selected from the group consisting of SEQ ID NOS: 116-117, 354, and 453-460; (ii) a VH CDR2 having an amino acid sequence selected from the group consisting of SEQ ID NOS: 118-119, 355, and 461-467; (iii) a VH CDR3 having an amino acid sequence selected from the group consisting of SEQ ID NOs: 120-160, 356, and 468-475; (iv) a VL CDR1 having an amino acid sequence selected from the group consisting of SEQ ID NOS: 163-195, 229, 357, and 490; (v) a VL CDR2 having an amino acid sequence selected from the group consisting of SEQ ID NOS: 196-217, 358, and 476-483; and (vi) a VL CDR3 having an amino acid sequence selected from the group consisting of SEQ ID NOS: 218-228, 230-253, 359, and 484-489. For example, the anti-LAG3 antibody or fragment thereof may comprise a VH CDR1 having an amino acid sequence of SEQ ID NO: 354; a VH CDR2 having an amino acid sequence of SEQ ID NO: 355 or 461; a VH CDR3 having an amino acid sequence of SEQ ID NO: 356 or 468; a VL CDR1 having an amino acid sequence of SEQ ID NO: 357 or 490; a VL CDR2 having an amino acid sequence of SEQ ID NO: 358; and a VL CDR3 having an amino acid sequence of SEQ ID NO: 359 or 488.

Also provided are antibodies and fragments that have specificity to the PD-L1 or LAG3 protein alone, or antibodies having additional specificity to one or more other antigens.

In one embodiment, provided is an antibody or antigen-binding fragment thereof having specificity to a human PD-L1 protein, comprising: (1) a VH CDR1 comprising an amino acid sequence selected from the group consisting of SEQ ID NO: 1 and 61-67; (2) a VH CDR2 comprising an amino acid sequence selected from the group consisting of SEQ ID NO: 2, 68-77, and 525-527; (3) a VH CDR3 comprising an amino acid sequence selected from the group consisting of SEQ ID NO: 3, 78-90, and 513-519; (4) a VL CDR1 comprising an amino acid sequence selected from the group consisting of SEQ ID NO: 4, 91-92, and 520-521; (5) a VL CDR2 comprising an amino acid sequence selected from the group consisting of SEQ ID NO: 5, and 93-105; and (6) a VL CDR3 comprising an amino acid sequence selected from the group consisting of SEQ ID NO: 6, 106-111, and 522-524.

In one embodiment, provided is an antibody or antigen-binding fragment thereof having specificity to a human LAG3 protein, comprising: (i) a VH CDR1 comprising an amino acid sequence selected from the group consisting of SEQ ID NO: 116-117, 354, and 453-460; (ii) a VH CDR2 comprising an amino acid sequence selected from the group consisting of SEQ ID NO: 118-119, 355, and 461-467; (iii) a VH CDR3 comprising an amino acid sequence selected from the group consisting of SEQ ID NO: 120-160, 356, and 468-475; (iv) a VL CDR1 comprising an amino acid sequence selected from the group consisting of SEQ ID NO: 163-195, 229, 357, and 490; (v) a VL CDR2 comprising an amino acid sequence selected from the group consisting of SEQ ID NO: 196-217, 358, and 476-483; and (vi) a VL CDR3 comprising an amino acid sequence selected from the group consisting of SEQ ID NO: 218-228, 230-253, 359, and 484-489.

Another embodiment provides a pharmaceutical composition comprising the bispecific antibody as described above. The pharmaceutical composition may further comprise a pharmaceutically acceptable carrier. The pharmaceutical composition may be used for treating and/or preventing a cancer or an infection.

Another embodiment provides a method of treating and/or preventing a cancer or an infection in a subject in need thereof, comprising administering to the subject a pharmaceutically effective amount of the bispecific antibody or the pharmaceutical composition. The method may further step of identifying the subject in need of treating and/or preventing a cancer or an infection, prior to the administering step.

Another embodiment provides a use of the bispecific antibody or the pharmaceutical composition in treating and/or preventing a cancer or an infection. Another embodiment

provides a use of the bispecific antibody in preparing a pharmaceutical composition for treating and/or preventing a cancer or an infection.

In the pharmaceutical compositions, methods and/or uses provided herein, the cancer may be a solid cancer or blood cancer, preferably a solid cancer.

Another embodiment provides a composition for detection of PD-L1, LAG3, or both thereof simultaneously, in a biological sample, the composition comprising the bispecific antibody. Another embodiment provides a method of detection of PD-L1, LAG3, or both thereof simultaneously, in a biological sample, the method comprising contacting the biological sample with the bispecific antibody; and detecting (measuring) an antigen-antibody reaction (binding) between the bispecific antibody and PD-L1, LAG3, or both thereof.

The method of detection may further comprise, after the detecting step, determining that PD-L1, LAG3, or both thereof are present in the biological sample when an antigen-antibody reaction is detected, and/or that PD-L1, LAG3, or both thereof are absent (not present) in the biological sample, when an antigen-antibody reaction is not detected.

Another embodiment provides a pharmaceutical composition for diagnosing a disease associated with PD-L1, LAG3, or both thereof, the composition comprising the bispecific antibody. In another embodiment, provided is a use of the bispecific antibody for diagnosing a disease associated with PD-L1, LAG3, or both thereof.

Another embodiment provides a method of diagnosing a disease associated with PD-L1, LAG3, or both thereof, the method comprising contacting a biological sample obtained from a patient with the bispecific antibody, and detecting antigen-antibody reaction or measuring a level of antigen-antibody reaction in the biological sample. In some embodiments, the method may further comprise contacting a normal sample with the bispecific antibody, and measuring a level of an antigen-antibody reaction in the normal sample. In addition, the method may further comprise comparing the level of the antigen-antibody reaction in the biological sample and in the normal sample, after the measuring step. In addition, after the detecting step or comparing step, the method may further comprise determining the patient as a patient with a disease associated with PD-L1, LAG3, or both thereof, when the antigen-antibody reaction is detected in the biological sample or the level of the antigen-antibody reaction in the biological sample is higher than that of the normal sample.

The disease associated with PD-L1, LAG3, or both thereof may be one associated with activation (e.g., abnormal activation or over-activation) and/or overproduction (overexpression) of PD-L1, LAG3, or both thereof. For example, the disease may be a cancer or an infection, as described above.

An embodiment provides a polynucleotide encoding the bispecific antibody. In particular, an embodiment provides a polynucleotide encoding a heavy chain of the bispecific antibody in an IgG-scFv form which comprises a full-length IgG and a scFv linked to a C-terminus and/or N-terminus of the full-length IgG. Other embodiment provides a polynucleotide encoding a light chain of the bispecific antibody in an IgG-scFv form. Another embodiment provides a recombinant vector comprising the polynucleotide encoding a heavy chain of the bispecific antibody, the polynucleotide encoding a light chain of the bispecific antibody, or both thereof. Another embodiment provides a recombinant cell transfected with the recombinant vector.

Another embodiment provides a method of preparing the bispecific antibody, comprising expressing the polynucleotide encoding a heavy chain of the bispecific antibody, the polynucleotide encoding a light chain of the bispecific antibody in a cell. The step of expressing the polynucleotide may be conducted by culturing the cell comprising the polynucleotide (for example, in a recombinant vector) under a condition allowing the expression of the polynucleotide. The method may further comprise isolating and/or purifying the bispecific antibody from the cell culture, after the step of expressing or culturing.

BRIEF DESCRIPTION OF THE DRAWINGS

FIG. 1 shows that HL1210-3 can bind to human PD-L1 with high affinity.

FIG. 2 shows that HL1210-3 can efficiently inhibit the binding of human PD-L1 to human PD1.

FIG. 3 shows the HL1210-3 antibody can highly efficiently inhibit the binding of PD-1 on PD-L1 expressed on mammalian cells.

FIG. 4 shows that the tested anti-PD-L1 antibodies can promote human T cell response.

FIG. 5 shows the binding kinetics of HL1210-3 to recombinant PD-L1.

FIGS. 6A-6E show that all tested humanized antibodies had comparable binding efficacy to human PD-L1 in contact to chimeric antibody.

FIGS. 7A-7C shows that all tested humanized antibodies can high efficiently bind to PD-L1 expressed on mammalian cells, comparable with chimeric antibody.

FIG. 8 shows that humanized antibody Hu1210-41 can bind to rhesus PD-L1 with lower affinity and cannot bind to rat and mouse PD-L1.

FIG. 9 shows that Hu1210-41 antibody can only specifically binding to B7-H1 (PD-L1), not B7-DC, B7-1, B7-2, B7-H2, PD-1, CD28, CTLA4, ICOS and BTLA.

FIG. 10 shows that Hu1210-41 can efficiently inhibit the binding of human PD-L1 to human PD1 and B7-1.

FIG. 11 shows that Hu1210-41 can efficiently inhibit the binding of human PD-L1 to human PD1 and B7-1.

FIG. 12 shows that the Hu1210-8, Hu1210-9, Hu1210-16, Hu1210-17, Hu1210-21 and Hu1210-36 humanized antibodies can dose dependently promote the IFNγ and IL-2 production in mix lymphocyte reaction.

FIG. 13 shows that the Hu1210-40, Hu1210-41 and Hu1210-17 humanized antibodies can dose dependently promote the IFNγ production in CMV recall assay.

FIG. 14 shows that Hu1210-31 can inhibit the tumor growth by 30%at 5mg/kg in HCC827-NSG-xenograft model.

FIG. 15 shows that Hu1210-41 antibody can dose-dependently inhibit the tumor growth in HCC827-NSG-xenograft model, while the tumor weight was also dose-dependently suppressed by Hu1210-41 antibody.

FIG. 16 plots, for each PD-L1 mutant, the mean binding value as a function of expression (control anti-PD-L1 mAb reactivity).

FIG. 17 illustrates the locations of Y134, K162, and N183, the residues (spheres) involved in binding to the anti-PD-L1 Hu1210-41 antibody.

FIG. 18 shows the results of a binding assay (to human PD-L1) for the derived antibodies.

FIG. 19 shows that antibody B6 more highly efficiently bound to PD-L1 expressed on mammalian cells, as compared to the parental antibody and Tecentriq™ (atezolizumab).

FIG. 20 shows the effects of the antibodies on IL2 production in Jurkat cells in which B6 also exhibited higher potency.

FIG. 21 shows that 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.

FIGS. 22A-22D show 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).

FIG. 23 shows 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 (10μg/ml, 3.333μg/ml, 1.111μg/ml, 0.370μg/ml, 0.123μg/ml, 0.041μg/ml, 0.014μg/ml and 0.005μg/ml).

FIG. 24 shows 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.

FIG. 25 shows 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).

FIG. 26 shows 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 2.1 were used in an in vitro Tregs suppression assay.

FIGS. 27A-27C show ELISA results showing EC50 of the antibody for binding to full extracellular domain of LAG3 (D1-D4 huFc) but not D1-D2 deleted LAG3 (ΔD1-D2 huFc), demonstrating that 122H, 147H and 170H are potent and selective binder for D1 and D2 domain of human LAG3.

FIGS. 28A-28C show that 122H, 147H and 170H antibodies dose dependently inhibited the binding of LAG3 to its receptor MHC class II molecules.

FIG. 29 shows that 122H, 147H and 170H mouse monoclonal antibodies dose dependently promoted IL2 production by Jurkat T cells.

FIG. 30 shows that Humanized monoclonal antibody 147H-13 dose dependently promoted the IL2 production by Jurkat T cells.

FIG. 31 shows binding curves of anti-LAG3 antibodies on Jurkat-LAG3 cells and activated CD4 T cell.

FIG. 32 schematically illustrates an anti-PD-L1/anti-LAG3 bispecific antibody according to an embodiment.

FIG. 33 shows graphs illustrating the binding of the anti-PD-L1/anti-LAG3 bispecific antibody according to an embodiment to human PD-L1 and human LAG3, measured by ELISA.

FIG. 34 shows the SEE assay results for the anti-PD-L1/anti-LAG3 bispecific antibody according to an embodiment. It also shows graphs illustrating the T-cell promoting activities of the anti-PD-L1/anti-LAG3 bispecific antibody according to an embodiment.

FIG. 35 shows a graph illustrating tumor growth inhibition effect of the anti-PD-L1/anti-LAG3 bispecific antibody according to an embodiment.

FIG. 36 shows graphs illustrating the T-cell promoting activities of the anti-PD-L1/anti-LAG3 bispecific antibody according to an embodiment.

FIG. 37 shows graphs illustrating the T-cell promoting activities of the anti-PD-L1/anti-LAG3 bispecific antibody according to an embodiment. FIG. 38 shows the binding of anti-LAG3 monoclonal antibody B3807 and control antibodies to the human LAG3 protein, through enzyme-linked immunosorbent assay (ELISA).

FIG. 39 shows the Biacore analysis result for B3807.

FIG. 40 shows the binding activities of B3807 to human LAG3 on Jurkat and PBMC cells.

FIG. 41 shows the inhibition of soluble LAG-3 (sLAG) binding to MHC class II receptor by B3807.

FIG. 42 shows the effects of the B3807 on IL2 production in Jurkat cells.

FIG. 43 shows the effects of the B3807, as well as in combination with anti-PD-L1 antibody, on IL2 production in primary T cells.

FIG. 44 shows the *in vivo* results of B3807, alone or in combination with anti-PD-1 or anti-PD-L1 antibodies, in inhibiting tumor growth.

FIG. 45 compares B3807 and B3807b in IL2 release and cell-based binding assays, and demonstrates their high level similarity.

FIG. 46 compares the Biacore assay results between B3807 and B3807b.

FIG. 47 demonstrates that B3807 effectively inhibited the binding between soluble LAG-3 and FGL1.

DETAILED DESCRIPTION

Definitions

It is to be noted that the term "a" or "an" entity refers to one or more of that entity for example, "an antibody," is understood to represent one or more antibodies. As such, the terms "a" (or "an"), "one or more," and "at least one" can be used interchangeably herein.

As used herein, the term "polypeptide" is intended to encompass a singular "polypeptide" as well as plural "polypeptides, " and refers to a molecule composed of monomers (amino acids) linearly linked by amide bonds (also known as peptide bonds) . The term "polypeptide" refers to any chain or chains of two or more amino acids, and does not refer to a specific length of the product. Thus, peptides, dipeptides, tripeptides, oligopeptides, "protein, " "amino acid chain, " or any other term used to refer to a chain or chains of two or more amino acids, are included within the definition of "polypeptide, " and the term "polypeptide" may be used instead of, or interchangeably with any of these terms. The term "polypeptide" is also intended to refer to the products of post-expression modifications of the polypeptide, including without limitation glycosylation, acetylation, phosphorylation, amidation, derivatization by known protecting/blocking groups, proteolytic cleavage, or modification by non-naturally occurring amino acids. A polypeptide may be derived from a natural biological source or produced by recombinant technology, but is not necessarily translated from a designated nucleic acid sequence. It may be generated in any manner, including by chemical synthesis.

The term "isolated" as used herein with respect to cells, nucleic acids, such as DNA or RNA, refers to molecules separated from other DNAs or RNAs, respectively, that are present in the natural source of the macromolecule. The term "isolated" as used herein also refers to a nucleic acid or peptide that is substantially free of cellular material, viral material, or culture medium when produced by recombinant DNA techniques, or chemical precursors or other chemicals when chemically synthesized. Moreover, an "isolated nucleic acid" is meant to include nucleic acid fragments which are not naturally occurring as fragments and would not be found in the natural state. The term "isolated" is also used herein to refer to cells or polypeptides which are isolated from other cellular proteins or tissues. Isolated polypeptides is meant to encompass both purified and recombinant polypeptides.

As used herein, the term "recombinant" as it pertains to polypeptides or polynucleotides intends a form of the polypeptide or polynucleotide that does not exist

naturally, a non-limiting example of which can be created by combining polynucleotides or polypeptides that would not normally occur together.

"Homology" or "identity" or "similarity" refers to sequence similarity between two peptides or between two nucleic acid molecules. Homology can be determined by comparing a position in each sequence which may be aligned for purposes of comparison. When a position in the compared sequence is occupied by the same base or amino acid, then the molecules are homologous at that position. A degree of homology between sequences is a function of the number of matching or homologous positions shared by the sequences. An "unrelated" or "non-homologous" sequence shares less than 40% identity, though preferably less than 25% identity, with one of the sequences of the present disclosure.

A polynucleotide or polynucleotide region (or a polypeptide or polypeptide region) has a certain percentage (for example, 60%, 65%, 70%, 75%, 80%, 85%, 90%, 95%, 98% or 99%) of "sequence identity" to another sequence means that, when aligned, that percentage of bases (or amino acids) are the same in comparing the two sequences. This alignment and the percent homology or sequence identity can be determined using software programs known in the art, for example those described in Ausubel et al. eds. (2007) Current Protocols in Molecular Biology. Preferably, default parameters are used for alignment. One alignment program is BLAST, using default parameters. In particular, programs are BLASTN and BLASTP, using the following default parameters: Genetic code = standard; filter = none; strand = both; cutoff = 60; expect = 10; Matrix = BLOSUM62; Descriptions = 50 sequences; sort by = HIGH SCORE; Databases = non-redundant, GenBank + EMBL + DDBJ + PDB + GenBank CDS translations + SwissProtein + SPupdate + PIR. Biologically equivalent polynucleotides are those having the above-noted specified percent homology and encoding a polypeptide having the same or similar biological activity.

The term "an equivalent nucleic acid or polynucleotide" refers to a nucleic acid having a nucleotide sequence having a certain degree of homology, or sequence identity, with the nucleotide sequence of the nucleic acid or complement thereof. A homolog of a double stranded nucleic acid is intended to include nucleic acids having a nucleotide sequence which has a certain degree of homology with or with the complement thereof. In one aspect, homologs of nucleic acids are capable of hybridizing to the nucleic acid or complement thereof. Likewise, "an equivalent polypeptide" refers to a polypeptide having a certain degree of homology, or sequence identity, with the amino acid sequence of a reference polypeptide. In some aspects, the sequence identity is at least 70%, at least 75%, at least 80%, at least 85%, at least 90%, at

least 95%, at least 98%, or at least 99%. In some aspects, the equivalent polypeptide or polynucleotide has one, two, three, four or five addition, deletion, substitution and their combinations thereof as compared to the reference polypeptide or polynucleotide. In some aspects, the equivalent sequence retains the activity (e.g., epitope-binding) or structure (e.g., salt-bridge) of the reference sequence.

Hybridization reactions can be performed under conditions of different "stringency." In general, a low stringency hybridization reaction is carried out at about 40°C in about 10xSSC or a solution of equivalent ionic strength/temperature. A moderate stringency hybridization is typically performed at about 50°C in about 6xSSC, and a high stringency hybridization reaction is generally performed at about 60°C in about 1xSSC. Hybridization reactions can also be performed under "physiological conditions" which is well known to one of skill in the art. A non-limiting example of a physiological condition is the temperature, ionic strength, pH and concentration of Mg^{2+} normally found in a cell.

A polynucleotide is composed of a specific sequence of four nucleotide bases: adenine (A); cytosine (C); guanine (G); thymine (T); and uracil (U) for thymine when the polynucleotide is RNA. Thus, the term "polynucleotide sequence" is the alphabetical representation of a polynucleotide molecule. This alphabetical representation can be input into databases in a computer having a central processing unit and used for bioinformatics applications such as functional genomics and homology searching. The term "polymorphism" refers to the coexistence of more than one form of a gene or portion thereof. A portion of a gene of which there are at least two different forms, i.e., two different nucleotide sequences, is referred to as a "polymorphic region of a gene." A polymorphic region can be a single nucleotide, the identity of which differs in different alleles.

The terms "polynucleotide" and "oligonucleotide" are used interchangeably and refer to a polymeric form of nucleotides of any length, either deoxyribonucleotides or ribonucleotides or analogs thereof. Polynucleotides can have any three-dimensional structure and may perform any function, known or unknown. The following are non-limiting examples of polynucleotides: a gene or gene fragment (for example, a probe, primer, EST or SAGE tag) , exons, introns, messenger RNA (mRNA) , transfer RNA, ribosomal RNA, ribozymes, cDNA, dsRNA, siRNA, miRNA, recombinant polynucleotides, branched polynucleotides, plasmids, vectors, isolated DNA of any sequence, isolated RNA of any sequence, nucleic acid probes and primers. A polynucleotide can comprise modified nucleotides, such as methylated nucleotides and nucleotide analogs. If present, modifications to the nucleotide structure can be imparted before or after assembly of the polynucleotide. The sequence of nucleotides can be interrupted

by non-nucleotide components. A polynucleotide can be further modified after polymerization, such as by conjugation with a labeling component. The term also refers to both double-and single-stranded molecules. Unless otherwise specified or required, any embodiment of this disclosure that is a polynucleotide encompasses both the double-stranded form and each of two complementary single-stranded forms known or predicted to make up the double-stranded form.

The term "encode" as it is applied to polynucleotides refers to a polynucleotide which is said to "encode" a polypeptide if, in its native state or when manipulated by methods well known to those skilled in the art, it can be transcribed and/or translated to produce the mRNA for the polypeptide and/or a fragment thereof. The antisense strand is the complement of such a nucleic acid, and the encoding sequence can be deduced therefrom.

As used herein, an "antibody" or "antigen-binding polypeptide" refers to a polypeptide or a polypeptide complex that specifically recognizes and binds to an antigen. An antibody can be a whole antibody and any antigen binding fragment or a single chain thereof. Thus the term "antibody" includes any protein or peptide containing molecule that comprises at least a portion of an immunoglobulin molecule having biological activity of binding to the antigen. Examples of such include, but are not limited to a complementarity determining region (CDR) of a heavy or light chain or a ligand binding portion thereof, a heavy chain or light chain variable region, a heavy chain or light chain constant region, a framework (FR) region, or any portion thereof, or at least one portion of a binding protein.

The terms "antibody fragment" or "antigen-binding fragment", as used herein, is a portion of an antibody such as $F(ab')_2$, $F(ab)_2$, Fab', Fab, Fv, scFv and the like. Regardless of structure, an antibody fragment binds with the same antigen that is recognized by the intact antibody. The term "antibody fragment" includes aptamers, spiegelmers, and diabodies. The term "antibody fragment" also includes any synthetic or genetically engineered protein that acts like an antibody by binding to a specific antigen to form a complex.

A "single-chain variable fragment" or "scFv" refers to a fusion protein of the variable regions of the heavy (V_H) and light chains (V_L) of immunoglobulins. In some aspects, the regions are connected with a short linker peptide of ten to about 25 amino acids. The linker can be rich in glycine for flexibility, as well as serine or threonine for solubility, and can either connect the N-terminus of the V_H with the C-terminus of the V_L, or vice versa. This protein retains the specificity of the original immunoglobulin, despite removal of the constant regions and the introduction of the linker. ScFv molecules are known in the art and are described, e.g., in US patent 5,892,019.

The term antibody encompasses various broad classes of polypeptides that can be distinguished biochemically. Those skilled in the art will appreciate that heavy chains are classified as gamma, mu, alpha, delta, or epsilon (γ, μ, α, δ, ε) with some subclasses among them (e.g., γl-γ4). It is the nature of this chain that determines the "class" of the antibody as IgG, IgM, IgA IgG, or IgE, respectively. The immunoglobulin subclasses (isotypes) e.g., IgG1, IgG2, IgG3, IgG4, IgG5, etc. are well characterized and are known to confer functional specialization. Modified versions of each of these classes and isotypes are readily discernable to the skilled artisan in view of the instant disclosure and, accordingly, are within the scope of the instant disclosure. All immunoglobulin classes are clearly within the scope of the present disclosure, the following discussion will generally be directed to the IgG class of immunoglobulin molecules. With regard to IgG, a standard immunoglobulin molecule comprises two identical light chain polypeptides of molecular weight approximately 23,000 Daltons, and two identical heavy chain polypeptides of molecular weight 53,000-70,000. The four chains are typically joined by disulfide bonds in a "Y" configuration wherein the light chains bracket the heavy chains starting at the mouth of the "Y" and continuing through the variable region.

Antibodies, antigen-binding polypeptides, variants, or derivatives thereof of the disclosure include, but are not limited to, polyclonal, monoclonal, multispecific, human, humanized, primatized, or chimeric antibodies, single chain antibodies, epitope-binding fragments, e.g., Fab, Fab' and $F(ab')_2$, Fd, Fvs, single-chain Fvs (scFv) , single-chain antibodies, disulfide-linked Fvs (sdFv) , fragments comprising either a VK or VH domain, fragments produced by a Fab expression library, and anti-idiotypic (anti-Id) antibodies (including, e.g., anti-Id antibodies to LIGHT antibodies disclosed herein) . Immunoglobulin or antibody molecules of the disclosure can be of any type (e.g., IgG, IgE, IgM, IgD, IgA, and IgY), class (e.g., IgGl, IgG2, IgG3, IgG4, IgAl and IgA2) or subclass of immunoglobulin molecule.

Light chains are classified as either kappa or lambda (K, λ). Each heavy chain class may be bound with either a kappa or lambda light chain. In general, the light and heavy chains are covalently bonded to each other, and the "tail" portions of the two heavy chains are bonded to each other by covalent disulfide linkages or non-covalent linkages when the immunoglobulins are generated either by hybridomas, B cells or genetically engineered host cells. In the heavy chain, the amino acid sequences run from an N-terminus at the forked ends of the Y configuration to the C-terminus at the bottom of each chain.

Both the light and heavy chains are divided into regions of structural and functional homology. The terms "constant" and "variable" are used functionally. In this regard, it will be appreciated that the variable domains of both the light (VK) and heavy (VH) chain portions determine antigen recognition and specificity. Conversely, the constant domains of the light chain (CK) and the heavy chain (CH1, CH2 or CH3) confer important biological properties such as secretion, transplacental mobility, Fc receptor binding, complement binding, and the like. By convention the numbering of the constant region domains increases as they become more distal from the antigen-binding site or amino-terminus of the antibody. The N-terminal portion is a variable region and at the C-terminal portion is a constant region; the CH3 and CK domains actually comprise the carboxy-terminus of the heavy and light chain, respectively.

As indicated above, the variable region allows the antibody to selectively recognize and specifically bind epitopes on antigens. That is, the VK domain and VH domain, or subset of the complementarity determining regions (CDRs) , of an antibody combine to form the variable region that defines a three dimensional antigen-binding site. This quaternary antibody structure forms the antigen-binding site present at the end of each arm of the Y. More specifically, the antigen-binding site is defined by three CDRs on each of the VH and VK chains (i.e. CDR-H1, CDR-H2, CDR-H3, CDR-L1, CDR-L2 and CDR-L3). In some instances, e.g., certain immunoglobulin molecules derived from camelid species or engineered based on camelid immunoglobulins, a complete immunoglobulin molecule may consist of heavy chains only, with no light chains. See, e.g., Hamers-Casterman et al., Nature 363: 446-448 (1993).

In naturally occurring antibodies, the six "complementarity determining regions" or "CDRs" present in each antigen-binding domain are short, non-contiguous sequences of amino acids that are specifically positioned to form the antigen-binding domain as the antibody assumes its three dimensional configuration in an aqueous environment. The remainder of the amino acids in the antigen-binding domains, referred to as "framework" regions, show less inter-molecular variability. The framework regions largely adopt a β-sheet conformation and the CDRs form loops which connect, and in some cases form part of, the β-sheet structure. Thus, framework regions act to form a scaffold that provides for positioning the CDRs in correct orientation by inter-chain, non-covalent interactions. The antigen-binding domain formed by the positioned CDRs defines a surface complementary to the epitope on the immunoreactive antigen. This complementary surface promotes the non-covalent binding of the antibody to its cognate epitope. The amino acids comprising the CDRs and the framework regions, respectively, can be readily identified for any given heavy or light chain variable region by one of ordinary skill in the art, since they have been precisely defined (see

www.bioinf.org.uk: Dr. Andrew C.R. Martin's Group; "Sequences of Proteins of Immunological Interest," Kabat, E., et al., U.S. Department of Health and Human Services, (1983); and Chothia and Lesk, J. MoI. Biol., 196: 901-917 (1987)).

In the case where there are two or more definitions of a term which is used and/or accepted within the art, the definition of the term as used herein is intended to include all such meanings unless explicitly stated to the contrary. A specific example is the use of the term "complementarity determining region" ("CDR") to describe the non-contiguous antigen combining sites found within the variable region of both heavy and light chain polypeptides. This particular region has been described by Kabat et al., U.S. Dept. of Health and Human Services, "Sequences of Proteins of Immunological Interest" (1983) and by Chothia et al., J. MoI. Biol. 196: 901-917 (1987), which are incorporated herein by reference in their entireties. The CDR definitions according to Kabat and Chothia include overlapping or subsets of amino acid residues when compared against each other. Nevertheless, application of either definition to refer to a CDR of an antibody or variants thereof is intended to be within the scope of the term as defined and used herein. The appropriate amino acid residues which encompass the CDRs as defined by each of the above cited references are set forth in the table below as a comparison. The exact residue numbers which encompass a particular CDR will vary depending on the sequence and size of the CDR. Those skilled in the art can routinely determine which residues comprise a particular CDR given the variable region amino acid sequence of the antibody.

[Table 1]

	Kabat	Chothia
CDR-H1	31-35	26-32
CDR-H2	50-65	52-58
CDR-H3	95-102	95-102
CDR-L1	24-34	26-32
CDR-L2	50-56	50-52
CDR-L3	89-97	91-96

Kabat et al. also defined a numbering system for variable domain sequences that is applicable to any antibody. One of ordinary skill in the art can unambiguously assign this system of "Kabat numbering" to any variable domain sequence, without reliance on any experimental data beyond the sequence itself. As used herein, "Kabat numbering" refers to the numbering system set forth by Kabat et al., U.S. Dept. of Health and Human Services, "Sequence of Proteins of Immunological Interest" (1983).

In addition to table above, the Kabat number system describes the CDR regions as follows: CDR-H1 begins at approximately amino acid 31 (i.e., approximately 9 residues after the first cysteine residue), includes approximately 5-7 amino acids, and ends at the next tryptophan residue. CDR-H2 begins at the fifteenth residue after the end of CDR-H1, includes approximately 16-19 amino acids, and ends at the next arginine or lysine residue. CDR-H3 begins at approximately the thirty third amino acid residue after the end of CDR-H2; includes 3-25 amino acids; and ends at the sequence W-G-X-G, where X is any amino acid. CDR-L1 begins at approximately residue 24 (i.e., following a cysteine residue); includes approximately 10-17 residues; and ends at the next tryptophan residue. CDR-L2 begins at approximately the sixteenth residue after the end of CDR-L1 and includes approximately 7 residues. CDR-L3 begins at approximately the thirty third residue after the end of CDR-L2 (i.e., following a cysteine residue); includes approximately 7-11 residues and ends at the sequence F or W-G-X-G, where X is any amino acid.

Antibodies disclosed herein may be from any animal origin including birds and mammals. Preferably, the antibodies are human, murine, donkey, rabbit, goat, guinea pig, camel, llama, horse, or chicken antibodies. In another embodiment, the variable region may be condricthoid in origin (e.g., from sharks).

As used herein, the term "heavy chain constant region" includes amino acid sequences derived from an immunoglobulin heavy chain. A polypeptide comprising a heavy chain constant region comprises at least one of: a CH1 domain, a hinge (e.g., upper, middle, and/or lower hinge region) domain, a CH2 domain, a CH3 domain, or a variant or fragment thereof. For example, an antigen-binding polypeptide for use in the disclosure may comprise a polypeptide chain comprising a CH1 domain; a polypeptide chain comprising a CH1 domain, at least a portion of a hinge domain, and a CH2 domain; a polypeptide chain comprising a CH1 domain and a CH3 domain; a polypeptide chain comprising a CH1 domain, at least a portion of a hinge domain, and a CH3 domain, or a polypeptide chain comprising a CH1 domain, at least a portion of a hinge domain, a CH2 domain, and a CH3 domain. In another embodiment, a polypeptide of the disclosure comprises a polypeptide chain comprising a CH3 domain. Further, an antibody for use in the disclosure may lack at least a portion of a CH2 domain (e.g., all or part of a CH2 domain) . As set forth above, it will be understood by one of ordinary skill in the art that the heavy chain constant region may be modified such that they vary in amino acid sequence from the naturally occurring immunoglobulin molecule.

The heavy chain constant region of an antibody disclosed herein may be derived from different immunoglobulin molecules. For example, a heavy chain constant region of a

polypeptide may comprise a CH1 domain derived from an IgG1 molecule and a hinge region derived from an IgG3 molecule. In another example, a heavy chain constant region can comprise a hinge region derived, in part, from an IgG1 molecule and, in part, from an IgG3 molecule. In another example, a heavy chain portion can comprise a chimeric hinge derived, in part, from an IgG1 molecule and, in part, from an IgG4 molecule.

As used herein, the term "light chain constant region" includes amino acid sequences derived from antibody light chain. Preferably, the light chain constant region comprises at least one of a constant kappa domain or constant lambda domain.

A "light chain-heavy chain pair" refers to the collection of a light chain and heavy chain that can form a dimer through a disulfide bond between the CL domain of the light chain and the CH1 domain of the heavy chain.

As previously indicated, the subunit structures and three dimensional configuration of the constant regions of the various immunoglobulin classes are well known. As used herein, the term "VH domain" includes the amino terminal variable domain of an immunoglobulin heavy chain and the term "CH1 domain" includes the first (most amino terminal) constant region domain of an immunoglobulin heavy chain. The CH1 domain is adjacent to the VH domain and is amino terminal to the hinge region of an immunoglobulin heavy chain molecule.

As used herein the term "CH2 domain" includes the portion of a heavy chain molecule that extends, e.g., from about residue 244 to residue 360 of an antibody using conventional numbering schemes (residues 244 to 360, Kabat numbering system; and residues 231-340, EU numbering system; see Kabat et al., U.S. Dept. of Health and Human Services, "Sequences of Proteins of Immunological Interest" (1983). The CH2 domain is unique in that it is not closely paired with another domain. Rather, two N-linked branched carbohydrate chains are interposed between the two CH2 domains of an intact native IgG molecule. It is also well documented that the CH3 domain extends from the CH2 domain to the C-terminal of the IgG molecule and comprises approximately 108 residues.

As used herein, the term "hinge region" includes the portion of a heavy chain molecule that joins the CH1 domain to the CH2 domain. This hinge region comprises approximately 25 residues and is flexible, thus allowing the two N-terminal antigen-binding regions to move independently. Hinge regions can be subdivided into three distinct domains: upper, middle, and lower hinge domains (Roux et al., J. Immunol 161: 4083 (1998)).

As used herein the term "disulfide bond" includes the covalent bond formed between two sulfur atoms. The amino acid cysteine comprises a thiol group that can form a disulfide bond or bridge with a second thiol group. In most naturally occurring IgG molecules, the CH1

and CK regions are linked by a disulfide bond and the two heavy chains are linked by two disulfide bonds at positions corresponding to 239 and 242 using the Kabat numbering system (position 226 or 229, EU numbering system).

As used herein, the term "chimeric antibody" will be held to mean any antibody wherein the immunoreactive region or site is obtained or derived from a first species and the constant region (which may be intact, partial or modified in accordance with the instant disclosure) is obtained from a second species. In certain embodiments the target binding region or site will be from a non-human source (e.g. mouse or primate) and the constant region is human.

As used herein, "percent humanization" is calculated by determining the number of framework amino acid differences (i.e., non-CDR difference) between the humanized domain and the germline domain, subtracting that number from the total number of amino acids, and then dividing that by the total number of amino acids and multiplying by 100.

By "specifically binds" or "has specificity to," it is generally meant that an antibody binds to an epitope via its antigen-binding domain, and that the binding entails some complementarity between the antigen-binding domain and the epitope. According to this definition, an antibody is said to "specifically bind" to an epitope when it binds to that epitope, via its antigen-binding domain more readily than it would bind to a random, unrelated epitope. The term "specificity" is used herein to qualify the relative affinity by which a certain antibody binds to a certain epitope. For example, antibody "A" may be deemed to have a higher specificity for a given epitope than antibody "B," or antibody "A" may be said to bind to epitope "C" with a higher specificity than it has for related epitope "D." Preferably, the antibody binds to an antigen (or epitope) 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.

As used herein, the terms "treat" or "treatment" may refer to both therapeutic treatment and prophylactic or preventative measures, wherein the object is to prevent or slow down (lessen) an undesired physiological change or disorder, such as the progression of cancer. Beneficial or desired clinical results include, but are not limited to, alleviation of symptoms, diminishment of extent of disease, stabilized (i.e., not worsening) state of disease, delay or slowing of disease progression, amelioration or palliation of the disease state, and remission (whether partial or total) , whether detectable or undetectable. "Treatment" can also mean prolonging survival as compared to expected survival if not receiving treatment. Those in need

of treatment include those already with the condition or disorder as well as those prone to have the condition or disorder or those in which the condition or disorder is to be prevented.

By "subject" or "individual" or "animal" or "patient" or "mammal," may refer to any subject, particularly a mammalian subject, for whom diagnosis, prognosis, or therapy is desired. Mammalian subjects include humans, domestic animals, farm animals, and zoo, sport, or pet animals such as dogs, cats, guinea pigs, rabbits, rats, mice, horses, cattle, cows, and so on.

As used herein, phrases such as "to a patient in need of treatment" or "a subject in need of treatment" includes subjects, such as mammalian subjects, that would benefit from administration of an antibody or composition of the present disclosure used, e.g., for detection, for a diagnostic procedure and/or for treatment.

The present disclosure provides an anti-PD-L1/anti-LAG3 bispecific antibody capable to effectively block the interactions between PD-L1 and its receptor PD-1 and between LAG3 and its ligand (e.g., a MHC class II molecule). The bispecific antibody may have high binding affinity to both of a PD-L1 protein (e.g., a human PD-L1 protein) and a LAG3 protein (e.g., a human LAG3 protein).

The anti-PD-L1/anti-LAG3 bispecific antibody may comprise an anti-PD-L1 antibody or an antigen-binding fragment thereof as a PD-L1 targeting moiety, which is capable of specifically recognizing and/or binding to a PD-L1 protein, and an anti-LAG3 antibody or an antigen-binding fragment thereof as a LAG3 targeting moiety, which is capable of specifically recognizing and/or binding to a LAG3 protein.

Anti-PD-L1 antibody

The anti-PD-L1/anti-LAG3 bispecific antibody may comprise an anti-PD-L1 antibody or an antigen-binding fragment thereof as a PD-L1 targeting moiety. The anti-PD-L1 antibody or antigen-binding fragment thereof may exhibit potent binding and inhibitory activities to PD-L1, and be useful for therapeutic and diagnostics uses.

The PD-L1 protein is a 40kDa type 1 transmembrane protein. The PD-L1 protein may be a human PD-L1 protein, and the human PD-L1 protein may be selected from the group consisting of proteins represented by GenBank Accession No. NP_001254635.1, NP_001300958.1, NP_054862.1, etc., but may not be limited thereto. The human PD-L1 protein includes an extracellular portion including an N-terminal immunoglobulin V (IgV) domain (amino acids 19-127) and a C-terminal immunoglobulin C (IgC) domain (amino acids 133-225). Unlike pre-existing anti-PD-L1 antibodies, which bind to the IgV domain of PD-L1,

thereby disrupting the binding between PD-1 and PD-L1, the anti-PD-L1 antibody or fragment thereof comprised in the bispecific antibody may not bind to an immunoglobulin V (IgV) domain of the PD-L1 protein but bind to the IgC domain of PD-L1, to effectively inhibit PD-L1, thereby improving therapeutic effects.

In particular, the anti-PD-L1 antibody or fragment thereof comprised in the bispecific antibody can specifically bind to an immunoglobulin C (IgC) domain of PD-L1 protein. In the case of human PD-L1 protein, the Ig C domain comprises or consists essentially of amino acid residues 133-225 of full-length of the human PD-L1 protein. More specifically, the anti-PD-L1 antibody or fragment thereof can bind to at least one selected from the amino acid residues Y134, K162, and N183 of human PD-L1 protein. In some embodiments, the anti-PD-L1 antibody or fragment thereof can bind to at least two selected from the amino acid residues Y134, K162, and N183 of human PD-L1 protein. In some embodiments, the anti-PD-L1 antibody or fragment thereof does not bind to an immunoglobulin V (IgV) domain of the PD-L1 protein, wherein the IgV domain consists of amino acid residues 19-127 of human PD-L1 protein.

In an embodiment, antibodies and fragments thereof are provided that are capable of specific binding to a human PD-L1 protein. These antibodies may be useful for therapeutic purposes such as treating various types of cancer, infections (inflammations), etc., and can also be used for diagnostic and prognostic purposes.

The anti-PD-L1 antibody or fragment thereof may comprise (1) a VH CDR1 having an amino acid sequence selected from the group consisting of SEQ ID NO: 1 and SEQ ID NO: 61-67; (2) a VH CDR2 having an amino acid sequence selected from the group consisting of SEQ ID NO: 2, SEQ ID NO: 68-77, and 525-527; (3) a VH CDR3 having an amino acid sequence selected from the group consisting of SEQ ID NO: 3, SEQ ID NO: 78-90 and SEQ ID NO: 513-519; (4) a VL CDR1 having an amino acid sequence selected from the group consisting of SEQ ID NO: 4, SEQ ID NO: 91-92, and SEQ ID NO: 520-521; (5) a VL CDR2 having an amino acid sequence selected from the group consisting of SEQ ID NO: 5 and SEQ ID NO: 93-105; and (6) a VL CDR3 having an amino acid sequence selected from the group consisting of SEQ ID NO: 6, SEQ ID NO: 106-111, and SEQ ID NO: 522-524.

[Table 2] CDRs of anti-PD-L1 antibodies

Name	Sequence	SEQ ID NO:
VH CDR1	SYDMS	1
	TYDMS	61
	CYDMS	62
	SEDMS	63

	SHDMS	64
	SWDMS	65
	SYDMT	66
	SYDMC	67
VH CDR2	**TISDGGGYIYYSDSVKG**	2
	TISDGGAYIYYSDSVKG	68
	TISDGGPYIYYSDSVKG	69
	TISDGGGFIYYSDSVKG	70
	TISDGGGHIYYSDSVKG	71
	TISDGGGWIYYSDSVKG	72
	TISDGGGYIYYSDTVKG	73
	TISDGGGYIYYSDCVKG	74
	TISDGGGYIYYSDSLKG	75
	TISDGGGYIYYSDSIKG	76
	TISDGGGYIYYSDSMKG	77
	TISDAGGYIYYSDSVKG	525
	TISDAGGYIYYRDSVKG	526
	TISDGGGYIYYRDSVKG	527
VH CDR3	**EFGKRYALDY**	3
	QFGKRYALDY	78
	DFGKRYALDY	79
	NFGKRYALDY	80
	EYGKRYALDY	81
	EHGKRYALDY	82
	EWGKRYALDY	83
	EFAKRYALDY	84
	EFPKRYALDY	85
	EFGRRYALDY	86
	EFGKKYALDY	87
	EFGKRFALDY	88
	EFGKRHALDY	89
	EFGKRWALDY	90
	EFGKRYALDS	513
	EIFNRYALDY	514
	ELPWRYALDY	515
	ELHFRYALDY	516
	ELYFRYALDY	517
	ELLHRYALDY	518
	ELRGRYALDY	519
VL CDR1	**KASQDVTPAVA**	4
	KATQDVTPAVA	91
	KACQDVTPAVA	92
	KAKQDVTPAVA	520

	KASQDVWPAVA	521
VL CDR2	**STSSRYT**	5
	TTSSRYT	93
	CTSSRYT	94
	SSSSRYT	95
	SMSSRYT	96
	SVSSRYT	97
	STTSRYT	98
	STCSRYT	99
	STSTRYT	100
	STSCRYT	101
	STSSKYT	102
	STSSRFT	103
	STSSRHT	104
	STSSRWT	105
VL CDR3	**QQHYTTPLT**	6
	EQHYTTPLT	106
	DQHYTTPLT	107
	NQHYTTPLT	108
	QEHYTTPLT	109
	QDHYTTPLT	110
	QNHYTTPLT	111
	MQHYTTPLT	522
	QQHSTTPLT	523
	QQHSDAPLT	524

In some embodiments, an antibody or fragment thereof includes no more than one, no more than two, or no more than three of the above substitutions. In some embodiments, the antibody or fragment thereof includes a VH CDR1 of SEQ ID NO: 1 or any one of SEQ ID NO: 61-67, a VH CDR2 of SEQ ID NO: 2, 525, 526 or 527, a VH CDR3 of SEQ ID NO: 3, a VL CDR1 of SEQ ID NO: 4, a VL CDR2 of SEQ ID NO: 5, and a VL CDR3 of SEQ ID NO: 6.

In some embodiments, the antibody or fragment thereof includes a VH CDR1 of SEQ ID NO: 1, a VH CDR2 of SEQ ID NO: 2 or any one of SEQ ID NO: 68- 77, 525, 526 or 527, a VH CDR3 of SEQ ID NO: 3, a VL CDR1 of SEQ ID NO: 4, a VL CDR2 of SEQ ID NO: 5, and a VL CDR3 of SEQ ID NO: 6.

In some embodiments, the antibody or fragment thereof includes a VH CDR1 of SEQ ID NO: 1, a VH CDR2 of SEQ ID NO: 2, 525, 526 or 527, a VH CDR3 of SEQ ID NO: 3 or

any one of SEQ ID NO: 78- 90 and 513-519, a VL CDR1 of SEQ ID NO: 4, a VL CDR2 of SEQ ID NO: 5, and a VL CDR3 of SEQ ID NO: 6.

In some embodiments, the antibody or fragment thereof includes a VH CDR1 of SEQ ID NO: 1, a VH CDR2 of SEQ ID NO: 2, 525, 526 or 527, a VH CDR3 of SEQ ID NO: 3, a VL CDR1 of SEQ ID NO: 4 or any one of SEQ ID NO: 91- 92 and 520-521, a VL CDR2 of SEQ ID NO: 5, and a VL CDR3 of SEQ ID NO: 6.

In some embodiments, the antibody or fragment thereof includes a VH CDR1 of SEQ ID NO: 1, a VH CDR2 of SEQ ID NO: 2, 525, 526 or 527, a VH CDR3 of SEQ ID NO: 3, a VL CDR1 of SEQ ID NO: 4, a VL CDR2 of SEQ ID NO: 5 or any one of SEQ ID NO: 93-105, and a VL CDR3 of SEQ ID NO: 6.

In some embodiments, the antibody or fragment thereof includes a VH CDR1 of SEQ ID NO: 1, a VH CDR2 of SEQ ID NO: 2, 525, 526 or 527, a VH CDR3 of SEQ ID NO: 3, a VL CDR1 of SEQ ID NO: 4, a VL CDR2 of SEQ ID NO: 5, and a VL CDR3 of SEQ ID NO: 6 or any one of SEQ ID NO: 106- 111 and 522-524.

For example, the anti-PD-L1 antibody or fragment thereof may comprise a VH CDR1 having an amino acid sequence of SEQ ID NO: 1; a VH CDR2 having an amino acid sequence of SEQ ID NO: 2, 525, 526 or 527; (3) a VH CDR3 having an amino acid sequence of SEQ ID NO: 3 or 515; a VL CDR1 having an amino acid sequence of SEQ ID NO: 4; a VL CDR2 having an amino acid sequence of SEQ ID NO: 5; and a VL CDR3 having an amino acid sequence of SEQ ID NO: 6.

In some embodiments, an anti-PD-L1 antibody or fragment thereof is provided that comprises a VH CDR1 having an amino acid sequence of SEQ ID NO: 1; a VH CDR2 having an amino acid sequence of SEQ ID NO: 525; a VH CDR3 having an amino acid sequence of SEQ ID NO: 3; a VL CDR1 having an amino acid sequence of SEQ ID NO: 4; a VL CDR2 having an amino acid sequence of SEQ ID NO: 5; and a VL CDR3 having an amino acid sequence of SEQ ID NO: 6.

In some embodiments, an anti-PD-L1 antibody or fragment thereof is provided that comprises a VH CDR1 having an amino acid sequence of SEQ ID NO: 1; a VH CDR2 having an amino acid sequence of SEQ ID NO: 526; a VH CDR3 having an amino acid sequence of SEQ ID NO: 515; a VL CDR1 having an amino acid sequence of SEQ ID NO: 4; a VL CDR2 having an amino acid sequence of SEQ ID NO: 5; and a VL CDR3 having an amino acid sequence of SEQ ID NO: 6.

Non-limiting examples of VH (heavy chain variable region) are provided in SEQ ID NOS: 7-26, 113, 493, 495, 497, 499, 501, 503, 505, 507, 509, and 511, wherein SEQ ID NO:

113 is the mouse VH, SEQ ID NOs: 7-26 are humanized ones, and SEQ ID NO: 493, 495, 497, 499, 501, 503, 505, 507, 509, and 511 is an affinity-matured one of the humanized antibodies. Further, among the humanized VHs, SEQ ID NO: 9-15, 17-21 and 23-26 include one or more back-mutations to the mouse version. Likewise, non-limiting examples of VL (VK; light chain (kappa type) variable region) are provided in SEQ ID NOS: 27-33, 494, 496, 498, 500, 502, 504, 506, 508, 510, and 512. SEQ ID NO: 28 and 30 are the originally derived, CDR-grafted, and humanized sequences as shown in the examples, and SEQ ID NO: 29 and 31-33 are humanized VL with back-mutations.

The back-mutations may be useful for retaining certain characteristics of the anti-PD-L1 antibodies. In some embodiments, the anti-PD-L1 antibodies of the present disclosure, in particular the human or humanized ones, may include one or more of the back-mutations. In some embodiments, the back-mutation (i.e., included amino acid at the specified position) in a heavy chain variable region (VH) is one or more selected from (a) Ser at position 44, (b) Ala at position 49, (c) Ala at position 53, (d) Ile at position 91, (e) Glu at position 1, (f) Val at position 37, (g) Thr at position 40 (h) Val at position 53, (i) Glu at position 54, (j) Asn at position 77, (k) Arg at position 94, and (l) Thr at position 108, of the heavy chain variable region, according to Kabat numbering, and combinations thereof. In some embodiments, the VH back-mutations are selected from (a) Ser at position 44, (b) Ala at position 49, (c) Ala at position 53, and/or (d) Ile at position 91, of the heavy chain variable region, according to Kabat numbering, and combinations thereof.

In some embodiments, the back-mutation in a light chain variable region (VL) is one or more selected from (a) Ser at position 22, (b) Gln at position 42, (c) Ser at position 43, (d) Asp at position 60, and (e) Thr at position 63, of the light chain variable region, according to Kabat numbering, and combinations thereof.

In some embodiments, the anti-PD-L1 antibody of the present disclosure or fragment thereof may comprise a VH selected from SEQ ID NO: 7-26, 113, 493, 495, 497, 499, 501, 503, 505, 507, 509, and 511, a VL selected from SEQ ID NO: 27-33, 494, 496, 498, 500, 502, 504, 506, 508, 510, and 512, or their respective biological equivalents as described above. A biological equivalent of the VH and/or VL may have an amino acid sequence that includes the designated amino acids (e.g., CDRs) while having sequence identity 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%. A biological equivalent of SEQ ID NO: 20, for instance, can be a VH that has an overall 80%, 85%, 90%, 95%, 96%, 97%, 98% or 99% sequence identity to SEQ ID NO: 20 but retains the

CDRs (SEQ ID NO: 1-6 or their variants), and optionally retains one or more, or all of the back-mutations.

Non-limiting examples of the antibody or fragment thereof may comprise a heavy chain variable region comprising or consisting essentially of the amino acid sequence of SEQ ID NO: 20 or 501, or a biological equivalent thereof, and a light chain variable region comprising or consisting essentially of the amino acid sequence of SEQ ID NO: 28 or 502, or a biological equivalent thereof.

In some embodiments, the anti-PD-L1 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 may be a kappa or lambda chain constant region. In some embodiments, the antibody is of an isotype of IgG, IgM, IgA, IgE or IgD, for example, human IgG, human IgM, human IgA, human IgE, or human IgD. In some embodiments, the isotype may be IgG, for example human IgG, such as, IgG1, IgG2, IgG3, or IgG4. In some embodiments, the fragment (antigen-binding fragment of the anti-PD-L1 antibody) may be any fragment comprising heavy chain CDRs and/or light chain CDRs of the antibody, and for example, it may be selected from the group consisting of Fab, Fab', $F(ab')_2$, Fd (comprising a heavy chain variable region and a CH1 domain), Fv (a heavy chain variable region and/or a light chain variable region), single-chain Fv (scFv; comprising or consisting essentially of a heavy chain variable region and a light chain variable region, in any order, and a peptide linker between the heavy chain variable region and the light chain variable region), single-chain antibodies, disulfide-linked Fvs (sdFv), and the like.

Without limitation, the anti-PD-L1 antibody or fragment thereof is a chimeric antibody, a humanized antibody, or a fully human antibody. In one aspect, antibody or fragment thereof is not naturally occurring, or chemically or recombinantly synthesized.

Given that each of these antibodies can bind to PD-L1 such as human PD-L1, the CDR sequences or V_H and V_L sequences can be "mixed and matched" to create other anti-LAG-3 binding molecules of the disclosure. Preferably, when the CDR sequences or V_H and V_L chains are mixed and matched, for example, 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.

Anti-LAG3 antibody

The anti-PD-L1/anti-LAG3 bispecific antibody may comprise an anti-LAG3 antibody or an antigen-binding fragment thereof as a LAG3 targeting moiety.

In an embodiment, antibodies and fragments thereof are provided that can specifically bind to LAG3 (e.g., human LAG3) protein; for example, the anti-LAG3 antibody or fragment thereof may bind to an extracellular domain of LAG-3.

For example, the human LAG3 protein may be selected from the group consisting of proteins represented by GenBank Accession No. NP_002277.4, etc., but may not be limited thereto. These anti-LAG3 antibodies may be useful for therapeutic purposes such as treating various types of cancer, infections (inflammations), etc., and can also be used for diagnostic and prognostic purposes.

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 002277.4. 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 002277.4 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 002277.4. 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.

As demonstrated in the experimental examples, some of the anti-LAG-3 antibodies disclosed 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 fragments thereof of the present disclosure are capable of reversing the inhibitory effect of regulatory T cells (T_{regs}) on effector T cells (T_{effs}). In some embodiments, the antibodies and fragments thereof of the present disclosure are capable of inhibiting the binding between LAG3 and Fibrinogen-like Protein 1 (FGL1).

These anti-LAG3 antibodies may be useful for therapeutic purposes such as treating various types of cancer, infections (inflammations), etc., and can also be used for diagnostic and prognostic purposes.

In an embodiment, an antibody or fragment thereof is provided that is capable of specificity to a human LAG3 protein. The anti-LAG3 antibody or fragment thereof may comprise (i) a VH CDR1 having an amino acid sequence selected from the group consisting of SEQ ID NOS: 116-117, 354, and 453-460; (ii) a VH CDR2 having an amino acid sequence selected from the group consisting of SEQ ID NOS: 118-119, 355, and 461-467; (iii) a VH CDR3 having an amino acid sequence selected from the group consisting of SEQ ID NOs: 120-160, 356, and 468-475; (iv) a VL CDR1 having an amino acid sequence selected from the group consisting of SEQ ID NOS: 163-195, 229, 357, and 490; (v) a VL CDR2 having an amino acid sequence selected from the group consisting of SEQ ID NOS: 196-217, 358, and 476-483; and (vi) a VL CDR3 having an amino acid sequence selected from the group consisting of SEQ ID NOS: 218-228, 230-253, 359, and 484-489. For example, the anti-LAG3 antibody or fragment thereof may comprise a VH CDR1 having an amino acid sequence of SEQ ID NO: 354; a VH CDR2 having an amino acid sequence of SEQ ID NO: 355 or 461; a VH CDR3 having an amino acid sequence of SEQ ID NO: 356 or 468; a VL CDR1 having an amino acid sequence of SEQ ID NO: 357 or 490; a VL CDR2 having an amino acid sequence of SEQ ID NO: 358; and a VL CDR3 having an amino acid sequence of SEQ ID NO: 359 or 488.

[Table 3] CDRs of anti-LAG3 antibodies

Name	Sequence	SEQ ID NO:
VH CDR1	SYAIS	116
	SYAMS	117
	GYTFTNYWLG	354
	GYTFENYWLG	453
	GYMFTNYWLG	454

	GYTFDNYWLG	455
	GYTFGNYWLG	456
	GYTFTNYWLW	457
	GYLFTNYWLG	458
	GYTFTNYWLS	459
	GFTFTNYWLG	460
VH CDR2	GIIPIFGTANYAQKFQG	118
	AISGSGGSTYYADSVKG	119
	DIYPGGDYINYNEKFKG	355
	DIYPGGDYIVYNEKFKG	461
	DIYPGGDIINYNEKFKG	462
	DIYPGGDVINYNEKFKG	463
	DIFPGGDYINYNEKFKG	464
	DIYPGGDLINYNEKFKG	465
	DIYPGGDHINYNEKFKG	466
	EIYPGGDYITYNEKFKG	467
VH CDR3	ARGSSWFDY	120
	ASSYHGGGYHRY	121
	TTSKYSGSALRY	122
	ARDRTGAFDY	123
	ARHETVAGSFDY	124
	ARTGYYGGNSGAFDI	125
	ARAGTGMDLVFNS	126
	ARGLARGDLNFGY	127
	TREPHFDY	128
	TTAAPGSYYLVFHY	129
	ARDAGPVGYYGMDV	130
	AGDGLYGSGSFGY	131
	AKDIRWFYGMDV	132
	ARHESGIAGGHFDY	133
	AKDIRWYYGMDV	134
	AKGVRGTYQIGYYGMDV	135
	ARQGTAMALDY	136
	VRDLQDWNYGGAAY	137
	ARDDYYYGQFDS	138
	AREITGTSYTALDS	139
	ARGHIDGQAAGDY	140
	AASTLRVPNPPY	141
	ARSGDRYDFWSGY	142
	TRGQDSTWYSSFDY	143
	AASTLRLPNPPY	144
	ATTQTSFYSHGMDV	145
	ARVRKTPFWGALDS	146
	ARGFTYGDFIFDY	147

	ARDVRGVTYLGMDV	148
	ARVRKTPFWGTLDS	149
	ARVRRTPFWGALDS	150
	AKRKGLGSPTDYYYGMDV	151
	VRPEYDTYYYGMDV	152
	AKGGGSYDY	153
	ARALNGMDV	154
	TRPLQGIAAADSYYYYAMDV	155
	ARLHSYLSEEFDP	156
	AKLSAVNTYIDD	157
	ARVTKTPFWGTLDY	158
	ARVSQSPVWGYFDY	159
	AKDGYYDFWSGYSDY	160
	PNLPGDY	356
	PNLPKDH	468
	PDLPGDY	469
	PGLPKDY	470
	PNLPKDY	471
	PNLPRDY	472
	PGLPRDY	473
	PGLPQDY	474
	PDLPKDY	475
VL CDR1	QANQDIHHYLN	161
	KSSQSVLYSSSNKNYLA	162
	KSSQSVLYSSNNKNYLA	163
	RSSQNLLHSDGYNYLN	164
	KSSQSVLYTSNNKNYLA	165
	QASQDINRYLS	166
	QASQDISNYLN	167
	QASQDISNYLN	167
	RASQTISSHLN	168
	RASQGIAGWLA	169
	RASQGVSSWLA	170
	KSSQSLFYHSNNHNYLA	171
	RASQGISSSLA	172
	QASRDISNSLS	173
	RASQSISRYLN	174
	RASRSISNWLA	175
	KSSQSVFYRSNQKNYLA	176
	RASQSVSSYLA	177
	RASRGISSWLA	178
	RASQGISSWLA	179
	RASQSISSYLN	180
	RASQAISNLLA	181

	RASQGISTWLA	182
	RASQGIASNLA	183
	RASQGVSSYLA	184
	RASQSIYTYLN	185
	RASQFVSDWLA	186
	RASQTISTWLA	187
	RASQGISSYLA	188
	RASQSIGYWLA	189
	RATQSISSWLA	190
	RASQGVRNWLA	191
	RASQSINNYLA	192
	RASQDITSWLA	193
	RASQGIYDYLA	194
	RASEGISGWLA	195
	RASQDIVNWLA	229
	RSSKSLLHSNGITYLY	357
	RSSKSLLHS**Q**GITYLY	490
VL CDR2	DASILQS	196
	WASTRES	197
	LGSNRAT	198
	DASNLET	199
	AASSLQS	200
	AASTLQS	201
	AAFSLQS	202
	GASSRAT	203
	GISSRAT	204
	AVSTLQS	205
	DISTLQN	206
	GASTLQS	207
	GASSLQS	208
	AASTLES	209
	DASSLQS	210
	KASNLQS	211
	TASTLQN	212
	RASSLQS	213
	AASHLQS	214
	DASTLQS	215
	AASNLER	216
	AASSLET	217
	QVSNLAS	358
	QVSNLAR	476
	QKSNLAS	477
	QVSNLAV	478
	QVSNLAL	479
	QVDNLAS	480

	QVSNLAT	481
	HVSNLAS	482
	QVSNRAS	483
VL CDR3	QQADSFPIT	218
	QQSYSTPWT	219
	QQYYSTPWT	220
	QQSFTTPWT	221
	QQYDNLPPT	222
	QQSYGSPVT	223
	QQGNSFPFT	224
	QQAKSFPLT	225
	QQVKSFPLT	226
	QQYYNTPWT	227
	QQTKNFPLT	228
	QQTKSFPLT	230
	QQSYNTPRT	231
	QQSYRAPWT	232
	QQANNFPLT	233
	QQGNSFPLT	234
	QQSKNFPVT	235
	QQANSFPLT	236
	QQLESYPLT	237
	QQYYSSPT	238
	QQLKTFPLT	239
	QQTNWFPLT	240
	QQAQSFPIT	241
	QQAHSFPLT	242
	LQDYHFPLT	243
	QQGHSFPLT	244
	QQSYIFPLT	245
	QQYDTYWT	246
	QQLNSYPLFT	247
	QQYSSYWT	248
	LQHNTYPFT	249
	QQGHSFPLT	250
	QQAHSFPFT	251
	QQANMFPLT	252
	QQADSFPFT	253
	AQNLELPWT	359
	GQNLELPWT	484
	AQNLEMPWT	485
	GQNLEMPWT	486
	AQYLEEPWT	487
	AQYLELPWT	488
	GQYLELPWT	489

In non-limiting examples, the antibody or fragment having specificity to LAG3 has a combination of VH CDR1, VH CDR2, VH CDR3, VL CDR1, VL CDR2, and VL CDR3 as shown in any of the antibodies listed in Table 27. For instance, the CDRs can be those from 147H 3807, which include a VH CDR1 of SEQ ID NO:354, a VH CDR2 of SEQ ID NO:461, a VH CDR3 of SEQ ID NO:468, a VL CDR1 of SEQ ID NO:490, a VL CDR2 of SEQ ID NO:358, and a VL CDR3 of SEQ ID NO:488. Variants of these antibodies are also provided, such as those having at least 75%, 80%, 85%, 90%, 95%, 98%, 99% or 99.5% sequence identity to the heavy chain/light chain variable regions and retaining the respective CDR sequences.

In one embodiment, for instance, provided is an antibody or antigen-binding fragment thereof, having specificity to a human LAG3 protein and comprising: a heavy chain variable region comprising the amino acid sequence of SEQ ID NO:443, or a polypeptide having at least 90% sequence identity to the amino acid sequence of SEQ ID NO:443 and having a VH CDR1 comprising the amino acid sequence of SEQ ID NO:354, a VH CDR2 comprising the amino acid sequence of SEQ ID NO:461, and a VH CDR3 comprising the amino acid sequence of SEQ ID NO:468, and a light chain variable region comprising the amino acid sequence of SEQ ID NO:444, or a polypeptide having at least 90% sequence identity to the amino acid sequence of SEQ ID NO:444 and having a VL CDR1 comprising the amino acid sequence of SEQ ID NO:490, a VL CDR2 comprising the amino acid sequence of SEQ ID NO:358, and a VL CDR3 comprising the amino acid sequence of SEQ ID NO:488.

In non-limiting examples of the anti-LAG3 antibody or fragment thereof,

(1) the heavy chain variable region may comprise or consist essentially of a polypeptide having an amino acid sequence selected from the group consisting of SEQ ID NOS: 254-302, 352, 360-373, 375, 377, 379, 381, 383, 385, 387, 389, 391, 393, 395, 397, 399, 401, 403, 405, 407, 409, 411, 413, 415, 417, 419, 421, 423, 425, 427, 429, 431, 433, 435, 437, 439, 441, 443, 445, 447, 449, 451 and 491, or a polypeptide having a sequence identity 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% to the above described amino acid sequences; and/or

(2) the light chain variable region may comprise or consist essentially of a polypeptide having an amino acid sequence selected from the group consisting of SEQ ID NOS: 303-351, 353, 374, 376, 378, 380, 382, 384, 386, 388, 390, 392, 394, 396, 398, 400, 402, 404, 406, 408, 410, 412, 414, 416, 418, 420, 422, 424, 426, 428, 430, 432, 434, 436, 438, 440, 442, 444, 446, 448, 450, 452 and 492, or a polypeptide having a sequence identity 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% to the above described amino acid sequences.

Non-limiting examples of the anti-LAG3 antibody or fragment thereof may comprise a heavy chain variable region comprising or consisting essentially of the amino acid sequence of SEQ ID NO: 352 or 443 and a light chain variable region comprising or consisting essentially of the amino acid sequence of SEQ ID NO: 353 or 444.

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.

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.

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.

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.

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-gamma) 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.

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 x 10^{-9} M or less, binds to a LAG-3 protein with a K_D of 3 x 10^{-9} M or less, binds to a LAG-3 protein with a K_D of 2 x 10^{-9} M or less, binds to a LAG-3 protein with a K_D of 125 x 10^{-9} M or less, binds to a LAG-3 protein with a K_D of 5 x 10^{-10} M or less, or binds to a LAG-3 protein with a K_D of 1 x 10^{-10} M or less.

In some embodiments, the anti-LAG3 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 may be a kappa or lambda chain constant region. In some embodiments, the antibody is of an isotype of IgG, IgM, IgA, IgE or IgD, for example, human IgG, human IgM, human IgA, human IgE, or human IgD. In some embodiments, the isotype may be IgG, for example human IgG, such as, IgG1, IgG2, IgG3, or IgG4. In some embodiments, the fragment (antigen-binding fragment of the anti-PD-L1 antibody) may be any fragment comprising heavy chain CDRs and/or light chain CDRs of the antibody, and for example, it may be selected from the group consisting of Fab, Fab', $F(ab')_2$, Fd (comprising a heavy chain variable region and a CH1 domain), Fv (a heavy chain variable region and/or a light chain variable region), single-chain Fv (scFv; comprising or consisting essentially of a heavy chain variable region and a light chain variable region, in any order, and a peptide linker between the heavy chain variable region and the light chain variable region), single-chain antibodies, disulfide-linked Fvs (sdFv), and the like.

Without limitation, the anti-LAG3 antibody or fragment thereof is a chimeric antibody, a humanized antibody, or a fully human antibody. In one aspect, antibody or fragment thereof is not naturally occurring, or chemically or recombinantly synthesized.

Given that each of these antibodies can bind to LAG-3 such as human LAG-3, the CDR sequences or 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 the CDRs sequences or V_H and V_L chains are mixed and matched, for example, 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.

Anti-PD-L1/anti-LAG3 bispecific antibody

In the bispecific antibody comprising the PD-L1 targeting moiety and the LAG3 targeting moiety, one of the PD-L1 targeting moiety and the LAG3 targeting moiety can be a full-length antibody, and the other can be an antigen-binding fragment (e.g., scFv) comprising heavy chain CDRs, light chain CDRs, or a combination thereof. The full-length antibody

targeting one of PD-L1 and LAG3 proteins, and the antigen-binding fragment targeting the other protein may be chemically linked (e.g., covalently linked) directly or via a peptide linker. The antigen-binding fragment (e.g., scFv) may be linked directly or via a peptide linker to N-terminus of the full-length antibody (e.g., N-terminus of a light chain or a heavy chain of the full-length antibody), C-terminus of the full-length antibody (e.g., C-terminus of a heavy chain (or Fc or CH3 domain) of the full-length antibody), or both thereof (see FIG. 32).

In an embodiment, the bispecific antibody may comprise a full-length anti-PD-L1 antibody, an antigen-binding fragment (e.g., scFv) of an anti-LAG3 antibody, and a peptide linker therebetween. In other embodiment, the bispecific antibody may comprise a full-length anti-LAG3 antibody, an antigen-binding fragment (e.g., scFv) of an anti-PD-L1 antibody, and a peptide linker therebetween.

In an embodiment, the scFv contained in the bispecific antibody may comprise a heavy chain variable region and a light chain variable region in any order. For example, the scFv contained in the bispecific antibody may comprise a heavy chain variable region and a light chain variable, in a direction from N-terminus to C-terminus, and optionally a peptide linker therebetween, or alternatively, the scFv contained in the bispecific antibody may comprise a light chain variable region and a heavy chain variable, in a direction from N-terminus to C-terminus, and optionally a peptide linker therebetween.

The use of a peptide linker for the bispecific antibody may lead to a high purity of the antibody.

As used herein, the term “peptide linker” may be those including any amino acids of 1 to 100, particularly 2 to 50, and any kinds of amino acids may be included without any restrictions. The peptide linker may include for example, Gly, Asn and/or Ser residues, and also include neutral amino acids such as Thr and/or Ala. Amino acid sequences suitable for the peptide linker may be those known in the relevant art. Meanwhile, a length of the peptide linker may be variously determined within such a limit that the functions of the fusion protein will not be affected. For instance, the peptide linker may be formed by including a total of about 1 to about 100, about 2 to about 50, or about 5 to about 25 of one or more selected from the group consisting of Gly, Asn, Ser, Thr, and Ala. In one embodiment, the peptide linker may be represented as $(G_mS_l)_n$ (m, l, and n, are independently an integer of about 1 to about 10, particularly an integer of about 2 to about 5). For example, the examples of the peptide liners are summarized as follows:

Linker Function	Examples			
	Fusion Protein	Linker		Ref.
		Type	Sequence[a]	
Increase Stability/Folding	scFv	flexible	$(GGGGS)_3$	[46]
	G-CSF-Tf	flexible	$(GGGGS)_3$	[20]
	HBsAg preS1	flexible	$(GGGGS)_3$	[85]
	Myc- Est2p	flexible	$(Gly)_8$	[30]
	albumin-ANF	flexible	$(Gly)_8$	[31]
	virus coat protein	rigid	$(EAAAK)_3$	[50]
	beta-glucanase-xylanase	rigid	$(EAAAK)_n$ (n=1-3)	[52]
Increase expression	hGH-Tf and Tf-hGH	rigid	$A(EAAAK)_4ALEA(EAAAK)_4A$	[18]
	G-CSF-Tf and Tf-G-CSF	rigid	$A(EAAAK)_4ALEA(EAAAK)_4A$	[18]
Improve biological activity	G-CSF-Tf	flexible	$(GGGGS)_3$	[20]
	G-CSF-Tf	rigid	$A(EAAAK)_4ALEA(EAAAK)_4A$	[20]
	hGH-Tf	rigid	$A(EAAAK)_4ALEA(EAAAK)_4A$	[40]
	HSA-IFN-α2b	flexible	GGGGS	[17]
	HSA-IFN-α2b	rigid	PAPAP	[17]
	HSA-IFN-α2b	rigid	AEAAAKEAAAKA	[17]
	PGA-rTHS	flexible	$(GGGGS)_n$ (n=1, 2, 4)	[55]
	interferon- -gp120	rigid	$(Ala\text{-}Pro)_n$ (10 – 34 aa)	[54]
	GSF-S-S-Tf	cleavable	disulfide	[39]
	IFN-α2b-HSA	cleavable	disulfide	[42]
Enable targeting	FIX-albumin	cleavable	VSQTSKLTR AETVFPDV[b]	[59]
	LAP-IFN-	cleavable	PLG LWA[c]	[64]
	MazE-MazF	cleavable	RVL AEA; EDVVCC SMSY; GGIEGR GS[c]	[68]
	Immunotoxins	cleavable	TRHRQPR GWE; AGNRVRR SVG; RRRRRRR R R[d]	[72]
	Immunotoxin	cleavable	GFLG[e]	[77]
Alter PK	G-CSF-Tf and hGH-Tf	dipeptide	LE	[79]
		rigid	$A(EAAAK)_4ALEA(EAAAK)_4A$	
		cleavable	Disulfide	

In another embodiment, both of the PD-L1 targeting moiety and the LAG3 targeting moiety may be a full-length antibody or an antigen-binding fragment comprising heavy chain CDRs, light chain CDRs, or a combination thereof.

In another embodiment, the bispecific antibody may be in a heterodimeric form, which comprises a first arm including a pair of a first heavy chain and a first light chain targeting one of PD-L1 and LAG3, and a second arm including a pair of a second heavy chain and a second light chain targeting the other one.

In an embodiment, the full-length antibody may be in a full-length immunoglobulin form (e.g., IgG, IgM, IgA, IgE or IgD, such as, human IgG, human IgM, human IgA, human IgE, or human IgD), and the antigen-binding fragment may be selected from the group consisting of Fab, Fab', $F(ab')_2$, Fd, Fv, scFv, single-chain antibodies, sdFv, and the like, as described above. For example, the full-length antibody may be in a full-length human IgG (human IgG1, human IgG2, human IgG3, or human IgG4) form, and the antigen-binding fragment may be scFv.

For example, an antibody described herein may comprise a flexible linker sequence, or may be modified to add a functional moiety (e.g., PEG, a drug, a toxin, or a label).

In some embodiments, a bi- or multi-specific antibody is provided, which includes anti-PD-L1 antibody or an antigen-binding fragment thereof and an anti-LAG3 antibody or an antigen-binding fragment thereof, wherein the anti-PD-L1 antibody or antigen-binding fragment thereof is capable of specifically binding to an immunoglobulin C (Ig C) domain of a human Programmed death-ligand 1 (PD-L1) protein, wherein the Ig C domain consists of amino acid residues 133-225; and the anti-LAG3 antibody or antigen-binding fragment thereof is capable of binding to a MHC class II molecule and/or FGL1.

In some embodiments, the anti-PD-L1 antibody or antigen-binding fragment thereof includes a VH CDR1 comprising an amino acid sequence selected from the group consisting of SEQ ID NO: 1 and 61-67; a VH CDR2 comprising an amino acid sequence selected from the group consisting of SEQ ID NO: 2, 68-77, and 525-527; a VH CDR3 comprising an amino acid sequence selected from the group consisting of SEQ ID NO: 3, 78-90, and 513-519; a VL CDR1 comprising an amino acid sequence selected from the group consisting of SEQ ID NO: 4, 91-92, and 520-521; a VL CDR2 comprising an amino acid sequence selected from the group consisting of SEQ ID NO: 5, and 93-105; and a VL CDR3 comprising an amino acid sequence selected from the group consisting of SEQ ID NO: 6, 106-111, and 522-524, and the anti-LAG3 antibody or antigen-binding fragment thereof includes a VH CDR1 comprising an amino acid sequence selected from the group consisting of SEQ ID NO: 116-117, 354, and 453-460; a VH

CDR2 comprising an amino acid sequence selected from the group consisting of SEQ ID NO: 118-119, 355, and 461-467; a VH CDR3 comprising an amino acid sequence selected from the group consisting of SEQ ID NO: 120-160, 356, and 468-475; a VL CDR1 comprising an amino acid sequence selected from the group consisting of SEQ ID NO: 163-195, 229, 357, and 490; a VL CDR2 comprising an amino acid sequence selected from the group consisting of SEQ ID NO: 196-217, 358, and 476-483; and a VL CDR3 comprising an amino acid sequence selected from the group consisting of SEQ ID NO: 218-228, 230-253, 359, and 484-489.

In some embodiments, the anti-PD-L1 antibody or antigen-binding fragment thereof includes a VH CDR1 having an amino acid sequence of SEQ ID NO: 1; a VH CDR2 having an amino acid sequence of SEQ ID NO: 525; a VH CDR3 having an amino acid sequence of SEQ ID NO: 3; a VL CDR1 having an amino acid sequence of SEQ ID NO: 4; a VL CDR2 having an amino acid sequence of SEQ ID NO: 5; and a VL CDR3 having an amino acid sequence of SEQ ID NO: 6, and the anti-LAG3 antibody or antigen-binding fragment thereof includes a VH CDR1 comprising the amino acid sequence of SEQ ID NO:354, a VH CDR2 comprising the amino acid sequence of SEQ ID NO:461, a VH CDR3 comprising the amino acid sequence of SEQ ID NO:468, a VL CDR1 comprising the amino acid sequence of SEQ ID NO:490, a VL CDR2 comprising the amino acid sequence of SEQ ID NO:358, and a VL CDR3 comprising the amino acid sequence of SEQ ID NO:488.

In some embodiments, the anti-PD-L1 antibody or antigen-binding fragment thereof includes a VH CDR1 having an amino acid sequence of SEQ ID NO: 1; a VH CDR2 having an amino acid sequence of SEQ ID NO: 526; a VH CDR3 having an amino acid sequence of SEQ ID NO: 515; a VL CDR1 having an amino acid sequence of SEQ ID NO: 4; a VL CDR2 having an amino acid sequence of SEQ ID NO: 5; and a VL CDR3 having an amino acid sequence of SEQ ID NO: 6, and the anti-LAG3 antibody or antigen-binding fragment thereof includes a VH CDR1 comprising the amino acid sequence of SEQ ID NO:354, a VH CDR2 comprising the amino acid sequence of SEQ ID NO:461, a VH CDR3 comprising the amino acid sequence of SEQ ID NO:468, a VL CDR1 comprising the amino acid sequence of SEQ ID NO:490, a VL CDR2 comprising the amino acid sequence of SEQ ID NO:358, and a VL CDR3 comprising the amino acid sequence of SEQ ID NO:488.Antibodies or variants described herein may comprise derivatives that are modified, e.g., by the covalent attachment of any type of molecule to the antibody such that covalent attachment does not prevent the antibody from binding to the antigen (e.g., an epitope). For example, but not by way of limitation, the antibodies can be modified, e.g., by at least one selected from the group consisting of glycosylation, acetylation, pegylation, phosphorylation, phosphorylation,

amidation, derivatization by known protecting/blocking groups, proteolytic cleavage, linkage to a cellular ligand or other protein, and the like. Any of numerous chemical modifications may be carried out by known techniques, including, but not limited to specific chemical cleavage, acetylation, formylation, metabolic synthesis of tunicamycin, etc. Additionally, the antibodies may contain one or more non-classical amino acids.

The antibodies or fragments thereof can be detectably labeled by tagging (coupling) with a conventional labeling material selected from chemiluminescent compounds, fluorescent compounds (e.g., fluorescence emitting metals), radioisotopes, dyes, etc. The presence of the tagged antibodies or fragments thereof can be detected by measuring a signal arising during a chemical reaction between the antibody (or fragment thereof) and the labeling material. Examples of particularly useful labeling material may be at least one selected from the group consisting of luminol, isoluminol, theromatic acridinium ester, imidazole, acridinium salt, oxalate ester, fluorescence emitting metals, and the like. For example, the fluorescence emitting metals may be ^{152}Eu, or others of the lanthanide series. These metals can be attached to the antibody using such metal chelating groups as diethylenetriaminepentacetic acid (DTPA) or ethylenediaminetetraacetic acid (EDTA).

In certain embodiments, the prepared bispecific antibodies will not elicit a deleterious immune response in the animal to be treated, e.g., in a human. In one embodiment, the bispecific antibody may be modified to reduce their immunogenicity using any conventional techniques. For example, the bispecific antibody may be a humanized, primatized, deimmunized, or chimeric antibody. These types of antibodies are derived from a non-human antibody, typically a murine or primate antibody, that retains or substantially retains the antigen-binding properties of the parent antibody, but which is less immunogenic in humans. This may be achieved by various methods, including (a) grafting the entire non-human variable domains onto human constant regions to generate chimeric antibodies; (b) grafting at least a part of one or more of the non-human complementarity determining regions (CDRs) into a human framework and constant regions with or without retention of critical framework residues; or (c) transplanting the entire non-human variable domains, but "cloaking" them with a human-like section by replacement of surface residues.

De-immunization can also be used to decrease the immunogenicity of an antibody. As used herein, the term "de-immunization" may include alteration of an antibody to modify T-cell epitopes (see, e.g., International Application Publication Nos. : WO/9852976 A1 and WO/0034317 A2). For example, variable heavy chain and variable light chain sequences from the starting antibody are analyzed and a human T-cell epitope "map" from each V (variable)

region showing the location of epitopes in relation to complementarity-determining regions (CDRs) and other key residues within the sequence is created. Individual T-cell epitopes from the T-cell epitope map are analyzed in order to identify alternative amino acid substitutions with a low risk of altering activity of the final antibody. A range of alternative variable heavy and variable light sequences are designed comprising combinations of amino acid substitutions and these sequences are subsequently incorporated into a range of binding polypeptides. Typically, between 12 and 24 variant antibodies are generated and tested for binding and/or function. Complete heavy and light chain genes comprising modified variable and human constant regions are then cloned into expression vectors and the subsequent plasmids introduced into cell lines for the production of whole antibody. The antibodies are then compared in appropriate biochemical and biological assays, and the optimal variant is identified.

The binding specificity and/or affinity of the bispecific antibody to each target protein can be determined by any conventional assay, for example, in vitro assays such as immunoprecipitation, radioimmunoassay (RIA), or enzyme-linked immunoabsorbent assay (ELISA), but not be limited thereto.

Alternatively, techniques described for the production of single-chain units (U.S. Pat. No. 4,694,778, etc.) can be adapted to produce single-chain units of the present disclosure. Single-chain units are formed by linking the heavy and light chain fragments of the Fv region via an amino acid bridge (peptide linker), resulting in a single-chain fusion peptide (scFv). Techniques for the assembly of functional Fv fragments in E. coli may also be used.

Examples of techniques which can be used to produce single-chain Fvs (scFvs) and antibodies include those described in U.S. Pat. Nos. 4,946,778, 5,258,498, etc.). For some uses, including in vivo use of antibodies in humans and in vitro detection assays, it may be preferable to use chimeric, humanized, or human antibodies. A chimeric antibody is a molecule in which different portions of the antibody are derived from different animal species, such as antibodies having a variable region derived from a murine monoclonal antibody and a human immunoglobulin constant region. Methods for producing chimeric antibodies are known in the art. See, e.g., U.S. Pat. Nos. 5,807,715, 4,816,567, and 4, 816,397, which are incorporated herein by reference in their entireties.

Humanized antibodies are antibody molecules derived from a non-human species antibody that bind the desired antigen having one or more complementarity determining regions (CDRs) from the non-human species and framework regions from a human immunoglobulin molecule. Often, framework residues in the human framework regions will

be substituted with the corresponding residue from the CDR donor antibody to alter, preferably improve, antigen-binding. These framework substitutions are identified by methods well known in the art, e.g., by modeling of the interactions of the CDR and framework residues to identify framework residues important for antigen-binding and sequence comparison to identify unusual framework residues at particular positions (See, e.g., Queen et al., U.S. Pat. No. 5,585,089, which are incorporated herein by reference in their entireties). Antibodies can be humanized using a variety of techniques known in the art including, for example, CDR-grafting (U.S. Pat. Nos. 5,225,539, 5,530,101, 5,585,089, etc., each of which is incorporated by reference in its entirety), veneering or resurfacing (EP 592,106; EP 519,596, each of which is incorporated by reference in its entirety), and chain shuffling (U.S. Pat. No. 5,565,332, which is incorporated by reference in its entirety).

Completely human antibodies are particularly desirable for therapeutic treatment of human patients. Human antibodies can be made by a variety of methods known in the art including phage display methods using antibody libraries derived from human immunoglobulin sequences. See also, U.S. Pat. Nos. 4,444,887, 4,716,111, etc., each of which is incorporated herein by reference in its entirety.

Human antibodies can also be produced using transgenic mice which are incapable of expressing functional endogenous immunoglobulins, but which can express human immunoglobulin genes. For example, the human heavy and light chain immunoglobulin gene complexes may be introduced randomly or by homologous recombination into mouse embryonic stem cells. Alternatively, the human variable region, constant region, and diversity region may be introduced into mouse embryonic stem cells in addition to the human heavy and light chain genes. The mouse heavy and light chain immunoglobulin genes may be rendered non-functional separately or simultaneously with the introduction of human immunoglobulin loci by homologous recombination. In particular, homozygous deletion of the JH region prevents endogenous antibody production. The modified embryonic stem cells are expanded and microinjected into blastocysts to produce chimeric mice. The chimeric mice are then bred to produce homozygous offspring that express human antibodies. The transgenic mice are immunized in the normal fashion with a selected antigen, e.g., all or a portion of a desired target polypeptide. Monoclonal antibodies directed against the antigen can be obtained from the immunized, transgenic mice using conventional hybridoma technology. The human immunoglobulin transgenes harbored by the transgenic mice rearrange during B-cell differentiation, and subsequently undergo class switching and somatic mutation. Thus, using

such a technique, it is possible to produce therapeutically useful IgG, IgA, IgM and IgE antibodies.

Completely human antibodies which recognize a selected epitope can also be generated using a technique referred to as "guided selection." In this approach a selected non-human monoclonal antibody, e.g., a mouse antibody, is used to guide the selection of a completely human antibody recognizing the same epitope.

In another embodiment, DNA encoding desired monoclonal antibodies may be readily isolated and sequenced using conventional procedures (e.g., by using oligonucleotide probes that are capable of binding specifically to genes encoding the heavy and light chains of murine antibodies). The isolated and subcloned hybridoma cells serve as a preferred source of such DNA. Once isolated, the DNA may be placed into expression vectors, which are then transfected into prokaryotic or eukaryotic host cells such as E. coli cells, simian COS cells, Chinese Hamster Ovary (CHO) cells or myeloma cells that do not otherwise produce immunoglobulins. More particularly, the isolated DNA (which may be synthetic as described herein) may be used to clone constant and variable region sequences for the manufacture antibodies as described in Newman et al., U.S. Pat. No. 5,658,570, which is incorporated by reference herein. Essentially, this entails extraction of RNA from the selected cells, conversion to cDNA, and amplification by PCR using Ig specific primers. Suitable primers for this purpose are also described in U.S. Pat. No. 5,658,570. As will be discussed in more detail below, transformed cells expressing the desired antibody may be grown up in relatively large quantities to provide clinical and commercial supplies of the immunoglobulin.

Additionally, using routine recombinant DNA techniques, one or more of the CDRs of the bispecific antibody may be inserted within framework regions, e.g., into human framework regions to humanize a non-human antibody. The framework regions may be naturally occurring or consensus framework regions, and preferably human framework regions (see, e.g., Chothia et al., J. Mol. Biol. 278: 457-479 (1998) for a listing of human framework regions). For example, the polynucleotide generated by the combination of the framework regions and CDRs encodes an antibody that specifically binds to at least one epitope of a desired polypeptide, e.g., LIGHT. Preferably, one or more amino acid substitutions may be made within the framework regions, and, preferably, the amino acid substitutions improve binding of the antibody to its antigen (or epitope). Additionally, such methods may be used to make amino acid substitutions or deletions of one or more variable region cysteine residues participating in an intrachain disulfide bond to generate antibody molecules lacking one or more intrachain disulfide bonds. Other

alterations to the polynucleotide are encompassed by the present disclosure and within the skill of the art.

In addition, techniques developed for the production of "chimeric antibodies" by splicing genes from a mouse antibody molecule, of appropriate antigen specificity, together with genes from a human antibody molecule of appropriate biological activity can be used. As used herein, a chimeric antibody is a molecule in which different portions are derived from different animal species, such as those having a variable region derived from a murine monoclonal antibody and a human immunoglobulin constant region.

Alternatively, antibody-producing cell lines may be selected and cultured using techniques well known to the skilled artisan. Such techniques are described in a variety of laboratory manuals and primary publications.

Additionally, standard techniques known to those of skill in the art can be used to introduce mutations in the nucleotide sequence encoding an antibody of the present disclosure, including, but not limited to, site-directed mutagenesis and PCR-mediated mutagenesis which result in amino acid substitutions. Preferably, the variants (including derivatives) encode less than 50 amino acid substitutions, less than 40 amino acid substitutions, less than 30 amino acid substitutions, less than 25 amino acid substitutions, less than 20 amino acid substitutions, less than 15 amino acid substitutions, less than 10 amino acid substitutions, less than 5 amino acid substitutions, less than 4 amino acid substitutions, less than 3 amino acid substitutions, or less than 2 amino acid substitutions relative to the reference variable heavy chain region, CDR-H1, CDR-H2, CDR-H3, variable light chain region, CDR-L1, CDR-L2, or CDR-L3. Alternatively, mutations can be introduced randomly along all or part of the coding sequence, such as by saturation mutagenesis, and the resultant mutants can be screened for biological activity to identify mutants that retain activity.

Therapeutic Use of the antibodies

The bispecific antibody provided herein is capable of simultaneously blocking the activities of PD-L1 and LAG3, thereby exhibiting improved effects in immunotherapies and/or cancer therapies, for example, by activating immune response (see FIG. 33). Given the ability of the bispecific 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 a composition or *in vitro* and *in vivo* methods of using the antibodies of the disclosure to stimulate, enhance or upregulate antigen-specific T cell responses.

An embodiment provides a pharmaceutical composition comprising the bispecific antibody or the anti-PD-L1 or anti-LAG3 antibody as described above. The pharmaceutical composition may further comprise a pharmaceutically acceptable carrier. The pharmaceutical composition may be used for stimulating an immune response (e.g., an antigen-specific T cell response), and/or treating and/or preventing a disease associated with PD-L1, LAG3, or both thereof.

Another embodiment provides a method of stimulating an immune response (e.g., an antigen-specific T cell response), and/or treating and/or preventing a disease associated with PD-L1, LAG3, or both thereof, in a subject in need thereof, comprising administering to the subject a pharmaceutically effective amount of the bispecific antibody, the anti-PD-L1 or anti-LAG3 antibody, or the pharmaceutical composition. The method may further step of identifying the subject in need of treating and/or preventing a disease associated with PD-L1, LAG3, or both thereof, prior to the administering step.

The disease associated with PD-L1, LAG3, or both thereof may be selected from cancers (or tumors), infectious diseases, autoimmune reactions, nervous system disorders, and the like.

In an embodiment, the subject may be selected from mammals including humans, for example, a mammal (e.g., a human) suffering from a cancer and/or infection mammalian cells. In other embodiment, the subject may be a cell separated (isolated) from a mammal, for example, a mammal suffering from the disease selected from cancers infectious diseases, autoimmune reactions, nervous system disorders, and the like (e.g., a cancer cell or a cell separated (isolated) from an infectious region in the mammal, or a T cell, such as a tumor-infiltrating T lymphocyte, a CD4+ T cell, a CD8+ T cell, or the combination thereof).

Another embodiment provides a use of the bispecific antibody, the anti-PD-L1 or anti-LAG3 antibody, or the pharmaceutical composition in treating and/or preventing a cancer or an infection. Another embodiment provides a use of the bispecific antibody, or the anti-PD-L1 or anti-LAG3 antibody, in preparing a pharmaceutical composition for treating and/or preventing a cancer or an infection.

In the pharmaceutical compositions, methods and/or uses provided herein, the disease associated with PD-L1, LAG3, or both thereof may be one associated with activation (e.g., abnormal activation or over-activation) and/or overproduction (overexpression) of PD-L1, LAG3, or both thereof. For example, the disease may be a cancer or an infection.

The cancer may be a solid cancer or blood cancer, preferably a solid cancer. The cancer may any tumor expressing PD-L1 protein, and may be selected from the group consisting of

bladder cancer, liver cancer, colon cancer, rectal cancer, endometrial cancer, leukemia, lymphoma, pancreatic cancer, lung cancer (e.g., small cell lung cancer, non-small cell lung cancer etc.), breast cancer, urethral cancer, head and neck cancer, gastrointestinal cancer, stomach cancer, oesophageal cancer, ovarian cancer, renal cancer, melanoma, prostate cancer, thyroid cancer, and the like, but may not be limited thereto. In some embodiments, the cancer is selected from the group consisting of bladder cancer, liver cancer, pancreatic cancer, non-small cell lung cancer, breast cancer, urethral cancer, colorectal cancer, head and neck cancer, squamous cell cancer, Merkel cell carcinoma, gastrointestinal cancer, stomach cancer, oesophageal cancer, ovarian cancer, renal cancer, small cell lung cancer, and the like. The cancer may be a primary or metastatic cancer.

A specific dosage and treatment regimen for any particular patient will depend upon a variety of factors, including the particular antibodies, variant or derivative thereof used, the patient's age, body weight, general health, sex, and diet, and the time of administration, rate of excretion, drug combination, and the severity of the particular disease being treated. Judgment of such factors by medical caregivers is within the ordinary skill in the art. The amount will also depend on the individual patient to be treated, the route of administration, the type of formulation, the characteristics of the compound used, the severity of the disease, and the desired effect. The amount used can be determined by pharmacological and pharmacokinetic principles well known in the art.

The administration of the bispecific antibody or the anti-PD-L1 or anti-LAG3 antibody may be conducted through at least one selected from the group consisting of intraperitoneal, intravenous, subcutaneous, intradermal, intramuscular, intranasal, epidural, and oral routes, but not be limited thereto. The bispecific antibody or the anti-PD-L1 or anti-LAG3 antibody or compositions may be administered by any convenient route, for example by infusion or bolus injection, by absorption through epithelial or mucocutaneous linings (e.g., oral mucosa, rectal and intestinal mucosa, etc.) and may be administered together with other biologically active agents. Thus, pharmaceutical compositions containing the antigen-binding polypeptides of the disclosure may be administered orally, parenterally, intracistemally, intravaginally, intraperitoneally, rectally, topically (as by powders, ointments, drops or transdermal patch), bucally, or as an oral or nasal spray.

The term "parenteral" as used herein refers to modes of administration which include intravenous, intramuscular, intraperitoneal, intrasternal, subcutaneous and intra-articular injection and infusion.

Administration can be systemic or local. In addition, it may be desirable to introduce the antibodies of the disclosure into the central nervous system by any suitable route, including intraventricular and intrathecal injection; intraventricular injection may be facilitated by an intraventricular catheter, for example, attached to a reservoir, such as an Ommaya reservoir. Pulmonary administration can also be employed, e.g., by use of an inhaler or nebulizer, and formulation with an aerosolizing agent.

It may be desirable to administer the bispecific antibodies, or the anti-PD-L1 or anti-LAG3 antibodies, or compositions of the disclosure locally to the area in need of treatment; this may be achieved by, for example, and not by way of limitation, local infusion during surgery, topical application, e.g., in conjunction, with a wound dressing after surgery, by injection, by means of a catheter, by means of a suppository, or by means of an implant, said implant being of a porous, non-porous, or gelatinous material, including membranes, such as sialastic membranes, or fibers. Preferably, when administering a protein, including an antibody, of the disclosure, care must be taken to use materials to which the protein does not absorb.

In another embodiment, the bispecific antibodies or the anti-PD-L1 or anti-LAG3 antibodies or composition can be delivered in a vesicle, in particular a liposome. In yet another embodiment, the bispecific antibodies or the anti-PD-L1 or anti-LAG3 antibodies or composition can be delivered in a controlled release system. In one embodiment, for the controlled release system, any pharmaceutically acceptable pumps, and/or polymeric materials may be used.

The pharmaceutically effective amount of the bispecific antibodies or the anti-PD-L1 or anti-LAG3 antibodies for treating, inhibiting, ameliorating, and/or preventing an inflammatory, immune or malignant disease, disorder, or condition, can be determined by standard clinical techniques. In addition, in vitro assays may optionally be employed to help identify optimal dosage ranges. The precise dose to be employed in the formulation will also depend on the route of administration, and the seriousness of the disease, disorder or condition, and should be decided according to the judgment of the practitioner and each patient's circumstances. Effective doses may be extrapolated from dose-response curves derived from in vitro or animal model test systems.

The methods of treating an infectious or malignant disease (e.g., cancer), condition or disorder comprising administration of the bispecific antibody or the anti-PD-L1 or anti-LAG3 antibody are typically tested in vitro, and then in vivo in an acceptable animal model, for the desired therapeutic or prophylactic activity, prior to use in humans. Suitable animal models, including transgenic animals, are well known to those of ordinary skill in the art. For example,

in vitro assays to demonstrate the therapeutic utility of the bispecific antibody or the anti-PD-L1 or anti-LAG3 antibody include the effect of the bispecific antibody or the anti-PD-L1 or anti-LAG3 antibody on a cell line or a patient tissue sample. The effect of the bispecific antibody or the anti-PD-L1 or anti-LAG3 antibody on the cell line and/or tissue sample can be determined utilizing techniques known to those of skill in the art, such as the assays disclosed elsewhere herein. In accordance with the disclosure, in vitro assays which can be used to determine whether administration of the bispecific antibody or the anti-PD-L1 or anti-LAG3 antibody is indicated, include in vitro cell culture assays in which a patient tissue sample is grown in culture, and exposed to or otherwise administered a compound, and the effect of such compound upon the tissue sample is observed.

Various delivery systems are known and can be used to administer an antibody of the disclosure or a polynucleotide encoding an antibody of the disclosure, e.g., encapsulation in liposomes, microparticles, microcapsules, recombinant cells capable of expressing the compound, receptor-mediated endocytosis, construction of a nucleic acid as part of a retroviral or other vector, etc.

The pharmaceutical compositions may comprise an effective amount of the bispecific antibody or the anti-PD-L1 or anti-LAG3 antibody, and an acceptable carrier. In some embodiments, the composition further includes a second anticancer agent (e.g., an immune checkpoint inhibitor).

In a specific embodiment, the term "pharmaceutically acceptable" may refer to approved by a regulatory agency of the Federal or a state government or listed in the U.S. Pharmacopeia or other generally recognized pharmacopeia for use in animals, and more particularly in humans. Further, a "pharmaceutically acceptable carrier" will generally be a non-toxic solid, semisolid or liquid filler, diluent, encapsulating material or formulation auxiliary of any type.

The term "carrier" may refer to a diluent, adjuvant, excipient, or vehicle with which the therapeutic is administered. Such pharmaceutical carriers can be sterile liquids, such as water and oils, including those of petroleum, animal, vegetable or synthetic origin, such as peanut oil, soybean oil, mineral oil, sesame oil and the like. Water is a preferred carrier when the pharmaceutical composition is administered intravenously. Saline solutions and aqueous dextrose and glycerol solutions can also be employed as liquid carriers, particularly for injectable solutions. Suitable pharmaceutical excipients include starch, glucose, lactose, sucrose, gelatin, malt, rice, flour, chalk, silica gel, sodium stearate, glycerol monostearate, talc, sodium chloride, dried skim milk, glycerol, propylene, glycol, water, ethanol and the like. The

composition, if desired, can also contain minor amounts of wetting or emulsifying agents, or pH buffering agents such as acetates, citrates or phosphates. Antibacterial agents such as benzyl alcohol or methyl parabens; antioxidants such as ascorbic acid or sodium bisulfite; chelating agents such as ethylenediaminetetraacetic acid; and agents for the adjustment of tonicity such as sodium chloride or dextrose are also envisioned. These compositions can take the form of solutions, suspensions, emulsion, tablets, pills, capsules, powders, sustained-release formulations and the like. The composition can be formulated as a suppository, with traditional binders and carriers such as triglycerides. Oral formulation can include standard carriers such as pharmaceutical grades of mannitol, lactose, starch, magnesium stearate, sodium saccharine, cellulose, magnesium carbonate, etc. Examples of suitable pharmaceutical carriers are described in Remington's Pharmaceutical Sciences by E.W. Martin, incorporated herein by reference. Such compositions will contain a therapeutically effective amount of the antigen-binding polypeptide, preferably in purified form, together with a suitable amount of carrier so as to provide the form for proper administration to the patient. The formulation should suit the mode of administration. The parental preparation can be enclosed in ampoules, disposable syringes or multiple dose vials made of glass or plastic.

In an embodiment, the composition is formulated in accordance with routine procedures as a pharmaceutical composition adapted for intravenous administration to human beings. Typically, compositions for intravenous administration are solutions in sterile isotonic aqueous buffer. Where necessary, the composition may also include a solubilizing agent and a local anesthetic such as lignocaine to ease pain at the site of the injection. Generally, the ingredients are supplied either separately or mixed together in unit dosage form, for example, as a dry lyophilized powder or water free concentrate in a hermetically sealed container such as an ampoule or sachette indicating the quantity of active agent. Where the composition is to be administered by infusion, it can be dispensed with an infusion bottle containing sterile pharmaceutical grade water or saline. Where the composition is administered by injection, an ampoule of sterile water for injection or saline can be provided so that the ingredients may be mixed prior to administration.

The compounds of the disclosure can be formulated as neutral or salt forms. Pharmaceutically acceptable salts include those formed with anions such as those derived from hydrochloric, phosphoric, acetic, oxalic, tartaric acids, etc., and those formed with cations such as those derived from sodium, potassium, ammonium, calcium, ferric hydroxides, isopropylamine, triethylamine, 2-ethylamino ethanol, histidine, procaine, etc.

Diagnostic Use of the antibody

Over-expression and/or over-activation of PD-L1 and/or LAG3 is observed in a biological sample (e.g., cells, tissues, blood, serum, etc.) from a patient suffering from a certain cancer and/or infection (for example, tumor cell or tissue, blood or serum from an infectious patient), and/or patients having PD-L1- and/or LAG3-over-expressing cells are likely responsive to treatments with the bispecific antibody or the anti-PD-L1 or anti-LAG3 antibody. Accordingly, the bispecific antibody or the anti-PD-L1 or anti-LAG3 antibody of the present disclosure can also be used for diagnostic and prognostic purposes.

An embodiment provides a pharmaceutical composition for diagnosing a disease associated with PD-L1, LAG3, or both thereof, the composition comprising the bispecific antibody or the anti-PD-L1 or anti-LAG3 antibody. In another embodiment, provided is a use of the bispecific antibody or the anti-PD-L1 or anti-LAG3 antibody for diagnosing a disease associated with PD-L1, LAG3, or both thereof.

Another embodiment provides a method of diagnosing a disease associated with PD-L1, LAG3, or both thereof, the method comprising contacting a biological sample obtained from a patient with the bispecific antibody or the anti-PD-L1 or anti-LAG3 antibody, and detecting antigen-antibody reaction or measuring a level of antigen-antibody reaction in the biological sample. In this method, when the antigen-antibody reaction is detected in the biological sample or the level of the antigen-antibody reaction in the biological sample is higher than that of a normal sample, the patient from whom the biological sample is obtained may be determined as a patient with a disease associated with PD-L1, LAG3, or both thereof. Therefore, in some embodiments, the method may further comprise contacting a normal sample with the bispecific antibody or the anti-PD-L1 or anti-LAG3 antibody, and measuring a level of an antigen-antibody reaction in the normal sample. In addition, the method may further comprise comparing the level of the antigen-antibody reaction in the biological sample and in the normal sample, after the measuring step. In addition, after the detecting step or comparing step, the method may further comprise determining the patient as a patient with a disease associated with PD-L1, LAG3, or both thereof, when the antigen-antibody reaction is detected in the biological sample or the level of the antigen-antibody reaction in the biological sample is higher than that of the normal sample.

The disease associated with PD-L1, LAG3, or both thereof may be one associated with activation (e.g., abnormal activation or over-activation) and/or overproduction (overexpression)

of PD-L1, LAG3, or both thereof. For example, the disease may be a cancer or an infection, as described above.

In the diagnosing composition and method, the biological sample may be at least one selected from the group consisting of a cell, a tissue, body fluid (e.g., blood, serum, lymph, etc.) and the like, obtained (separated) from a patient to be diagnosed. The normal sample may be at least one selected from the group consisting of a cell, a tissue, body fluid (e.g., blood, serum, lymph, urine, etc.) and the like, obtained (separated) from a patient having no disease associated with PD-L1, LAG3, or both thereof. The patient may be selected from a mammal, such as a human. Upon optional pre-treatment of the sample, the sample can be incubated with the bispecific antibody or the anti-PD-L1 or anti-LAG3 antibody of the present disclosure under conditions allowing the antibody to interact with a PD-L1 and/or LAG3 protein potentially present in the sample.

Presence and/or level (concentration) of the PD-L1 and/or LAG3 protein in the sample can be used for identifying a patient who is suitable for a treatment with the bispecific antibody or the anti-PD-L1 or anti-LAG3 antibody, or a patient who is responsive or susceptive to the treatment with the bispecific antibody or the anti-PD-L1 or anti-LAG3 antibody.

An embodiment provides a pharmaceutical composition identifying a patient who is suitable for a treatment with the bispecific antibody or the anti-PD-L1 or anti-LAG3 antibody, or a patient who is responsive or susceptive to the treatment with the bispecific antibody or the anti-PD-L1 or anti-LAG3 antibody, the composition comprising the bispecific antibody or the anti-PD-L1 or anti-LAG3 antibody. In another embodiment, provided is a use of the bispecific antibody or the anti-PD-L1 or anti-LAG3 antibody for identifying a patient who is suitable for a treatment with the bispecific antibody or the anti-PD-L1 or anti-LAG3 antibody, or a patient who is responsive or susceptive to the treatment with the bispecific antibody or the anti-PD-L1 or anti-LAG3 antibody. Another embodiment provides a method of identifying a patient who is suitable for a treatment with the bispecific antibody or the anti-PD-L1 or anti-LAG3 antibody, or a patient who is responsive or susceptive to the treatment with the bispecific antibody or the anti-PD-L1 or anti-LAG3 antibody, the method comprising contacting a biological sample obtained from a patient with the bispecific antibody or the anti-PD-L1 or anti-LAG3 antibody, and detecting antigen-antibody reaction or measuring a level of antigen-antibody reaction in the biological sample.

An embodiment provides a composition for detection of PD-L1, LAG3, or both thereof simultaneously, in a biological sample, the composition comprising the bispecific antibody or the anti-PD-L1 or anti-LAG3 antibody. Another embodiment provides a method of detection

of PD-L1, LAG3, or both thereof simultaneously, in a biological sample, the method comprising contacting the biological sample with the bispecific antibody or the anti-PD-L1 or anti-LAG3 antibody; and detecting (measuring) an antigen-antibody reaction (binding) between the bispecific antibody or the anti-PD-L1 or anti-LAG3 antibody and PD-L1, LAG3, or both thereof.

In the detecting composition and the detecting method, the term "detection of PD-L1, LAG3, or both thereof" may refer to, but not be limited to, detection of presence (and/or absence) and/or level of PD-L1, LAG3, or both thereof in the biological sample.

In the method of detection, when an antigen-antibody reaction is detected, it can be determined that PD-L1, LAG3, or both thereof are present in the biological sample, and when an antigen-antibody reaction is not detected, it can be determined that PD-L1, LAG3, or both thereof are absent (not present) in the biological sample. Therefore, the method of detection may further comprise, after the detecting step, determining that PD-L1, LAG3, or both thereof are present in the biological sample when an antigen-antibody reaction is detected, and/or that PD-L1, LAG3, or both thereof are absent (not present) in the biological sample, when an antigen-antibody reaction is not detected.

In the method of detection, the level of PD-L1, LAG3, or both thereof may be determined according to the degree of the antigen-antibody reaction (e.g., the amount of antigen-antibody complex formed by the antigen-antibody reaction, the intensity of any signal obtained by the antigen-antibody reaction, and the like, which can be measured by any conventional means).

The biological sample may comprise at least one selected from the group consisting of a cell (e.g., a tumor cell), a tissue (e.g., a tumor tissue), body fluid (e.g., blood, serum, etc.), and the like, obtained or isolated from a mammal such as a human. The steps of the method of detection may be conducted *in virto*.

In the diagnosing method and/or detecting method, the step of detecting the antigen-antibody reaction or measuring a level of the antigen-antibody reaction may be performed by any general method known to the relevant art, such as general enzymatic reactions, fluorescent reactions, luminescent reactions, and/or detection of radiation. For example, the step may be performed by a method selected from, but not limited to, the group consisting of immunochromatography, immunohistochemistry (IHC), enzyme linked immunosorbent assay (ELISA), radioimmunoassay (RIA), enzyme immunoassay (EIA), fluorescence immunoassay (FIA), luminescence immunoassay (LIA), western blotting, microarray, flow cytometry, surface plasmon resonance (SPR), and the like, but not be limited thereto.

Polynucleotides Encoding the Antibodies and Methods of Preparing the Antibodies

An embodiment provides a polynucleotide encoding the bispecific antibody or the anti-PD-L1 or anti-LAG3 antibody. In particular, an embodiment provides a polynucleotide encoding a heavy chain of the bispecific antibody in an IgG-scFv form. Other embodiment provides a polynucleotide encoding a light chain of the bispecific antibody in the IgG-scFv form. The IgG-scFv form may refer to a kind of a bispecific antibody comprising a full-length IgG antibody targeting (binding to) one of PD-L1 and LAG3 proteins and a scFv fragment targeting (binding to) the other one, wherein the scFv is linked to a C-terminus and/or N-terminus of the full-length IgG antibody directly (without a peptide linker) or via a peptide linker.

In an embodiment, when the bispecific antibody in an IgG-scFv form comprises a full-length IgG antibody against PD-L1 and a scFv fragment against LAG3, the polynucleotide encoding a heavy chain of the bispecific antibody may encode a heavy chain of the full-length IgG antibody against PD-L1 and a scFv fragment against LAG3 that is linked to a C-terminus and/or N-terminus of the full-length IgG antibody directly or via a peptide linker; and the polynucleotide encoding a light chain of the bispecific antibody may encode a light chain of the full-length IgG antibody against PD-L1.

In another embodiment, when the bispecific antibody in an IgG-scFv form comprises a full-length IgG antibody against LAG3 and a scFv fragment against PD-L1, the polynucleotide encoding a heavy chain of the bispecific antibody may encode a heavy chain of the full-length IgG antibody against LAG3 and a scFv fragment against PD-L1 that is linked to a C-terminus and/or N-terminus of the full-length IgG antibody directly or via a peptide linker; and the polynucleotide encoding a light chain of the bispecific antibody may encode a light chain of the full-length IgG antibody against LAG3.

Another embodiment provides a recombinant vector comprising the polynucleotide encoding a heavy chain of the bispecific antibody, the polynucleotide encoding a light chain of the bispecific antibody, or both thereof. Another embodiment provides a recombinant cell transfected with the recombinant vector.

Another embodiment provides a method of preparing the bispecific antibody, comprising expressing the polynucleotide encoding a heavy chain of the bispecific antibody, the polynucleotide encoding a light chain of the bispecific antibody in a cell. The step of expressing the polynucleotide may be conducted by culturing the cell comprising the

polynucleotide (for example, in a recombinant vector) under a condition allowing the expression of the polynucleotide. The method may further comprise isolating and/or purifying the bispecific antibody from the cell culture, after the step of expressing or culturing.

EXAMPLES

Hereafter, the present invention will be described in detail by examples.

The following examples are intended merely to illustrate the invention and are not construed to restrict the invention.

Example 1: Preparation of anti-PD-L1 monoclonal antibodies

1.1. Preparation of Anti-human-PD-L1 mouse monoclonal antibodies and analysis thereof

Anti-human-PD-L1 mouse monoclonal antibodies were generated using the hybridoma technology.

Antigen: human PD-L1-Fc protein and human PD-L1 highly expressed CHOK1 cell line (PDL1-CHOK1 cell line).

Immunization: To generate mouse monoclonal antibodies to human PD-L1, 6-8 week female BALB/c mice were firstly immunized with $1.5x10^7$ PDL1-CHOK1 cells. Day 14 and 33 post first immunization, the immunized mice were re-immunized with $1.5x10^7$ PDL1-CHOK1 cells respectively. To select mice producing antibodies that bound PD-L1 protein, sera from immunized mice were tested by ELISA. Briefly, microtiter plates were coated with human PD-L1 protein at 1 μg/ml in PBS, 100μl/well at room temperature (RT) overnight, then blocked with 100μl/well of 5% BSA. Dilutions of plasma from immunized mice were added to each well and incubated for 1-2 hours at RT. The plates were washed with PBS/Tween and then incubate with anti-mouse IgG antibody conjugated with Horse Radish Peroxidase (HRP) for 1 hour at RT. After washing, the plates were developed with ABTS substrate and analyzed by spectrophotometer at OD 405nm. Mice with sufficient titers of anti-PDL1 IgG were boosted with 50μg human PDL1-Fc protein at Day 54 post-immunization. The resulting mice were used for fusions. The hybridoma supernatants were tested for anti-PD-L1 IgGs by ELISA.

The amino acid and polynucleotide sequences of the variable regions of Hybridoma HL1210-3 are provided in Table 5 below.

[Table 5] HL1210-3 variable sequences

Name	Sequence	SEQ ID NO:
HL1210-3 VH	GAAGTGAAACTGGTGGAGTCTGGGGGAGACTTAGTGAAGC CTGGAGGGTCCCTGAAACTCTCCTGTGCAGCCTCTGGATT CACTTTCAGTAGCTATGACATGTCTTGGGTTCGCCAGACT CCGGAGAAGAGTCTGGAGTGGGTCGCAACCATTAGTGATG GTGGTGGTTACATCTACTATTCAGACAGTGTGAAGGGGCG ATTTACCATCTCCAGAGACAATGCCAAGAACAACCTGTAC CTGCAAATGAGCAGTCTGAGGTCTGAGGACACGGCCTTGT ATATTTGTGCAAGAGAATTTGGTAAGCGCTATGCTTTGGA CTACTGGGGTCAAGGAACCTCAGTCACCGTCTCCTCA	112
HL1210-3 VH	EVKLVESGGDLVKPGGSLKLSCAASGFTFSSYDMSWVRQT PEKSLEWVATISDGGGYIYYSDSVKGRFTISRDNAKNNLY LQMSSLRSEDTALYICAREFGKRYALDYWGQGTSVT	113
HL1210-3 VL	GACATTGTGATGACCCAGTCTCACAAATTCATGTCCACAT CGGTAGGAGACAGGGTCAGCATCTCCTGCAAGGCCAGTCA GGATGTGACTCCTGCTGTCGCCTGGTATCAACAGAAGCCA GGACAATCTCCTAAACTACTGATTTACTCCACATCCTCCC GGTACACTGGAGTCCCTGATCGCTTCACTGGCAGTGGATC TGGGACGGATTTCACTTTCACCATCAGCAGTGTGCAGGCT GAAGACCTGGCAGTTTATTACTGTCAGCAACATTATACTA CTCCGCTCACGTTCGGTGCTGGGACCAAGCTGGAGCTGAA A	114
HL1210-3 VL	DIVMTQSHKFMSTSVGDRVSISCKASQDVTPAVAWYQQKP GQSPKLLIYSTSSRYTGVPDRFTGSGSGTDFTFTISSVQA EDLAVYYCQQHYTTPLTFGAGTKLELK	115

1.2. Activities of HL1210-3 mouse mAb

To evaluate the binding activity of hybridoma clone HL1210-3, the purified mAb from this clone were subjected to ELISA test. Briefly, microtiter plates were coated with human PD-L1-Fc protein at 0.1μg/ml in PBS, 100μl/well at 4°C overnight, then blocked with 100μl/well of 5% BSA. Three-fold dilutions of HL1210-3 antibodies starting from 0.2μg/ml were added to each well and incubated for 1-2 hours at RT. The plates were washed with PBS/Tween and then incubate with goat-anti-mouse IgG antibody conjugated with Horse Radish Peroxidase (HRP) for 1 hour at RT. After washing, the plates were developed with TMB substrate and analyzed by spectrophotometer at OD 450-630nm. As shown in FIG. 1, HL1210-3 can bind to human PD-L1 with high activity (EC_{50}=5.539ng/ml).

To evaluate the activity of HL1210-3 mouse mAb to block human PD-L1 binding to its receptor PD-1, a receptor blocking assay was performed by using recombinant human PD-L1.

To evaluate the blocking effect of HL1210-3 mouse mAb on recombinant human PD-L1 to bind to its receptor PD-1, the ELISA based receptor blocking assay was employed. Briefly, microtiter plates were coated with human PD-L1-Fc protein at 1μg/ml in PBS, 100μl/well at 4°C overnight, then blocked with 100μl/well of 5% BSA. 50μl biotin-labeled human PD-1-Fc protein and 3-fold dilutions of HL1210-3 antibodies starting from 2μg/ml at 50μl were added to each well and incubated for 1 hour at 37°C. The plates were washed with PBS/Tween and then incubated with Streptavidin-HRP for 1 hour at 37°C. After washing, the plates were developed with TMB substrate and analyzed by spectrophotometer at OD 450-630nm. As shown in FIG. 2, HL1210-3 can efficiently inhibit the binding of human PD-L1 to human PD1 at IC_{50}=0.7835nM.

In addition, a receptor blocking assay was also performed by using mammalian cell expressed human PD-L1.

To evaluate the blocking effect of HL1210-3 mouse mAb on human PD-L1 expressed on mammalian cells to bind to its receptor PD-1, the FACS-based receptor blocking assay was used. Briefly, PDL1-CHOK1 cells were firstly incubated with 3-fold serious diluted HL1210-3 mouse mAb starting at 20μg/ml at RT for 1 hour. After wash by FACS buffer (PBS with 2% FBS), the biotin-labeled huPD-1 was added to each well and incubated at RT for 1 hour. Then, the Streptavidin-PE was added to each well for 0.5 hour post twice wash with FACS buffer. The mean florescence intensity (MFI) of PE was evaluated by FACSAriaIII. As shown in FIG. 3, the HL1210-3 antibody can highly efficiently inhibit the binding of PD-1 on PD-L1 expressed on mammalian cells at IC50 of 2.56nM with 92.6%top inhibition rate.

$$\%\ \text{of inhibition} = \left(1 - \frac{\text{MFI of testing antibody}}{\text{MFI of vehicle contorl}}\right) \times 100\%$$

1.3. Effects of HL1210-3 mouse mAb

To evaluate the effect of HL1210-3 mouse mAb to promote human T cell immune response, the response of human T cells 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 the DCs and serial dilutions of anti-PD-L1 blocking antibody. At day 5 post-inoculation, the culture supernatant was assayed for IFNγ production. The results indicated that the HL1210-3 antibodies can dose-dependently promote IFNγ production, suggesting anti-PD-L1 antibody can promote human T cell response (FIG. 4).

1.4. Binding affinity of HL1210-3 mouse mAb

The binding of the HL1210-3 antibodies to recombinant PD-L1 protein (human PD-L1-his taq) was tested with BIACORE ™ using a capture method. The HL1210-3 mouse mAb was captured using anti-mouse Fc antibody coated on a CM5 chip. A series dilution of human PD-L1-his taq protein was injected over captured antibody for 3 mins at a flow rate of 25μg/ml. The antigen was allowed to dissociate for 900s. All the experiment were carried out on a Biacore T200. Data analysis was carried out using Biacore T200 evaluation software. The results are shown in FIG. 5 and Table 6 below.

[Table 6] Binding Kinetics of HL1210-3 to recombinant human PD-L1

Antibody	ka (1/Ms)	kd (1/s)	KD (M)
HL1210-3	1.61E+05	4.69E-05	2.93E-10

1.5. Humanization of the HL1210-3 mouse mAb

The mAb HL1210-3 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 HL1210-3 were compared against the available database of human Ig gene (IgG1) sequences to find the overall best-matching human germline Ig gene sequences. For the light chain, the closest human match was the O18/Jk2 and KV1-39*01/KJ2*04 gene, and for the heavy chain the closest human match was the VH3-21 gene. VH3-11, VH3-23, VH3-7*01 and VH3-48 genes were also selected due to their close matches.

Humanized variable domain sequences were then designed where the CDR1 (SEQ ID NO. 4) , 2 (SEQ ID NO. 5) and 3 (SEQ ID NO. 6) of the HL1210-3 light chain were grafted onto framework sequences of the O18/Jk2 and KV1-39*01/KJ2*04 gene, and the CDR1 (SEQ ID NO. 1) , 2 (SEQ ID NO. 2) , and 3 (SEQ ID NO. 3) sequences of the HL1210-3 VH were grafted onto framework sequences of the VH3-21, VH3-11, VH3-23, VH3-48 or VH3-7*01 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 light chain, 22S, 43S, 60D, 63T and 42Q (Kabat numbering, see Table 7) in framework were identified. In the case of the heavy chain, 1E, 37V, 40T, 44S, 49A, 77N, 91I, 94R and 108T in the framework was involved in back-mutations.

Table 7. Humanization Design

VH Design I: VH3-21/JH6	
Construct	**Mutation**

Hu1210 VH	Chimera
Hu1210 VH.1	CDR-grafted
Hu1210 VH.1a	S49A
Hu1210 VH.1b	S49A, G44S, Y91I
VH Design II: VH3-11/JH6	
Hu1210 VH.2	CDR-grafted, Q1E
Hu1210 VH.2a	Q1E, S49A
Hu1210 VH.2b	Q1E, I37V, S49A, G44S, Y91I
VH Design III: VH3-23/JH6	
Hu1210 VH.3	CDR-grafted, K94R
Hu1210 VH.3a	G44S, S49A, Y91I, K94R
VH Design IV: VH3-48/JH6	
Hu1210 VH.4	CDR-grafted
Hu1210 VH.4a	S49A
Hu1210 VH.4b	S49A, G44S, Y91I
Hu1210 VH.4c	D52E, S49A, G44S, Y91I
Hu1210 VH.4d	G53A, S49A, G44S, Y91I
Hu1210 VH.4e	G53V, S49A, G44S, Y91I
VH Design V： VH3-7*01/ HJ1*01	
Hu1210 VH.5	CDR-grafted
Hu1210 VH.5a	H91I
Hu1210 VH.5b	H91I, H108T
Hu1210 VH.5c	H91I, H77N
Hu1210 VH.5d	H91I, H77N, H40T
VK Design I: 018/Jk2	
Construct	**Mutation**
Hu1210 Vk	Chimera
Hu1210 Vk.1	CDR-grafted
Hu1210 Vk.1a	A43S
VK Design II: KV1-39*01/KJ2*04	
Hu1210 Vk.2	CDR-grafted
Hu1210 Vk.2a	L60D, L63T
Hu1210 Vk.2b	L60D, L63T, L42Q, L43S
Hu1210 Vk.2c	L60D, L63T, L42Q, L43S, T22S

The amino acid and nucleotide sequences of some of the humanized antibody are listed in Table 8 below.

[Table 8] Humanized antibody sequences (bold indicates CDR)

Name	Amino Acid Sequence	SEQ ID NO:
HL1210-VH	EVKLVESGGDLVKPGGSLKLSCAASGFTFS**SYDMS**WVRQTPEKSLEW VA**TISDGGGYIYYSDSVKG**RFTISRDNAKNNLYLQMSSLRSEDTALYI CAR**EFGKRYALDY**WGQGTSVTVSS	7
Hu1210 VH.1	EVQLVESGGGLVKPGGSLRLSCAASGFTFS**SYDMS**WVRQAPGKGLEW VS**TISDGGGYIYYSDSVKG**RFTISRDNAKNSLYLQMNSLRAEDTAVY YCAR**EFGKRYALDY**WGQGTTVTVSS	8
Hu1210 VH.1a	EVQLVESGGGLVKPGGSLRLSCAASGFTFS**SYDMS**WVRQAPGKGLEW VA**TISDGGGYIYYSDSVKG**RFTISRDNAKNSLYLQMNSLRAEDTAVY YCAR**EFGKRYALDY**WGQGTTVTVSS	9
Hu1210 VH.1b	EVQLVESGGGLVKPGGSLRLSCAASGFTFS**SYDMS**WVRQAPGKSLEW VA**TISDGGGYIYYSDSVKG**RFTISRDNAKNSLYLQMNSLRAEDTAVYI CAR**EFGKRYALDY**WGQGTTVTVSS	10
Hu1210 VH.2	EVQLVESGGGLVKPGGSLRLSCAASGFTFS**SYDMS**WIRQAPGKGLEW VS**TISDGGGYIYYSDSVKG**RFTISRDNAKNSLYLQMNSLRAEDTAVY YCAR**EFGKRYALDY**WGQGTTVTVSS	11
Hu1210 VH.2a	EVQLVESGGGLVKPGGSLRLSCAASGFTFS**SYDMS**WIRQAPGKGLEW VA**TISDGGGYIYYSDSVKG**RFTISRDNAKNSLYLQMNSLRAEDTAVY YCAR**EFGKRYALDY**WGQGTTVTVSS	12
Hu1210 VH.2b	EVQLVESGGGLVKPGGSLRLSCAASGFTFS**SYDMS**WVRQAPGKSLEW VA**TISDGGGYIYYSDSVKG**RFTISRDNAKNSLYLQMNSLRAEDTAVYI CAR**EFGKRYALDY**WGQGTTVTVSS	13
Hu1210 VH.3	EVQLLESGGGLVQPGGSLRLSCAASGFTFS**SYDMS**WVRQAPGKGLEW VS**TISDGGGYIYYSDSVKG**RFTISRDNSKNTLYLQMNSLRAEDTAVYY CAR**EFGKRYALDY**WGQGTTVTVSS	14
Hu1210 VH.3a	EVQLLESGGGLVQPGGSLRLSCAASGFTFS**SYDMS**WVRQAPGKSLEW VA**TISDGGGYIYYSDSVKG**RFTISRDNSKNTLYLQMNSLRAEDTAVYI CAR**EFGKRYALDY**WGQGTTVTVSS	15
Hu1210 VH.4	EVQLVESGGGLVQPGGSLRLSCAASGFTFS**SYDMS**WVRQAPGKGLEW VS**TISDGGGYIYYSDSVKG**RFTISRDNAKNSLYLQMNSLRDEDTAVY YCAR**EFGKRYALDY**WGQGTTVTVSS	16
Hu1210 VH.4a	EVQLVESGGGLVQPGGSLRLSCAASGFTFS**SYDMS**WVRQAPGKGLEW VA**TISDGGGYIYYSDSVKG**RFTISRDNAKNSLYLQMNSLRDEDTAVY YCAR**EFGKRYALDY**WGQGTTVTVSS	17
Hu1210 VH.4b	EVQLVESGGGLVQPGGSLRLSCAASGFTFS**SYDMS**WVRQAPGKSLEW VA**TISDGGGYIYYSDSVKG**RFTISRDNAKNSLYLQMNSLRDEDTAVYI CAR**EFGKRYALDY**WGQGTTVTVSS	18
Hu1210 VH.4c	EVQLVESGGGLVQPGGSLRLSCAASGFTFS**SYDMS**WVRQAPGKSLEW VA**TISEGGGYIYYSDSVKG**RFTISRDNAKNSLYLQMNSLRDEDTAVYI CAR**EFGKRYALDY**WGQGTTVTVSS	19
Hu1210 VH.4d	EVQLVESGGGLVQPGGSLRLSCAASGFTFS**SYDMS**WVRQAPGKSLEW VA**TISDAGGYIYYSDSVKG**RFTISRDNAKNSLYLQMNSLRDEDTAVYI CAR**EFGKRYALDY**WGQGTTVTVSS	20
Hu1210 VH.4e	EVQLVESGGGLVQPGGSLRLSCAASGFTFS**SYDMS**WVRQAPGKSLEW VA**TISDVGGYIYYSDSVKG**RFTISRDNAKNSLYLQMNSLRDEDTAVYI CAR**EFGKRYALDY**WGQGTTVTVSS	21
Hu1210 VH.5	EVQLVESGGGLVQPGGSLRLSCAASGFTFS**SYDMS**WVRQAPGKGLEW	22

	VA**TISDGGGYIYYSDSVKG**RFTISRDNAKNSLYLQMNSLRAEDTAVY YCAR**EFGKRYALDY**WGQGTLVTVSS	
HU1210 VH.5a	EVQLVESGGGLVQPGGSLRLSCAASGFTFS**SYDMS**WVRQAPGKGLEW VA**TISDGGGYIYYSDSVKG**RFTISRDNAKNSLYLQMNSLRAEDTAVYI CAR**EFGKRYALDY**WGQGTLVTVSS	23
HU1210 VH.5b	EVQLVESGGGLVQPGGSLRLSCAASGFTFS**SYDMS**WVRQAPGKGLEW VA**TISDGGGYIYYSDSVKG**RFTISRDNAKNSLYLQMNSLRAEDTAVYI CAR**EFGKRYALDY**WGQGTTVTVSS	24
HU1210 VH.5C	EVQLVESGGGLVQPGGSLRLSCAASGFTFS**SYDMS**WVRQAPGKGLEW VA**TISDGGGYIYYSDSVKG**RFTISRDNAKNNLYLQMNSLRAEDTAVYI CAR**EFGKRYALDY**WGQGTLVTVSS	25
HU1210 VH.5d	EVQLVESGGGLVQPGGSLRLSCAASGFTFS**SYDMS**WVRQTPEKSLEW VA**TISDGGGYIYYSDSVKG**RFTISRDNAKNNLYLQMNSLRAEDTAVYI CAR**EFGKRYALDY**WGQGTLVTVSS	26
HL1210-VK	DIVMTQSHKFMSTSVGDRVSISC**KASQDVTPAVA**WYQQKPGQSPKLLI Y**STSSRYT**GVPDRFTGSGSGTDFTFTISSVQAEDLAVYYC**QQHYTTPL T**FGAGTKLELK	27
Hu1210 VK.1	DIQMTQSPSSLSASVGDRVTITC**KASQDVTPAVA**WYQQKPGKAPKLLI Y**STSSRYT**GVPSRFSGSGSGTDFTFTISSLQPEDIATYYC**QQHYTTPLT** FGQGTKLEIK	28
Hu1210 VK.1a	DIQMTQSPSSLSASVGDRVTITC**KASQDVTPAVA**WYQQKPGKSPKLLI Y**STSSRYT**GVPSRFSGSGSGTDFTFTISSLQPEDIATYYC**QQHYTTPLT** FGQGTKLEIK	29
Hu1210 Vk.2	DIQMTQSPSSLSASVGDRVTITC**KASQDVTPAVA**WYQQKPGKAPKLLI Y**STSSRYT**GVPSRFSGSGSGTDFTLTISSLQPEDFATYYC**QQHYTTPLT** FGQGTKLEIKR	30
Hu1210 Vk.2a	DIQMTQSPSSLSASVGDRVTITC**KASQDVTPAVA**WYQQKPGKAPKLLI Y**STSSRYT**GVPDRFTGSGSGTDFTLTISSLQPEDFATYYC**QQHYTTPLT** FGQGTKLEIKR	31
Hu1210 Vk.2b	DIQMTQSPSSLSASVGDRVTITC**KASQDVTPAVA**WYQQKPGQSPKLLI Y**STSSRYT**GVPDRFTGSGSGTDFTLTISSLQPEDFATYYC**QQHYTTPLT** FGQGTKLEIKR	32
Hu1210 Vk.2c	DIQMTQSPSSLSASVGDRVTISC**KASQDVTPAVA**WYQQKPGQSPKLLI Y**STSSRYT**GVPDRFTGSGSGTDFTLTISSLQPEDFATYYC**QQHYTTPLT** FGQGTKLEIKR	33

Name	**Nucleic Acid Sequence**	**SEQ ID NO:**
HL1210 VH	GAGGTGAAGCTGGTGGAGAGCGGCGGAGATCTGGTGAAGCCTGGC GGCAGCCTGAAGCTGAGCTGTGCCGCCAGCGGCTTCACCTTCAGCA GCTACGACATGAGCTGGGTGAGGCAGACCCCCGAGAAGAGCCTGG AGTGGGTGGCCACCATCAGCGATGGCGGCGGCTACATCTACTACAG CGACAGCGTGAAGGGCAGGTTCACCATCAGCAGGGACAACGCCAA GAACAACCTGTACCTGCAGATGAGCAGCCTGAGGAGCGAGGACAC CGCCCTGTACATCTGCGCCAGGGAGTTCGGCAAGAGGTACGCCCTG GACTACTGGGGACAGGGCACCAGCGTGACCGTGAGCAGC	34
Hu1210 VH.1	GAGGTGCAGCTGGTGGAGAGCGGAGGAGGACTGGTGAAGCCCGG AGGCAGCCTGAGACTGAGCTGCGCTGCCAGCGGCTTCACCTTCAGC AGCTACGACATGAGCTGGGTGAGACAGGCCCCTGGCAAAGGCCTG GAGTGGGTGAGCACCATCTCCGATGGCGGCGGCTACATCTATTACTC CGACAGCGTGAAGGGCAGGTTCACCATCAGCAGGGACAACGCCAA GAACAGCCTGTACCTGCAGATGAACAGCCTGAGGGCCGAGGACAC CGCCGTGTACTACTGCGCCAGGGAGTTCGGCAAAAGGTACGCCCTG GACTACTGGGGCCAGGGCACAACCGTGACCGTGAGCAGC	35

Hu1210 VH.1a	GAGGTGCAGCTGGTGGAGAGCGGAGGAGGACTGGTGAAGCCCGG AGGCAGCCTGAGACTGAGCTGCGCTGCCAGCGGCTTCACCTTCAGC AGCTACGACATGAGCTGGGTGAGACAGGCCCCTGGCAAAGGCCTG GAGTGGGTGGCCACCATCTCCGATGGCGGCGGCTACATCTATTACTC CGACAGCGTGAAGGGCAGGTTCACCATCAGCAGGGACAACGCCAA GAACAGCCTGTACCTGCAGATGAACAGCCTGAGGGCCGAGGACAC CGCCGTGTACTACTGCGCCAGGGAGTTCGGCAAAAGGTACGCCCTG GACTACTGGGGCCAGGGCACAACCGTGACCGTGAGCAGC	36
Hu1210 VH.1b	GAGGTGCAGCTGGTGGAGAGCGGAGGAGGACTGGTGAAGCCCGG AGGCAGCCTGAGACTGAGCTGCGCTGCCAGCGGCTTCACCTTCAGC AGCTACGACATGAGCTGGGTGAGACAGGCCCCTGGCAAAAGCCTG GAGTGGGTGGCCACCATCTCCGATGGCGGCGGCTACATCTATTACTC CGACAGCGTGAAGGGCAGGTTCACCATCAGCAGGGACAACGCCAA GAACAGCCTGTACCTGCAGATGAACAGCCTGAGGGCCGAGGACAC CGCCGTGTACATCTGCGCCAGGGAGTTCGGCAAAAGGTACGCCCTG GACTACTGGGGCCAGGGCACAACCGTGACCGTGAGCAGC	37
Hu1210 VH.2	GAGGTGCAGCTGGTGGAGAGCGGAGGAGGACTGGTGAAGCCCGG AGGCAGCCTGAGACTGAGCTGCGCTGCCAGCGGCTTCACCTTCAGC AGCTACGACATGAGCTGGATCAGACAGGCCCCTGGCAAAGGCCTG GAGTGGGTGAGCACCATCTCCGATGGCGGCGGCTACATCTATTACTC CGACAGCGTGAAGGGCAGGTTCACCATCAGCAGGGACAACGCCAA GAACAGCCTGTACCTGCAGATGAACAGCCTGAGGGCCGAGGACAC CGCCGTGTACTACTGCGCCAGGGAGTTCGGCAAAAGGTACGCCCTG GACTACTGGGGCCAGGGCACAACCGTGACCGTGAGCAGC	38
Hu1210 VH.2a	GAGGTGCAGCTGGTGGAGAGCGGAGGAGGACTGGTGAAGCCCGG AGGCAGCCTGAGACTGAGCTGCGCTGCCAGCGGCTTCACCTTCAGC AGCTACGACATGAGCTGGATCAGACAGGCCCCTGGCAAAGGCCTG GAGTGGGTGGCCACCATCTCCGATGGCGGCGGCTACATCTATTACTC CGACAGCGTGAAGGGCAGGTTCACCATCAGCAGGGACAACGCCAA GAACAGCCTGTACCTGCAGATGAACAGCCTGAGGGCCGAGGACAC CGCCGTGTACTACTGCGCCAGGGAGTTCGGCAAAAGGTACGCCCTG GACTACTGGGGCCAGGGCACAACCGTGACCGTGAGCAGC	39
Hu1210 VH.2b	GAGGTGCAGCTGGTGGAGAGCGGAGGAGGACTGGTGAAGCCCGG AGGCAGCCTGAGACTGAGCTGCGCTGCCAGCGGCTTCACCTTCAGC AGCTACGACATGAGCTGGGTGAGACAGGCCCCTGGCAAAAGCCTG GAGTGGGTGGCCACCATCTCCGATGGCGGCGGCTACATCTATTACTC CGACAGCGTGAAGGGCAGGTTCACCATCAGCAGGGACAACGCCAA GAACAGCCTGTACCTGCAGATGAACAGCCTGAGGGCCGAGGACAC CGCCGTGTACATCTGCGCCAGGGAGTTCGGCAAAAGGTACGCCCTG GACTACTGGGGCCAGGGCACAACCGTGACCGTGAGCAGC	40
Hu1210 VH.3	GAGGTGCAGCTGCTGGAGAGCGGAGGAGGACTGGTGCAACCCGGA GGCAGCCTGAGACTGAGCTGCGCTGCCAGCGGCTTCACCTTCAGCA GCTACGACATGAGCTGGGTGAGACAGGCCCCTGGCAAAGGCCTGG AGTGGGTGAGCACCATCTCCGATGGCGGCGGCTACATCTATTACTCC GACAGCGTGAAGGGCAGGTTCACCATCAGCAGGGACAACAGCAAG AACACCCTGTACCTGCAGATGAACAGCCTGAGGGCCGAGGACACC GCCGTGTACTACTGCGCCAGGGAGTTCGGCAAAAGGTACGCCCTGG ACTACTGGGGCCAGGGCACAACCGTGACCGTGAGCAGC	41
Hu1210 VH.3a	GAGGTGCAGCTGCTGGAGAGCGGAGGAGGACTGGTGCAACCCGGA GGCAGCCTGAGACTGAGCTGCGCTGCCAGCGGCTTCACCTTCAGCA GCTACGACATGAGCTGGGTGAGACAGGCCCCTGGCAAAAGCCTGG AGTGGGTGGCCACCATCTCCGATGGCGGCGGCTACATCTATTACTCC GACAGCGTGAAGGGCAGGTTCACCATCAGCAGGGACAACAGCAAG AACACCCTGTACCTGCAGATGAACAGCCTGAGGGCCGAGGACACC GCCGTGTACATCTGCGCCAGGGAGTTCGGCAAAAGGTACGCCCTGG ACTACTGGGGCCAGGGCACAACCGTGACCGTGAGCAGC	42
Hu1210 VH.4	GAGGTGCAGCTGGTGGAGAGCGGAGGAGGACTGGTGCAACCCGGA GGCAGCCTGAGACTGAGCTGCGCTGCCAGCGGCTTCACCTTCAGCA	43

	GCTACGACATGAGCTGGGTGAGACAGGCCCCTGGCAAAGGCCTGG AGTGGGTGAGCACCATCTCCGATGGCGGCGGCTACATCTATTACTCC GACAGCGTGAAGGGCAGGTTCACCATCAGCAGGGACAACGCCAAG AACAGCCTGTACCTGCAGATGAACAGCCTGAGGGATGAGGACACC GCCGTGTACTACTGCGCCAGGGAGTTCGGCAAAAGGTACGCCCTGG ACTACTGGGGCCAGGGCACAACCGTGACCGTGAGCAGC	
Hu1210 VH.4a	GAGGTGCAGCTGGTGGAGAGCGGAGGAGGACTGGTGCAACCCGGA GGCAGCCTGAGACTGAGCTGCGCTGCCAGCGGCTTCACCTTCAGCA GCTACGACATGAGCTGGGTGAGACAGGCCCCTGGCAAAGGCCTGG AGTGGGTGGCCACCATCTCCGATGGCGGCGGCTACATCTATTACTCC GACAGCGTGAAGGGCAGGTTCACCATCAGCAGGGACAACGCCAAG AACAGCCTGTACCTGCAGATGAACAGCCTGAGGGATGAGGACACC GCCGTGTACTACTGCGCCAGGGAGTTCGGCAAAAGGTACGCCCTGG ACTACTGGGGCCAGGGCACAACCGTGACCGTGAGCAGC	44
Hu1210 VH.4b	GAGGTGCAGCTGGTGGAGAGCGGAGGAGGACTGGTGCAACCCGGA GGCAGCCTGAGACTGAGCTGCGCTGCCAGCGGCTTCACCTTCAGCA GCTACGACATGAGCTGGGTGAGACAGGCCCCTGGCAAAAGCCTGG AGTGGGTGGCCACCATCTCCGATGGCGGCGGCTACATCTATTACTCC GACAGCGTGAAGGGCAGGTTCACCATCAGCAGGGACAACGCCAAG AACAGCCTGTACCTGCAGATGAACAGCCTGAGGGATGAGGACACC GCCGTGTACATCTGCGCCAGGGAGTTCGGCAAAAGGTACGCCCTGG ACTACTGGGGCCAGGGCACAACCGTGACCGTGAGCAGC	45
Hu1210 VH.4c	GAGGTGCAGCTGGTGGAGAGCGGAGGAGGACTGGTGCAACCCGGA GGCAGCCTGAGACTGAGCTGCGCTGCCAGCGGCTTCACCTTCAGCA GCTACGACATGAGCTGGGTGAGACAGGCCCCTGGCAAAAGCCTGG AGTGGGTGGCCACCATCTCCGAAGGCGGCGGCTACATCTATTACTCC GACAGCGTGAAGGGCAGGTTCACCATCAGCAGGGACAACGCCAAG AACAGCCTGTACCTGCAGATGAACAGCCTGAGGGATGAGGACACC GCCGTGTACATCTGCGCCAGGGAGTTCGGCAAAAGGTACGCCCTGG ACTACTGGGGCCAGGGCACAACCGTGACCGTGAGCAGC	46
Hu1210_VH.4d	GAGGTGCAGCTGGTGGAGAGCGGAGGAGGACTGGTGCAACCCGGA GGCAGCCTGAGACTGAGCTGCGCTGCCAGCGGCTTCACCTTCAGCA GCTACGACATGAGCTGGGTGAGACAGGCCCCTGGCAAAAGCCTGG AGTGGGTGGCCACCATCTCCGATGCGGGCGGCTACATCTATTACTCC GACAGCGTGAAGGGCAGGTTCACCATCAGCAGGGACAACGCCAAG AACAGCCTGTACCTGCAGATGAACAGCCTGAGGGATGAGGACACC GCCGTGTACATCTGCGCCAGGGAGTTCGGCAAAAGGTACGCCCTGG ACTACTGGGGCCAGGGCACAACCGTGACCGTGAGCAGC	47
Hu1210_VH.4e	GAGGTGCAGCTGGTGGAGAGCGGAGGAGGACTGGTGCAACCCGGA GGCAGCCTGAGACTGAGCTGCGCTGCCAGCGGCTTCACCTTCAGCA GCTACGACATGAGCTGGGTGAGACAGGCCCCTGGCAAAAGCCTGG AGTGGGTGGCCACCATCTCCGATGTTGGCGGCTACATCTATTACTCC GACAGCGTGAAGGGCAGGTTCACCATCAGCAGGGACAACGCCAAG AACAGCCTGTACCTGCAGATGAACAGCCTGAGGGATGAGGACACC GCCGTGTACATCTGCGCCAGGGAGTTCGGCAAAAGGTACGCCCTGG ACTACTGGGGCCAGGGCACAACCGTGACCGTGAGCAGC	48
Hu1210 VH.5	GAGGTGCAGCTGGTGGAGTCCGGAGGAGGCCTGGTGCAACCTGGA GGCTCCCTGAGGCTGTCCTGTGCCGCTTCCGGCTTCACCTTCAGCTC CTACGATATGAGCTGGGTGAGGCAGGCTCCTGGAAAGGGCCTGGAG TGGGTGGCCACCATCTCCGACGGAGGCGGCTACATCTACTACTCCG ACTCCGTGAAGGGCAGGTTCACCATCTCCCGGGACAACGCCAAGA ACTCCCTGTACCTGCAGATGAACTCTCTCAGGGCTGAGGACACCGC CGTGTATTACTGCGCCAGGGAGTTTGGCAAGAGGTACGCCCTGGAT TACTGGGGCCAGGGCACACTGGTGACAGTGAGCTCC	49
Hu1210 VH.5a	GAGGTGCAGCTGGTGGAGTCCGGAGGAGGCCTGGTGCAACCTGGA GGCTCCCTGAGGCTGTCCTGTGCCGCTTCCGGCTTCACCTTCAGCTC CTACGATATGAGCTGGGTGAGGCAGGCTCCTGGAAAGGGCCTGGAG TGGGTGGCCACCATCTCCGACGGAGGCGGCTACATCTACTACTCCG	50

	ACTCCGTGAAGGGCAGGTTCACCATCTCCCGGGACAACGCCAAGA ACTCCCTGTACCTGCAGATGAACTCTCTCAGGGCTGAGGACACCGC CGTGTATATCTGCGCCAGGGAGTTTGGCAAGAGGTACGCCCTGGATT ACTGGGGCCAGGGCACACTGGTGACAGTGAGCTCC	
Hu1210 VH.5b	GAGGTGCAGCTGGTGGAGTCCGGAGGAGGCCTGGTGCAACCTGGA GGCTCCCTGAGGCTGTCCTGTGCCGCTTCCGGCTTCACCTTCAGCTC CTACGATATGAGCTGGGTGAGGCAGGCTCCTGGAAAGGGCCTGGAG TGGGTGGCCACCATCTCCGACGGAGGCGGCTACATCTACTACTCCG ACTCCGTGAAGGGCAGGTTCACCATCTCCCGGGACAACGCCAAGA ACAACCTGTACCTGCAGATGAACTCTCTCAGGGCTGAGGACACCGC CGTGTATATCTGCGCCAGGGAGTTTGGCAAGAGGTACGCCCTGGATT ACTGGGGCCAGGGCACACTGGTGACAGTGAGCTCC	51
Hu1210 VH.5c	GAGGTGCAGCTGGTGGAGTCCGGAGGAGGCCTGGTGCAACCTGGA GGCTCCCTGAGGCTGTCCTGTGCCGCTTCCGGCTTCACCTTCAGCTC CTACGATATGAGCTGGGTGAGGCAGACCCCTGAGAAGAGCCTGGAG TGGGTGGCCACCATCTCCGACGGAGGCGGCTACATCTACTACTCCG ACTCCGTGAAGGGCAGGTTCACCATCTCCCGGGACAACGCCAAGA ACAACCTGTACCTGCAGATGAACTCTCTCAGGGCTGAGGACACCGC CGTGTATATCTGCGCCAGGGAGTTTGGCAAGAGGTACGCCCTGGATT ACTGGGGCCAGGGCACACTGGTGACAGTGAGCTCC	52
Hu1210_VH.5d	GAGGTGCAGCTGGTGGAGTCCGGAGGAGGCCTGGTGCAACCTGGA GGCTCCCTGAGGCTGTCCTGTGCCGCTTCCGGCTTCACCTTCAGCTC CTACGATATGAGCTGGGTGAGGCAGGCTCCTGGAAAGGGCCTGGAG TGGGTGGCCACCATCTCCGACGGAGGCGGCTACATCTACTACTCCG ACTCCGTGAAGGGCAGGTTCACCATCTCCCGGGACAACGCCAAGA ACTCCCTGTACCTGCAGATGAACTCTCTCAGGGCTGAGGACACCGC CGTGTATATCTGCGCCAGGGAGTTTGGCAAGAGGTACGCCCTGGATT ACTGGGGCCAGGGCACAACCGTGACAGTGAGCTCC	53
HL1210 VK	GACATCGTGATGACCCAGAGCCACAAGTTCATGAGCACCAGCGTGG GCGATAGGGTGAGCATCAGCTGCAAGGCCAGCCAGGATGTGACCCC TGCCGTGGCCTGGTACCAGCAGAAGCCCGGCCAGAGCCCCAAGCT GCTGATCTACAGCACCAGCAGCAGGTACACCGGCGTGCCCGACAGG TTCACAGGAAGCGGCAGCGGCACCGACTTCACCTTCACCATCAGCA GCGTGCAGGCCGAGGACCTGGCCGTGTACTACTGCCAGCAGCACTA CACCACCCCTCTGACCTTCGGCGCCGGCACCAAGCTGGAGCTGAAG	54
Hu1210 VK.1	GACATCCAGATGACCCAGAGCCCTAGCAGCCTGAGCGCTAGCGTGG GCGACAGGGTGACCATCACCTGCAAGGCCAGCCAGGATGTGACCC CTGCCGTGGCCTGGTACCAGCAGAAGCCCGGCAAGGCCCCCAAGC TGCTGATCTACAGCACCAGCAGCAGGTACACCGGCGTGCCCAGCAG GTTTAGCGGAAGCGGCAGCGGCACCGACTTCACCTTCACCATCAGC AGCCTGCAGCCCGAGGACATCGCCACCTACTACTGCCAGCAGCACT ACACCACCCCTCTGACCTTCGGCCAGGGCACCAAGCTGGAGATCAA G	55
Hu1210 VK.1a	GACATCCAGATGACCCAGAGCCCTAGCAGCCTGAGCGCTAGCGTGG GCGACAGGGTGACCATCACCTGCAAGGCCAGCCAGGATGTGACCC CTGCCGTGGCCTGGTACCAGCAGAAGCCCGGCAAGTCCCCCAAGCT GCTGATCTACAGCACCAGCAGCAGGTACACCGGCGTGCCCAGCAGG TTTAGCGGAAGCGGCAGCGGCACCGACTTCACCTTCACCATCAGCA GCCTGCAGCCCGAGGACATCGCCACCTACTACTGCCAGCAGCACTA CACCACCCCTCTGACCTTCGGCCAGGGCACCAAGCTGGAGATCAAG	56
Hu1210 VK.2	GACATTCAGATGACCCAGTCCCCTAGCAGCCTGTCCGCTTCCGTGG GCGACAGGGTGACCATCACCTGCAAGGCCAGCCAGGACGTGACAC CTGCTGTGGCCTGGTATCAACAGAAGCCTGGCAAGGCTCCTAAGCT CCTGATCTACAGCACATCCTCCCGGTACACCGGAGTGCCCTCCAGGT TTAGCGGCAGCGGCTCCGGCACCGATTTCACCCTGACCATTTCCTCC CTGCAGCCCGAGGACTTCGCCACCTACTACTGCCAGCAGCACTACA CCACACCCCTGACCTTCGGCCAGGGCACCAAGCTGGAGATCAAGC GG	57

Hu1210 VK.2a	GACATTCAGATGACCCAGTCCCCTAGCAGCCTGTCCGCTTCCGTGG GCGACAGGGTGACCATCACCTGCAAGGCCAGCCAGGACGTGACAC CTGCTGTGGCCTGGTATCAACAGAAGCCTGGCAAGGCTCCTAAGCT CCTGATCTACAGCACATCCTCCCGGTACACCGGAGTGCCCGACAGG TTTACCGGCAGCGGCTCCGGCACCGATTTCACCCTGACCATTTCCTC CCTGCAGCCCGAGGACTTCGCCACCTACTACTGCCAGCAGCACTAC ACCACACCCCTGACCTTCGGCCAGGGCACCAAGCTGGAGATCAAG CGG	58
Hu1210 VK.2b	GACATTCAGATGACCCAGTCCCCTAGCAGCCTGTCCGCTTCCGTGG GCGACAGGGTGACCATCACCTGCAAGGCCAGCCAGGACGTGACAC CTGCTGTGGCCTGGTATCAACAGAAGCCTGGCCAGAGCCCTAAGCT CCTGATCTACAGCACATCCTCCCGGTACACCGGAGTGCCCGACAGG TTTACCGGCAGCGGCTCCGGCACCGATTTCACCCTGACCATTTCCTC CCTGCAGCCCGAGGACTTCGCCACCTACTACTGCCAGCAGCACTAC ACCACACCCCTGACCTTCGGCCAGGGCACCAAGCTGGAGATCAAG CGG	59
Hu1210 VK.2c	GACATTCAGATGACCCAGTCCCCTAGCAGCCTGTCCGCTTCCGTGG GCGACAGGGTGACCATCAGCTGCAAGGCCAGCCAGGACGTGACAC CTGCTGTGGCCTGGTATCAACAGAAGCCTGGCCAGAGCCCTAAGCT CCTGATCTACAGCACATCCTCCCGGTACACCGGAGTGCCCGACAGG TTTACCGGCAGCGGCTCCGGCACCGATTTCACCCTGACCATTTCCTC CCTGCAGCCCGAGGACTTCGCCACCTACTACTGCCAGCAGCACTAC ACCACACCCCTGACCTTCGGCCAGGGCACCAAGCTGGAGATCAAG CGG	60

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 the 40 humanized antibodies (see Table 9).

[Table 9] Humanized antibodies with their VH an VL regions

VH / Vk	**Hu1210 VH.1**	**Hu1210 VH.1a**	**Hu1210 VH.1b**	**Hu1210 VH.2**	**Hu1210 VH.2a**	**Hu1210 VH 2.b**	**Hu1210 VH**
Hu1210 Vk.1	Hu1210-1	Hu1210-2	Hu1210-3	Hu1210-4	Hu1210-5		
Hu1210 Vk.1a	Hu1210-7	Hu1210-8	Hu1210-9	Hu1210-10	Hu1210-11		
Hu1210 Vk							H1210 chimera

VH / Vk	**Hu1210 VH.3**	**Hu1210 VH.3a**	**Hu1210 VH.4**	**Hu1210 VH.4a**	**Hu1210 VH.4b**
Hu1210 Vk.1	Hu1210-13	Hu1210-14	Hu1210-15	Hu1210-16	Hu1210-17
Hu1210 Vk.1a	Hu1210-18	Hu1210-19	Hu1210-20	Hu1210-21	Hu1210-22

VH	**Hu1210 VH.5**	**HU1210 VH.5a**	**HU1210 VH.5b**	**HU1210 VH.5c**	**HU1210 VH.5d**
Hu1210 Vk.2	Hu1210-23	Hu1210-27	Hu1210-31	Hu1210-32	Hu1210-36
Hu1210 Vk.2a	Hu1210-24	Hu1210-28		Hu1210-33	Hu1210-37
Hu1210 Vk.2b	Hu1210-25	Hu1210-29		Hu1210-34	Hu1210-38
Hu1210 Vk.2c	Hu1210-26	Hu1210-30		Hu1210-35	Hu1210-39

VH Vk	Hu1210 VH.4c	Hu1210 VH.4d	Hu1210 VH.4e
Hu1210 Vk.1	Hu1210-40	Hu1210-41	Hu1210-42

1.6. Antigen binding properties of humanized PD-L1 antibodies

To evaluate the antigen binding activity, the humanized antibodies were subjected to ELISA test. Briefly, microtiter plates were coated with human PD-L1-Fc protein at 0.1μg/ml in PBS, 100μl/well at 4°C overnight, then blocked with 100μl/well of 5% BSA. Five-fold dilutions of humanized antibodies starting from 10μg/ml were added to each well and incubated for 1-2 hours at RT. The plates were washed with PBS/Tween and then incubate with goat-anti-mouse IgG antibody conjugated with Horse Radish Peroxidase (HRP) for 1 hour at RT. After washing, the plates were developed with TMB substrate and analyzed by spectrophotometer at OD 450-630nm. As shown in FIGS. 6A-6E, all the humanized antibodies show comparable binding efficacy to human PD-L1 in contact to chimeric antibody.

To evaluate the antigen binding property, the humanized antibodies were analyzed for its binding to mammalian expressed PD-L1 by FACS. Briefly, PDL1-CHOK1 cells were firstly incubated with 5-fold serious diluted humanized antibodies starting at 2μg/ml at RT for 1 hour. After wash by FACS buffer (PBS with 2%FBS), the alexa 488-anti-human IgG antibody was added to each well and incubated at RT for 1 hour. The MFI of Alexa 488 was evaluated by FACSAriaIII. As shown in the FIGS. 7A-7C, all the humanized antibodies can high efficiently bind to PD-L1 expressed on mammalian cells, which was comparable with chimeric antibody.

To explore the binding kinetics of the humanized antibody, this example performed the affinity ranking by using Octet Red 96. As shown in Table 10, hu1210-3, hu1210-8, hu1210-9, hu1210-14, hu1210-17, hu1210-1 and Hu1210-22 show better affinity, which is comparable with chimeric antibody.

[Table 10] Affinity ranking of humanized antibodies

Antibody	KD (M)	Kon (1/Ms)	kdis(1/s)	Antibody	KD (M)	Kon (1/Ms)	kdis(1/s)
Hu1210 (mIgG)	7.16E-09	3.94E+05	2.83E-03	**Hu1210-11**	4.18E-09	7.54E+04	3.15E-04
H1210 chi mera	1.07E-09	1.62E+05	1.73E-04	**Hu1210-13**	4.36E-09	8.38E+04	3.66E-04
Hu1210-1	4.25E-09	7.10E+04	3.02E-04	**Hu1210-14**	2.34E-09	8.41E+04	1.97E-04
Hu1210-2	3.23E-09	7.78E+04	2.51E-04	**Hu1210-15**	4.45E-09	7.87E+04	3.50E-04
Hu1210-3	2.64E-09	8.62E+04	2.28E-04	**Hu1210-16**	3.14E-09	8.41E+04	2.64E-04
Hu1210-4	7.68E-09	7.12E+04	5.46E-04	**Hu1210-17**	2.20E-09	8.17E+04	1.80E-04
Hu1210-5	4.83E-09	7.93E+04	3.83E-04	**Hu1210-18**	4.50E-09	7.92E+04	3.57E-04

Hu1210-7	4.78E-09	8.45E+04	4.04E-04	**Hu1210-19**	2.50E-09	9.03E+04	2.25E-04
Hu1210-8	1.64E-09	7.72E+04	1.27E-04	**Hu1210-20**	4.51E-09	8.87E+04	4.00E-04
Hu1210-9	2.33E-09	8.37E+04	1.95E-04	**Hu1210-21**	3.12E-09	9.39E+04	2.93E-04
Hu1210-10	7.03E-09	8.59E+04	6.04E-04	**Hu1210-22**	2.56E-09	9.00E+04	2.30E-04

The binding of the humanized antibodies to recombinant PD-L1 protein (human PD-L1-his taq) was tested by BIACORE™ using a capture method. The HL1210-3 mouse mAb were captured using anti-mouse Fc antibody coated on a CM5 chip. A series dilution of human PD-L1-his taq protein was injected over captured antibody for 3 mins at a flow rate of 25μg/ml. The antigen was allowed to dissociate for 900s. All the experiments were carried out on a Biacore T200. Data analysis was carried out using Biacore T200 evaluation software and is shown in Table 11 below.

[Table 11] Affinity by Biacore

Antibody	ka (1/Ms)	kd (1/s)	KD (M)
Hu1210-8	9.346E+4	7.169E-5	7.671E-10
Hu1210-9	9.856E+4	4.528E-5	4.594E-10
Hu1210-14	1.216E+5	5.293E-5	4.352E-10
Hu1210-16	9.978E+4	6.704E-5	6.720E-10
Hu1210-17	1.101E+5	2.128E-5	1.933E-10
Hu1210-28	1.289E+5	1.080E-4	8.378E-10
Hu1210-31	1.486E+5	1.168E-4	7.862E-10
Hu1210-36	1.461E+5	7.852E-5	5.376E-10
Hu1210-40	8.77E+04	1.31E-04	1.49E-09
Hu1210-41	9.17E+04	3.46E-05	3.78E-10
Hu1210-42	8.68E+04	7.53E-05	8.67E-10
1210 Chimera	1.236E+5	3.265E-5	2.642E-10

1.7. Cross species activity

To evaluate the binding of humanized antibodies to huPD-L1, Mouse PD-L1, Rat PD-L1, Rhesus PD-L1, the antibodies were performed for the ELISA testing. Briefly, microtiter plates were coated with human, mouse, rat and rhesus PD-L1-Fc protein at 1 μg/ml in PBS, 100μl/well at 4℃ overnight, then blocked with 100μl/well of 5%BSA. Three-fold dilutions of humanized antibodies starting from 1 μg/ml were added to each well and incubated for 1-2 hours at RT. The plates were washed with PBS/Tween and then incubate with goat-anti-mouse IgG antibody conjugated with Horse Radish Peroxidase (HRP) for 1 hour at RT. After washing, the plates were developed with TMB substrate and analyzed by spectrophotometer at OD 450-630nm. The Hu1210-41 antibody can bind to rhesus PD-L1 with lower affinity and cannot bind to rat and mouse PD-L1 (FIG. 8 & Table 12).

[Table 12]

	Human	Rhesus	Rat	Mouse
EC50	0.215nM	0.628nM	No binding	No binding

To evaluate the binding of humanized anti-PD-L1 antibody to human B7 family and other immune checkpoint, the antibody was evaluate for its binding to B7-H1 (PD-L1) , B7-DC, B7-1, B7-2, B7-H2, PD-1, CD28, CTLA4, ICOS and BTLA by ELISA. As shown in FIG. 9, the Hu1210-41 antibody can only specifically bind to B7-H1 (PD-L1).

1.8. Activity of humanized anit-PD-L1 antibodies to block human PD-L1 to PD-1

Cell based receptor blocking assay

To evaluate the blocking effect of humanized antibodies on human PD-L1 expressed on mammalian cells to bind to its receptor PD-1, the FACS-based receptor blocking assay was employed. Briefly, PDL1-CHOK1 cells were firstly incubated with 3-fold serious diluted HL1210-3 mouse mAb starting at 20μg/ml at RT for 1 hour. After wash by FACS buffer (PBS with 2% FBS) , the biotin-labeled huPD-1 were added to each well and incubated at RT for 1 hour. Then, the Streptavidin-PE was added to each well for 0.5 hour post twice wash with FACS buffer. The mean florescence intensity (MFI) of PE was evaluated by FACSAriaIII.

$$\%\ \text{of inhibition} = \left(1 - \frac{\text{MFI of testing antibody}}{\text{MFI of vehicle contorl}}\right) \times\ 100\%$$

As shown in Table 13 below, Hu1210-3, Hu1210-9, Hu1210-8, Hu1210-14, Hu1210-17, Hu1210-19 and Hu1210-22 antibodies show comparable efficacy with chimeric antibody to blocking the binding of PD-L1 to PD-1.

[Table 13] PD-1 receptor blocking assay

	Bio-PD1(30μg/ml)	
	TOP	EC50
H1210 chimera	87.16	3.961
Hu1210-8	86.35	4.194
Hu1210-9	85.7	4.038
Hu1210-16	88.02	5.436
Hu1210-17	80.88	4.424
Hu1210-3	84.28	3.693
Hu1210-14	79.56	3.572
Hu1210-19	87.45	4.52
Hu1210-22	85.83	4.505
Hu1210-27	103.9	11.48
Hu1210-31	92.91	6.179
Hu1210-36	91.75	8.175

Receptor blocking assay by using recombinant human PD-L1

There are two receptors i.e. PD-1 and B7-1 for human PD-L1. To explore the blocking property of humanized PD-L1 antibody to these two proteins, the protein based receptor blocking assay was employed here. Briefly, microtiter plates were coated with human PD-L1-Fc protein at 1μg/ml in PBS, 100μl/well at 4°C overnight, then blocked with 200μl/well of 5% BSA at 37°C for 2 hr. 50μl biotin-labeled human PD-1-Fc or B7-1 protein and 5-fold dilutions of PD-L1 antibodies starting from 100nM at 50μl were added to each well and incubated for 1 hour at 37°C. The plates were washed with PBS/Tween and then incubate with Streptavidin-HRP for 1 hour at 37°C. After washing, the plates were developed with TMB substrate and analyzed by spectrophotometer at OD 450nm. As shown in FIG. 10 and 11, Hu1210-41 can efficiently inhibit the binding of human PD-L1 to human PD1 and B7-1.

1.9. Activity of humanized anti-PD-L1 antibody to promote human T cell immune response

Mixed lymphocyte Reaction assay

To evaluate the in vitro function of humanized antibodies, the response of human T cells 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 the DCs and serial dilutions of anti-PD-L1 blocking antibody. At day 5 post-inoculation, the culture supernatant was assayed for IL-2 and IFNγ production. The results indicated that the Hu1210-8, Hu1210-9, Hu1210-16 and Hu1210-17 antibodies can dose-dependently promote IL-2 and IFNγ production, suggesting anti-PD-L1 antibodies can promote human T cell response.

CMV recall assay

To evaluate the in vitro function of humanized antibodies, the response of human T cells assessed in CMV recall assay. Human PBMCs were stimulated with 1μg/ml CMV antigen in the presence of serious diluted humanized antibodies. As shown in FIG. 12 and 13 the Hu1210-40, Hu1210-41 and Hu1210-17 can dose dependently promote the IFNγ production.

1.10. Tumor growth inhibition by anti-PD-L1 mAb.

Cells from the human lung adenocarcinoma cell line HCC827 will be grafted into NOD scid gamma (NSG) mice. NSG mice are NOD scid gamma deficient and the most immunodeficient mice making them ideal recipients for human tumor cell and PBMC grafting. 10 days post-graft, human PBMCs will be transplanted into the tumor-bearing mice. Approximately 20 days post-graft, once the tumor volume has reached 100-150mm^3, PD-L1

antibody will be administered to the mice every other day at 5 mg/kg. Tumor volume will be monitored every other day in conjunction with antibody administration. As shown in FIG. 14, Hu1210-31 can inhibit the tumor growth by 30% at 5mg/kg. Hu1210-41 antibody can dose-dependently inhibit the tumor growth, while the tumor weight was also dose-dependently suppressed by Hu1210-41 antibody (FIG. 15).

1.11. Computer Simulation of Further Variation and Optimization of the Humanized Antibodies

It was contemplated that certain amino acid residues within the CDR regions or the framework regions could be changed to further improve or retain the activity and/or stability of the antibodies. Variants were tested, with a computational tool (VectorNTI, available at www. ebi. ac. uk/tools/msa/clustalo/), with respect to their structural, conformational and functional properties, and those (within the CDR regions) that showed promises are listed in the tables blow.

[Table 14] VH and VL CDRs and their variants suitable for inclusion in humanized antibodies

Name	Sequence	SEQ ID NO:
VH CDR1	**SYDMS**	1
	TYDMS	61
	CYDMS	62
	SFDMS	63
	SHDMS	64
	SWDMS	65
	SYDMT	66
	SYDMC	67
VH CDR2	**TISDGGGYIYYSDSVKG**	2
	TISDGGAYIYYSDSVKG	68
	TISDGGPYIYYSDSVKG	69
	TISDGGGFIYYSDSVKG	70
	TISDGGGHIYYSDSVKG	71
	TISDGGGWIYYSDSVKG	72
	TISDGGGYIYYSDTVKG	73
	TISDGGGYIYYSDCVKG	74
	TISDGGGYIYYSDSLKG	75
	TISDGGGYIYYSDSIKG	76
	TISDGGGYIYYSDSMKG	77
VH CDR3	**EFGKRYALDY**	3
	QFGKRYALDY	78

	DFGKRYALDY	79
	NFGKRYALDY	80
	EYGKRYALDY	81
	EHGKRYALDY	82
	EWGKRYALDY	83
	EFAKRYALDY	84
	EFPKRYALDY	85
	EFGRRYALDY	86
	EFGKKYALDY	87
	EFGKRFALDY	88
	EFGKRHALDY	89
	EFGKRWALDY	90
VL CDR1	**KASQDVTPAVA**	4
	KATQDVTPAVA	91
	KACQDVTPAVA	92
VL CDR2	**STSSRYT**	5
	TTSSRYT	93
	CTSSRYT	94
	SSSSRYT	95
	SMSSRYT	96
	SVSSRYT	97
	STTSRYT	98
	STCSRYT	99
	STSTRYT	100
	STSCRYT	101
	STSSKYT	102
	STSSRFT	103
	STSSRHT	104
	STSSRWT	105
VL CDR3	**QQHYTTPLT**	6
	EQHYTTPLT	106
	DQHYTTPLT	107
	NQHYTTPLT	108
	QEHYTTPLT	109
	QDHYTTPLT	110
	QNHYTTPLT	111

(in Table 14, hotspot mutation residues and their substitutes are underlined)

1.12. Identification of PD-L1 Epitope

This study was conducted to identify amino acid residues involved in the binding of PD-L1 to the antibodies of the present disclosure.

An alanine-scan library of PD-L1 was constructed. Briefly, 217 mutant clones of PD-L1 were generated on Integral Molecular's protein engineering platform. Binding of Hu1210-41 Fab to each variant in the PD-L1 mutation library was determined, in duplicate, by high-throughput flow cytometry. Each raw data point had background fluorescence subtracted and was normalized to reactivity with PD-L1 wild-type (WT). For each PD-L1 variant, the mean binding value was plotted as a function of expression (control anti-PD-L1 mAb reactivity). To identify preliminary critical clones (circles with crosses), thresholds (dashed lines) of >70% WT binding to control MAb and <30%WT reactivity to Hu1210-41 Fab were applied (FIG. 16). Y134, K162, and N183 of PDL1 were identified as required residues for Hu1210-41 binding. The low reactivity of N183A clone with Hu1210-41 Fab suggests that it is the major energetic contributor to Hu1210-41 binding, with lesser contributions by Y134 and K162.

The critical residues (spheres) were identified on a 3D PD-L1 structure, as illustrated in FIG. 17. These residues, Y134, K162, and N183, therefore, constitute an epitope of PD-L1 responsible for binding to antibodies of various embodiments of the present disclosure.

It is interesting to note that Y134, K162, and N183 are all located within the IgC domain of the PD-L1 protein. Both PD-1 and PD-L1's extracellular portions have an IgV domain and an IgC domain. It is commonly known that PD-L1 binds to PD-1 through bindings between their IgV domains. Unlike such conventional antibodies, however, Hu1210-41 binds to the IgC domain, which would have been expected to be ineffective in inhibiting PD-1/PD-L1 binding. This different epitope of Hu1210-41, surprisingly, likely contributes to the excellent activities of Hu1210-41.

1.13. Antibody engineering of anti-PDL1 antibody

Examples 1.13-1.15 attempted to identify further improved antibodies based on Hu1210-41 using mutagenesis.

Four sub-libraries were constructed for antibody engineering of anti-PD-L1 monoclonal antibody, using either of the following strategies. In strategy 1, mutagenesis of heavy chain variable domain VH CDR3 or VL-CDR3 was perform by highly random mutation. In strategy 2, two CDR combination libraries composed of (VH-CDR3, VL-CDR3 and VL-CDR1) or (VH-CDR1, VH-CDR2 and VL-CDR2) were generated by CDR walking with controlled mutation rates.

Bio-Panning: the phage panning methods were adapted by shortening the incubation/binding time prior to the harsh washing condition. Briefly, 100 µl magnetic streptavidin beads (Invitrogen, USA) were blocked with 1 ml of MPBS for 1 hr at room

temperature. In another tube, library phage was pre-incubated (5 x 10^11~12 for each round) with 100 µl magnetic streptavidin beads in 1 ml of MPBS to remove unwanted binders. Magnet particle concentrator was used to separate the phage and beads. The biotinylated PD-L1 protein was added to the phage and incubated 2h at room temperature, and gently mixed using an over-head shaker. Beads carrying phage from the solution were separated in the magnetic particle concentrator and the supernatant was discarded. The beads were washed with fresh wash buffer, ten times with PBST and ten times with PBS (pH7.4). 0.8ml, 0.25% Trypsin in PBS (Sigma, USA) was added and incubated for 20 min at 37°C to elute the phage. The output phage was titrated and rescued for next round panning, decreasing antigen concentration round by round.

ELISA screening and On/off rate ranking

Clones were picked and induced from the desired panning output; phage ELISA was conducted for primary screening; positive clones were analyzed by sequencing; unique hotspots were found. Table 15 shows the mutations identified. As shown below, the FGK residues in the CDRH3 are hotpot residues producing improved antibodies.

[Table 15] Mutations in the CDRs

	CDR-H1 (SEQ No.)	CDR-H2 (SEQ No.)	CDR-H3 (SEQ No.)
WT*	SYDMS (1)	TISDAGGYIYYRDSVKG (526)	E**FGK**RYALD**Y** (3)
B3	SYDMS (1)	TISDAGGYIYY**R**DSVKG (526)	EFGKRYALDY (3)
C4	SYDMS (1)	TISDAGGYIYY**R**DSVKG (526)	EFGKRYALD**S** (513)
B1	SYDMS (1)	TISDAGGYIYY**R**DSVKG (526)	E**IFN**RYALDY (514)
B6	SYDMS (1)	TISDAGGYIYY**R**DSVKG (526)	E**LPW**RYALDY (515)
C3	SYDMS (1)	TISDAGGYIYY**R**DSVKG (526)	E**LHF**RYALDY (516)
C6	SYDMS (1)	TISDAGGYIYY**R**DSVKG (526)	E**LYF**RYALDY (517)
A1	SYDMS (1)	TISDAGGYIYY**R**DSVKG (526)	E**LLH**RYALDY (518)
A2	SYDMS (1)	TISDAGGYIYY**R**DSVKG (526)	E**LRG**RYALDY (519)
A3	SYDMS (1)	TISDAGGYIYY**R**DSVKG (526)	EFGKRYALDY (3)
	CDR-L1 (SEQ No.)	**CDR-L2 (SEQ No.)**	**CDR-L3 (SEQ No.)**
WT*	KA**S**QDV**T**PAVA (4)	STSSRYT (5)	**Q**QH**YTT**PLT (6)
B3	KA**K**QDVTPAVA (520)	STSSRYT (5)	**M**QHYTTPLT (522)
C4	KASQDV**W**PAVA (521)	STSSRYT (5)	QQH**S**TTPLT (523)
B1	KASQDVTPAVA (4)	STSSRYT (5)	QQHYTTPLT (6)
B6	KASQDVTPAVA (4)	STSSRYT (5)	QQHYTTPLT (6)
C3	KASQDVTPAVA (4)	STSSRYT (5)	QQHYTTPLT (6)
C6	KASQDVTPAVA (4)	STSSRYT (5)	QQHYTTPLT (6)
A1	KASQDVTPAVA (4)	STSSRYT (5)	QQHYTTPLT (6)
A2	KASQDVTPAVA (4)	STSSRYT (5)	QQHYTTPLT (6)
A3	KASQDVTPAVA (4)	STSSRYT (5)	QQH**SDA**PLT (524)

(* WT differs from Hu1210-41 by a S60R (Kabat numbering) substitution in the heavy chain to improve affinity.)

The amino acid sequences of the variable regions of these antibodies are shown in Table 16 below.

[Table 16] Antibody sequences

Name	Sequence	SEQ ID NO:
WT-VH	EVQLVESGGGLVQPGGSLRLSCAASGFTFSSYDMSWVRQAPGKSLE WVATISDAGGYIYYRDSVKGRFTISRDNAKNSLYLQMNSLRDEDTA VYICAREFGKRYALDYWGQGTTVTVSS	493
WT-Vk	DIQMTQSPSSLSASVGDRVTITCKASQDVTPAVAWYQQKPGKAPKLL IYSTSSRYTGVPSRFSGSGSGTDFTFTISSLQPEDIATYYCQQHYTTPL TFGQGTKLEIK	494
B3-VH	EVQLVESGGGLVQPGGSLRLSCAASGFTFSSYDMSWVRQAPGKSLE WVATISDAGGYIYYRDSVKGRFTISRDNAKNSLYLQMNSLRDEDTA VYICAREFGKRYALDYWGQGTTVTVSS	495
B3-Vk	DIQMTQSPSSLSASVGDRVTITCKAKQDVTPAVAWYQQKPGKAPKL LIYSTSSRYTGVPSRFSGSGSGTDFTFTISSLQPEDIATYYCMQHYTTP LTFGQGTKLEIK	496
C4-VH	EVQLVESGGGLVQPGGSLRLSCAASGFTFSSYDMSWVRQAPGKSLE WVATISDAGGYIYYRDSVKGRFTISRDNAKNSLYLQMNSLRDEDTA VYICAREFGKRYALDSWGQGTTVTVSS	497
C4-Vk	DIQMTQSPSSLSASVGDRVTITCKASQDVWPAVAWYQQKPGKAPKL LIYSTSSRYTGVPSRFSGSGSGTDFTFTISSLQPEDIATYYCQQHSTTP LTFGQGTKLEIK	498
B1-VH	EVQLVESGGGLVQPGGSLRLSCAASGFTFSSYDMSWVRQAPGKSL EWVATISDAGGYIYYRDSVKGRFTISRDNAKNSLYLQMNSLRDEDT AVYICAREIFNRYALDYWGQGTTVTVSS	499
B1-Vk	DIQMTQSPSSLSASVGDRVTITCKASQDVTPAVAWYQQKPGKAPKL LIYSTSSRYTGVPSRFSGSGSGTDFTFTISSLQPEDIATYYCQQHYTT PLTFGQGTKLEIK	500
B6-VH	EVQLVESGGGLVQPGGSLRLSCAASGFTFSSYDMSWVRQAPGKSL EWVATISDAGGYIYYRDSVKGRFTISRDNAKNSLYLQMNSLRDEDT AVYICARELPWRYALDYWGQGTTVTVSS	501
B6-Vk	DIQMTQSPSSLSASVGDRVTITCKASQDVTPAVAWYQQKPGKAPKL LIYSTSSRYTGVPSRFSGSGSGTDFTFTISSLQPEDIATYYCQQHYTT PLTFGQGTKLEIK	502
C3-VH	EVQLVESGGGLVQPGGSLRLSCAASGFTFSSYDMSWVRQAPGKSL EWVATISDAGGYIYYRDSVKGRFTISRDNAKNSLYLQMNSLRDEDT AVYICARELHFRYALDYWGQGTTVTVSS	503
C3-Vk	DIQMTQSPSSLSASVGDRVTITCKASQDVTPAVAWYQQKPGKAPKL LIYSTSSRYTGVPSRFSGSGSGTDFTFTISSLQPEDIATYYCQQHYTT PLTFGQGTKLEIK	504
C6-VH	EVQLVESGGGLVQPGGSLRLSCAASGFTFSSYDMSWVRQAPGKSL EWVATISDAGGYIYYRDSVKGRFTISRDNAKNSLYLQMNSLRDEDT AVYICARELYFRYALDYWGQGTTVTVSS	505
C6-Vk	DIQMTQSPSSLSASVGDRVTITCKASQDVTPAVAWYQQKPGKAPKL LIYSTSSRYTGVPSRFSGSGSGTDFTFTISSLQPEDIATYYCQQHYTT PLTFGQGTKLEIK	506
A1-VH	EVQLVESGGGLVQPGGSLRLSCAASGFTFSSYDMSWVRQAPGKSL EWVATISDAGGYIYYRDSVKGRFTISRDNAKNSLYLQMNSLRDEDT AVYICARELLHRYALDYWGQGTTVTVSS	507
A1-Vk	DIQMTQSPSSLSASVGDRVTITCKASQDVTPAVAWYQQKPGKAPKL LIYSTSSRYTGVPSRFSGSGSGTDFTFTISSLQPEDIATYYCQQHYTT PLTFGQGTKLEIK	508
A2-VH	EVQLVESGGGLVQPGGSLRLSCAASGFTFSSYDMSWVRQAPGKSL EWVATISDAGGYIYYRDSVKGRFTISRDNAKNSLYLQMNSLRDEDT AVYICARELRGRYALDYWGQGTTVTVSS	509
A2-Vk	DIQMTQSPSSLSASVGDRVTITCKASQDVTPAVAWYQQKPGKAPKL LIYSTSSRYTGVPSRFSGSGSGTDFTFTISSLQPEDIATYYCQQHYTT PLTFGQGTKLEIK	510
A3-VH	EVQLVESGGGLVQPGGSLRLSCAASGFTFSSYDMSWVRQAPGKSL EWVATISDAGGYIYYRDSVKGRFTISRDNAKNSLYLQMNSLRDEDT AVYICAREFGKRYALDYWGQGTTVTVSS	511

A3-Vk	DIQMTQSPSSLSASVGDRVTITCKASQDVTPAVAWYQQKPGKAPKL LIYSTSSRYTGVPSRFSGSGSGTDFTFTISSLQPEDIATYYCQQHSDA PLTFGQGTKLEIK	512

1.14. Antigen binding properties of the PD-L1 antibodies

As shown in Tables 15 and 16, totally 9 unique clones were characterized and converted into full-length IgG.

Binding property to recombinant human PD-L1

To evaluate the antigen binding activity, the antibodies were subjected to ELISA test. Briefly, microtiter plates were coated with human PD-L1-Fc protein at 2 μg/ml in PBS, 100μl/well at 4°C overnight, then blocked with 100μl/well of 5% BSA. 4-fold dilutions of humanized antibodies starting from 10 μg/ml were added to each well and incubated for 1-2 hours at RT. The plates were washed with PBS/Tween and then incubate with goat-anti-mouse IgG antibody conjugated with Horse Radish Peroxidase (HRP) for 1 hour at RT. After washing, the plates were developed with TMB substrate and analyzed by spectrophotometer at OD 450-630nm. As shown in FIG. 18, all the humanized antibodies showed excellent binding efficacy to human PD-L1, and B6 and C3 behaved better than the parental clone WT.

Binding property to mammalian expressed human PD-L1

To evaluate the antigen binding property, the antibodies were analyzed for its binding to mammalian expressed PD-L1 by FACS. Briefly, PDL1- Raji cells were firstly incubated with 5-fold serious diluted humanized antibodies starting at 2 g/ml at RT for 1 hour. After wash by FACS buffer (PBS with 2% FBS), the Alexa 488-anti-human IgG antibody was added to each well and incubated at RT for 1 hour. The MFI of Alexa 488 was evaluated by FACSAriaIII. As shown in the FIG. 19, B6 highly efficiently bound to PD-L1 expressed on mammalian cells, which was more potent than the parental antibody WT.

Affinity ranking of humanized antibodies by Biacore

To explore the binding kinetics of the humanized antibody, this example performed the affinity ranking using Biacore. As shown Table 17, B6, C3, C6, A1 and A3 showed better affinity than the parent antibody WT.

[Table 17] Affinity ranking

Antibody	ka (1/Ms)	kd (1/s)	KD (M)
WT	1.77E+05	4.64E-04	2.63E-09
B3	1.19E+05	2.96E-04	2.49E-09
C4	1.13E+05	5.06E-04	4.50E-09
B1	1.63E+05	2.61E-04	1.60E-09
B6	2.42E+05	2.46E-04	1.02E-09

C3	2.18E+05	2.99E-04	1.37E-09
C6	2.06E+05	3.34E-04	1.63E-09
A1	2.03E+05	2.76E-04	1.36E-09
A2	1.87E+05	4.75E-04	2.55E-09
A3	2.18E+05	3.24E-04	1.49E-09

1.15. Anti-PDL1 antibody cell-based function

To test the ability of anti-PDL1 antibodies to stimulate T cell response, hPD-1-expressed Jurkat cells were used. Briefly, Jurkat is human T cell leukemia cell line that can produce IL2 upon TCR stimulation. In this assay, Jurkat cells transfected with human PD-1 gene by lentivirus were used as the responder cells. The Raji-PDL1 cells were used as the antigen presenting cells (APC). Staphylococcal Enterotoxins (SE) are used to stimulate TCR signal. In this system, ectopically expressed huPDL1 can suppress SE stimulated IL-2 production by Jurkat cells, while anti-PDL1 antibodies can reverse IL-2 production. In short, APCs (2.5×10^4) were co-cultured with PD-1 expressing Jurkat T cells (1×10^5) in the presence of SE stimulation. Anti-PDL1 antibodies (starting from 100nM and 1:4 serially diluted for 8 dose) were added at the beginning of the culture. 48hr later, culture supernatant was evaluated for IL2 production by ELISA. As shown in FIG. 20, the B6 monoclonal antibody was more potent than parental antibody WT.

Example 2. Preparation of anti-LAG3 monoclonal antibodies

2.1. Screening of full human monoclonal antibodies against LAG-3

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. 21). Consequently, the D1-D2 domains are critical for LAG-3 function.

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.

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.

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.

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, ΔD1D2-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

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.

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.

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 18] heavy chain variable regions

Antibody No.	**VH**	**SEQ ID NO:**
NLAG3-HDB169-T03	QVQLVQSGAEVKKPGSSVKVSCKASGGTFSSYAISWVRQAPGQGLEWMGG IIPIFGTANYAQKFQGRVTITADESTSTAYMELSSLRSEDTAVYYCARGS SWFDYWGQGTLVTVSS	254
NLAG3-HDB169-T05	QVQLVQSGAEVKKPGSSVKVSCKASGGTFSSYAISWVRQAPGQGLEWMGG IIPIFGTANYAQKFQGRVTITADESTSTAYMELSSLRSEDTAVYYCASSY HGGGYHRYWGQGTLVTVSS	255
NLAG3-HDB169-T06	QVQLVQSGAEVKKPGSSVKVSCKASGGTFSSYAISWVRQAPGQGLEWMGG IIPIFGTANYAQKFQGRVTITADESTSTAYMELSSLRSEDTAVYYCTTSK YSGSALRYWGQGTLVTVSS	256
NLAG3-HDB169-T07	QVQLVQSGAEVKKPGSSVKVSCKASGGTFSSYAISWVRQAPGQGLEWMGG IIPIFGTANYAQKFQGRVTITADESTSTAYMELSSLRSEDTAVYYCARDR TGAFDYWGQGTLVTVSS	257
NLAG3-HDB169-T08	QVQLVQSGAEVKKPGSSVKVSCKASGGTFSSYAISWVRQAPGQGLEWMGG IIPIFGTANYAQKFQGRVTITADESTSTAYMELSSLRSEDTAVYYCARHE TVAGSFDYWGQGTLVTVSS	258
NLAG3-HDB169-T10	QVQLVQSGAEVKKPGSSVKVSCKASGGTFSSYAISWVRQAPGQGLEWMGG IIPIFGTANYAQKFQGRVTITADESTSTAYMELSSLRSEDTAVYYCARTG YYGGNSGAFDIWGQGTMVTVSS	259
NLAG3-HDB169-T13	QVQLVQSGAEVKKPGSSVKVSCKASGGTFSSYAISWVRQAPGQGLEWMGG IIPIFGTANYAQKFQGRVTITADESTSTAYMELSSLRSEDTAVYYCARAG TGMDLVFNSWGQGTLVTVSS	260
NLAG3-HDB169-T23	QVQLVQSGAEVKKPGSSVKVSCKASGGTFSSYAISWVRQAPGQGLEWMGG IIPIFGTANYAQKFQGRVTITADESTSTAYMELSSLRSEDTAVYYCARGL ARGDLNFGYWGQGTLVTVSS	261
NLAG3-HDB169-S24	QVQLVQSGAEVKKPGSSVKVSCKASGGTFSSYAISWVRQAPGQGLEWMGG IIPIFGTANYAQKFQGRVTITADESTSTAYMELSSLRSEDTAVYYCTREP HFDYWGQGTLVTVSS	262
NLAG3-HDB169-S27	QVQLVQSGAEVKKPGSSVKVSCKASGGTFSSYAISWVRQAPGQGLEWMGG IIPIFGTANYAQKFQGRVTITADESTSTAYMELSSLRSEDTAVYYCTTAA	263

	PGSYYLVFHYWGQGTLVTVSS	
NLAG3-HDB169-S31	QVQLVQSGAEVKKPGSSVKVSCKASGGTFSSYAISWVRQAPGQGLEWMGG IIPIFGTANYAQKFQGRVTITADESTSTAYMELSSLRSEDTAVYYCARDA GPVGYYGMDVWGQGTTVTVSS	264
NLAG3-HDB169-S32	QVQLVQSGAEVKKPGSSVKVSCKASGGTFSSYAISWVRQAPGQGLEWMGG IIPIFGTANYAQKFQGRVTITADESTSTAYMELSSLRSEDTAVYYCAGDG LYGSGSFGYWGQGTPVTVSS	265
NLAG3-HDB169-S61	QVQLVQSGAEVKKPGSSVKVSCKASGGTFSSYAISWVRQAPGQGLEWMGG IIPIFGTANYAQKFQGRVTITADESTSTAYMELSSLRSEDTAVYYCAKDI RWFYGMDVWGQGTTVTVSSw	266
NLAG3-HDB169-S64	QVQLVQSGAEVKKPGSSVKVSCKASGGTFSSYAISWVRQAPGQGLEWMGG IIPIFGTANYAQKFQGRVTITADESTSTAYMELSSLRSEDTAVYYCARHE SGIAGGHFDYWGQGTLVTVSS	267
NLAG3-HDB169-S86	QVQLVQSGAEVKKPGSSVKVSCKASGGTFSSYAISWVRQAPGQGLEWMGG IIPIFGTANYAQKFQGRVTITADESTSTAYMELSSLRSEDTAVYYCARDA GPVGYYGMDVWGQGTTVTVS	268
NLAG3-HDB169-S87	QVQLVQSGAEVKKPGSSVKVSCKASGGTFSSYAISWVRQAPGQGLEWMGG IIPIFGTANYAQKFQGRVTITADESTSTAYMELSSLRSEDTAVYYCAKDI RWYYGMDVWGQGTTVTVSS	269
NLAG3-HDB169-T94	QVQLVQSGAEVKKPGSSVKVFCKASGGTFSSYAISWVRQAPGQGLEWMGG IIPIFGTANYAQKFQGRVTITADESTSTAYMELSSLRSEDTAVYYCAKGV RGTYQIGYYGMDVWGQGTTVTVSS	270
NLAG3-HDB169-T97	QVQLVQSGAEVKKPGSSVKVSCKASGGTFSSYAISWVRQAPGQGLEWMGG IIPIFGTANYAQKFQGRVTITADESTSTAYMELSSLRSEDTAVYYCARQG TAMALDYWGQGTLVTVSS	271
NLAG3-HDB169-T99	QVQLVQSGAEVKKPGSSVKVSCKASGGTFSSYAISWVRQAPGQGLEWMGG IIPIFGTANYAQKFQGRVTITADESTSTAYMELSSLRSEDTAVYYCVRDL QDWNYGGAAYWGQGTLVTVSS	272
NLAG3-HDB169-S103	QVQLVQSGAEVKKPGSSVKVSCKASGGTFSSYAISWVRQAPGQGLEWMGG IIPIFGTANYAQKFQGRVTITADESTSTAYMELSSLRSEDTAVYYCARDD YYYGQFDSWGQGTLVTVSS	273
NLAG3-HDB169-S107	QVQLVQSGAEVKKPGSSVKVSCKASGGTFSSYAISWVRQAPGQGLEWMGG IIPIFGTANYAQKFQGRVTITADESTSTAYMELSSLRSEDTAVYYCAREI TGTSYTALDSWGQGTLVTVSS	274
NLAG3-HDB169-S109	QVQLVQSGAEVKKPGSSVKVSCKASGGTFSSYAISWVRQAPGQGLEWMGG IIPIFGTANYAQKFQGRVTITADESTSTAYMELSSLRSEDTAVYYCARGH IDGQAAGDYWGQGTLVTVSS	275
NLAG3-HDB169-S119	QVQLVQSGAEVKKPGSSVKVSCKASGGTFSSYAISWVRQAPGQGLEWMGG IIPIFGTANYAQKFQGRVTITADESTSTAYMELSSLRSEDTAVYYCAAST LRVPNPPYWGQGTLVTVSS	276
NLAG3-HDB169-S120	QVQLVQSGAEVKKPGSSVKVSCKASGGTFSSYAISWVRQAPGQGLEWMGG IIPIFGTANYAQKFQGRVTITADESTSTAYMELSSLRSEDTAVYYCARSG DRYDFWSGYWGQGTLVTVSS	277
NLAG3-HDB169-S127	QVQLVQSGAEVKKPGSSVKVSCKASGGTFSSYAISWVRQAPGQGLEWMGG	278

	IIPIFGTANYAQKFQGRVTITADESTSTAYMELSSLRSEDTAVYYCAAST LRVPNPPYWGQGTLVTVSS	
NLAG3-HDB169-S128	QVQLVQSGAEVKKPGSSVKVSCKASGGTFSSYAISWVRQAPGQGLEWMG G IIPIFGTANYAQKFQGRVTITADESTSTAYMELSSLRSEDTAVYYCARDA GPVGYYGMDVWGQGTMVTVSS	279
NLAG3-HDB169-S136	QVQLVQSGAEVKKPGSSVKVSCKASGGTFSSYAISWVRQAPGQGLEWMG G IIPIFGTANYAQKFQGRVTITADESTSTAYMELSSLRSEDTAVYYCTRGQ DSTWYSSFDYWGQGTLVTVSS	280
NLAG3-HDB169-S139	QVQLVQSGAEVKKPGSSVKVSCKASGGTFSSYAISWVRQAPGQGLEWMG G IIPIFGTANYAQKFQGRVTITADESTSTAYMELSSLRSEDTAVYYCAAST LRLPNPPYWGQGTLVTVSS	281
NLAG3-HDB169-S150	QVQLVQSGAEVKKPGSSVKVSCKASGGTFSSYAISWVRQAPGQGLEWMG G IIPIFGTANYAQKFQGRVTITADESTSTAYMELSSLRSEDTAVYYCATTQ TSFYSHGMDVWGQGTTVTVSS	282
NLAG3-HDB169-S157	QLLESGGGLVQPGGSLRLSCAASGFTFSSYAMSWVRQAPGKGLEWVSAIS GSGGSTYYADSVKGRFTISRDNSKNTLYLQMNSLRAEDTAVYYCARVRKT PFWGALDSWGRGTLVTVSS	283
NLAG3-HDB169-S164	QVQLVQSGAEVKKPGSSVKVSCKASGGTFSSYAISWVRQAPGQGLEWMG G IIPIFGTANYAQKFQGRVTITADESTSTAYMELSSLRSEDTAVYYCARGF TYGDFIFDYWGQGTLVTVSS	284
NLAG3-HDB169-S177	QVQLVQSGAEVKKPGSSVKVSCKASGGTFSSYAISWVRQAPGQGLEWMG G IIPIFGTANYAQKFQGRVTITADESTSTAYMELSSLRSEDTAVYYCARDV RGVTYLGMDVWGQGTTVTVSS	285
NLAG3-HDB323-S20	QLLESGGGLVQPGGSLRLSCAASGFTFSSYAMSWVRQAPGKGLEWVSAIS GSGGSTYYADSVKGRFTISRDNSKNTLYLQMNSLRAEDTAVYYCARVRKT PFWGTLDSWGRGTLVTVSS	286
NLAG3-HDB323-S21	QLLESGGGLVQPGGSLRLSCAASGFTFSSYAMSWVRQAPGKGLEWVSAIS GSGGSTYYADSVKGRFTISRDNSKNTLYLQMNSLRAEDTAVYYCARVRRT PFWGALDSWGRGTLVTVSS	287
NLAG3-HDB323-S32	QLLESGGGLVQPGGSLRLSCAASGFTFSSYAMSWVRQAPGKGLEWVSAIS GSGGSTYYADSVKGRFTISRDNSKNTLYLQMNSLRAEDTAVYYCARVRKT PFWGALDSWGRGTLVTVSS	288
NLAG3-HDB323-S35	QLLESGGGLVQPGGSLRLSCAASGFTFSSYAMSWVRQAPGKGLEWVSAIS GSGGSTYYADSVKGRFTISRDNSKNTLYLQMNSLRAEDTAVYYCAKRKGL GSPTDYYYGMDVWGQGTTVTVSS	289
NLAG3-HDB323-S52	QLLESGGGLVQPGGSLRLSCAASGFTFSSYAMSWVRQAPGKGLEWVSAIS GSGGSTYYADSVKGRFTISRDNSKNTLYLQMNSLRAEDTAVYYCARVRKT PFWGALDSWGRGTLVTVSS	290
NLAG3-HDB323-S55	QLLESGGGLVQPGGSLRLSCAASGFTFSSYAMSWVRQAPGKGLEWVSAIS GSGGSTYYADSVKGRFTISRDNSKNTLYLQMNSLRAEDTAVYYCARVRKT PFWGTLDSWGRGSLVTVSS	291
NLAG3-HDB323-T89	QLLESGGGLVQPGGSLRLSCAASGFTFSSYAMSWVRQAPGKGLEWVSAIS GSGGSTYYADSVKGRFTISRDNSKNTLYLQMNSLRAEDTAVYYCVRPEYD TYYYGMDVWGQGTTVTVSS	292
NLAG3-HDB323-T92	QLLESGGGLVQPGGSLRLSCAASGFTFSSYAMSWVRQAPGKGLEWVSAIS GSGGSTYYADSVKGRFTISRDNSKNTLYLQMNSLRAEDTAVYYCAKGGGS YDYWGQGTLVTVSS	293
NLAG3-HDB323-T94	QLLESGGGLVQPGGSLRLSCAASGFTFSSYAMSWVRQAPGKGLEWVSAIS GSGGSTYYADSVKGRFTISRDNSKNTLYLQMNSLRAEDTAVYYCARALNG MDVWGQGTMVTVSS	294
NLAG3-HDB323-S102	QLLESGGGLVQPGGSLRLSCAASGFTFSSYAMSWVRQAPGKGLEWVSAIS GSGGSTYYADSVKGRFTISRDNSKNTLYLQMNSLRAEDTAVYYCTRPLQG IAAADSYYYYAMDVWGQGTTVTVSS	295

NLAG3-HDB323-S103	QLLESGGGLVQPGGSLRLSCAASGFTFSSYAMSWVRQAPGKGLEWVSAIS GSGGSTYYADSVKGRFTISRDNSKNTLYLQMNSLRAEDTAVYYCARLHSY LSEEFDPWGQGTLVTVSS	296
NLAG3-HDB323-S107	QLLESGGGLVQPGGSLRLSCAASGFTFSSYAMSWVRQAPGKGLEWVSAIS GSGGSTYYADSVKGRFTISRDNSKNTLYLQMNSLRAEDTAVYYCARVRKT PFWGALDSWGRGTLVTVSS	297
NLAG3-HDB323-S114	QLLESGGGLVQPGGSLRLSCAASGFTFSSYAMSWVRQAPGKGLEWVSAIS GSGGSTYYADSVKGRFTISRDNSKNTLYLQMNSLRAEDTAVYYCAKLSAV NTYIDDWGQGTLVTVSS	298
NLAG3-HDB323-S135	QLLESGGGLVQPGGSLRLSCAASGFTFSSYAMSWVRQAPGKGLEWVSAIS GSGGSTYYADSVKGRFTISRDNSKNTLYLQMNSLRAEDTAVYYCARVTKT PFWGTLDYWGQGTLVTVSS	299
NLAG3-HDB323-S143	QLLESGGGLVQPGGSLRLSCAASGFTFSSYAMSWVRQAPGKGLEWVSAIS GSGGSTYYADSVKGRFTISRDNSKNTLYLQMNSLRAEDTAVYYCARVRRT PFWGALDSWGRGTLVTVSS	300
NLAG3-HDB323-S146	QLLESGGGLVQPGGSLRLSCAASGFTFSSYAMSWVRQAPGKGLEWVSAIS GSGGSTYYADSVKGRFTISRDNSKNTLYLQMNSLRAEDTAVYYCARVSQS PVWGYFDYWGQGMLVTVSS	301
NLAG3-HDB323-S161	QLLESGGGLVQPGGSLRLSCAASGFTFSSYAMSWVRQAPGKGLEWVSAIS GSGGSTYYADSVKGRFTISRDNSKNTLYLQMNSLRAEDTAVYYCAKDGYY DFWSGYSDYWGQGTLVTVSS	302

[Table 19] Heavy Chain CDRs

Antibody No.	**CDR H1**	**SEQ ID NO:**	**CDR H2**	**SEQ ID NO:**	**CDR H3**	**SEQ ID NO:**
NLAG3-HDB169-T03	SYAIS	116	GIIPIFGTANYAQKFQG	118	ARGSSWFDY	120
NLAG3-HDB169-T05	SYAIS	116	GIIPIFGTANYAQKFQG	118	ASSYHGGGYHRY	121
NLAG3-HDB169-T06	SYAIS	116	GIIPIFGTANYAQKFQG	118	TTSKYSGSALRY	122
NLAG3-HDB169-T07	SYAIS	116	GIIPIFGTANYAQKFQG	118	ARDRTGAFDY	123
NLAG3-HDB169-T08	SYAIS	116	GIIPIFGTANYAQKFQG	118	ARHETVAGSFDY	124
NLAG3-HDB169-T10	SYAIS	116	GIIPIFGTANYAQKFQG	118	ARTGYYGGNSGAFDI	125
NLAG3-HDB169-T13	SYAIS	116	GIIPIFGTANYAQKFQG	118	ARAGTGMDLVFNS	126
NLAG3-HDB169-T23	SYAIS	116	GIIPIFGTANYAQKFQG	118	ARGLARGDLNFGY	127
NLAG3-HDB169-S24	SYAIS	116	GIIPIFGTANYAQKFQG	118	TREPHFDY	128
NLAG3-HDB169-S27	SYAIS	116	GIIPIFGTANYAQKFQG	118	TTAAPGSYYLVFHY	129
NLAG3-HDB169-S31	SYAIS	116	GIIPIFGTANYAQKFQG	118	ARDAGPVGYYGMDV	130

NLAG3-HDB169-S32	SYAIS	116	GIIPIFGTANYAQKFQG	118	AGDGLYGSGSFGY	131
NLAG3-HDB169-S61	SYAIS	116	GIIPIFGTANYAQKFQG	118	AKDIRWFYGMDV	132
NLAG3-HDB169-S64	SYAIS	116	GIIPIFGTANYAQKFQG	118	ARHESGIAGGHFDY	133
NLAG3-HDB169-S86	SYAIS	116	GIIPIFGTANYAQKFQG	118	ARDAGPVGYYGMDV	130
NLAG3-HDB169-S87	SYAIS	116	GIIPIFGTANYAQKFQG	118	AKDIRWYYGMDV	134
NLAG3-HDB169-T94	SYAIS	116	GIIPIFGTANYAQKFQG	118	AKGVRGTYQIGYYGMDV	135
NLAG3-HDB169-T97	SYAIS	116	GIIPIFGTANYAQKFQG	118	ARQGTAMALDY	136
NLAG3-HDB169-T99	SYAIS	116	GIIPIFGTANYAQKFQG	118	VRDLQDWNYGGAAY	137
NLAG3-HDB169-S103	SYAIS	116	GIIPIFGTANYAQKFQG	118	ARDDYYYGQFDS	138
NLAG3-HDB169-S107	SYAIS	116	GIIPIFGTANYAQKFQG	118	AREITGTSYTALDS	139
NLAG3-HDB169-S109	SYAIS	116	GIIPIFGTANYAQKFQG	118	ARGHIDGQAAGDY	140
NLAG3-HDB169-S119	SYAIS	116	GIIPIFGTANYAQKFQG	118	AASTLRVPNPPY	141
NLAG3-HDB169-S120	SYAIS	116	GIIPIFGTANYAQKFQG	118	ARSGDRYDFWSGY	142
NLAG3-HDB169-S127	SYAIS	116	GIIPIFGTANYAQKFQG	118	AASTLRVPNPPY	141
NLAG3-HDB169-S128	SYAIS	116	GIIPIFGTANYAQKFQG	118	ARDAGPVGYYGMDV	130
NLAG3-HDB169-S136	SYAIS	116	GIIPIFGTANYAQKFQG	118	TRGQDSTWYSSFDY	143
NLAG3-HDB169-S139	SYAIS	116	GIIPIFGTANYAQKFQG	118	AASTLRLPNPPY	144
NLAG3-HDB169-S150	SYAIS	116	GIIPIFGTANYAQKFQG	118	ATTQTSFYSHGMDV	145
NLAG3-HDB169-S157	SYAIS	116	GIIPIFGTANYAQKFQG	118	ARVRKTPFWGALDS	146
NLAG3-HDB169-	SYAIS	116	GIIPIFGTANYAQKFQG	118	ARGFTYGDFIFDY	147

S164						
NLAG3-HDB169-S177	SYAIS	116	GIIPIFGTANYAQKFQG	118	ARDVRGVTYLGMDV	148
NLAG3-HDB323-S20	SYAMS	117	AISGSGGSTYYADSVKG	119	ARVRKTPFWGTLDS	149
NLAG3-HDB323-S21	SYAMS	117	AISGSGGSTYYADSVKG	119	ARVRRTPFWGALDS	150
NLAG3-HDB323-S32	SYAMS	117	AISGSGGSTYYADSVKG	119	ARVRKTPFWGALDS	146
NLAG3-HDB323-S35	SYAMS	117	AISGSGGSTYYADSVKG	119	AKRKGLGSPTDYYYGMDV	151
NLAG3-HDB323-S52	SYAMS	117	AISGSGGSTYYADSVKG	119	ARVRKTPFWGALDS	146
NLAG3-HDB323-S55	SYAMS	117	AISGSGGSTYYADSVKG	119	ARVRKTPFWGTLDS	149
NLAG3-HDB323-T89	SYAMS	117	AISGSGGSTYYADSVKG	119	VRPEYDTYYYGMDV	152
NLAG3-HDB323-T92	SYAMS	117	AISGSGGSTYYADSVKG	119	AKGGGSYDY	153
NLAG3-HDB323-T94	SYAMS	117	AISGSGGSTYYADSVKG	119	ARALNGMDV	154
NLAG3-HDB323-S102	SYAMS	117	AISGSGGSTYYADSVKG	119	TRPLQGIAAADSYYYYAMDV	155
NLAG3-HDB323-S103	SYAMS	117	AISGSGGSTYYADSVKG	119	ARLHSYLSEEFDP	156
NLAG3-HDB323-S107	SYAMS	117	AISGSGGSTYYADSVKG	119	ARVRKTPFWGALDS	146
NLAG3-HDB323-S114	SYAMS	117	AISGSGGSTYYADSVKG	119	AKLSAVNTYIDD	157
NLAG3-HDB323-S135	SYAMS	117	AISGSGGSTYYADSVKG	119	ARVTKTPFWGTLDY	158
NLAG3-HDB323-S143	SYAMS	117	AISGSGGSTYYADSVKG	119	ARVRRTPFWGALDS	150
NLAG3-HDB323-S146	SYAMS	117	AISGSGGSTYYADSVKG	119	ARVSQSPVWGYFDY	159
NLAG3-HDB323-S161	SYAMS	117	AISGSGGSTYYADSVKG	119	AKDGYYDFWSGYSDY	160

[Table 20] Light chain variable regions

Antibody No.	VL	SEQ ID NO:
NLAG3-HDB169-T03	DIQLTQSPSSLSAFVGDRVTITCQANQDIHHYLNWYQQKPGKAPKLLIYD ASILQSGVPSRFSGSGSGTDFTLTISSLQPEDFATYFCQQADSFPITFGQ GTRLEIKR	303
NLAG3-HDB169-T05	EIVLTQSPDSLAVSLGERATINCKSSQSVLYSSSNKNYLAWYQQKPGQ PP KLLIYWASTRESGVPDRFSGSGSGTDFTLTISSLQPEDFATYYCQQSYS T PWTFGPGTKLEIKR	304
NLAG3-HDB169-T06	DIQMTQSPDSLAVSLGERATINCKSSQSVLYSSNNKNYLAWYQQKPG HPP KLLVYWASTRESGVPARFSASGSGTDFTLAISNLQAEDVAVYYCQQY YST PWTFGQGTKVEIKR	305
NLAG3-HDB169-T07	EIVLTQSPLSLPVTPGEPASISCRSSQNLLHSDGYNYLNWYLQKPGQSP Q LLIYLGSNRATGVPDRFSGSGSGTDFTLTISSLQPEDFATYYCQQSYST P WTFGQGTKVEIKR	306
NLAG3-HDB169-T08	DIVMTQSPDSLAVSLGERATINCKSSQSVLYTSNNKNYLAWYQQKPG QPP KLLIYWASTRESGVPDRFSGSGSGTDFTLTISSLQAEDVAIYYCQQYY ST PWTFGQGTKLEIKR	307
NLAG3-HDB169-T10	AIQLTQSPDSLAVSLGERATINCKSSQSVLYSSNNKNYLAWYQQKPGQ PP KLLIYWASTRESGVPDRFSGSGSGTDFTLTISSLQAEDSATYYCQQSFT T PWTFGQGTKVEIKR	308
NLAG3-HDB169-T13	DIQMTQSPSSLSASVGDRVTITCQASQDINRYLSWYQQKPGKAPKLLIYD ASNLETGVPSRFSGSASGTDFTFAISSLQPEDIATYYCQQYDNLPPTFG Q GTRLEIKR	309
NLAG3-HDB169-T23	EIVMTQSPSSLSASVGDRVTITCQASQDISNYLNWYQQKPGKAPKLLIYA ASSLQSGVPSRFSGSGSGTDFTLTISSLQPEDFASYYCQQSYGSPVTFG Q GTKLEIKR	310
NLAG3-HDB169-S24	EIVMTQSPSSLSASVGDRVTITCQASQDISNYLNWYQQKPGKAPKLLIYD ASNLETGVPSRFSGSGSGTEFTLTISSLRPEDFATYFCQQADSFPITFGQ GTRLEIKR	311
NLAG3-HDB169-S27	DIQLTQSPSSLSASVGDRVTITCRASQTISSHLNWYQQKPGKAPKVLIYA ASSLQSGVPSRFSGSGSGTEFTLTISSLQPDDFATYYCQQGNSFPFTFG P GTKVEIKR	312
NLAG3-HDB169-S31	AIRMTQSPSTLSASVGDRVTITCRASQGIAGWLAWYQQKPGKAPKLLIYA ASSLQSGVPSRFSGSASGTDFTLTISNLQPEDFATYYCQQAKSFPLTFG G GTKVEIKR	313
NLAG3-HDB169-S32	DIVMTQSPDSLAVSLGERATINCKSSQSVLYSSNNKNYLAWYQQKPG QPP KLLIYWASTRESGVPDRFSGTGSGTDFTLTISSLQAEDVAVYYCQQSY	314

	ST PWTFGQGTKLEIK	
NLAG3-HDB169-S61	DIVMTQSPSSVSAFVGDRVTITCRASQGVSSWLAWFQQKPGKAPKLLIYA ASTLQSGVPSRFSGRGYGTEFTLTISSLQPEDLATYYCQQVKSFPLTFGG GTKVDIKR	315
NLAG3-HDB169-S64	DIVMTQSPDSLAVSLGERATINCKSSQSLFYHSNNHNYLAWYQQKPGQPP KLLIYWASTRQSGVPDRFTGSGSGTDFTLTISSLQAEDVAVYYCQQYYNT PWTFGQGTKVEIKR	316
NLAG3-HDB169-S86	AIRMTQSPSTLSASVGDRVTITCRASQGIAGWLAWYQQKPGKAPKLLIYA ASSLQSGVPSRFSGSASGTDFTLTISNLQPEDFATYYCQQAKSFPLTFGG GTKVEIKR	317
NLAG3-HDB169-S87	DIVMTQSPSSVSAFVGDRVTITCRASQGVSSWLAWFQQKPGKAPKLLIYA ASTLQSGVPSRFSGRGYGTEFTLTISSLQPEDLATYYCQQVKSFPLTFGG GTKVDIKR	318
NLAG3-HDB169-T94	DIVMTQSPSSLSASVGDRVTITCRASQGISSSLAWYQQKPGKAPNLLIYT ASTLQNGVPSRFSGSGSGTDFTLTISGLQPEDFATYYCQQTKNFPLTFGQ GTRLEIKR	319
NLAG3-HDB169-T97	EIVLTQSPDSLAVSLGERATINCKSSQSVLYSSNNKNYLAWYQQRPGQPP KLLISWASTRESGVPDRFSGSGSGADFSLTISSLQAEDVAVYYCQQYYST PWTFGQGTKLEIKR	320
NLAG3-HDB169-T99	VIWMTQSPSSLSASVGDSVTITCQASRDISNSLSWHQQKPGKAPKLLIYA ASSLQSGVPSRFSGSGSGTDFTLTISSLQPEDFATYYCQQTKSFPLTFGG GTKVEIKR	321
NLAG3-HDB169-S103	EIVMTQSPSSLSASVGDRVTISCRASQSISRYLNWYQQKPGQAPKLLIYA AFSLQSGVPSRFSGSGSGTDFTLTISSLQPEDFATYYCQQSYNTPRTFGQ GTKLEIKR	322
NLAG3-HDB169-S107	DVVMTQSPSTVSASVGDRITITCRASRSISNWLAWYQQKPGKAPKLLIYA ASSLQSGVPSRFSGSGSGTDFTLTISSLQPEDFATYYCQQAKSFPLTFGG GTKVEIK	323
NLAG3-HDB169-S109	DIQLTQSPDSLAVSLGERATINCKSSQSVFYRSNQKNYLAWYQQKPGQTP RLLIYGASSRATGIPDRFSGSGSGTDFTLTISSLQPEDFATYYCQQSYRA PWTFGQGTKVEIKR	324
NLAG3-HDB169-S119	EIVLTQSPGTLSLSPGERATLSCRASQSVSSYLAWYQQKPGQAPRLLIYG ISSRATGIPDRFSGSGSGTDFTLTISSLQPEDFATYYCQQANNFPLTFGG GTKLEIKR	325
NLAG3-HDB169-S120	EIVLTQSPSSVSASVGDRVTITCRASRGISSWLAWYQQKPGKAPKLLIYA ASTLQSGVPSRFSGSGSGTDFTLTISSLQPEDFATYYCQQAKSFPLTFGG	326

	GTKVEIKR	
NLAG3-HDB169-S127	EIVLTQSPGTLSLSPGERATLSCRASQSVSSYLAWYQQKPGQAPRLLIYG ISSRATGIPDRFSGSGSGTDFTLTISSLQPEDFATYYCQQANNFPLTFGG GTKLEIKR	327
NLAG3-HDB169-S128	AIQMTQSPSSLSASVGDRVTITCRASQGISSWLAWYQQKPGKAPKLLIYA ASSLQSGVPSRFSGSGSGTDFTLTISRLQPEDFATYYCQQAKSFPLTFGG GTKVEIKR	328
NLAG3-HDB169-S136	AIRMTQSPSSLSASVGDRVTITCRASQSISSYLNWYQQKPGKAPNLLIYA VSTLQSGVPSRFSGSGSGTVFTLTISSLQPEDFATYFCQQGNSFPLTFGG GTKVEIKR	329
NLAG3-HDB169-S139	DIQLTQSPSTLSASVGDRVTITCRASQAISNLLAWYQQKPGKPPNLLIYD ISTLQNGVPSRFSGSGSGTDFTLTINSLQPEDFAIYYCQQSKNFPVTFGG GTKVEIKR	330
NLAG3-HDB169-S150	DIQLTQSPSSVSASVGDRVTITCRASQGISSWLAWYQQKPGKAPKLLIYG ASTLQSGVPSRFSGSGSGADYTLTISSLQPEDFATYYCQQANSFPLTFAG GTKLEIKR	331
NLAG3-HDB169-S157	DIQLTQSPSSLSASPGDRVTITCRASQGISTWLAWYQQKPGNAPKLLIYA ASSLQSGVPSRFSGSKSGTEYTLTISSLQPEDFATYYCQQLESYPLTFGG GTKVEIKR	332
NLAG3-HDB169-S164	AIRMTQSPDSLVVSLGERATINCKSSQSVLYSSNNKNYLAWYQQKPGQPP KLLIYWASTRESGVPDRFSGSGSGTDFTLSISSLQAEDVAVYYCQQYYSS PTFGGGTKVEIKR	333
NLAG3-HDB169-S177	DVVMTQSPFFLSASVGDRVTITCRASQGIASNLAWYQQKPGKAPKLLIYA ASTLQSGVPSRFTGSGSGTEFTLTVTSLQPEDFATYYCQQLKTFPLTFGG GTKVEIKR	334
NLAG3-HDB323-S20	VIWMTQSPSSLSASVGDRVTITCRASQGVSSYLAWYQQKPGKAPKLLIYA ASSLQSGVPSRFSGSGSGTEFTLTISSLQPEDFATYYCQQTNWFPLTFGP GTRLEIKR	335
NLAG3-HDB323-S21	DIQMTQSPSSLSTSAGDTVTITCRASQSIYTYLNWYQQKPGKAPNLLIYG ASSLQSGVPSRFSGSGSGTDFTLTISSLQPEDFATYYCQQAQSFPITFGQ GTRLEIKR	336
NLAG3-HDB323-S32	VIWMTQSPSSVSASVGDRVTITCRASQGISSWLAWYQQKPGKAPKLLIYA ASSLQSGVPSRFSGSGSGTDFTLTISSLQPEDFATYYCQQAHSFPLTFGG GTKVEIKR	337
NLAG3-HDB323-S35	AIQLTQSPSTLSASVGDRVTITCRASQFVSDWLAWYQQKPGKAPKLLIYA ASTLQSGVPSRFSGSGSGTDFTLTISSLQPEDLATYYCLQDYHFPLTFGG GTKLEIKR	338

NLAG3-HDB323-S52	DVVMTQSPSSVSASVGDRVTITCRASQDIVNWLAWYQQKPGKAPKLLIYA ASTLESGAPSRFSASGSGTDFTLTISSLQPDDFATYYCQQGHSFPLTFGP GTKLEIKR	339
NLAG3-HDB323-S55	DIVMTQSPSSLSASVGDRVTITCRASQSIYTYLNWYQQKPGKAPKLLIYD ASSLQSGVPSRFSGSGYGTEFTLTISGLQPEDFATYYCQQSYIFPLTFGR GTKVEIKR	340
NLAG3-HDB323-T89	AIRMTQSPSFVSASVGDRVTIACRASQTISTWLAWYQQKPGKAPKVLISK ASNLQSGVPSRFSGSGSGTEFTLTISSLQPDDFATYYCQQYDTYWTFGQG TKVEIKR	341
NLAG3-HDB323-T92	AIRMTQSPSFVSASVGDRVTIACRASQTISTWLAWYQQKPGKAPKVLISK ASNLQSGVPSRFSGSGSGTEFTLTISSLQPDDFATYYCQQYDTYWTFGQG TKVEIKR	342
NLAG3-HDB323-T94	DIVMTQSPSFVSASVGDTVTITCRASQGISSYLAWYQQKPGKAPKLLIYA ASTLQSGVPSRFSGSGSGTEFTLTISSLQPEDFATYYCQQLNSYPLFTFG PGTKVEIKR	343
NLAG3-HDB323-S102	DIQMTQSPSTLSASVGDRVTITCRASQSIGYWLAWYQQKPGKAPKLLIYR ASSLQSGVPSRFSGSGSATEFTLTITSLQPDDFATYFCQQYSSYWTFGQG TKVEIKR	344
NLAG3-HDB323-S103	EIVLTQSPSSLSASVGDTVTITCRATQSISSWLAWYQQKPGKAPQRLISG ASTLQSGVPSRFSGSGSGTEFTLTISGLQPEDFATYYCLQHNTYPFTFGQ GTKVEIKR	345
NLAG3-HDB323-S107	DIVMTQSPSSVSASVGDRVTITCRASQGVRNWLAWYQQKPGKAPKLLIYA ASHLQSGVPSRFSGSGSGTDFTLTISSLQTDDFATYYCQQGHSFPLTFGG GTKVEIKR	346
NLAG3-HDB323-S114	DIVMTQSPSSVSASVGDRVTITCRASQGVRNWLAWYQQKPGKAPKLLIYA ASHLQSGVPSRFSGSGSGTDFTLTISSLQTDDFATYYCQQGHSFPLTFGG GTKVEIKR	347
NLAG3-HDB323-S135	VIWMTQSPSTLSASVGDRVTITCRASQSINNYLAWYQQKPGKAPKLLIYD ASTLQSGVPSRFSGGGSGTDFTLTINSLQPDDFASYYCQQAHSFPFTFGG GTKLEIKR	348
NLAG3-HDB323-S143	EIVMTQSPSSVSASVGDRVTITCRASQDITSWLAWYQQKPGKAPKLLIYA ASTLESGVPSRFSGSGSGTDFTLTITGLQPEDFATYYCQQANMFPLTFGG GTKVEIKR	349
NLAG3-HDB323-S146	AIRMTQSPSSLSASVGDRVTITCRASQGIYDYLAWYQQKPGKAPSLLIYA ASNLERGVPSRFSGSGSGKYFILTISSLQPEDFATYYCQQANSFPLTFGG GTKVEIKR	350

NLAG3-HDB323-S161	AIQLTQSPSSLSASVGDRVTITCRASEGISGWLAWYQQIPGKAPKLLIYA ASSLETGVPSRFSGSGYGTDFTLTISSLQPEDFATYYCQQADSFPFTFGP GTKVEIKR	351

[Table 21] Light Chain CDRs

Antibody No.	**CDR L1**	**SEQ ID NO:**	**CDR L2**	**SEQ ID NO:**	**CDR L3**	**SEQ ID NO:**
NLAG3-HDB169-T03	QANQDIHHYLN	161	DASILQS	196	QQADSFPIT	218
NLAG3-HDB169-T05	KSSQSVLYSSSNKNYLA	162	WASTRES	197	QQSYSTPWT	219
NLAG3-HDB169-T06	KSSQSVLYSSNNKNYLA	163	WASTRES	197	QQYYSTPWT	220
NLAG3-HDB169-T07	RSSQNLLHSDGYNYLN	164	LGSNRAT	198	QQSYSTPWT	219
NLAG3-HDB169-T08	KSSQSVLYTSNNKNYLA	165	WASTRES	197	QQYYSTPWT	220
NLAG3-HDB169-T10	KSSQSVLYSSNNKNYLA	163	WASTRES	197	QQSFTTPWT	221
NLAG3-HDB169-T13	QASQDINRYLS	166	DASNLET	199	QQYDNLPPT	222
NLAG3-HDB169-T23	QASQDISNYLN	167	AASSLQS	200	QQSYGSPVT	223
NLAG3-HDB169-S24	QASQDISNYLN	167	DASNLET	199	QQADSFPIT	218
NLAG3-HDB169-S27	RASQTISSHLN	168	AASSLQS	200	QQGNSFPFT	224
NLAG3-HDB169-S31	RASQGIAGWLA	169	AASSLQS	200	QQAKSFPLT	225
NLAG3-HDB169-S32	KSSQSVLYSSNNKNYLA	163	WASTRES	197	QQSYSTPWT	219
NLAG3-HDB169-S61	RASQGVSSWLA	170	AASTLQS	201	QQVKSFPLT	226
NLAG3-HDB169-S64	KSSQSLFYHSNNHNYLA	171	WASTRQS	#N/A	QQYYNTPWT	227
NLAG3-HDB169-S86	RASQGIAGWLA	169	AASSLQS	200	QQAKSFPLT	225
NLAG3-HDB169-S87	RASQGVSSWLA	170	AASTLQS	201	QQVKSFPLT	226
NLAG3-HDB169-T94	RASQGISSSLA	172	TASTLQN	212	QQTKNFPLT	228
NLAG3-HDB169-T97	KSSQSVLYSSNNKNYLA	163	WASTRES	197	QQYYSTPWT	220
NLAG3-HDB169-T99	QASRDISNSLS	173	AASSLQS	200	QQTKSFPLT	230
NLAG3-HDB169-S103	RASQSISRYLN	174	AAFSLQS	202	QQSYNTPRT	231
NLAG3-HDB169-S107	RASRSISNWLA	175	AASSLQS	200	QQAKSFPLT	225
NLAG3-HDB169-S109	KSSQSVFYRSNQKNYLA	176	GASSRAT	203	QQSYRAPWT	232
NLAG3-HDB169-S119	RASQSVSSYLA	177	GISSRAT	204	QQANNFPLT	233
NLAG3-	RASRGISSWLA	178	AASTLQS	201	QQAKSFPLT	225

HDB169-S120						
NLAG3-HDB169-S127	RASQSVSSYLA	177	GISSRAT	204	QQANNFPLT	233
NLAG3-HDB169-S128	RASQGISSWLA	179	AASSLQS	200	QQAKSFPLT	225
NLAG3-HDB169-S136	RASQSISSYLN	180	AVSTLQS	205	QQGNSFPLT	234
NLAG3-HDB169-S139	RASQAISNLLA	181	DISTLQN	206	QQSKNFPVT	235
NLAG3-HDB169-S150	RASQGISSWLA	179	GASTLQS	207	QQANSFPLT	236
NLAG3-HDB169-S157	RASQGISTWLA	182	AASSLQS	200	QQLESYPLT	237
NLAG3-HDB169-S164	KSSQSVLYSSNNKNYLA	163	WASTRES	197	QQYYSSPT	238
NLAG3-HDB169-S177	RASQGIASNLA	183	AASTLQS	201	QQLKTFPLT	239
NLAG3-HDB323-S20	RASQGVSSYLA	184	AASSLQS	200	QQTNWFPLT	240
NLAG3-HDB323-S21	RASQSIYTYLN	185	GASSLQS	208	QQAQSFPIT	241
NLAG3-HDB323-S32	RASQGISSWLA	179	AASSLQS	200	QQAHSFPLT	242
NLAG3-HDB323-S35	RASQFVSDWLA	186	AASTLQS	201	LQDYHFPLT	243
NLAG3-HDB323-S52	RASQDIVNWLA	229	AASTLES	209	QQGHSFPLT	244
NLAG3-HDB323-S55	RASQSIYTYLN	185	DASSLQS	210	QQSYIFPLT	245
NLAG3-HDB323-T89	RASQTISTWLA	187	KASNLQS	211	QQYDTYWT	246
NLAG3-HDB323-T92	RASQTISTWLA	187	KASNLQS	211	QQYDTYWT	246
NLAG3-HDB323-T94	RASQGISSYLA	188	AASTLQS	201	QQLNSYPLFT	247
NLAG3-HDB323-S102	RASQSIGYWLA	189	RASSLQS	213	QQYSSYWT	248
NLAG3-HDB323-S103	RATQSISSWLA	190	GASTLQS	207	LQHNTYPFT	249
NLAG3-HDB323-S107	RASQGVRNWLA	191	AASHLQS	214	QQGHSFPLT	244
NLAG3-HDB323-S114	RASQGVRNWLA	191	AASHLQS	214	QQGHSFPLT	250
NLAG3-HDB323-S135	RASQSINNYLA	192	DASTLQS	215	QQAHSFPFT	251
NLAG3-HDB323-S143	RASQDITSWLA	193	AASTLES	209	QQANMFPLT	252
NLAG3-HDB323-S146	RASQGIYDYLA	194	AASNLER	216	QQANSFPLT	236
NLAG3-HDB323-S161	RASEGISGWLA	195	AASSLET	217	QQADSFPFT	253

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

To evaluate the capability of the anti-LAG-3 antibodies to bind to human, rat, and mouse LAG3 the antibodies identified in Example 2.1 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 10μg/ml, 3.333μg/ml, 1.111μg/ml, 0.370μg/ml, 0.123μg/ml, 0.041μg/ml, 0.014μg/ml, 0.005μg/ml, 0.0015μg/ml and 0.0005μg/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 (FIGS. 22A-22D).

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

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 2.1 were serially diluted with FACS buffer. To assess binding, LAG-3 antibodies at various concentrations (10μg/ml, 3.333μg/ml, 1.111μg/ml, 0.370μg/ml, 0.123μg/ml, 0.041μg/ml, 0.014μg/ml and 0.005μg/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. 23, the S27, S31, T99 and S119 antibodies can dose-dependently bind to LAG3 expressed on the activated human $CD4^+$ T cells.

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

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

6μg/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. 24, the S27, S31, S119 and T99 antibodies can dose dependently inhibit the binding of LAG3 to its receptor MHC class II molecules.

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

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. 25, 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.

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

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} ($CD4^+CD25CD127^{hi}$) 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. 26, 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 T_{effs} by T_{regs}.

2.7. LAG-3 antibody BIACORE Analysis

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 30μl/min. The dissociation phase was 900s or 550s. The results are shown in Table 22 below. The Biacore results for the anti-LAG3 antibodies have shown that these anti-LAG3 antibodies are high affinity binder to human LAG3.

[Table 22]

	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

2.8. Generation of mouse monoclonal antibodies against human LAG3

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

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 ΔD1-D2 huFc respectively). The LAG-3 fusion proteins were prepared using standard recombinant DNA techniques.

Immunizations:

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 25μg of antigen followed by a subsequent boost with 25μg 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.

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 ΔD1-D2 huFc was served as a counter screen. In short, D1-D4 huFc or ΔD1-D2 huFc was coated at 0.5ug/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 2.4. 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.

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

2.9. Binding properties of anti-LAG3 mouse monoclonal antibodies

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

D1-D2 specific binders:

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 ΔD1-D2 huFc antigens. Briefly, D1-D4 huFc or ΔD1-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 serial dilution 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.

The results of the ELISA are summarized in FIGS. 27A-27C, which show strong binding to full extracellular domain of LAG3 (D1-D4 huFc) but not D1-D2 deleted LAG3 (ΔD1-D2 huFc), confirm that 122H, 147H and 170H are potent and selective binder for D1 and D2 domain of human LAG3.

2.10. Functional properties of anti-LAG3 mouse monoclonal antibodies

Blocking the binding of LAG3 to its receptor

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 6ug/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 FIGS. 28A-28C, 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

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 20μg/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. 29, 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.

2.11. 147H mouse mAb humanization design

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 Vκ 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 23] Mouse antibody sequences

Antibody chain or domain	**Sequences (CDR residues with VH and VL are underlined)**	**SEQ ID NO:**
147H VH	QVQLQQSGSELVRPGTSVKISCKASGYTFTNYWLGWIKQRPGHG LEWIGDIYPGGDYINYNEKFKGKATLSADTSSSTAYMQLSSLTSED SAVYFCARPNLPGDYWGQGTSVTVSS	352
147H VL	DIVMTQAAFSNPVTLGTSASISCRSSKSLLHSNGITYLYWYLQKPG QSPQLLIYQVSNLASGVPGRFSGSGSGTDFTLRISRVEAEDVGVY YCAQNLELPWTFGGGTKLEIK	353
CDRH1	GYTFTNYWLG	354
CDRH2	DIYPGGDYINYNEKFKG	355
CDRH3	PNLPGDY	356
CDRL1	RSSKSLLHSNGITYLY	357
CDRL2	QVSNLAS	358
CDRL3	AQNLELPWT	359

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 24] Humanized antibodies and back mutations

Antibody chain	Sequences (CDR underlined; back mutations bold and underlined)	SEQ ID NO:
147H-1 VH	QVQLVQSGAEVKKPGASVKVSCKASGYTFTNYWLGWVRQAPGQ GLEWMGDIYPGGDYINYNEKFKGRVTMTRDTSISTAYMELSRLRS DDTAVYYCARPNLPGDYWGQGTTVTVSS	360
147H-2 VH	QVQLVQSGAEVKKPGASVKVSCKASGYTFTNYWLGWVRQAPGQ GLEWMGDIYPGGDYINYNEKFKGRVTMT**A**DTSISTAYMELSRLRS DDTAVYYCARPNLPGDYWGQGTTVTVSS	361
147H-3 VH	QVQLVQSGAEVKKPGASVKVSCKASGYTFTNYWLGWVRQAPGQ GLEWMGDIYPGGDYINYNEKFKGRVT**L**T**A**DTSISTAYMELSRLRSD DTAVYYCARPNLPGDYWGQGTTVTVSS	362
147H-4 VH	QVQLVQSGAEVKKPGASVKVSCKASGYTFTNYWLGWVRQAPGQ GLEWMGDIYPGGDYINYNEKFKG**KA**T**L**T**A**DTSISTAYMELSRLRS DDTAVYYCARPNLPGDYWGQGTTVTVSS	363
147H-5 VH	QVQLVQSGAEVKKPGASVKVSCKASGYTFTNYWLGWVRQAPGQ GLEW**I**GDIYPGGDYINYNEKFKG**KA**T**L**T**A**DTSISTAYMELSRLRSD DTAVYYCARPNLPGDYWGQGTTVTVSS	364
147H-6 VH	QVQLVQSGAEVKKPGASVKVSCKASGYTFTNYWLGW**IK**QAPGQ GLEW**I**GDIYPGGDYINYNEKFKG**KA**T**L**T**A**DTSISTAYMELSRLRSD DTAVYYCARPNLPGDYWGQGTTVTVSS	365
147H-7 VH	QVQLVQSGAEVKKPGASVKVSCKASGYTFTNYWLGW**IK**QAPGQ GLEW**I**GDIYPGGDYINYNEKFKG**KA**T**L**T**A**DTSISTAYMELSRLRSD DTAVY**F**CARPNLPGDYWGQGTTVTVSS	366
147H-8 VH	**E**VQLVQSGAEVKKPGASVKVSCKASGYTFTNYWLGWVRQAPGQ GLEWMGDIYPGGDYINYNEKFKGRVTMTRDTSISTAYMELSRLRS DDTAVYYCARPNLPGDYWGQGTTVTVSS	367
147H-9 VH	**E**VQLVQSGAEVKKPGASVKVSCKASGYTFTNYWLGWVRQAPGQ GLEWMGDIYPGGDYINYNEKFKGRVTMT**A**DTSISTAYMELSRLRS DDTAVYYCARPNLPGDYWGQGTTVTVSS	368
147H-10 VH	**E**VQLVQSGAEVKKPGASVKVSCKASGYTFTNYWLGWVRQAPGQ GLEWMGDIYPGGDYINYNEKFKGRVT**L**T**A**DTSISTAYMELSRLRSD DTAVYYCARPNLPGDYWGQGTTVTVSS	369
147H-11 VH	**E**VQLVQSGAEVKKPGASVKVSCKASGYTFTNYWLGWVRQAPGQ GLEWMGDIYPGGDYINYNEKFKG**KA**T**L**T**A**DTSISTAYMELSRLRS DDTAVYYCARPNLPGDYWGQGTTVTVSS	370
147H-12 VH	**E**VQLVQSGAEVKKPGASVKVSCKASGYTFTNYWLGWVRQAPGQ GLEW**I**GDIYPGGDYINYNEKFKG**KA**T**L**T**A**DTSISTAYMELSRLRSD DTAVYYCARPNLPGDYWGQGTTVTVSS	371
147H-13 VH	**E**VQLVQSGAEVKKPGASVKVSCKASGYTFTNYWLGW**IK**QAPGQG LEW**I**GDIYPGGDYINYNEKFKG**KA**T**L**T**A**DTSISTAYMELSRLRSDD TAVYYCARPNLPGDYWGQGTTVTVSS	372
147H-14 VH	**E**VQLVQSGAEVKKPGASVKVSCKASGYTFTNYWLGW**IK**QAPGQG LEW**I**GDIYPGGDYINYNEKFKG**KA**T**L**T**A**DTSISTAYMELSRLRSDD TAVY**F**CARPNLPGDYWGQGTTVTVSS	373

147H VL	DIVMTQSPLSLPVTPGEPASISCRSSKSLLHSNGITYLYWYLQKPGQ SPQLLIYQVSNLASGVPDRFSGSGSGTDFTLKISRVEAEDVGVYYC AQNLELPWTFGGGTKVEIK	374

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.

2.12. Binding properties of anti-LAG3 147H humanized monoclonal antibodies

Affinity ranking of humanized antibodies by Octet® RED96 System

To explore the binding kinetics of the humanized antibody, this example performed the affinity ranking by using Octet Red 96. As shown in Table 25 below, 147H, 147H-6, 147H-7, 147H-13 and 147H-14 show better affinity.

[Table 25]

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

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 Table 26, 147H-6, 147H-7, 147H-13 and 147H-14 showed comparable affinity with 147H chimeric antibody.

[Table 26]

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-7	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

2.13. Functional properties of anti-LAG3 mouse monoclonal antibodies

Stimulation of human T cell response by anti-LAG3 antibodies

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. 30, 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.

2.14. Affinity maturation of anti-LAG3 147H humanized monoclonal antibodies

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.

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 27] Antibody affinity maturation

No.	Sequence (CDR underlined, mutation bold)
147H 3421	VH (SEQ ID NO: 375) EVQLVQSGAEVKKPGASVKVSCKASGYTFTNYWLGWIKQAPGQGLEWIGDIYPGG DYINYNEKFKGKATLTADTSISTAYMELSRLRSDDTAVYYCARPNLP**KDH**WGQGTT VTVSS VL (SEQ ID NO: 376) DIVMTQSPLSLPVTPGEPASISCRSSKSLLHSNAITYLYWYLQKPGQSPQLLIYQVSNL

	ASGVPDRFSGSGSGTDFTLKISRVEAEDVGVYYCAQNLELPWTFGGGTKVEIK
147H 3422	VH (SEQ ID NO: 377) EVQLVQSGAEVKKPGASVKVSCKASGYTFTNYWLGWIKQAPGQGLEWIGDIYPGG DYINYNEKFKGKATLTADTSISTAYMELSRLRSDDTAVYYCARP**D**LPGDYWGQGTT VTVSS VL (SEQ ID NO: 378) DIVMTQSPLSLPVTPGEPASISCRSSKSLLHSNAITYLYWYLQKPGQSPQLLIYQVSNL ASGVPDRFSGSGSGTDFTLKISRVEAEDVGVYYCAQNLELPWTFGGGTKVEIK
147H 3423	VH (SEQ ID NO: 379) EVQLVQSGAEVKKPGASVKVSCKASGYTFTNYWLGWIKQAPGQGLEWIGDIYPGG DYINYNEKFKGKATLTADTSISTAYMELSRLRSDDTAVYYCARP**G**LP**K**DYWGQGTT VTVSS VL (SEQ ID NO: 380) DIVMTQSPLSLPVTPGEPASISCRSSKSLLHSNAITYLYWYLQKPGQSPQLLIYQVSNL ASGVPDRFSGSGSGTDFTLKISRVEAEDVGVYYCAQNLELPWTFGGGTKVEIK
147H 3424	VH (SEQ ID NO: 381) EVQLVQSGAEVKKPGASVKVSCKASGYTFTNYWLGWIKQAPGQGLEWIGDIYPGG DYINYNEKFKGKATLTADTSISTAYMELSRLRSDDTAVYYCARPNLP**K**DYWGQGTT VTVSS VL (SEQ ID NO: 382) DIVMTQSPLSLPVTPGEPASISCRSSKSLLHSNAITYLYWYLQKPGQSPQLLIYQVSNL ASGVPDRFSGSGSGTDFTLKISRVEAEDVGVYYCAQNLELPWTFGGGTKVEIK
147H 3425	VH (SEQ ID NO: 383) EVQLVQSGAEVKKPGASVKVSCKASGYTFTNYWLGWIKQAPGQGLEWIGDIYPGG DYINYNEKFKGKATLTADTSISTAYMELSRLRSDDTAVYYCARPNLP**R**DYWGQGTT VTVSS VL (SEQ ID NO: 384) DIVMTQSPLSLPVTPGEPASISCRSSKSLLHSNAITYLYWYLQKPGQSPQLLIYQVSNL ASGVPDRFSGSGSGTDFTLKISRVEAEDVGVYYCAQNLELPWTFGGGTKVEIK
147H 3426	VH (SEQ ID NO: 385) EVQLVQSGAEVKKPGASVKVSCKASGYTFTNYWLGWIKQAPGQGLEWIGDIYPGG DYINYNEKFKGKATLTADTSISTAYMELSRLRSDDTAVYYCARP**G**LP**R**DYWGQGTT VTVSS VL (SEQ ID NO: 386) DIVMTQSPLSLPVTPGEPASISCRSSKSLLHSNAITYLYWYLQKPGQSPQLLIYQVSNL ASGVPDRFSGSGSGTDFTLKISRVEAEDVGVYYCAQNLELPWTFGGGTKVEIK
147H 3427	VH (SEQ ID NO: 387) EVQLVQSGAEVKKPGASVKVSCKASGYTFTNYWLGWIKQAPGQGLEWIGDIYPGG DYINYNEKFKGKATLTADTSISTAYMELSRLRSDDTAVYYCARP**G**LP**Q**DYWGQGTT VTVSS VL (SEQ ID NO: 388) DIVMTQSPLSLPVTPGEPASISCRSSKSLLHSNAITYLYWYLQKPGQSPQLLIYQVSNL ASGVPDRFSGSGSGTDFTLKISRVEAEDVGVYYCAQNLELPWTFGGGTKVEIK
147H 3428	VH (SEQ ID NO: 389) EVQLVQSGAEVKKPGASVKVSCKASGYTFTNYWLGWIKQAPGQGLEWIGDIYPGG DYINYNEKFKGKATLTADTSISTAYMELSRLRSDDTAVYYCARP**D**LP**K**DYWGQGTT VTVSS VL (SEQ ID NO: 390) DIVMTQSPLSLPVTPGEPASISCRSSKSLLHSNAITYLYWYLQKPGQSPQLLIYQVSNL ASGVPDRFSGSGSGTDFTLKISRVEAEDVGVYYCAQNLELPWTFGGGTKVEIK
147H 3429	VH (SEQ ID NO: 391) EVQLVQSGAEVKKPGASVKVSCKASGYTFTNYWLGWIKQAPGQGLEWIGDIYPGG DYINYNEKFKGKATLTADTSISTAYMELSRLRSDDTAVYYCARPNLPGDYWGQGTT VTVSS VL (SEQ ID NO: 392) DIVMTQSPLSLPVTPGEPASISCRSSKSLLHSNAITYLYWYLQKPGQSPQLLIYQVSNL ASGVPDRFSGSGSGTDFTLKISRVEAEDVGVYYC**G**QNLELPWTFGGGTKVEIK

147H 3430	VH (SEQ ID NO: 393) EVQLVQSGAEVKKPGASVKVSCKASGYTFTNYWLGWIKQAPGQGLEWIGDIYPGG DYINYNEKFKGKATLTADTSISTAYMELSRLRSDDTAVYYCARPNLPGDYWGQGTT VTVSS VL (SEQ ID NO: 394) DIVMTQSPLSLPVTPGEPASISCRSSKSLLHSNAITYLYWYLQKPGQSPQLLIYQVSNL ASGVPDRFSGSGSGTDFTLKISRVEAEDVGVYYCAQNLE**M**PWTFGGGTKVEIK
147H 3431	VH (SEQ ID NO: 395) EVQLVQSGAEVKKPGASVKVSCKASGYTFTNYWLGWIKQAPGQGLEWIGDIYPGG DYINYNEKFKGKATLTADTSISTAYMELSRLRSDDTAVYYCARPNLPGDYWGQGTT VTVSS VL (SEQ ID NO: 396) DIVMTQSPLSLPVTPGEPASISCRSSKSLLHSNAITYLYWYLQKPGQSPQLLIYQVSNL ASGVPDRFSGSGSGTDFTLKISRVEAEDVGVYYC**G**QNLE**M**PWTFGGGTKVEIK
147H 3432	VH (SEQ ID NO: 397) EVQLVQSGAEVKKPGASVKVSCKASGYTFTNYWLGWIKQAPGQGLEWIGDIYPGG DYINYNEKFKGKATLTADTSISTAYMELSRLRSDDTAVYYCARPNLPGDYWGQGTT VTVSS VL (SEQ ID NO: 398) DIVMTQSPLSLPVTPGEPASISCRSSKSLLHSNAITYLYWYLQKPGQSPQLLIYQVSNL ASGVPDRFSGSGSGTDFTLKISRVEAEDVGVYYCAQ**Y**LE**E**PWTFGGGTKVEIK
147H 3433	VH (SEQ ID NO: 399) EVQLVQSGAEVKKPGASVKVSCKASGYTFTNYWLGWIKQAPGQGLEWIGDIYPGG DYINYNEKFKGKATLTADTSISTAYMELSRLRSDDTAVYYCARPNLPGDYWGQGTT VTVSS VL (SEQ ID NO: 400) DIVMTQSPLSLPVTPGEPASISCRSSKSLLHSNAITYLYWYLQKPGQSPQLLIYQVSNL ASGVPDRFSGSGSGTDFTLKISRVEAEDVGVYYCAQ**Y**LELPWTFGGGTKVEIK
147H 3508	VH (SEQ ID NO: 401) EVQLVQSGAEVKKPGASVKVSCKASGYTFTNYWLGWIKQAPGQGLEWIGDIYPGG DYINYNEKFKGKATLTADTSISTAYMELSRLRSDDTAVYYCARPNLP**K**D**H**WGQGTT VTVSS VL (SEQ ID NO: 402) DIVMTQSPLSLPVTPGEPASISCRSSKSLLHSNAITYLYWYLQKPGQSPQLLIYQVSNL ASGVPDRFSGSGSGTDFTLKISRVEAEDVGVYYC**G**QNLELPWTFGGGTKVEIK
147H 3549	VH (SEQ ID NO: 403) EVQLVQSGAEVKKPGASVKVSCKASGYTFTNYWLGWIKQAPGQGLEWIGDIYPGG DYINYNEKFKGKATLTADTSISTAYMELSRLRSDDTAVYYCARPNLP**K**D**H**WGQGTT VTVSS VL (SEQ ID NO: 404) DIVMTQSPLSLPVTPGEPASISCRSSKSLLHSNAITYLYWYLQKPGQSPQLLIYQVSNL ASGVPDRFSGSGSGTDFTLKISRVEAEDVGVYYCAQ**Y**LE**E**PWTFGGGTKVEIK
147H 3550	VH (SEQ ID NO: 405) EVQLVQSGAEVKKPGASVKVSCKASGYTFTNYWLGWIKQAPGQGLEWIGDIYPGG DYINYNEKFKGKATLTADTSISTAYMELSRLRSDDTAVYYCARPNLP**K**D**H**WGQGTT VTVSS VL (SEQ ID NO: 406) DIVMTQSPLSLPVTPGEPASISCRSSKSLLHSNAITYLYWYLQKPGQSPQLLIYQVSNL ASGVPDRFSGSGSGTDFTLKISRVEAEDVGVYYCAQ**Y**LELPWTFGGGTKVEIK
147H 3663	VH (SEQ ID NO: 407) EVQLVQSGAEVKKPGASVKVSCKASGYTF**E**NYWLGWIKQAPGQGLEWIGDIYPGG DYI**V**YNEKFKGKATLTADTSISTAYMELSRLRSDDTAVYYCARPNLPGDYWGQGTT VTVSS VL (SEQ ID NO: 408) DIVMTQSPLSLPVTPGEPASISCRSSKSLLHSNAITYLYWYLQKPGQSPQLLIYQVSNL A**R**GVPDRFSGSGSGTDFTLKISRVEAEDVGVYYCAQNLELPWTFGGGTKVEIK
147H 3664	VH (SEQ ID NO: 409) EVQLVQSGAEVKKPGASVKVSCKASGY**M**FTNYWLGWIKQAPGQGLEWIGDIYPGG DYINYNEKFKGKATLTADTSISTAYMELSRLRSDDTAVYYCARPNLPGDYWGQGTT VTVSS

	VL (SEQ ID NO: 410) DIVMTQSPLSLPVTPGEPASISCRSSKSLLHSNAITYLYWYLQKPGQSPQLLIYQ**K**SNL ASGVPDRFSGSGSGTDFTLKISRVEAEDVGVYYCAQNLELPWTFGGGTKVEIK
147H 3665	VH (SEQ ID NO: 411) EVQLVQSGAEVKKPGASVKVSCKASGYTF**D**NYWLGWIKQAPGQGLEWIGDIYPGG D**I**INYNEKFKGKATLTADTSISTAYMELSRLRSDDTAVYYCARPNLPGDYWGQGTTV TVSS VL (SEQ ID NO: 412) DIVMTQSPLSLPVTPGEPASISCRSSKSLLHSNAITYLYWYLQKPGQSPQLLIYQVSNL A**V**GVPDRFSGSGSGTDFTLKISRVEAEDVGVYYCAQNLELPWTFGGGTKVEIK
147H 3666	VH (SEQ ID NO: 413) EVQLVQSGAEVKKPGASVKVSCKASGYTF**G**NYWLGWIKQAPGQGLEWIGDIYPGG D**V**INYNEKFKGKATLTADTSISTAYMELSRLRSDDTAVYYCARPNLPGDYWGQGTT VTVSS VL (SEQ ID NO: 414) DIVMTQSPLSLPVTPGEPASISCRSSKSLLHSNAITYLYWYLQKPGQSPQLLIYQVSNL A**L**GVPDRFSGSGSGTDFTLKISRVEAEDVGVYYCAQNLELPWTFGGGTKVEIK
147H 3667	VH (SEQ ID NO: 415) EVQLVQSGAEVKKPGASVKVSCKASGYTFTNYWL**W**WIKQAPGQGLEWIGDI**F**PGG DYINYNEKFKGKATLTADTSISTAYMELSRLRSDDTAVYYCARPNLPGDYWGQGTT VTVSS VL (SEQ ID NO: 416) DIVMTQSPLSLPVTPGEPASISCRSSKSLLHSNAITYLYWYLQKPGQSPQLLIYQV**D**N LASGVPDRFSGSGSGTDFTLKISRVEAEDVGVYYCAQNLELPWTFGGGTKVEIK
147H 3668	VH (SEQ ID NO: 417) EVQLVQSGAEVKKPGASVKVSCKASGYTFTNYWLGWIKQAPGQGLEWIGDIYPGG DYI**V**YNEKFKGKATLTADTSISTAYMELSRLRSDDTAVYYCARPNLPGDYWGQGTT VTVSS VL (SEQ ID NO: 418) DIVMTQSPLSLPVTPGEPASISCRSSKSLLHSNAITYLYWYLQKPGQSPQLLIYQVSNL A**T**GVPDRFSGSGSGTDFTLKISRVEAEDVGVYYCAQNLELPWTFGGGTKVEIK
147H 3669	VH (SEQ ID NO: 419) EVQLVQSGAEVKKPGASVKVSCKASGY**L**FTNYWLGWIKQAPGQGLEWIGDIYPGG DYI**V**YNEKFKGKATLTADTSISTAYMELSRLRSDDTAVYYCARPNLPGDYWGQGTT VTVSS VL (SEQ ID NO: 420) DIVMTQSPLSLPVTPGEPASISCRSSKSLLHSNAITYLYWYLQKPGQSPQLLIYQVSNL ASGVPDRFSGSGSGTDFTLKISRVEAEDVGVYYCAQNLELPWTFGGGTKVEIK
147H 3670	VH (SEQ ID NO: 421) EVQLVQSGAEVKKPGASVKVSCKASGYTFTNYWLGWIKQAPGQGLEWIGDIYPGG DYINYNEKFKGKATLTADTSISTAYMELSRLRSDDTAVYYCARPNLPGDYWGQGTT VTVSS VL (SEQ ID NO: 422) DIVMTQSPLSLPVTPGEPASISCRSSKSLLHSNAITYLYWYLQKPGQSPQLLIY**H**VSNL ASGVPDRFSGSGSGTDFTLKISRVEAEDVGVYYCAQNLELPWTFGGGTKVEIK
147H 3675	VH (SEQ ID NO: 423) EVQLVQSGAEVKKPGASVKVSCKASGYTFTNYWL**W**WIKQAPGQGLEWIGDIYPGG D**L**INYNEKFKGKATLTADTSISTAYMELSRLRSDDTAVYYCARPNLPGDYWGQGTT VTVSS VL (SEQ ID NO: 424) DIVMTQSPLSLPVTPGEPASISCRSSKSLLHSNAITYLYWYLQKPGQSPQLLIY**H**VSNL ASGVPDRFSGSGSGTDFTLKISRVEAEDVGVYYCAQNLELPWTFGGGTKVEIK
147H 3676	VH (SEQ ID NO: 425) EVQLVQSGAEVKKPGASVKVSCKASGYTFTNYWL**S**WIKQAPGQGLEWIGDIYPGG D**H**INYNEKFKGKATLTADTSISTAYMELSRLRSDDTAVYYCARPNLPGDYWGQGTT VTVSS VL (SEQ ID NO: 426) DIVMTQSPLSLPVTPGEPASISCRSSKSLLHSNAITYLYWYLQKPGQSPQLLIYQVSNL ASGVPDRFSGSGSGTDFTLKISRVEAEDVGVYYCAQNLELPWTFGGGTKVEIK
147H 3677	VH (SEQ ID NO: 427)

	EVQLVQSGAEVKKPGASVKVSCKASGYTFTNYWLWWIKQAPGQGLEWIGEIYPGG DYITYNEKFKGKATLTADTSISTAYMELSRLRSDDTAVYYCARPNLPGDYWGQGTT VTVSS VL (SEQ ID NO: 428) DIVMTQSPLSLPVTPGEPASISCRSSKSLLHSNAITYLYWYLQKPGQSPQLLIYQVSNR ASGVPDRFSGSGSGTDFTLKISRVEAEDVGVYYCAQNLELPWTFGGGTKVEIK
147H 3678	VH (SEQ ID NO: 429) EVQLVQSGAEVKKPGASVKVSCKASGYTFTNYWLGWIKQAPGQGLEWIGDIYPGG DYINYNEKFKGKATLTADTSISTAYMELSRLRSDDTAVYYCARPNLPGDYWGQGTT VTVSS VL (SEQ ID NO: 430) DIVMTQSPLSLPVTPGEPASISCRSSKSLLHSNAITYLYWYLQKPGQSPQLLIYQVDN LASGVPDRFSGSGSGTDFTLKISRVEAEDVGVYYCAQNLELPWTFGGGTKVEIK
147H 3679	VH (SEQ ID NO: 431) EVQLVQSGAEVKKPGASVKVSCKASGFTFTNYWLGWIKQAPGQGLEWIGDIYPGG DYIVYNEKFKGKATLTADTSISTAYMELSRLRSDDTAVYYCARPNLPGDYWGQGTT VTVSS VL (SEQ ID NO: 432) DIVMTQSPLSLPVTPGEPASISCRSSKSLLHSNAITYLYWYLQKPGQSPQLLIYQVSNL ASGVPDRFSGSGSGTDFTLKISRVEAEDVGVYYCAQNLELPWTFGGGTKVEIK
147H 3790	VH (SEQ ID NO: 433) EVQLVQSGAEVKKPGASVKVSCKASGYTFTNYWLGWIKQAPGQGLEWIGDIYPGG DYINYNEKFKGKATLTADTSISTAYMELSRLRSDDTAVYYCARPNLPKDHWGQGTT VTVSS VL (SEQ ID NO: 434) DIVMTQSPLSLPVTPGEPASISCRSSKSLLHSNAITYLYWYLQKPGQSPQLLIYQVSNL ATGVPDRFSGSGSGTDFTLKISRVEAEDVGVYYCAQNLELPWTFGGGTKVEIK
147H 3791	VH (SEQ ID NO: 435) EVQLVQSGAEVKKPGASVKVSCKASGYTFTNYWLGWIKQAPGQGLEWIGDIYPGG DYIVYNEKFKGKATLTADTSISTAYMELSRLRSDDTAVYYCARPNLPGDYWGQGTT VTVSS VL (SEQ ID NO: 436) DIVMTQSPLSLPVTPGEPASISCRSSKSLLHSNAITYLYWYLQKPGQSPQLLIYQVSNL ASGVPDRFSGSGSGTDFTLKISRVEAEDVGVYYCGQNLELPWTFGGGTKVEIK
147H 3792	VH (SEQ ID NO: 437) EVQLVQSGAEVKKPGASVKVSCKASGYTFTNYWLGWIKQAPGQGLEWIGDIYPGG DYIVYNEKFKGKATLTADTSISTAYMELSRLRSDDTAVYYCARPNLPGDYWGQGTT VTVSS VL (SEQ ID NO: 438) DIVMTQSPLSLPVTPGEPASISCRSSKSLLHSNAITYLYWYLQKPGQSPQLLIYQVSNL ASGVPDRFSGSGSGTDFTLKISRVEAEDVGVYYCAQYLELPWTFGGGTKVEIK
147H 3793	VH (SEQ ID NO: 439) EVQLVQSGAEVKKPGASVKVSCKASGYLFTNYWLGWIKQAPGQGLEWIGDIYPGG DYIVYNEKFKGKATLTADTSISTAYMELSRLRSDDTAVYYCARPNLPGDYWGQGTT VTVSS VL (SEQ ID NO: 440) DIVMTQSPLSLPVTPGEPASISCRSSKSLLHSNAITYLYWYLQKPGQSPQLLIYQVSNL ASGVPDRFSGSGSGTDFTLKISRVEAEDVGVYYCGQNLELPWTFGGGTKVEIK
147H 3794	VH (SEQ ID NO: 441) EVQLVQSGAEVKKPGASVKVSCKASGYLFTNYWLGWIKQAPGQGLEWIGDIYPGG DYIVYNEKFKGKATLTADTSISTAYMELSRLRSDDTAVYYCARPNLPGDYWGQGTT VTVSS VL (SEQ ID NO: 442) DIVMTQSPLSLPVTPGEPASISCRSSKSLLHSNAITYLYWYLQKPGQSPQLLIYQVSNL ASGVPDRFSGSGSGTDFTLKISRVEAEDVGVYYCAQYLELPWTFGGGTKVEIK
147H 3807	VH (SEQ ID NO: 443) EVQLVQSGAEVKKPGASVKVSCKASGYTFTNYWLGWIKQAPGQGLEWIGDIYPGG DYIVYNEKFKGKATLTADTSISTAYMELSRLRSDDTAVYYCARPNLPKDHWGQGTT VTVSS VL (SEQ ID NO: 444)

	DIVMTQSPLSLPVTPGEPASISCRSSKSLLHSQGITYLYWYLQKPGQSPQLLIYQVSNL ASGVPDRFSGSGSGTDFTLKISRVEAEDVGVYYCAQ**Y**LELPWTFGGGTKVEIK
147H 3807b	VH (SEQ ID NO: 491) EVQLVQSGAEVKKPGASVKVSCKASGYTFTNYWLGWIKQAPGQGLEWIGDIYPGG DYI**V**YNEKFKGKATLTADTSISTAYMELSRLRSDDTAVYYCARPNLP**K**D**H**WGQGTT VTVSS VL (SEQ ID NO: 492) DIVMTQSPLSLPVTPGEPASISCRSSKSLLHS**NA**ITYLYWYLQKPGQSPQLLIYQVSNL ASGVPDRFSGSGSGTDFTLKISRVEAEDVGVYYCAQ**Y**LELPWTFGGGTKVEIK
147H 3808	VH (SEQ ID NO: 445) EVQLVQSGAEVKKPGASVKVSCKASGYTFTNYWLGWIKQAPGQGLEWIGDIYPGG DYI**V**YNEKFKGKATLTADTSISTAYMELSRLRSDDTAVYYCARPNLP**K**D**H**WGQGTT VTVSS VL (SEQ ID NO: 446) DIVMTQSPLSLPVTPGEPASISCRSSKSLLHSNAITYLYWYLQKPGQSPQLLIYQVSNL ASGVPDRFSGSGSGTDFTLKISRVEAEDVGVYYC**G**Q**Y**LELPWTFGGGTKVEIK
147H 3809	VH (SEQ ID NO: 447) EVQLVQSGAEVKKPGASVKVSCKASGY**L**FTNYWLGWIKQAPGQGLEWIGDIYPGG DYI**V**YNEKFKGKATLTADTSISTAYMELSRLRSDDTAVYYCARPNLP**K**D**H**WGQGTT VTVSS VL (SEQ ID NO: 448) DIVMTQSPLSLPVTPGEPASISCRSSKSLLHSNAITYLYWYLQKPGQSPQLLIYQVSNL ASGVPDRFSGSGSGTDFTLKISRVEAEDVGVYYCAQ**Y**LELPWTFGGGTKVEIK
147H 3810	VH (SEQ ID NO: 449) EVQLVQSGAEVKKPGASVKVSCKASGY**L**FTNYWLGWIKQAPGQGLEWIGDIYPGG DYI**V**YNEKFKGKATLTADTSISTAYMELSRLRSDDTAVYYCARPNLP**K**D**H**WGQGTT VTVSS VL (SEQ ID NO: 450) DIVMTQSPLSLPVTPGEPASISCRSSKSLLHSNAITYLYWYLQKPGQSPQLLIYQVSNL A**T**GVPDRFSGSGSGTDFTLKISRVEAEDVGVYYCAQ**Y**LELPWTFGGGTKVEIK
147H 3811	VH (SEQ ID NO: 451) EVQLVQSGAEVKKPGASVKVSCKASGY**L**FTNYWLGWIKQAPGQGLEWIGDIYPGG DYI**V**YNEKFKGKATLTADTSISTAYMELSRLRSDDTAVYYCARPNLP**K**D**H**WGQGTT VTVSS VL (SEQ ID NO: 452) DIVMTQSPLSLPVTPGEPASISCRSSKSLLHSNAITYLYWYLQKPGQSPQLLIYQVSNL A**T**GVPDRFSGSGSGTDFTLKISRVEAEDVGVYYC**G**Q**Y**LELPWTFGGGTKVEIK

[Table 28] Summary of mutations and mutated CDR regions:

	Original sequence (SEQ ID NO:__)	**Example substitutions (based on kabat numbering)**	**Example mutated sequences (SEQ ID NO:__)**
CDRH1	G**Y**T**F**T**N**YWL**G** (354)	Y27: F T28: M, L T30: E, D, G 527: W, S	GYTF**E**NYWLG (453) GY**M**FTNYWLG (454) GYTF**D**NYWLG (455) GYTF**G**NYWLG (456) GYTFTNYWL**W** (457) GY**L**FTNYWLG (458) GYTFTNYWL**S** (459) G**F**TFTNYWLG (460)
CDRH2	**D**I**Y**PGGD**Y**I**N**YNEKFKG (355)	D50: E Y52: F Y56: I, V, L, H N58: V, T	DIYPGGDYI**V**YNEKFKG (461) DIYPGGD**I**INYNEKFKG (462) DIYPGGD**V**INYNEKFKG (463) DI**F**PGGDYINYNEKFKG (464) DIYPGGD**L**INYNEKFKG (465) DIYPGGD**H**INYNEKFKG (466) **E**IYPGGDYI**T**YNEKFKG (467)
CDRH3	P**N**LP**G**D**Y** (356)	N96: D, G	PNLP**K**D**H** (468)

		G99: K, R, Q Y102: H	P**D**LPGDY (469) P**G**LP**K**DY (470) PNLP**K**DY (471) PNLP**R**DY (472) P**G**LP**R**DY (473) P**G**LP**Q**DY (474) P**D**LP**K**DY (475)
CDRL1	RSSKSLLHS**N**GITYLY (357)	N28: Q	RSSKSLLHS**Q**GITYLY (490)
CDRL2	**QVS**N**L**A**S** (358)	Q50: H V51: K S52: D L54: R S56: R, V, L, T	QVSNLA**R** (476) Q**K**SNLAS (477) QVSNLA**V** (478) QVSNLA**L** (479) QV**D**NLAS (480) QVSNLA**T** (481) **H**VSNLAS (482) QVSN**R**AS (483)
CDRL3	**A**QN**L**E**L**PWT (359)	A89: G N91: Y L94: M, E	**G**QNLELPWT (484) AQNLE**M**PWT (485) **G**QNLE**M**PWT (486) AQ**Y**LE**E**PWT (487) AQ**Y**LELPWT (488) **G**Q**Y**LELPWT (489)

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

The binding kinetics of affinity matured antibodies to recombinant his-tag human LAG3-ECD protein was examined by Biacore T200, as stated in Example 2.7. The results were shown in Table below. The Biacore results showed that these anti-LAG3 antibodies had better affinity than parent 147H-13.

[Table 29]

	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 3807b	5.1E-10	4.0E+06	2.0E-03
147H 3808	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

To confirm the capability of affinity matured anti-LAG-3 antibodies binding to human LAG3, 2 antibodies with highest affinity (B3807b and B3810) along with parent antibody 147H-13 were evaluated using ELISA, which was described in Example 2.2. EC50 of B3807b, B3810 along with parent antibody was showed in table below. Both B3807b and B3810 showed superior binding capability than parent antibody 147H-13.

[Table 30]

Name	EC50 （nM）
147H-13	6.5
147H 3807b	0.41
147H 3810	0.49

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 B3807b 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. 31 is a graph showing the binding curve of anti-LAG3 antibodies. EC50 of tested antibodies was showed below.

[Table 31]

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

2.16. Characterization of monoclonal antibody 147H 3807 (B3807)

A. Binding of B3807 to LAG3 protein

This example evaluated the capability of the anti-LAG-3 antibody 147H 3807 (B3807) to bind to the human LAG3 protein. The streptavidin was coated to an ELISA plate at 2μg/ml with 100μl/well. 100μl of Bio-LAG3 at 1.0μg/ml was subsequently incubated with streptavidin at RT for 1hr. B3807, along with a positive control 25F7 and a negative control IgG, were serially diluted with ELISA diluent buffer. To assess binding, the antibodies at various concentrations were added to LAG3 protein-coated plate for 1.5hr RT. The resulting plates were washed and then labeled with anti-human IgG(Fab)-HRP antibody.

As shown in FIG. 38, both B3807 and 25F7 bound to human LAG3 in a dose-dependent manner, with B3807 showing a higher potency and lower EC50 (0.06 nM vs. 0.22nM for 25F7).

B. Biacore analysis

The binding of B3807 to recombinant His-tag human LAG3-ECD protein was examined by Biacore T200 using a capture method. B3807 was captured using protein A which was immobilized on CM5 sensor chip. Serial concentrations of his-tag human LAG3-ECD protein (0-12nM) 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 FIG. 39, demonstrating that B3807 is binding to human LAG3 with high affinity

C. Jurkat cell and PBMC-based binding assays

To further confirm that B3807 could bind to cell-derived human LAG3, both inducible human LAG3 expressed Jurkat cells and activated PBMCs were used to test the binding capability of B3807. In brief, Jurkat cells were resuspended in FACS buffer. B3807, 25F7 and isotype control were 3-fold serially diluted in FACS buffer with a dose ranging from 20nM to 9 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 the 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 1μg/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 are presented in FIG. 40. EC50 of tested antibodies are also showed in the figure. In both tests, B3807 exhibited stronger binding capability than the control antibody 25F7.

D. Blocking of LAG3 binding to MHC class II

To measure the ability of B3807 to block the interaction between human LAG3 and MHCII, the LAG3 and MHC II binding assay (Cisbio, 64ICP03PEG) was performed utilizing homogeneous TR-FRET technology, following the protocol provided by the kit manufacturer. B3807 was 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 B3807 was 0.41nM (FIG. 41) demonstrating potent blocking activity.

E. Stimulation of human T cell response

To test the ability of anti-LAG3 antibodies to stimulate T cell response, hLAG3-expressed Jurkat cells were used. 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. B3807 was 3-fold diluted and added to the cells at a final concentration ranging from 100nM to 50pm. 48 hours later, IL2 from the culture medium was measured using a homogeneous TR-FRET assay (PerkinElmer, TRF1221M). FIG. 42 shows the curve of B3807 and 25F7 in stimulating IL2 release, in which B3807 outperformed 25F7 by a great margin.

F. IL2 release in primary T cells

The antibodies' ability to stimulate T cell response was also tested with hLAG3-expressed primary T cells. At all four tested doses, B3807 outperformed 25F7 (FIG. 43, left panel). When used with an anti-PD-L1 antibody together, the IL2 release profile (FIG. 43, right panel) demonstrated the synergistic effect between the anti-LAG3 antibody B3807 and the anti-PD-L1 antibody.

G. Combinatory effects with anti-PD1/anti-PD-L1 antibodies in tumor regression

Humanized mice that expressed the extracellular domains of human LAG3 were used. As shown in FIG. 44, left panel, B3807 and 25F7 exhibited some effect in inhibiting the tumor growth when combo with anti-PD-1 antibody.

In the right panel of FIG. 44, however, it is apparent that both B3807 and 25F7 had significant synergistic effect when used together with Tecentriq, a commercially available anti-PD-L1 antibody.

H. Comparison of B3807 with B3807b

The activities of B3807 and B3807b were compared for their ability in promoting IL2 release in Jurkat cells (see experimental procedure in Example 2.16(E)) and in binding to LAG3 on Jurkat cells (see experimental procedure in Example 2.16(C)).

The comparison results are presented in FIG. 45. In both experiments, B3807 and B3807b exhibited highly similar activity profiles, demonstrating that the sequence difference in CDRL1 between these two antibodies did not impact their activities.

Also, as shown in FIG. 46, the Biacore data (see experimental procedure in Example 2.16(B)) further demonstrate that the great similarity between these two antibodies. B3807 was used in the following examples for further testing and preparing bispecific antibodies.

Example 3. Preparation of anti-PD-L1/anti-LAG3 bispecific antibodies

Hu1210-41 (Hu1210 VH.4dxHu1210 Vk.1, see Table 8; hereinafter, "H12") and B6 (see Table 16) clones among the anti-PD-L1 clones prepared in Example 1 and 147H (also called as "147", see Table 23) and 147H 3807 (also called as "147(H3807)"; see Table 27) clones among the anti-LAG3 clones prepared in Example 2 were exemplarily selected, to prepare anti-PD-L1/anti-LAG3 bispecific antibodies in a full-length IgG X scFv form. When PD-L1 is placed in full IgG part, IgG1 with ADCC reduced mutant backbone (N297A mutation; US Patent. No. 7332581, 8219149, etc.) was used, and when LAG3 is placed in full IgG part, IgG4 was used with S241P mutation (Angal et al., *Mol. Immunol.* 30:105-108).

A DNA segment 1 having a nucleotide sequence encoding a heavy chain of an IgG antibody of the anti-PD-L1/anti-LAG3 bispecific antibody was inserted into pcDNA 3.4 (Invitrogen, A14697; plasmid 1), and a DNA segment 2 having a nucleotide sequence encoding a light chain of an IgG antibody of the anti-PD-L1/anti-LAG3 bispecific antibody was inserted into pcDNA 3.4 (Invitrogen, A14697; plasmid 2). Thereafter, a DNA segment 3 encoding a scFv was fused at a part of the DNA segment 1 corresponding to the c-terminus of the Fc region of the IgG antibody inserted into the plasmid 1, using a DNA segment 4 encoding a linker peptide having 10 amino acid lengths consisting of (GGGGS)2, to construct vectors for the expression of bispecific antibodies.

The sequences of the heavy chain, light chain, scFv and DNA segments were summarized in Tables 32 and 33:

[Table 32] Bispecific antibody comprising the anti-PD-L1 clone in IgG form and the anti-LAG3 clone in scFv form (PD-L1xLAG3)

H12x147 (bispecific antibody comprising the anti-PD-L1 H12 clone in IgG form and the anti-LAG3 147 clone in scFv form)				
			Amino acid sequence (N'→C')	Nucleotide Sequence (5'→3')
Heavy Chain	Heavy chain of H12		EVQLVESGGGLVQP GGSLRLSCAASGFT FSSYDMSWVRQAP GKSLEWVATISDAG GYIYYSDSVKGRFTI SRDNAKNSLYLQM NSLRDEDTAVYICA REFGKRYALDYWG QGTTVTVSSASTKG PSVFPLAPSSKSTSG GTAALGCLVKDYFP EPVTVSWNSGALTS GVHTFPAVLQSSGL YSLSSVVTVPSSSLG TQTYICNVNHKPSN TKVDKKVEPKSCDK THTCPPCPAPELLGG PSVFLFPPKPKDTLM ISRTPEVTCVVVDVS HEDPEVKFNWYVD GVEVHNAKTKPREE QYASTYRVVSVLTV LHQDWLNGKEYKC KVSNKALPAPIEKTI SKAKGQPREPQVYT LPPSREEMTKNQVS LTCLVKGFYPSDIA VEWESNGQPENNY KTTPPVLDSDGSFFL YSKLTVDKSRWQQ GNVFSCSVMHEALH NHYTQKSLSLSPGK (SEQ ID NO:528)	GAGGTGCAGCTGGTGGAGAGCGGAGGAG GACTGGTGCAACCCGGAGGCAGCCTGAG ACTGAGCTGCGCTGCCAGCGGCTTCACCT TCAGCAGCTACGACATGAGCTGGGTGAG ACAGGCCCCTGGCAAAAGCCTGGAGTGG GTGGCCACCATCTCCGATGCGGGCGGCTA CATCTATTACTCCGACAGCGTGAAGGGCA GGTTCACCATCAGCAGGGACAACGCCAA GAACAGCCTGTACCTGCAGATGAACAGC CTGAGGGATGAGGACACCGCCGTGTACA TCTGCGCCAGGGAGTTCGGCAAAAGGTA CGCCCTGGACTACTGGGGCCAGGGCACA ACCGTGACCGTGAGCAGCgctAgcAccAAgG GCCCCTCTGTGTTCCCTCTGGCCCCTTCCT CTAAATCCACCTCTGGCGGAACCGCTGCT CTGGGCTGTCTGGTCAAGGACTACTTCCC TGAGCCCGTGACCGTGTCTTGGAATTCTG GCGCTCTGACCAGCGGAGTGCACACCTTT CCAGCTGTGCTGCAGTCCTCCGGCCTGTA CTCTCTGTCCTCTGTCGTGACAGTGCCTTC CAGCTCTCTGGGCACCCAGACCTACATCT GCAACGTGAACCACAAGCCCTCCAACAC CAAGGTGGACAAGAAGGTGGAACCCAAG TCCTGCGACAAGACCCACACCTGTCCTCC ATGTCCTGCTCCAGAACTGCTGGGCGGAC CCTCCGTGTTCCTGTTCCCTCCAAAGCCT AAGGACACCCTGATGATCTCCCGGACCCC TGAAGTGACCTGCGTGGTGGTGGATGTGT CCCACGAGGATCCCGAAGTGAAGTTCAA TTGGTACGTGGACGGCGTGGAAGTGCAC AACGCCAAGACCAAGCCTAGAGAGGAAC AGTACgccTCCACCTACCGGGTGGTGTCCG TGCTGACCGTTCTGCACCAGGATTGGCTG AACGGCAAAGAGTACAAGTGCAAGGTGT CCAACAAGGCCCTGCCTGCCCCTATCGAA AAGACCATCTCTAAGGCCAAGGGCCAGC CCCGGGAACCTCAAGTGTACACCTTGCCT CCCAGCCGGGAAGAGATGACCAAGAACC AGGTGTCCCTGACCTGCCTGGTTAAGGGC TTCTACCCCTCCGATATCGCCGTGGAATG GGAGTCTAATGGCCAGCCTGAGAACAAC TACAAGACCACACCTCCTGTGCTGGACTC CGACGGCTCATTCTTCCTGTACTCCAAGC TGACCGTGGACAAGTCCAGATGGCAGCA GGGCAACGTGTTCTCCTGCTCCGTGATGC ACGAGGCCCTGCACAATCACTACACCCA GAAGTCCCTGTCTCTGTCCCCTGGCAAAG GCTCCGGATCTGGTTCTGGATCCGGAAGC
	Linker		GSGSGSGSGSGSGS GSGS (SEQ ID NO:529)	
	scFv of 147	VL	DIVMTQSPLSLPVTP GEPASISC**RSSKSLL** **HSNGITYLY**WYLQ KPGQSPQLLIY**QVS** **NLAS**GVPDRFSGSG SGTDFTLKISRVEAE DVGVYYC**AQNLEL** **PWT**FGCGTKVEIKR	

			(SEQ ID NO:530)	GGTTCTGGCAGCGGCTCTGGATCTGACAT CGTGATGACCCAGTCTCCACTGAGCCTGC CTGTGACACCTGGCGAGCCTGCTTCCATC TCCTGCCGGTCCTCTAAGTCCCTGCTGCA CTCTAACGGCATCACCTACCTGTACTGGT ATCTGCAGAAGCCCGGCCAGTCTCCTCAG CTGCTGATCTACCAGGTGTCCAACCTGGC TTCTGGCGTGCCCGATAGATTCTCCGGTA GCGGATCTGGAACCGACTTCACCCTGAAG ATCTCCAGAGTGGAAGCCGAGGACGTGG GCGTGTACTACTGTGCCCAGAACCTGGAA CTGCCCTGGACCTTTGGCTGTGGCACCAA GGTGGAAATCAAGAGAGGCGGCGGAGGA TCTGGCGGAGGTGGAAGCGGAGGCGGAG GAAGCGGTGGCGGCGGATCTGAAGTTCA GTTGGTTCAGTCTGGCGCCGAAGTGAAGA AACCTGGCGCCTCTGTGAAGGTGTCCTGC AAGGCTTCCGGCTACACCTTTACCAACTA CTGGCTCGGCTGGATCAAGCAGGCCCCTG GACAGTGTCTGGAATGGATCGGCGACAT CTACCCTGGCGGCGACTACATCAACTACA ACGAGAAGTTCAAGGGCAAAGCTACCCT GACCGCCGACACCTCTATCTCCACCGCCT ACATGGAACTGTCCCGGCTGAGATCTGAC GACACCGCCGTGTACTATTGCGCCAGACC TAACCTGCCTGGCGACTATTGGGGCCAGG GCACAACAGTGACCGTGTCCTCTTAA (SEQ ID NO:533)
		Linker	GGGGSGGGGSGGG GSGGGGS (SEQ ID NO:531)	
		VH	EVQLVQSGAEVKKP GASVKVSCKAS**GY** **TFTNYWLG**WIKQA PGQCLEWIG**DIYPG** **GDYINYNEKFKG**K ATLTADTSISTAYM ELSRLRSDDTAVYY CAR**PNLPGDY**WGQ GTTVTVSS* (SEQ ID NO:532)	
Light chain	Light chain of H12		DIQMTQSPSSLSASV GDRVTITCKASQDV TPAVAWYQQKPGK APKLLIYSTSSRYTG VPSRFSGSGSGTDFT FTISSLQPEDIATYY CQQHYTTPLTFGQG TKLEIKRTVAAPSVF IFPPSDEQLKSGTAS VVCLLNNFYPREAK VQWKVDNALQSGN SQESVTEQDSKDST YSLSSTLTLSKADYE KHKVYACEVTHQG LSSPVTKSFNRGEC* (SEQ ID NO:534)	GACATCCAGATGACCCAGAGCCCTAGCA GCCTGAGCGCTAGCGTGGGCGACAGGGT GACCATCACCTGCAAGGCCAGCCAGGAT GTGACCCCTGCCGTGGCCTGGTACCAGCA GAAGCCCGGCAAGGCCCCCAAGCTGCTG ATCTACAGCACCAGCAGCAGGTACACCG GCGTGCCCAGCAGGTTTAGCGGAAGCGG CAGCGGCACCGACTTCACCTTCACCATCA GCAGCCTGCAGCCCGAGGACATCGCCAC CTACTACTGCCAGCAGCACTACACCACCC CTCTGACCTTCGGCCAGGGCACCAAGCTG GAGATCAAGAGAACCGTGGCCGCTCCCT CCGTGTTCATCTTCCCACCATCTGACGAG CAGCTGAAGTCCGGCACCGCTTCTGTCGT GTGCCTGCTGAACAACTTCTACCCTCGGG AAGCCAAGGTGCAGTGGAAGGTGGACAA TGCCCTGCAGTCCGGCAACTCCCAAGAGT CTGTGACCGAGCAGGACTCCAAGGACAG CACCTACTCCCTGTCCTCTACCCTGACCCT GTCCAAGGCCGACTACGAGAAGCACAAG GTGTACGCCTGCGAAGTGACCCACCAGG GACTGTCTAGCCCCGTGACCAAGTCCTTC AACAGAGGCGAGTGCTGA (SEQ ID NO:535)
H12x147(H3807) (bispecific antibody comprising the anti-PD-L1 H12 clone in IgG form and the anti-LAG3 147(H3807) clone in scFv form)				
			Amino acid sequence (N'→C')	Nucleotide Sequence (5'→3')
Heavy Chain	Heavy chain of H12		EVQLVESGGGLVQP GGSLRLSCAASGFT FSSYDMSWVRQAP	GAGGTGCAGCTGGTGGAGAGCGGAGGAG GACTGGTGCAACCCGGAGGCAGCCTGAG ACTGAGCTGCGCTGCCAGCGGCTTCACCT

			GKSLEWVATISDAG GYIYYSDSVKGRFTI SRDNAKNSLYLQM NSLRDEDTAVYICA REFGKRYALDYWG QGTTVTVSSASTKG PSVFPLAPSSKSTSG GTAALGCLVKDYFP EPVTVSWNSGALTS GVHTFPAVLQSSGL YSLSSVVTVPSSSLG TQTYICNVNHKPSN TKVDKKVEPKSCDK THTCPPCPAPELLGG PSVFLFPPKPKDTLM ISRTPEVTCVVVDVS HEDPEVKFNWYVD GVEVHNAKTKPREE QYASTYRVVSVLTV LHQDWLNGKEYKC KVSNKALPAPIEKTI SKAKGQPREPQVYT LPPSREEMTKNQVS LTCLVKGFYPSDIA VEWESNGQPENNY KTTPPVLDSDGSFFL YSKLTVDKSRWQQ GNVFSCSVMHEALH NHYTQKSLSLSPGK (SEQ ID NO:528)	TCAGCAGCTACGACATGAGCTGGGTGAG ACAGGCCCCTGGCAAAAGCCTGGAGTGG GTGGCCACCATCTCCGATGCGGGCGGCTA CATCTATTACTCCGACAGCGTGAAGGGCA GGTTCACCATCAGCAGGGACAACGCCAA GAACAGCCTGTACCTGCAGATGAACAGC CTGAGGGATGAGGACACCGCCGTGTACA TCTGCGCCAGGGAGTTCGGCAAAAGGTA CGCCCTGGACTACTGGGGCCAGGGCACA ACCGTGACCGTGAGCAGCgctAgcAccAAgG GCCCCTCTGTGTTCCCTCTGGCCCCTTCCT CTAAATCCACCTCTGGCGGAACCGCTGCT CTGGGCTGTCTGGTCAAGGACTACTTCCC TGAGCCCGTGACCGTGTCTTGGAATTCTG GCGCTCTGACCAGCGGAGTGCACACCTTT CCAGCTGTGCTGCAGTCCTCCGGCCTGTA CTCTCTGTCCTCTGTCGTGACAGTGCCTTC CAGCTCTCTGGGCACCCAGACCTACATCT GCAACGTGAACCACAAGCCCTCCAACAC CAAGGTGGACAAGAAGGTGGAACCCAAG TCCTGCGACAAGACCCACACCTGTCCTCC ATGTCCTGCTCCAGAACTGCTGGGCGGAC CCTCCGTGTTCCTGTTCCCTCCAAAGCCT AAGGACACCCTGATGATCTCCCGGACCCC TGAAGTGACCTGCGTGGTGGTGGATGTGT CCCACGAGGATCCCGAAGTGAAGTTCAA TTGGTACGTGGACGGCGTGGAAGTGCAC AACGCCAAGACCAAGCCTAGAGAGGAAC AGTACgccTCCACCTACCGGGTGGTGTCCG TGCTGACCGTTCTGCACCAGGATTGGCTG AACGGCAAAGAGTACAAGTGCAAGGTGT CCAACAAGGCCCTGCCTGCCCCTATCGAA AAGACCATCTCTAAGGCCAAGGGCCAGC
	Linker		GSGSGSGSGSGSGS GSGS (SEQ ID NO:529)	CCCGGGAACCTCAAGTGTACACCTTGCCT CCCAGCCGGGAAGAGATGACCAAGAACC AGGTGTCCCTGACCTGCCTGGTTAAGGGC TTCTACCCCTCCGATATCGCCGTGGAATG GGAGTCTAACGGCCAGCCCGAGAACAAC TACAAGACCACCCCTCCTGTGCTGGACTC CGACGGCTCATTCTTCCTGTACTCCAAGC TGACCGTGGACAAGTCTCGGTGGCAGCA GGGCAACGTGTTCTCCTGCTCTGTGATGC ACGAGGCCCTGCACAACCACTACACCCA GAAGTCCCTGTCCCTGTCTCCCGGCAAAG GCTCCGGATCTGGTTCTGGATCCGGAAGC GGTTCTGGCAGCGGCTCTGGATCTGACAT TGTGATGACCCAGAGCCCCCTGAGCCTCC CCGTGACCCCTGGAGAACCCGCCAGCAT AAGCTGCAGATCCTCCAAAAGCCTGCTGC ACTCCCAGGGAATAACCTACCTGTATTGG TACCTGCAGAAACCCGGCCAATCCCCCCA ACTCCTGATATACCAAGTGTCCAACCTGG CCTCCGGCGTGCCCGACAGATTCTCCGGC TCCGGCAGCGGTACCGACTTCACCCTCAA AATCTCCAGAGTGGAAGCAGAAGACGTC GGCGTGTACTACTGCGCCCAGTACCTGGA ACTGCCCTGGACCTTCGGCtgtGGCACCAA GGTGGAAATCAAGAGAGGCGGCGGAGGA AGCGGAGGCGGCGGTTCTGGTGGTGGCG GTAGCGGAGGTGGTGGATCTGAGGTGCA
	scFv of 147(H3 807)	VL	DIVMTQSPLSLPVTP GEPASISCRSSKSLL HSQGITYLYWYLQK PGQSPQLLIY**QVSN** **LAS**GVPDRFSGSGS GTDFTLKISRVEAED VGVYYCAQYLELP WTFGCGTKVEIKR (SEQ ID NO:536)	
		Linker	GGGGSGGGGSGGG GSGGGGS (SEQ ID NO:531)	
		VH	EVQLVQSGAEVKKP GASVKVSCKAS**GY** **TFTNYWLG**WIKQA PGQCLEWIG**DIYPG** **GDYIVYNEKFKG**K ATLTADTSISTAYM ELSRLRSDDTAVYY CAR**PNLPKDH**WGQ GTTVTVSS* (SEQ ID NO:537)	

				GCTGGTGCAGAGCGGAGCAGAGGTGAAG AAGCCAGGGGCCAGCGTGAAGGTGAGCT GTAAGGCTAGTGGGTACACATTTACAAAC TATTGGCTGGGATGGATTAAGCAGGCCCC AGGCCAAtgcCTGGAGTGGATAGGAGACA TATACCCCGGAGGAGACTATATCGTGTAC AACGAGAAGTTCAAGGGCAAGGCCACAC TCACCGCTGATACAAGCATCAGCACCGCC TACATGGAGCTGAGCCGACTGAGAAGCG ACGACACAGCAGTGTATTACTGCGCCAG ACCCAACCTGCCCAAGGACCACTGGGGA CAAGGCACCACCGTGACCGTGAGCAGCtg a (SEQ ID NO:538)
Light chain	Light chain of H12		DIQMTQSPSSLSASV GDRVTITCKASQDV TPAVAWYQQKPGK APKLLIYSTSSRYTG VPSRFSGSGSGTDFT FTISSLQPEDIATYY CQQHYTTPLTFGQG TKLEIKRTVAAPSVF IFPPSDEQLKSGTAS VVCLLNNFYPREAK VQWKVDNALQSGN SQESVTEQDSKDST YSLSSTLTLSKADYE KHKVYACEVTHQG LSSPVTKSFNRGEC* (SEQ ID NO:534)	GACATCCAGATGACCCAGAGCCCTAGCA GCCTGAGCGCTAGCGTGGGCGACAGGGT GACCATCACCTGCAAGGCCAGCCAGGAT GTGACCCCTGCCGTGGCCTGGTACCAGCA GAAGCCCGGCAAGGCCCCCAAGCTGCTG ATCTACAGCACCAGCAGCAGGTACACCG GCGTGCCCAGCAGGTTTAGCGGAAGCGG CAGCGGCACCGACTTCACCTTCACCATCA GCAGCCTGCAGCCCGAGGACATCGCCAC CTACTACTGCCAGCAGCACTACACCACCC CTCTGACCTTCGGCCAGGGCACCAAGCTG GAGATCAAGAGAACCGTGGCCGCTCCCT CCGTGTTCATCTTCCCACCATCTGACGAG CAGCTGAAGTCCGGCACCGCTTCTGTCGT GTGCCTGCTGAACAACTTCTACCCTCGGG AAGCCAAGGTGCAGTGGAAGGTGGACAA TGCCCTGCAGTCCGGCAACTCCCAAGAGT CTGTGACCGAGCAGGACTCCAAGGACAG CACCTACTCCCTGTCCTCTACCCTGACCCT GTCCAAGGCCGACTACGAGAAGCACAAG GTGTACGCCTGCGAAGTGACCCACCAGG GACTGTCTAGCCCCGTGACCAAGTCCTTC AACAGAGGCGAGTGCTGA (SEQ ID NO:535)
B6x147 (bispecific antibody comprising the anti-PD-L1 B6 clone in IgG form and the anti-LAG3 147 clone in scFv form)				
			Amino acid sequence (N'→C')	Nucleotide Sequence (5'→3')
Heavy Chain	Heavy chain of B6		EVQLVESGGGLVQP GGSLRLSCAASGFT FSSYDMSWVRQAP GKSLEWVATISDAG GYIYYRDSVKGRFTI SRDNAKNSLYLQM NSLRDEDTAVYICA RELPWRYALDYWG QGTTVTVSSASTKG PSVFPLAPSSKSTSG GTAALGCLVKDYFP EPVTVSWNSGALTS GVHTFPAVLQSSGL YSLSSVVTVPSSSLG TQTYICNVNHKPSN TKVDKKVEPKSCDK THTCPPCPAPELLGG	GAAGTGCAGCTGGTTGAATCTGGCGGCG GATTGGTTCAGCCTGGCGGATCTCTGAGA CTGTCTTGTGCCGCCTCCGGCTTCACCTTC TCCAGCTACGATATGTCCTGGGTCCGACA GGCCCCTGGCAAGTCTTTGGAATGGGTCG CCACCATCTCTGACGCTGGCGGCTACATC TACTACCGGGACTCTGTGAAGGGCAGATT CACCATCAGCCGGGACAACGCCAAGAAC TCCCTGTACCTGCAGATGAACAGCCTGCG CGACGAGGATACCGCCGTGTACATCTGTG CTAGAGAGCTGCCTTGGAGATACGCCCTG GATTATTGGGGCCAGGGCACCACAGTGA CCGTGTCCTCTGCTTCTACCAAGGGACCC AGCGTGTTCCCTCTGGCTCCTTCCAGCAA GTCTACCTCTGGCGGAACAGCTGCTCTGG GCTGCCTGGTCAAGGACTACTTTCCTGAG CCTGTGACAGTGTCCTGGAACTCTGGCGC

			PSVFLFPPKPKDTLM ISRTPEVTCVVVDVS HEDPEVKFNWYVD GVEVHNAKTKPREE QYASTYRVVSVLTV LHQDWLNGKEYKC KVSNKALPAPIEKTI SKAKGQPREPQVYT LPPSREEMTKNQVS LTCLVKGFYPSDIA VEWESNGQPENNY KTTPPVLDSDGSFFL YSKLTVDKSRWQQ GNVFSCSVMHEALH NHYTQKSLSLSPGK (SEQ ID NO:539)	TCTGACATCTGGCGTGCACACCTTTCCAG CAGTGCTGCAGTCCTCCGGCCTGTACTCT CTGTCCTCTGTCGTGACCGTGCCTTCCAG CTCTCTGGGCACCCAGACCTACATCTGCA ACGTGAACCACAAGCCCTCCAACACCAA GGTGGACAAGAAGGTGGAACCCAAGTCC TGCGACAAGACCCACACCTGTCCTCCATG TCCTGCTCCAGAACTGCTGGGCGGACCCT CCGTGTTCCTGTTCCCTCCAAAGCCTAAG GACACCCTGATGATCTCCCGGACCCCTGA AGTGACCTGCGTGGTGGTGGATGTGTCCC ACGAGGATCCCGAAGTGAAGTTCAATTG GTACGTGGACGGCGTGGAAGTGCACAAC GCCAAGACCAAGCCTAGAGAGGAACAGT ACgccTCCACCTACCGGGTGGTGTCCGTGC TGACCGTTCTGCACCAGGATTGGCTGAAC GGCAAAGAGTACAAGTGCAAGGTGTCCA ACAAGGCCCTGCCTGCCCCTATCGAAAAG ACCATCTCTAAGGCCAAGGGCCAGCCCC GGGAACCTCAAGTGTACACCTTGCCTCCC AGCCGGGAAGAGATGACCAAGAACCAGG TGTCCCTGACCTGCCTGGTTAAGGGCTTC TACCCCTCCGATATCGCCGTGGAATGGGA GTCTAATGGCCAGCCTGAGAACAACTAC AAGACCACACCTCCTGTGCTGGACTCCGA CGGCTCATTCTTCCTGTACTCCAAGCTGA CCGTGGACAAGTCCAGATGGCAGCAGGG CAACGTGTTCTCCTGCTCCGTGATGCACG AGGCCCTGCACAATCACTACACCCAGAA GTCCCTGTCTCTGTCCCCTGGCAAAGGCT CCGGATCTGGTTCTGGATCCGGAAGCGGT TCTGGCAGCGGCTCTGGATCTGACATCGT GATGACCCAGTCTCCACTGAGCCTGCCTG TGACACCTGGCGAGCCTGCTTCCATCTCC TGCCGGTCCTCTAAGTCCCTGCTGCACTC TAACGGCATCACCTACCTGTACTGGTATC TGCAGAAGCCCGGCCAGTCTCCTCAGCTG CTGATCTACCAGGTGTCCAACCTGGCTTC TGGCGTGCCCGATAGATTCTCCGGTAGCG GATCTGGAACCGACTTCACCCTGAAGATC TCCAGAGTGGAAGCCGAGGACGTGGGCG TGTACTACTGTGCCCAGAACCTGGAACTG CCCTGGACCTTTGGCTGTGGCACCAAGGT GGAAATCAAGAGAGGCGGCGGAGGATCT GGCGGAGGTGGAAGCGGAGGCGGAGGA AGCGGTGGCGGCGGATCTGAAGTTCAGTT GGTTCAGTCTGGCGCCGAAGTGAAGAAA CCTGGCGCCTCTGTGAAGGTGTCCTGCAA GGCTTCCGGCTACACCTTTACCAACTACT GGCTCGGCTGGATCAAGCAGGCCCCTGG ACAGTGTCTGGAATGGATCGGCGACATCT ACCCTGGCGGCGACTACATCAACTACAAC GAGAAGTTCAAGGGCAAAGCTACCCTGA CCGCCGACACCTCTATCTCCACCGCCTAC ATGGAACTGTCCCGGCTGAGATCTGACGA CACCGCCGTGTACTATTGCGCCAGACCTA ACCTGCCTGGCGACTATTGGGGCCAGGGC ACAACAGTGACCGTGTCCTCTTAA (SEQ ID NO:540)
	Linker		GSGSGSGSGSGSGS GSGS (SEQ ID NO:529)	
	scFv of 147	VL	DIVMTQSPLSLPVTP GEPASISC**RSSKSLL HSNGITYLY**WYLQ KPGQSPQLLIY**QVS NLAS**GVPDRFSGSG SGTDFTLKISRVEAE DVGVYYC**AQNLEL PWT**FGCGTKVEIKR (SEQ ID NO:530)	
		Linker	GGGGSGGGGSGGG GSGGGGS (SEQ ID NO:531)	
		VH	EVQLVQSGAEVKKP GASVKVSCKAS**GY TFTNYWLG**WIKQA PGQCLEWIG**DIYPG GDYINYNEKFKG**K ATLTADTSISTAYM ELSRLRSDDTAVYY CAR**PNLPGDY**WGQ GTTVTVSS* (SEQ ID NO:532)	

Light chain	Light chain of B6	DIQMTQSPSSLSASV GDRVTITCKASQDV TPAVAWYQQKPGK APKLLIYSTSSRYTG VPSRFSGSGSGTDFT FTISSLQPEDIATYY CQQHYTTPLTFGQG TKLEIKRTVAAPSVF IFPPSDEQLKSGTAS VVCLLNNFYPREAK VQWKVDNALQSGN SQESVTEQDSKDST YSLSSTLTLSKADYE KHKVYACEVTHQG LSSPVTKSFNRGEC* (SEQ ID NO:534)	GACATCCAGATGACCCAGAGCCCTAGCA GCCTGAGCGCTAGCGTGGGCGACAGGGT GACCATCACCTGCAAGGCCAGCCAGGAT GTGACCCCTGCCGTGGCCTGGTACCAGCA GAAGCCCGGCAAGGCCCCCAAGCTGCTG ATCTACAGCACCAGCAGCAGGTACACCG GCGTGCCCAGCAGGTTTAGCGGAAGCGG CAGCGGCACCGACTTCACCTTCACCATCA GCAGCCTGCAGCCCGAGGACATCGCCAC CTACTACTGCCAGCAGCACTACACCACCC CTCTGACCTTCGGCCAGGGCACCAAGCTG GAGATCAAGAGAACCGTGGCCGCTCCCT CCGTGTTCATCTTCCCACCATCTGACGAG CAGCTGAAGTCCGGCACCGCTTCTGTCGT GTGCCTGCTGAACAACTTCTACCCTCGGG AAGCCAAGGTGCAGTGGAAGGTGGACAA TGCCCTGCAGTCCGGCAACTCCCAAGAGT CTGTGACCGAGCAGGACTCCAAGGACAG CACCTACTCCCTGTCCTCTACCCTGACCCT GTCCAAGGCCGACTACGAGAAGCACAAG GTGTACGCCTGCGAAGTGACCCACCAGG GACTGTCTAGCCCCGTGACCAAGTCCTTC AACAGAGGCGAGTGCTGA (SEQ ID NO:535)
B6x147(H3807) (bispecific antibody comprising the anti-PD-L1 B6 clone in IgG form and the anti-LAG3 147(H3807) clone in scFv form)			
		Amino acid sequence (N'→C')	Nucleotide Sequence (5'→3')
Heavy Chain	Heavy chain of B6	EVQLVESGGGLVQP GGSLRLSCAASGFT FSSYDMSWVRQAP GKSLEWVATISDAG GYIYYRDSVKGRFTI SRDNAKNSLYLQM NSLRDEDTAVYICA RELPWRYALDYWG QGTTVTVSSASTKG PSVFPLAPSSKSTSG GTAALGCLVKDYFP EPVTVSWNSGALTS GVHTFPAVLQSSGL YSLSSVVTVPSSSLG TQTYICNVNHKPSN TKVDKKVEPKSCDK THTCPPCPAPELLGG PSVFLFPPKPKDTLM ISRTPEVTCVVVDVS HEDPEVKFNWYVD GVEVHNAKTKPREE QYASTYRVVSVLTV LHQDWLNGKEYKC KVSNKALPAPIEKTI SKAKGQPREPQVYT LPPSREEMTKNQVS LTCLVKGFYPSDIA VEWESNGQPENNY KTTPPVLDSDGSFFL YSKLTVDKSRWQQ	GAAGTGCAGCTGGTTGAATCTGGCGGCG GATTGGTTCAGCCTGGCGGATCTCTGAGA CTGTCTTGTGCCGCCTCCGGCTTCACCTTC TCCAGCTACGATATGTCCTGGGTCCGACA GGCCCCTGGCAAGTCTTTGGAATGGGTCG CCACCATCTCTGACGCTGGCGGCTACATC TACTACCGGGACTCTGTGAAGGGCAGATT CACCATCAGCCGGGACAACGCCAAGAAC TCCCTGTACCTGCAGATGAACAGCCTGCG CGACGAGGATACCGCCGTGTACATCTGTG CTAGAGAGCTGCCTTGGAGATACGCCCTG GATTATTGGGGCCAGGGCACCACAGTGA CCGTGTCCTCTGCTTCTACCAAGGGACCC AGCGTGTTCCCTCTGGCTCCTTCCAGCAA GTCTACCTCTGGCGGAACAGCTGCTCTGG GCTGCCTGGTCAAGGACTACTTTCCTGAG CCTGTGACAGTGTCCTGGAACTCTGGCGC TCTGACATCTGGCGTGCACACCTTTCCAG CAGTGCTGCAGTCCTCCGGCCTGTACTCT CTGTCCTCTGTCGTGACCGTGCCTTCCAG CTCTCTGGGCACCCAGACCTACATCTGCA ACGTGAACCACAAGCCCTCCAACACCAA GGTGGACAAGAAGGTGGAACCCAAGTCC TGCGACAAGACCCACACCTGTCCTCCATG TCCTGCTCCAGAACTGCTGGGCGGACCCT CCGTGTTCCTGTTCCCTCCAAAGCCTAAG GACACCCTGATGATCTCCCGGACCCCTGA AGTGACCTGCGTGGTGGTGGATGTGTCCC ACGAGGATCCCGAAGTGAAGTTCAATTG GTACGTGGACGGCGTGGAAGTGCACAAC

			GNVFSCSVMHEALH NHYTQKSLSLSPGK (SEQ ID NO:539)	GCCAAGACCAAGCCTAGAGAGGAACAGT ACgccTCCACCTACCGGGTGGTGTCCGTGC TGACCGTTCTGCACCAGGATTGGCTGAAC GGCAAAGAGTACAAGTGCAAGGTGTCCA ACAAGGCCCTGCCTGCCCCTATCGAAAAG ACCATCTCTAAGGCCAAGGGCCAGCCCC GGGAACCTCAAGTGTACACCTTGCCTCCC AGCCGGGAAGAGATGACCAAGAACCAGG TGTCCCTGACCTGCCTGGTTAAGGGCTTC TACCCCTCCGATATCGCCGTGGAATGGGA GTCTAACGGCCAGCCCGAGAACAACTAC AAGACCACCCCTCCTGTGCTGGACTCCGA CGGCTCATTCTTCCTGTACTCCAAGCTGA CCGTGGACAAGTCTCGGTGGCAGCAGGG CAACGTGTTCTCCTGCTCTGTGATGCACG AGGCCCTGCACAACCACTACACCCAGAA GTCCCTGTCCCTGTCTCCCGGCAAAGGCT CCGGATCTGGTTCTGGATCCGGAAGCGGT TCTGGCAGCGGCTCTGGATCTGACATTGT GATGACCCAGAGCCCCCTGAGCCTCCCCG TGACCCCTGGAGAACCCGCCAGCATAAG CTGCAGATCCTCCAAAAGCCTGCTGCACT CCCAGGGAATAACCTACCTGTATTGGTAC CTGCAGAAACCCGGCCAATCCCCCCAACT CCTGATATACCAAGTGTCCAACCTGGCCT CCGGCGTGCCCGACAGATTCTCCGGCTCC GGCAGCGGTACCGACTTCACCCTCAAAAT CTCCAGAGTGGAAGCAGAAGACGTCGGC GTGTACTACTGCGCCCAGTACCTGGAACT GCCCTGGACCTTCGGCtgtGGCACCAAGGT GGAAATCAAGAGAGGCGGCGGAGGAAGC GGAGGCGGCGGTTCTGGTGGTGGCGGTA GCGGAGGTGGTGGATCTGAGGTGCAGCT GGTGCAGAGCGGAGCAGAGGTGAAGAAG CCAGGGGCCAGCGTGAAGGTGAGCTGTA AGGCTAGTGGGTACACATTTACAAACTAT TGGCTGGGATGGATTAAGCAGGCCCCAG GCCAAtgcCTGGAGTGGATAGGAGACATA TACCCCGGAGGAGACTATATCGTGTACAA CGAGAAGTTCAAGGGCAAGGCCACACTC ACCGCTGATACAAGCATCAGCACCGCCTA CATGGAGCTGAGCCGACTGAGAAGCGAC GACACAGCAGTGTATTACTGCGCCAGACC CAACCTGCCCAAGGACCACTGGGGACAA GGCACCACCGTGACCGTGAGCAGCtga (SEQ ID NO:541)
		scFv of 147(H3 807)	VL	DIVMTQSPLSLPVTP GEPASISCRSSKSLL HSQGITYLYWYLQK PGQSPQLLIY**QVSN LAS**GVPDRFSGSGS GTDFTLKISRVEAED VGVYYCAQYLELP WTFGCGTKVEIKR (SEQ ID NO:536)
			Linker	GGGGSGGGGSGGG GSGGGGS (SEQ ID NO:531)
			VH	EVQLVQSGAEVKKP GASVKVSCKAS**GY TFTNYWLG**WIKQA PGQCLEWIG**DIYPG GDYIVYNEKFKG**K ATLTADTSISTAYM ELSRLRSDDTAVYY CAR**PNLPKDH**WGQ GTTVTVSS* (SEQ ID NO:537)
Light chain	Light chain of B6			DIQMTQSPSSLSASV GDRVTITCKASQDV TPAVAWYQQKPGK APKLLIYSTSSRYTG VPSRFSGSGSGTDFT FTISSLQPEDIATYY CQQHYTTPLTFGQG TKLEIKRTVAAPSVF IFPPSDEQLKSGTAS VVCLLNNFYPREAK VQWKVDNALQSGN SQESVTEQDSKDST YSLSSTLTLSKADYE	GACATCCAGATGACCCAGAGCCCTAGCA GCCTGAGCGCTAGCGTGGGCGACAGGGT GACCATCACCTGCAAGGCCAGCCAGGAT GTGACCCCTGCCGTGGCCTGGTACCAGCA GAAGCCCGGCAAGGCCCCCAAGCTGCTG ATCTACAGCACCAGCAGCAGGTACACCG GCGTGCCCAGCAGGTTTAGCGGAAGCGG CAGCGGCACCGACTTCACCTTCACCATCA GCAGCCTGCAGCCCGAGGACATCGCCAC CTACTACTGCCAGCAGCACTACACCACCC CTCTGACCTTCGGCCAGGGCACCAAGCTG GAGATCAAGAGAACCGTGGCCGCTCCCT CCGTGTTCATCTTCCCACCATCTGACGAG CAGCTGAAGTCCGGCACCGCTTCTGTCGT

		KHKVYACEVTHQG LSSPVTKSFNRGEC* (SEQ ID NO:534)	GTGCCTGCTGAACAACTTCTACCCTCGGG AAGCCAAGGTGCAGTGGAAGGTGGACAA TGCCCTGCAGTCCGGCAACTCCCAAGAGT CTGTGACCGAGCAGGACTCCAAGGACAG CACCTACTCCCTGTCCTCTACCCTGACCCT GTCCAAGGCCGACTACGAGAAGCACAAG GTGTACGCCTGCGAAGTGACCCACCAGG GACTGTCTAGCCCCGTGACCAAGTCCTTC AACAGAGGCGAGTGCTGA (SEQ ID NO:535)

[Table 33] Bispecific antibody comprising the anti-LAG3 clone in IgG form and the anti-PD-L1 clone in scFv form (LAG3xPD-L1)

147xH12 (bispecific antibody comprising the anti-LAG3 147 clone in IgG form and the anti-PD-L1 H12 clone in scFv form)				
			Amino acid sequence (N'→C')	Nucleotide Sequence (5'→3')
Heavy Chain	Heavy chain of 147		EVQLVQSGAEVKKP GASVKVSCKAS**GY** **TFTNYWLG**WIKQA PGQGLEWIG**DIYPG** **GDYINYNEKFKG**K ATLTADTSISTAYM ELSRLRSDDTAVYY CAR**PNLPGDY**WGQ GTTVTVSSASTKGP SVFPLAPCSRSTSES TAALGCLVKDYFPE PVTVSWNSGALTSG VHTFPAVLQSSGLY SLSSVVTVPSSSLGT KTYTCNVDHKPSNT KVDKRVESKYGPPC PPCPAPEFLGGPSVF LFPPKPKDTLMISRT PEVTCVVVDVSQED PEVQFNWYVDGVE VHNAKTKPREEQFN STYRVVSVLTVLHQ DWLNGKEYKCKVS NKGLPSSIEKTISKA KGQPREPQVYTLPP SQEEMTKNQVSLTC LVKGFYPSDIAVEW ESNGQPENNYKTTP PVLDSDGSFFLYSRL TVDKSRWQEGNVF SCSVMHEALHNHY TQKSLSLSLGK (SEQ ID NO:542)	GAGGTGCAGCTGGTGCAGAGCGGAGCAG AGGTGAAGAAGCCAGGGGCCAGCGTGAA GGTGAGCTGTAAGGCTAGTGGGTACACA TTTACAAACTATTGGCTGGGATGGATTAA GCAGGCCCCAGGCCAAGGACTGGAGTGG ATAGGAGACATATACCCCGGAGGAGACT ATATCAATTACAACGAGAAGTTCAAGGG CAAGGCCACACTCACCGCTGATACAAGC ATCAGCACCGCCTACATGGAGCTGAGCC GACTGAGAAGCGACGACACAGCAGTGTA TTACTGCGCCAGACCCAACCTGCCCGGCG ACTACTGGGGACAAGGCACCACCGTGAC CGTGTCTTCCgctAgcAccAAgggcccctcgtgttcc ctctggccccAtgctcccggtccAcctccgAgtccAccgccgct ctgggctgtctggtgAAggActActtcctgAgcccgtgAccg tgAgctggAActctggcgccctgAcctccggcgtgcAcAcct tccctgccgtgctgcAgtcctccggcctgtActcctgtcctccgtg gtgAccgtgccttcctcctccctgggcAccAAgAcctAcAcc tgcAAcgtggAccAcAAgccttccAAcAccAAggtggA cAAgcgggtggAgtccAAgtAcggccctccttgccctccctg ccctgcccctgAgttcctgggcggAccctccgtgttcctgttcctc ctAAgcctAAggAcAccctgAtgAtctcccggAcccctgA ggtgAcctgcgtggtggtggAcgtgtcccAggAAgAtcctg AggtccAgttcAAttggtAcgtggAtggcgtggAggtgcAc AAcgccAAgAccAAgcctcgggAggAAcAgttcAAct ccAcctAccgggtggtgtctgtgctgAccgtgctgcAccAgg ActggctgAAcggcAAggAAtAcAAgtgcAAggtcAg cAAcAAgggcctgccctcctccAtcgAgAAAAccAtctc cAAggccAAgggccAgcctcgcgAgcctcAggtgtAcAc cctgcctcctAgccAggAAgAgAtgAccAagAAtcAgg tgtccctgAcAtgcctggtgAAgggcttctAcccttccgAtAtc gccgtggAgtgggAgAgcAAcggccAgccAgAgAAc AActAcAAgAccAccctcctgtgctggActccgAcggct ccttcttcctgtActccAggctgAccgtggAcAAgtcccggtg gcAggAAggcAAcgtcttttcctgctccgtgAtgcAcgAgg ccctgcAcAAccActAcAcccAgAAgtccctgtccctgtct ctgggcAAgGGTGGAGGTGGGTCTGGGGGTG GCGGGTCAGGTGGAGGAGGTTCAGACAT CCAGATGACCCAGAGCCCTAGCAGCCTG
	Linker		GGGGSGGGGSGGG GS (SEQ ID NO:543)	
	scFv of H12	VL	DIQMTQSPSSLSASV GDRVTITCKASQDV TPAVAWYQQKPGK	

			APKLLIYSTSSRYTG VPSRFSGSGSGTDFT FTISSLQPEDIATYY CQQHYTTPLTFGCG TKLEIKR (SEQ ID NO:544)	AGCGCTAGCGTGGGCGACAGGGTGACCA TCACCTGCAAGGCCAGCCAGGATGTGAC CCCTGCCGTGGCCTGGTACCAGCAGAAGC CCGGCAAGGCCCCCAAGCTGCTGATCTAC AGCACCAGCAGCAGGTACACCGGCGTGC CCAGCAGGTTTAGCGGAAGCGGCAGCGG CACCGACTTCACCTTCACCATCAGCAGCC TGCAGCCCGAGGACATCGCCACCTACTAC TGCCAGCAGCACTACACCACCCCTCTGAC CTTCGGCtgtGGCACCAAGCTGGAGATCAA GAGAGGTGGAGGCGGCTCAGGGGGGGGT GGATCAGGGGGAGGAGGATCAGGGGGAG GCGGTAGTGAGGTGCAGCTGGTGGAGAG CGGAGGAGGACTGGTGCAACCCGGAGGC AGCCTGAGACTGAGCTGCGCTGCCAGCG GCTTCACCTTCAGCAGCTACGACATGAGC TGGGTGAGACAGGCCCCTGGCAAAtgtCTG GAGTGGGTGGCCACCATCTCCGATGCGG GCGGCTACATCTATTACTCCGACAGCGTG AAGGGCAGGTTCACCATCAGCAGGGACA ACGCCAAGAACAGCCTGTACCTGCAGAT GAACAGCCTGAGGGATGAGGACACCGCC GTGTACATCTGCGCCAGGGAGTTCGGCAA AAGGTACGCCCTGGACTACTGGGGCCAG GGCACAACCGTGACCGTGAGCAGCtga (SEQ ID NO:546)
		Linker	GGGGSGGGGSGGG GSGGGGS (SEQ ID NO:531)	
		VH	EVQLVESGGGLVQP GGSLRLSCAASGFT FSSYDMSWVRQAP GKCLEWVATISDAG GYIYYSDSVKGRFTI SRDNAKNSLYLQM NSLRDEDTAVYICA REFGKRYALDYWG QGTTVTVSS (SEQ ID NO:545)	
Light chain	Light chain of 147		DIVMTQSPLSLPVTP GEPASISC**RSSKSLL HSNGITYLY**WYLQ KPGQSPQLLIY**QVS NLAS**GVPDRFSGSG SGTDFTLKISRVEAE DVGVYYC**AQNLEL PWT**FGGGTKVEIKR TVAAPSVFIFPPSDE QLKSGTASVVCLLN NFYPREAKVQWKV DNALQSGNSQESVT EQDSKDSTYSLSSTL TLSKADYEKHKVY ACEVTHQGLSSPVT KSFNRGEC (SEQ ID NO:547)	GACATTGTGATGACCCAGAGCCCCCTGAG CCTCCCCGTGACCCCTGGAGAACCCGCCA GCATAAGCTGCAGATCCTCCAAAAGCCTG CTGCACTCCAACGGAATAACCTACCTGTA TTGGTACCTGCAGAAACCCGGCCAATCCC CCCAACTCCTGATATACCAAGTGTCCAAC CTGGCCTCCGGCGTGCCCGACAGATTCTC CGGCTCCGGCAGCGGTACCGACTTCACCC TCAAAATCTCCAGAGTGGAAGCAGAAGA CGTCGGCGTGTACTACTGCGCCCAGAATC TGGAACTGCCCTGGACCTTCGGCGGCGGC ACCAAGGTGGAAATCAAGAGAACCGTGG CCGCTCCCTCCGTGTTCATCTTCCCACCAT CTGACGAGCAGCTGAAGTCCGGCACCGC TTCTGTCGTGTGCCTGCTGAACAACTTCT ACCCTCGGGAAGCCAAGGTGCAGTGGAA GGTGGACAATGCCCTGCAGTCCGGCAACT CCCAAGAGTCTGTGACCGAGCAGGACTC CAAGGACAGCACCTACTCCCTGTCCTCTA CCCTGACCCTGTCCAAGGCCGACTACGAG AAGCACAAGGTGTACGCCTGCGAAGTGA CCCACCAGGGACTGTCTAGCCCCGTGACC AAGTCCTTCAACAGAGGCGAGTGCTGA (SEQ ID NO:548)
147xB6 (bispecific antibody comprising the anti-LAG3 147 clone in IgG form and the anti-PD-L1 B6 clone in scFv form)				
			Amino acid sequence (N'→C')	Nucleotide Sequence (5'→3')
Heavy Chain	Heavy chain of 147		EVQLVQSGAEVKKP GASVKVSCKAS**GY TFTNYWLG**WIKQA PGQGLEWIG**DIYPG GDYINYNEKFKG**K	GAGGTGCAGCTGGTGCAGAGCGGAGCAG AGGTGAAGAAGCCAGGGGCCAGCGTGAA GGTGAGCTGTAAGGCTAGTGGGTACACA TTTACAAACTATTGGCTGGGATGGATTAA GCAGGCCCCAGGCCAAGGACTGGAGTGG

			ATLTADTSISTAYM ELSRLRSDDTAVYY CAR**PNLPGDY**WGQ GTTVTVSSASTKGP SVFPLAPCSRSTSES TAALGCLVKDYFPE PVTVSWNSGALTSG VHTFPAVLQSSGLY SLSSVVTVPSSSLGT KTYTCNVDHKPSNT KVDKRVESKYGPPC PPCPAPEFLGGPSVF LFPPKPKDTLMISRT PEVTCVVVDVSQED PEVQFNWYVDGVE VHNAKTKPREEQFN STYRVVSVLTVLHQ DWLNGKEYKCKVS NKGLPSSIEKTISKA KGQPREPQVYTLPP SQEEMTKNQVSLTC LVKGFYPSDIAVEW ESNGQPENNYKTTP PVLDSDGSFFLYSRL TVDKSRWQEGNVF SCSVMHEALHNHY TQKSLSLSLGK (SEQ ID NO:542)	ATAGGAGACATATACCCCGGAGGAGACT ATATCAATTACAACGAGAAGTTCAAGGG CAAGGCCACACTCACCGCTGATACAAGC ATCAGCACCGCCTACATGGAGCTGAGCC GACTGAGAAGCGACGACACAGCAGTGTA TTACTGCGCCAGACCCAACCTGCCCGGCG ACTACTGGGGACAAGGCACCACCGTGAC CGTGTCTTCCgctAgcAccAAgggcccctcgtgttcc ctctggccccAtgctcccggtccAcctccgAgtccAccgccgct ctgggctgtctggtgAAggActActtccctgAgcccgtgAccg tgAgctggAActctggcgccctgAcctccggcgtgcAcAcct tccctgccgtgctgcAgtcctccggcctgtActcctgtcctccgtg gtgAccgtgccttcctcctccctgggcAccAAgAcctAcAcc tgcAAcgtggAccAcAAgccttccAAcAccAAggtggA cAAgcgggtggAgtccAAgtAcggccctccttgccctcctg ccctgcccctgAgttcctgggcggAccctccgtgttcctgttcctc ctAAgcctAAggAcAccctgAtgAtctcccggAccccctgA ggtgAcctgcgtggtggtggAcgtgtcccAggAAgAtcctg AggtccAgttcAAttggtAcgtggAtggcgtggAggtgcAc AAcgccAAgAccAAgcctcgggAggAAcAgttcAAct ccAcctAccgggtggtgtctgtgctgAccgtgctgcAccAgg ActggctgAAcggcAAggAAtAcAAgtgcAAggtcAg cAAcAAgggcctgccctcctccAtcgAgAAAAccAtctc cAAggccAAgggccAgcctcgcgAgcctcAggtgtAcAc cctgcctcctAgccAggAAgAgAtgAccAagAAtcAgg tgtccctgAcAtgcctggtgAAgggcttctAcccttccgAtAT CGCCGTGGAATGGGAGAGCAATGGCCAG CCTGAGAACAACTACAAGACAACCCCTC CTGTGCTGGACTCCGACGGCTCCTTCTTT CTGTACTCTCGCCTGACCGTGGACAAGTC CAGATGGCAAGAGGGCAACGTGTTCTCCT GCTCCGTGATGCACGAGGCCCTGCACAAT CACTACACCCAGAAGTCCCTGTCTCTGTC CCTCGGAAAAGGCGGCGGAGGATCTGGC GGAGGCGGTAGCGGTGGTGGCGGATCTG ATATTCAGATGACCCAGTCTCCTTCCAGC CTGTCCGCTTCTGTGGGCGACAGAGTGAC CATCACATGCAAGGCCAGCCAGGATGTG ACCCCTGCTGTGGCTTGGTATCAGCAGAA GCCTGGCAAGGCCCCTAAGCTGCTGATCT ACTCCACCTCCTCCAGATACACAGGCGTG CCCTCCAGATTCTCCGGCTCTGGCTCTGG CACCGACTTTACCTTTACAATCTCCAGCC TGCAGCCTGAGGACATTGCCACCTACTAC TGCCAGCAGCACTACACCACACCTCTGAC CTTTGGCTGCGGCACCAAGCTGGAAATCA AGAGAGGTGGCGGAGGAAGCGGAGGCG GCGGTTCAGGTGGCGGTGGTTCAGGCGGT GGTGGATCTGAAGTTCAGCTGGTGGAATC TGGCGGCGGATTGGTTCAACCAGGCGGCT CTCTGAGACTGTCTTGTGCCGCTTCCGGC TTCACCTTCTCCAGCTACGACATGTCCTG GGTCCGACAGGCCCCTGGAAAGTGTCTG GAATGGGTCGCCACCATCTCTGACGCTGG CGGCTACATCTACTACCGGGACTCTGTGA AGGGCAGATTCACCATCAGCCGGGACAA TGCCAAGAACTCCCTGTACCTGCAGATGA ACAGTCTGCGCGACGAGGACACCGCCGT GTACATCTGTGCTAGAGAGCTGCCTTGGC
	Linker		GGGGSGGGGSGGG GS (SEQ ID NO:543)	
	scFv of B6	VL	DIQMTQSPSSLSASV GDRVTITCKASQDV TPAVAWYQQKPGK APKLLIYSTSSRYTG VPSRFSGSGSGTDFT FTISSLQPEDIATYY CQQHYTTPLTFGCG TKLEIKR (SEQ ID NO:544)	
		Linker	GGGGSGGGGSGGG GSGGGGS (SEQ ID NO:531)	
		VH	EVQLVESGGGLVQP GGSLRLSCAASGFT FSSYDMSWVRQAP GKCLEWVATISDAG GYIYYRDSVKGRFTI SRDNAKNSLYLQM NSLRDEDTAVYICA RELPWRYALDYWG QGTTVTVSS* (SEQ ID NO:549)	

			GCTACGCCCTGGATTATTGGGGCCAGGGC ACAACAGTGACAGTGTCCTCTTGA (SEQ ID NO:550)
Light chain	Light chain of 147	DIVMTQSPLSLPVTP GEPASISC**RSSKSLL** **HSNGITYLY**WYLQ KPGQSPQLLIY**QVS** **NLAS**GVPDRFSGSG SGTDFTLKISRVEAE DVGVYYC**AQNLEL** **PWT**FGGGTKVEIKR TVAAPSVFIFPPSDE QLKSGTASVVCLLN NFYPREAKVQWKV DNALQSGNSQESVT EQDSKDSTYSLSSTL TLSKADYEKHKVY ACEVTHQGLSSPVT KSFNRGEC (SEQ ID NO:547)	GACATTGTGATGACCCAGAGCCCCCTGAG CCTCCCCGTGACCCCTGGAGAACCCGCCA GCATAAGCTGCAGATCCTCCAAAAGCCTG CTGCACTCCAACGGAATAACCTACCTGTA TTGGTACCTGCAGAAACCCGGCCAATCCC CCCAACTCCTGATATACCAAGTGTCCAAC CTGGCCTCCGGCGTGCCCGACAGATTCTC CGGCTCCGGCAGCGGTACCGACTTCACCC TCAAAATCTCCAGAGTGGAAGCAGAAGA CGTCGGCGTGTACTACTGCGCCCAGAATC TGGAACTGCCCTGGACCTTCGGCGGCGGC ACCAAGGTGGAAATCAAGAGAACCGTGG CCGCTCCCTCCGTGTTCATCTTCCCACCAT CTGACGAGCAGCTGAAGTCCGGCACCGC TTCTGTCGTGTGCCTGCTGAACAACTTCT ACCCTCGGGAAGCCAAGGTGCAGTGGAA GGTGGACAATGCCCTGCAGTCCGGCAACT CCCAAGAGTCTGTGACCGAGCAGGACTC CAAGGACAGCACCTACTCCCTGTCCTCTA CCCTGACCCTGTCCAAGGCCGACTACGAG AAGCACAAGGTGTACGCCTGCGAAGTGA CCCACCAGGGACTGTCTAGCCCCGTGACC AAGTCCTTCAACAGAGGCGAGTGCTGA (SEQ ID NO:548)
147(H3807)xH12 (bispecific antibody comprising the anti-LAG3 147(H3807) clone in IgG form and the anti-PD-L1 H12 clone in scFv form)			
		Amino acid sequence (N'→C')	Nucleotide Sequence (5'→3')
Heavy Chain	Heavy chain of 147(H3807)	EVQLVQSGAEVKKP GASVKVSCKAS**GY** **TFTNYWLG**WIKQA PGQGLEWIG**DIYPG** **GDYIVYNEKFKG**K ATLTADTSISTAYM ELSRLRSDDTAVYY CAR**PNLPKDH**WGQ GTTVTVSSASTKGP SVFPLAPCSRSTSES TAALGCLVKDYFPE PVTVSWNSGALTSG VHTFPAVLQSSGLY SLSSVVTVPSSSLGT KTYTCNVDHKPSNT KVDKRVESKYGPPC PPCPAPEFLGGPSVF LFPPKPKDTLMISRT PEVTCVVVDVSQED PEVQFNWYVDGVE VHNAKTKPREEQFN STYRVVSVLTVLHQ DWLNGKEYKCKVS NKGLPSSIEKTISKA KGQPREPQVYTLPP SQEEMTKNQVSLTC LVKGFYPSDIAVEW ESNGQPENNYKTTP	GAGGTGCAGCTGGTGCAGAGCGGAGCAG AGGTGAAGAAGCCAGGGGCCAGCGTGAA GGTGAGCTGTAAGGCTAGTGGGTACACA TTTACAAACTATTGGCTGGGATGGATTAA GCAGGCCCCAGGCCAAGGACTGGAGTGG ATAGGAGACATATACCCCGGAGGAGACT ATATCgtgTACAACGAGAAGTTCAAGGGC AAGGCCACACTCACCGCTGATACAAGCA TCAGCACCGCCTACATGGAGCTGAGCCG ACTGAGAAGCGACGACACAGCAGTGTAT TACTGCGCCAGACCCAACCTGCCCAAGG ACCACTGGGGACAAGGCACCACCGTGAC CGTGTCTTCCgctAgcAccAAgggcccctcgtgttcc ctctggccccAtgctcccggtccAcctccgAgtccAccgccgct ctgggctgtctggtgAAggActActtccctgAgcccgtgAccg tgAgctggAActctggcgccctgAcctccggcgtgcAcAcct tccctgccgtgctgcAgtcctccggcctgtActccctgtcctccgtg gtgAccgtgccttcctcctccctgggcAccAAgAcctAcAcc tgcAAcgtggAccAcAAgccttccAAcAccAAggtggA cAAgcgggtggAgtccAAgtAcggccctccttgccctccctg ccctgcccctgAgttcctgggcggAccctccgtgttcctgttccctc ctAAgcctAAggAcAccctgAtgAtctcccggAcccctgA ggtgAcctgcgtggtggtggAcgtgtcccAggAAgAtcctg AggtccAgttcAAttggtAcgtggAtggcgtggAggtgcAc AAcgccAAgAccAAgcctcgggAggAAcAgttcAAct ccAcctAccgggtggtgtctgtgctgAccgtgctgcAccAgg ActggctgAAcggcAAggAAtAcAAgtgcAAggtcAg cAAcAAgggcctgccctcctccAtcgAgAAAAccAtctc

			PVLDSDGSFFLYSRL TVDKSRWQEGNVF SCSVMHEALHNHY TQKSLSLSLGK (SEQ ID NO:551)	cAAggccAAgggccAgcctcgcgAgcctcAggtgtAcAc cctgcctcctAgccAggAAgAgAtgAccAagAAtcAgg tgtccctgAcAtgcctggtgAAgggcttctAcccttccgAtAtc gccgtggAgtgggAgAgcAAcggccAgccAgAgAAc AActAcAAgAccAcccctcctgtgctggActccgAcggct ccttcttcctgtActccAggctgAccgtggAcAAgtcccggtg gcAggAAggcAAcgtcttttcctgctccgtgAtgcAcgAgg ccctgcAcAAccActAcAcccAgAAgtccctgtcctgtct ctgggcAAgGGTGGAGGTGGGTCTGGGGGTG GCGGGTCAGGTGGAGGAGGTTCAGACAT CCAGATGACCCAGAGCCCTAGCAGCCTG AGCGCTAGCGTGGGCGACAGGGTGACCA TCACCTGCAAGGCCAGCCAGGATGTGAC CCCTGCCGTGGCCTGGTACCAGCAGAAGC CCGGCAAGGCCCCCAAGCTGCTGATCTAC AGCACCAGCAGCAGGTACACCGGCGTGC CCAGCAGGTTTAGCGGAAGCGGCAGCGG CACCGACTTCACCTTCACCATCAGCAGCC TGCAGCCCGAGGACATCGCCACCTACTAC TGCCAGCAGCACTACACCACCCCTCTGAC CTTCGGCtgtGGCACCAAGCTGGAGATCAA GAGAGGTGGAGGCGGCTCAGGGGGGGGT GGATCAGGGGGAGGAGGATCAGGGGGAG GCGGTAGTGAGGTGCAGCTGGTGGAGAG CGGAGGAGGACTGGTGCAACCCGGAGGC AGCCTGAGACTGAGCTGCGCTGCCAGCG GCTTCACCTTCAGCAGCTACGACATGAGC TGGGTGAGACAGGCCCCTGGCAAAtgtCTG GAGTGGGTGGCCACCATCTCCGATGCGG GCGGCTACATCTATTACTCCGACAGCGTG AAGGGCAGGTTCACCATCAGCAGGGACA ACGCCAAGAACAGCCTGTACCTGCAGAT GAACAGCCTGAGGGATGAGGACACCGCC GTGTACATCTGCGCCAGGGAGTTCGGCAA AAGGTACGCCCTGGACTACTGGGGCCAG GGCACAACCGTGACCGTGAGCAGCtga (SEQ ID NO:552)
	Linker		GGGGSGGGGSGGG GS (SEQ ID NO:543)	
	scFv of H12	VL	DIQMTQSPSSLSASV GDRVTITCKASQDV TPAVAWYQQKPGK APKLLIYSTSSRYTG VPSRFSGSGSGTDFT FTISSLQPEDIATYY CQQHYTTPLTFGCG TKLEIKR (SEQ ID NO:544)	
		Linker	GGGGSGGGGSGGG GSGGGGS (SEQ ID NO:531)	
		VH	EVQLVESGGGLVQP GGSLRLSCAASGFT FSSYDMSWVRQAP GKCLEWVATISDAG GYIYYSDSVKGRFTI SRDNAKNSLYLQM NSLRDEDTAVYICA REFGKRYALDYWG QGTTVTVSS (SEQ ID NO:545)	
Light chain	Light chain of 147(H3807)		DIVMTQSPLSLPVTP GEPASISC**RSSKSLL HSQGITYLY**WYLQ KPGQSPQLLIY**QVS NLAS**GVPDRFSGSG SGTDFTLKISRVEAE DVGVYYCAQYLEL PWTFGGGTKVEIKR TVAAPSVFIFPPSDE QLKSGTASVVCLLN NFYPREAKVQWKV DNALQSGNSQESVT EQDSKDSTYSLSSTL TLSKADYEKHKVY ACEVTHQGLSSPVT KSFNRGEC* (SEQ ID NO:553)	GACATTGTGATGACCCAGAGCCCCCTGAG CCTCCCCGTGACCCCTGGAGAACCCGCCA GCATAAGCTGCAGATCCTCCAAAAGCCTG CTGCACTCCcagGGAATAACCTACCTGTAT TGGTACCTGCAGAAACCCGGCCAATCCCC CCAACTCCTGATATACCAAGTGTCCAACC TGGCCTCCGGCGTGCCCGACAGATTCTCC GGCTCCGGCAGCGGTACCGACTTCACCCT CAAAATCTCCAGAGTGGAAGCAGAAGAC GTCGGCGTGTACTACTGCGCCCAGtacCTG GAACTGCCCTGGACCTTCGGCGGCGGCAC CAAGGTGGAAATCAAGAGAACCGTGGCC GCTCCCTCCGTGTTCATCTTCCCACCATCT GACGAGCAGCTGAAGTCCGGCACCGCTT CTGTCGTGTGCCTGCTGAACAACTTCTAC CCTCGGGAAGCCAAGGTGCAGTGGAAGG TGGACAATGCCCTGCAGTCCGGCAACTCC CAAGAGTCTGTGACCGAGCAGGACTCCA AGGACAGCACCTACTCCCTGTCCTCTACC CTGACCCTGTCCAAGGCCGACTACGAGA AGCACAAGGTGTACGCCTGCGAAGTGAC CCACCAGGGACTGTCTAGCCCCGTGACCA AGTCCTTCAACAGAGGCGAGTGCTGA

			(SEQ ID NO:554)
147(H3807)xB6 (bispecific antibody comprising the anti-LAG3 147(H3807) clone in IgG form and the anti-PD-L1 B6 clone in scFv form)			
		Amino acid sequence (N'→C')	Nucleotide Sequence (5'→3')
Heavy Chain	Heavy chain of 147(H3807)	EVQLVQSGAEVKKP GASVKVSCKAS**GY** **TFTNYWLG**WIKQA PGQGLEWIG**DIYPG** **GDYIVYNEKFKG**K ATLTADTSISTAYM ELSRLRSDDTAVYY CAR**PNLPKDH**WGQ GTTVTVSSASTKGP SVFPLAPCSRSTSES TAALGCLVKDYFPE PVTVSWNSGALTSG VHTFPAVLQSSGLY SLSSVVTVPSSSLGT KTYTCNVDHKPSNT KVDKRVESKYGPPC PPCPAPEFLGGPSVF LFPPKPKDTLMISRT PEVTCVVVDVSQED PEVQFNWYVDGVE VHNAKTKPREEQFN STYRVVSVLTVLHQ DWLNGKEYKCKVS NKGLPSSIEKTISKA KGQPREPQVYTLPP SQEEMTKNQVSLTC LVKGFYPSDIAVEW ESNGQPENNYKTTP PVLDSDGSFFLYSRL TVDKSRWQEGNVF SCSVMHEALHNHY TQKSLSLSLGK (SEQ ID NO:551)	GAGGTGCAGCTGGTGCAGAGCGGAGCAG AGGTGAAGAAGCCAGGGGCCAGCGTGAA GGTGAGCTGTAAGGCTAGTGGGTACACA TTTACAAACTATTGGCTGGGATGGATTAA GCAGGCCCCAGGCCAAGGACTGGAGTGG ATAGGAGACATATACCCCGGAGGAGACT ATATCgtgTACAACGAGAAGTTCAAGGGC AAGGCCACACTCACCGCTGATACAAGCA TCAGCACCGCCTACATGGAGCTGAGCCG ACTGAGAAGCGACGACACAGCAGTGTAT TACTGCGCCAGACCCAACCTGCCCAAGG ACCACTGGGGACAAGGCACCACCGTGAC CGTGTCTTCCgctAgcAccAAgggcccctcgtgttcc ctctggccccAtgctcccggtccAcctcgAgtccAccgccgct ctgggctgtctggtgAAggActActtcctgAgcccgtgAccg tgAgctggAActctggcgccctgAcctccggcgtgcAcAcct tcctgccgtgctgcAgtcctccggcctgtActcctgtcctcgtg gtgAccgtgccttcctcctcctgggcAccAAgAcctAcAcc tgcAAcgtggAccAcAAgccttccAAcAccAAggtggA cAAgcgggtggAgtccAAgtAcggccctccttgccctcctg ccctgcccctgAgttcctgggcggAccctcgtgttcctgttcctc ctAAgcctAAggAcAccctgAtgAtctcccggAccccctgA ggtgAcctgcgtggtggtggAcgtgtcccAggAAgAtcctg AggtccAgttcAAttggtAcgtggAtggcgtggAggtgcAc AAcgccAAgAccAAgcctcgggAggAAcAgttcAAct ccAcctAccgggtggtgtctgtgctgAccgtgctgcAccAgg ActggctgAAcggcAAggAAtAcAAgtgcAAggtcAg cAAcAAgggcctgccctcctccAtcgAgAAAAccAtctc cAAggccAAgggccAgcctcgcgAgcctcAggtgtAcAc cctgcctcctAgccAggAAgAgAtgAccAagAAtcAgg tgtccctgAcAtgcctggtgAAgggcttctAcccttccgAtAT CGCCGTGGAATGGGAGAGCAATGGCCAG CCTGAGAACAACTACAAGACAACCCCTC CTGTGCTGGACTCCGACGGCTCCTTCTTT CTGTACTCTCGCCTGACCGTGGACAAGTC CAGATGGCAAGAGGGCAACGTGTTCTCCT GCTCCGTGATGCACGAGGCCCTGCACAAT CACTACACCCAGAAGTCCCTGTCTCTGTC CCTCGGAAAAGGCGGCGGAGGATCTGGC GGAGGCGGTAGCGGTGGTGGCGGATCTG ATATTCAGATGACCCAGTCTCCTTCCAGC CTGTCCGCTTCTGTGGGCGACAGAGTGAC CATCACATGCAAGGCCAGCCAGGATGTG ACCCCTGCTGTGGCTTGGTATCAGCAGAA GCCTGGCAAGGCCCCTAAGCTGCTGATCT ACTCCACCTCCTCCAGATACACAGGCGTG CCCTCCAGATTCTCCGGCTCTGGCTCTGG CACCGACTTTACCTTTACAATCTCCAGCC TGCAGCCTGAGGACATTGCCACCTACTAC TGCCAGCAGCACTACACCACACCTCTGAC CTTTGGCTGCGGCACCAAGCTGGAAATCA
	Linker	GGGGSGGGGSGGG GS (SEQ ID NO:543)	
	scFv of B6 — VL	DIQMTQSPSSLSASV GDRVTITCKASQDV TPAVAWYQQKPGK APKLLIYSTSSRYTG VPSRFSGSGSGTDFT FTISSLQPEDIATYY CQQHYTTPLTFGCG TKLEIKR (SEQ ID NO:544)	
	Linker	GGGGSGGGGSGGG GSGGGGS (SEQ ID NO:531)	
	VH	EVQLVESGGGLVQP GGSLRLSCAASGFT FSSYDMSWVRQAP	

			GKCLEWVATISDAG GYIYYRDSVKGRFTI SRDNAKNSLYLQM NSLRDEDTAVYICA RELPWRYALDYWG QGTTVTVSS* (SEQ ID NO:549)	AGAGAGGTGGCGGAGGAAGCGGAGGCG GCGGTTCAGGTGGCGGTGGTTCAGGCGGT GGTGGATCTGAAGTTCAGCTGGTGGAATC TGGCGGCGGATTGGTTCAACCAGGCGGCT CTCTGAGACTGTCTTGTGCCGCTTCCGGC TTCACCTTCTCCAGCTACGACATGTCCTG GGTCCGACAGGCCCCTGGAAAGTGTCTG GAATGGGTCGCCACCATCTCTGACGCTGG CGGCTACATCTACTACCGGGACTCTGTGA AGGGCAGATTCACCATCAGCCGGGACAA TGCCAAGAACTCCCTGTACCTGCAGATGA ACAGTCTGCGCGACGAGGACACCGCCGT GTACATCTGTGCTAGAGAGCTGCCTTGGC GCTACGCCCTGGATTATTGGGGCCAGGGC ACAACAGTGACAGTGTCCTCTTGA (SEQ ID NO:555)
Light chain	Light chain of 147(H3807)		DIVMTQSPLSLPVTP GEPASISCRSSKSLL HSQGITYLYWYLQK PGQSPQLLIY**QVSN** **LAS**GVPDRFSGSGS GTDFTLKISRVEAED VGVYYCAQYLELP WTFGGGTKVEIKRT VAAPSVFIFPPSDEQ LKSGTASVVCLLNN FYPREAKVQWKVD NALQSGNSQESVTE QDSKDSTYSLSSTLT LSKADYEKHKVYA CEVTHQGLSSPVTK SFNRGEC* (SEQ ID NO:553)	GACATTGTGATGACCCAGAGCCCCCTGAG CCTCCCCGTGACCCCTGGAGAACCCGCCA GCATAAGCTGCAGATCCTCCAAAAGCCTG CTGCACTCCcagGGAATAACCTACCTGTAT TGGTACCTGCAGAAACCCGGCCAATCCCC CCAACTCCTGATATACCAAGTGTCCAACC TGGCCTCCGGCGTGCCCGACAGATTCTCC GGCTCCGGCAGCGGTACCGACTTCACCCT CAAAATCTCCAGAGTGGAAGCAGAAGAC GTCGGCGTGTACTACTGCGCCCAGtacCTG GAACTGCCCTGGACCTTCGGCGGCGGCAC CAAGGTGGAAATCAAGAGAACCGTGGCC GCTCCCTCCGTGTTCATCTTCCCACCATCT GACGAGCAGCTGAAGTCCGGCACCGCTT CTGTCGTGTGCCTGCTGAACAACTTCTAC CCTCGGGAAGCCAAGGTGCAGTGGAAGG TGGACAATGCCCTGCAGTCCGGCAACTCC CAAGAGTCTGTGACCGAGCAGGACTCCA AGGACAGCACCTACTCCCTGTCCTCTACC CTGACCCTGTCCAAGGCCGACTACGAGA AGCACAAGGTGTACGCCTGCGAAGTGAC CCACCAGGGACTGTCTAGCCCCGTGACCA AGTCCTTCAACAGAGGCGAGTGCTGA (SEQ ID NO:554)

The constructed vectors were transiently expressed in ExpiCHO-S™ cells (Thermo Fisher, A29127) using (ExpiFectamine™CHO Kit, Thermo, A29129), cultured in ExpiCHO™ Expression medium (Thermo, A29100-01) under the conditions of 30 to 37°C for 7 to 15 days in a CO_2 incubator equipped with rotating shaker. Plasmid DNA (250 μg) and ExpiFectamin CHO Reagent (800 μL) were mixed with Opti-MEM® I medium (20 mL final volume) and allowed to stand at room temperature for 5 min. The mixed solution was added to 6 x 10^6 ExpiCHO cells cultured in ExpiCHO Expression Medium and gently mixed in a shaker incubator at 37°C with a humidified atmosphere of 8% CO_2 in air. At 18 hours post-transfection, 1.5 mL of ExpiFectamin CHO Transfection Enhancer 1 and 60 mL of ExpiFectamin CHO Transfection Feed were added to each flask.

Each BsAb was purified from the cell culture supernatant by recombinant Protein A affinity chromatography (Hitrap Mabselect Sure, GE Healthcare, 28-4082-55) and gel filtration chromatography with a HiLoad 26/200 Superdex200 prep grade column (GE Healthcare, 28-9893-36). SDS-PAGE (NuPage 4-12% Bis-Tris gel, NP0321) and size exclusion HPLC (Agilent, 1200 series) analysis with SE-HPLC column (SWXL SE-HPLC column, TOSOH, G3000SWXL) were performed to detect and confirm the size and purity of each BsAb. Purified proteins were concentrated in PBS by ultrafiltration using a Amicon Ultra 15 30K device (Merck, UFC903096), and protein concentrations were estimated using a nanodrop (Thermo, Nanodrop One). When a two-vector system is applied, the ratio between light to heavy chain could be 1:1 to 1:3 by weight. Alternatively, a one-vector system that contains both chains in one single vector can also be used.

The prepared anti-PD-L1/anti-LAG3 bispecific antibodies are named as H12x147, H12x147(H3807), B6x147, and B6x147(H3807), 147xH12, 147(H3807)xH12, 147xB6, and 147(H3807)xB6, respectively, wherein the former refers to the clone in the IgG form and the latter refers to the clone in the scFv form.

Example 4. Characterization of bispecific antibodies H12x147 and 147xH12

4.1. Binding of the bispecific antibodies

To evaluate the binding activity to PD-L1 and LAG3 of the bispecific antibodies (BsAb; H12x147 and 147xH12) prepared in Example 3, the BsAb were subjected to ELISA test. Briefly, microtiter plates were coated with each of human PD-L1-Fc protein (Sinobio, 10084-H02H) and human LAG3-His protein (Sinobio, 16498-H08H) at 0.5 μg/ml in PBS, 100μl/well at 4°C overnight, then blocked with 100μl/well of 5% BSA. Four-fold dilutions of each of the BsAbs starting from 100 nM were added to each well and incubated for 1-2 hours at RT. The plates were washed with PBS/Tween and then incubate with goat-anti-human IgG antibody conjugated with Horse Radish Peroxidase (HRP) (Pierce, cat# 31413) for 1 hour at RT. After washing, the plates were developed with TMB substrate and analyzed by spectrophotometer at OD 450-630nm. The results are shown in FIG. 33. As shown in FIG. 33, all the BsAbs tested can bind to both of human PD-L1 and human LAG3 proteins with high activities.

4.2. Binding affinity of bispecific antibodies

The binding affinities of bispecific antibodies PD-L1 and LAG3 of the bispecific antibodies (BsAb; 147xH12, 147H3807xB6 and B6x147H3807) prepared in Example 3 to PD-L1 protein and human LAG3 protein were tested with BIACORE ™ using a capture method.

The results are shown in Table 34.

[Table 34]

Antibody	Human PD-L1 (KD (M))	Human LAG3 (KD (M))
147xH12	2.74E-08	1.35E-08
147H3807xB6	5.94E-09	1.63E-09
B6x147H3807	1.18E-09	8.87E-09

As shown in Table 34 and FIG. 33, the bispecific antibody tested display relatively high binding affinities to both of human PD-L1 and human LAG3 proteins.

In addition, SEE assay was conducted, and the obtained results are shown in FIG. 34, the results indicating that the bispecific antibody tested inhibits the binding between MHC II and LAG3, thereby increasing T cell activity by MHC II and TCR.

4.3. Activity of the bispecific antibodies to promote human T cell immune response

To test the ability of bispecific antibodies to stimulated T cell response, Jurkat cell activation assay was used. Jurkat cells transfected with human Lag3 and Pd1 by lentivirus were used as the responder cells. Raji cells which overexpressed PDL1 was used as the antigen presenting cells (APC). Staphylococcal enterotoxins E (SEE) are superantigen, which was used as the stimulator in this assay. In this system, ectopically expressed huLAG3 and huPD-1 can suppress SE stimulated IL-2 production by Jurkat cells, while anti-LAG3 and anti-PD-L1 antibodies can reverse IL-2 production. In short, Raji (1×10^4) were co-cultured with Jurkat T cells (1×10^5) in the presence of superantigen. Bispecific antibodies and their counterpart monoantibodies (starting from 100nM diluted for 6 dose) were added to the mixed culture. 48hrs later, supernatant was collected for IL2 production. As shown in FIG. 34 (upper panel), bispecific antibodies (147xH12 (labeled as 147-H12) and H12X147 (labeled as H12-147)) can dose dependently promote IL2 production by Jurkat cells.

To further evaluate *in vitro* function of bispecific antibodies towards primary T cells, mixed lymphocyte reaction was performed. Human dendritic cells (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 the DCs and serially diluted antibodies. 5 days

after mixed culture, the culture supernatant was assayed for IFN production. The results in FIG. 34 (lower panel) indicated that both bispecific antibodies (147XH12 (labeled as A3L1) and H12X147 (labeled as L1A3) can significantly promote IFN production.

4.4. Tumor growth inhibition of the bispecific antibodies (*In vivo* assay)

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), H12 (anti-PD-L1 antibody, 5mg/kg), 147H (anti-LAG3 antibody, 5mg/kg) and 147xH12 (6.6mg/kg) every other day for 8 doses, starting from day 10. Tumor volumes were monitored by caliper measurement twice per week for the duration of the experiment (29 days). Neither H12 nor 147H showed tumor inhibition at 5mg/kg. By contrast, 147xH12 demonstrated robust inhibition of MC38 tumor growth, with a TGI of 67.7% at the end of the study (FIG. 35).

Example 5. Characterization of bispecific antibodies 147xH12 and 147(H3807)xH12

5.1. Binding of the bispecific antibodies

To evaluate the binding activity to LAG3 of the bispecific antibodies (BsAb; 147xH12 and 147(H3807)xH12) prepared in Example 3, the BsAbs were subjected to ELISA test. Briefly, microtiter plates were coated with human LAG3-His protein (Sinobio, 16498-H08H) at 0.5 μg/ml in PBS, 100μl/well at 4°C overnight, then blocked with 100μl/well of 5% BSA. Four-fold dilutions of each of the BsAbs starting from 100 nM were added to each well and incubated for 1-2 hours at RT. The plates were washed with PBS/Tween and then incubate with goat-anti-human IgG antibody conjugated with Horse Radish Peroxidase (HRP) (Pierce, cat# 31413) for 1 hour at RT. After washing, the plates were developed with TMB substrate and analyzed by spectrophotometer at OD 450-630nm. As shown in FIG. 33, the BsAbs 147(H3807)xH12 displays more improved binding activity to human LAG3 protein.

5.2. Activity of the bispecific antibodies to promote human T cell immune response

The effect of bispecific antibodies prepared in Example 3 was further studied using

PBMCs from healthy donors. In brief, human DCs were differentiated from CD14+ monocytes for 7 days. Purified CD4+ T cells isolated from another donor was stimulated by anti-CD3/CD28 for 2 days. Serially diluted antibodies were then added to DC and T cell co-culture in the presence of superantigen and incubated for 5 days and the culture medium was collected for IL-2 level. As showed in FIG 36, bispecific antibodies could significantly stimulate IL-2 production in primary CD4+ T cells, which was superior than combination of their corresponding monoantibodies. Data are shown as mean values from triplicate wells ± SD.

Moreover, the effect of bispecific antibodies prepared in Example 3 was studied using PBMCs from healthy donors. In brief, human DCs were differentiated from CD14+ monocytes for 5 days, followed by LPS treatment for maturation. Pan T cells were isolated from another donor PBMC. Serially diluted antibodies were then added to mature DC and T cell co-culture and incubated for 5 days and the culture medium was collected for IFNγ level. As showed in FIG 37, bispecific antibodies could significantly stimulate IFNγ production in primary pan T cells, which was superior than combination of their corresponding monoantibodies. Data are shown as mean values from duplicate wells ±SD.

5.3. Developability of bispecific antibodies

The developability regarding the physicochemical properties to PD-L1 and LAG-3 bispecific antibodies (BsAb; B6x147H3807 and 147(H3807)xB6) was assessed. The quality attributes for the BsAbs were evaluated by several analytical methods. Briefly, the purity was measured by Size exclusion-high performance liquid chromatography (SE-HPLC) and both of the BsAbs showed the high purity over 99%. The thermal stability by Protein thermal shift (PTS) with fluorescence labeled Real time-polymerase chain reaction (RT-PCR) was analyzed. Their melting temperature was observed over 67°C which indicated that the test articles have stable structural integrity. To evaluate solubility of the molecules, the proteins were concentrated to 20 mg/mL using ultrafiltration (Amicon Ultra-15 spin concentrator). As a result, the visible particles were not observed by visual inspection and no increment of aggregates was confirmed by SE-HPLC. The Isoelectric point (pI) of each bsabs measured by capillary isoelectric focusing (cIEF) were 8.26 and 8.35, respectively. This pI range is appropriate to proceed downstream process and formulation development. Overall, as shown in Table 19. It showed that the tested BsAbs(B6x147H3807 and 147(H3807)xB6) have proper physicochemical properties for the successful development.

[Table 35]

Content	Method	B6x147H3807	147(H3807)xB6
Purity	SEC	99.8	99.8
Thermal Stability	PTS	61.8	62.0
		77.7	71.6
Solubility	Visual inspection	Easy to concentrate up to 20 mg/mL, clear	Easy to concentrate up to 20 mg/mL, clear
pI	cIEF	8.56	7.65

Example 6. The effect of B3807 on inhibition of the binding of FGL1 to LAG3

This example tested the anti-LAG3 antibody B3807's activity in inhibiting the binding between LAG3 and Fibrinogen-like Protein 1 (FGL1).

It was recently reported that Fibrinogen-like Protein 1 (FGL1) is another functional ligand of LAG3, apart from MHC-II (Cell. 2019;176:1-14). FGL-1 is secreted from liver and highly produced by cancer cells. FGL-1 inhibits antigen-specific T cell activation and inversely, blockade of FGL-1 potentiates anti-tumor response. Interaction between FGL-1 and LAG3 may represent another mechanism for immune evasion.

Recombinant FGL-1 were coated on a 96 well plated at a concentration of 1μg/ml and incubated overnight at 4°C. Serially diluted anti-LAG3 antibody B3807 (starting from 10μg/ml and 1:3 dilution) and biotin-labeled LAG3-ECD (2μg/ml) were incubated with FGL-1 coated wells at room temperature for 2 hours. After extensive washing with the wash buffer, streptavidin-HRP was added. As shown in FIG. 47, B3807 dose-dependently inhibited the binding of FGL-1 to LAG3 protein.

* * *

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.

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

1. An anti-PD-L1/anti-LAG3 bispecific antibody, comprising an anti-PD-L1 antibody or an antigen-binding fragment thereof and an anti-LAG3 antibody or an antigen-binding fragment thereof, wherein

the anti-PD-L1 antibody or antigen-binding fragment thereof is capable of specifically binding to an immunoglobulin C (Ig C) domain of a human Programmed death-ligand 1 (PD-L1) protein, wherein the Ig C domain consists of amino acid residues 133-225; and

the anti-LAG3 antibody or antigen-binding fragment thereof comprises a VH CDR1 having an amino acid sequence selected from the group consisting of SEQ ID NOS: 116-117, 354, and 453-460; a VH CDR2 having an amino acid sequence selected from the group consisting of SEQ ID NOS: 118-119, 355, and 461-467; a VH CDR3 having an amino acid sequence selected from the group consisting of SEQ ID NOs: 120-160, 356, and 468-475; a VL CDR1 having an amino acid sequence selected from the group consisting of SEQ ID NOS: 163-195, 229, 357, and 490; a VL CDR2 having an amino acid sequence selected from the group consisting of SEQ ID NOS: 196-217, 358, and 476-483; and a VL CDR3 having an amino acid sequence selected from the group consisting of SEQ ID NOS: 218-228, 230-253, 359, and 484-489.

2. The anti-PD-L1/anti-LAG3 bispecific antibody of claim 1, wherein the anti-PD-L1 antibody or antigen-binding fragment thereof is capable of binding to at least one of amino acid residues Y134, K162, or N183 of the PD-L1 protein.

3. The anti-PD-L1/anti-LAG3 bispecific antibody of claim 2, wherein the anti-PD-L1 antibody or antigen-binding fragment thereof is capable of binding to amino acid residues Y134, K162, and N183 of the PD-L1 protein.

4. The anti-PD-L1/anti-LAG3 bispecific antibody of any of claims 1-3, wherein the anti-PD-L1 antibody or antigen-binding fragment thereof does not bind to an immunoglobulin V (Ig V) domain of the PD-L1 protein, wherein the Ig V domain consists of amino acid residues 19-127.

5. The anti-PD-L1/anti-LAG3 bispecific antibody of any of claims 1-4, wherein each of the anti-PD-L1 antibody or antigen-binding fragment thereof and the anti-LAG3 antibody or

antigen-binding fragment thereof is independently a chimeric antibody, a humanized antibody, or a fully human antibody.

6. An anti-PD-L1/anti-LAG3 bispecific antibody, comprising an anti-PD-L1 antibody or an antigen-binding fragment thereof and an anti-LAG3 antibody or an antigen-binding fragment thereof, wherein

the anti-PD-L1 antibody or antigen-binding fragment thereof comprises:

(1) a VH CDR1 having an amino acid sequence selected from the group consisting of SEQ ID NO: 1 and SEQ ID NO: 61-67;

(2) a VH CDR2 having an amino acid sequence selected from the group consisting of SEQ ID NO: 2, SEQ ID NO: 68-77, and 525-527;

(3) a VH CDR3 having an amino acid sequence selected from the group consisting of SEQ ID NO: 3, SEQ ID NO: 78-90 and SEQ ID NO: 513-519;

(4) a VL CDR1 having an amino acid sequence selected from the group consisting of SEQ ID NO: 4, SEQ ID NO: 91-92, and SEQ ID NO: 520-521;

(5) a VL CDR2 having an amino acid sequence selected from the group consisting of SEQ ID NO: 5 and SEQ ID NO: 93-105; and

(6) a VL CDR3 having an amino acid sequence selected from the group consisting of SEQ ID NO: 6, SEQ ID NO: 106-111, and SEQ ID NO: 522-524, and

the anti-LAG3 antibody or antigen-binding fragment thereof comprises:

(i) a VH CDR1 having an amino acid sequence selected from the group consisting of SEQ ID NOS: 116-117, 354, and 453-460;

(ii) a VH CDR2 having an amino acid sequence selected from the group consisting of SEQ ID NOS: 118-119, 355, and 461-467;

(iii) a VH CDR3 having an amino acid sequence selected from the group consisting of SEQ ID NOs: 120-160, 356, and 468-475;

(iv) a VL CDR1 having an amino acid sequence selected from the group consisting of SEQ ID NOS: 163-195, 229, 357, and 490;

(v) a VL CDR2 having an amino acid sequence selected from the group consisting of SEQ ID NOS: 196-217, 358, and 476-483; and

(vi) a VL CDR3 having an amino acid sequence selected from the group consisting of SEQ ID NOS: 218-228, 230-253, 359, and 484-489.

7. The anti-PD-L1/anti-LAG3 bispecific antibody of claim 6, wherein each of the anti-PD-L1 antibody or antigen-binding fragment thereof and the anti-LAG3 antibody or antigen-binding fragment thereof is independently a chimeric antibody, a humanized antibody, or a fully human antibody.

8. The anti-PD-L1/anti-LAG3 bispecific antibody of claim 8, wherein the anti-PD-L1 antibody or antigen-binding fragment thereof and the anti-LAG3 antibody or antigen-binding fragment thereof are humanized antibodies.

9. The anti-PD-L1/anti-LAG3 bispecific antibody of any one of claims 6-8, wherein the anti-PD-L1 antibody or antigen-binding fragment thereof comprises a heavy chain variable region comprising an amino acid sequence selected from the group consisting of SEQ ID NOS: 7-26, 113, 493, 495, 497, 499, 501, 503, 505, 507, 509, and 511, or a polypeptide having at least 90% sequence identity to an amino acid sequence selected from the group consisting of SEQ ID NOS: 7-26, 113, 493, 495, 497, 499, 501, 503, 505, 507, 509, and 511.

10. The anti-PD-L1/anti-LAG3 bispecific antibody of any one of claims 6-9, wherein the anti-PD-L1 antibody or antigen-binding fragment thereof comprises a light chain variable region comprising an amino acid sequence selected from the group consisting of SEQ ID NOS: 27-33, 494, 496, 498, 500, 502, 504, 506, 508, 510, and 512, or a peptide having at least 90% sequence identity to an amino acid sequence selected from the group consisting of SEQ ID NOS: 27-33, 494, 496, 498, 500, 502, 504, 506, 508, 510, and 512.

11. The anti-PD-L1/anti-LAG3 bispecific antibody of any one of claims 6-10, wherein the anti-LAG3 antibody or antigen-binding fragment thereof comprises a heavy chain variable region comprising an amino acid sequence selected from the group consisting of SEQ ID NOS: 254-302, 352, 360-373, 375, 377, 379, 381, 383, 385, 387, 389, 391, 393, 395, 397, 399, 401, 403, 405, 407, 409, 411, 413, 415, 417, 419, 421, 423, 425, 427, 429, 431, 433, 435, 437, 439, 441, 443, 445, 447, 449, 451 and 491, or a polypeptide having at least 90% sequence identity to an amino acid sequence selected from the group consisting of SEQ ID NOS: 254-302, 352, 360-373, 375, 377, 379, 381, 383, 385, 387, 389, 391, 393, 395, 397, 399, 401, 403, 405, 407, 409, 411, 413, 415, 417, 419, 421, 423, 425, 427, 429, 431, 433, 435, 437, 439, 441, 443, 445, 447, 449, 451 and 491.

12. The anti-PD-L1/anti-LAG3 bispecific antibody of any one of claims 6-11, wherein the anti-LAG3 antibody or antigen-binding fragment thereof comprises a light chain variable region comprising an amino acid sequence selected from the group consisting of SEQ ID NOS: 303-351, 353, 374, 376, 378, 380, 382, 384, 386, 388, 390, 392, 394, 396, 398, 400, 402, 404, 406, 408, 410, 412, 414, 416, 418, 420, 422, 424, 426, 428, 430, 432, 434, 436, 438, 440, 442, 444, 446, 448, 450, 452 and 492, or a peptide having at least 90% sequence identity to an amino acid sequence selected from the group consisting of SEQ ID NOS: 303-351, 353, 374, 376, 378, 380, 382, 384, 386, 388, 390, 392, 394, 396, 398, 400, 402, 404, 406, 408, 410, 412, 414, 416, 418, 420, 422, 424, 426, 428, 430, 432, 434, 436, 438, 440, 442, 444, 446, 448, 450, 452 and 378.

13. The anti-PD-L1/anti-LAG3 bispecific antibody of any one of claims 1-12, which is in the form of IgG-scFv form.

14. A pharmaceutical composition for treating or preventing a disease associated with PD-L1, LAG3, or both thereof, the composition comprising the anti-PD-L1/anti-LAG3 bispecific antibody of any one of claims 1-12 and a pharmaceutically acceptable carrier.

15. The pharmaceutical composition of claim 14, wherein the disease associated with PD-L1, LAG3, or both thereof is cancer or infection.

16. The pharmaceutical composition of claim 15, wherein the cancer is a solid tumor.

17. The pharmaceutical composition of claim 15, wherein the cancer is selected from the group consisting of bladder cancer, liver cancer, colon cancer, rectal cancer, endometrial cancer, leukemia, lymphoma, pancreatic cancer, small cell lung cancer, non-small cell lung cancer, breast cancer, urethral cancer, head and neck cancer, gastrointestinal cancer, stomach cancer, oesophageal cancer, ovarian cancer, renal cancer, melanoma, prostate cancer and thyroid cancer.

18. An antibody or antigen-binding fragment thereof, having specificity to a human PD-L1 protein and comprising:

(1) a VH CDR1 comprising an amino acid sequence selected from the group consisting of SEQ ID NO: 1 and 61-67;

(2) a VH CDR2 comprising an amino acid sequence selected from the group consisting of SEQ ID NO: 2, 68-77, and 525-527;

(3) a VH CDR3 comprising an amino acid sequence selected from the group consisting of SEQ ID NO: 3, 78-90, and 513-519;

(4) a VL CDR1 comprising an amino acid sequence selected from the group consisting of SEQ ID NO: 4, 91-92, and 520-521;

(5) a VL CDR2 comprising an amino acid sequence selected from the group consisting of SEQ ID NO: 5, and 93-105; and

(6) a VL CDR3 comprising an amino acid sequence selected from the group consisting of SEQ ID NO: 6, 106-111, and 522-524.

19. An antibody or antigen-binding fragment thereof, having specificity to a human LAG3 protein and comprising:

(i) a VH CDR1 comprising an amino acid sequence selected from the group consisting of SEQ ID NO: 116-117, 354, and 453-460;

(ii) a VH CDR2 comprising an amino acid sequence selected from the group consisting of SEQ ID NO: 118-119, 355, and 461-467;

(iii) a VH CDR3 comprising an amino acid sequence selected from the group consisting of SEQ ID NO: 120-160, 356, and 468-475;

(iv) a VL CDR1 comprising an amino acid sequence selected from the group consisting of SEQ ID NO: 163-195, 229, 357, and 490;

(v) a VL CDR2 comprising an amino acid sequence selected from the group consisting of SEQ ID NO: 196-217, 358, and 476-483; and

(vi) a VL CDR3 comprising an amino acid sequence selected from the group consisting of SEQ ID NO: 218-228, 230-253, 359, and 484-489.

20. The antibody or fragment of claim 19, wherein the VH CDR1 comprises the amino acid sequence of SEQ ID NO:354, the VH CDR2 comprises the amino acid sequence of SEQ ID NO:461, the VH CDR3 comprises the amino acid sequence of SEQ ID NO:468, the VL CDR1 comprises the amino acid sequence of SEQ ID NO:490, the VL CDR2 comprises the amino acid sequence of SEQ ID NO:358, and the VL CDR3 comprises the amino acid sequence of SEQ ID NO:488.

21. The antibody or fragment of claim 20, which comprises a heavy chain variable region comprising the amino acid sequence of SEQ ID NO:443, or a polypeptide having at least 90% sequence identity to the amino acid sequence of SEQ ID NO:443.

22. The antibody or fragment of claim 20 or 21, which comprises a light chain variable region comprising the amino acid sequence of SEQ ID NO:444, or a polypeptide having at least 90% sequence identity to the amino acid sequence of SEQ ID NO:444.

23. The antibody or fragment of any one of claims 19-22, which is capable of inhibiting the binding of a human LAG protein to a MHC class II molecule or Fibrinogen-like Protein 1 (FGL1).

24. An antibody or antigen-binding fragment thereof, having specificity to a human LAG3 protein and comprising:

a heavy chain variable region comprising the amino acid sequence of SEQ ID NO:443, or a polypeptide having at least 90% sequence identity to the amino acid sequence of SEQ ID NO:443 and having a VH CDR1 comprising the amino acid sequence of SEQ ID NO:354, a VH CDR2 comprising the amino acid sequence of SEQ ID NO:461, and a VH CDR3 comprising the amino acid sequence of SEQ ID NO:468, and

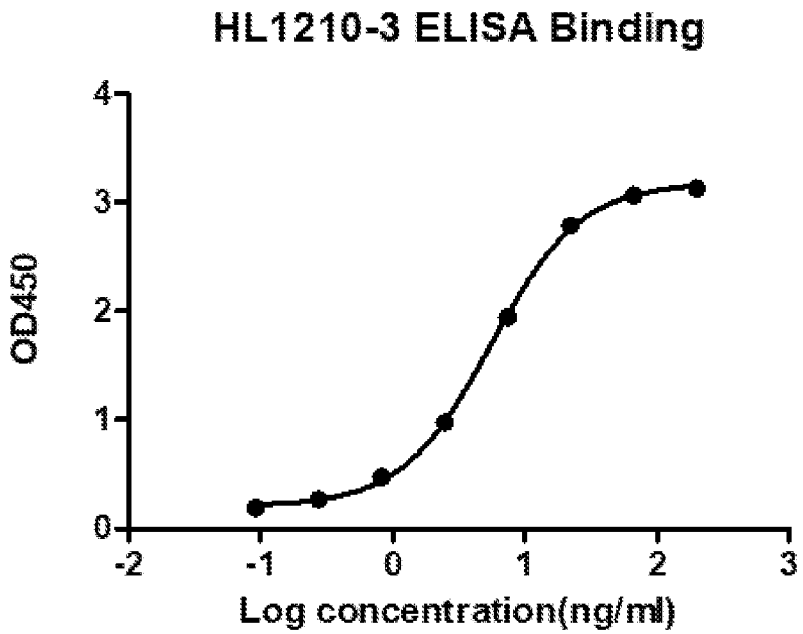
a light chain variable region comprising the amino acid sequence of SEQ ID NO:444, or a polypeptide having at least 90% sequence identity to the amino acid sequence of SEQ ID NO:444 and having a VL CDR1 comprising the amino acid sequence of SEQ ID NO:490, a VL CDR2 comprising the amino acid sequence of SEQ ID NO:358, and a VL CDR3 comprising the amino acid sequence of SEQ ID NO:488.

25. The antibody or fragment of any one of claims 18-24, which is humanized.

26. One or more polynucleotides that encode the antibody or fragment of any one of claims 18-24.

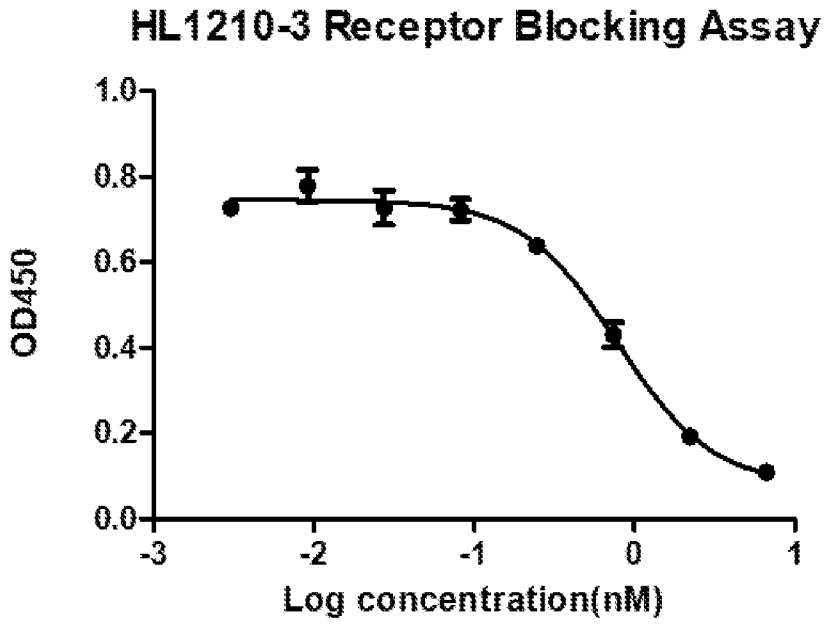
27. A pharmaceutical composition for treating or preventing cancer, comprising the antibody or fragment of any one of claims 18-24 and a pharmaceutically acceptable carrier.

28. The pharmaceutical composition of claim 27, wherein the cancer is selected from the group consisting of bladder cancer, liver cancer, colon cancer, rectal cancer, endometrial cancer, leukemia, lymphoma, pancreatic cancer, small cell lung cancer, non-small cell lung cancer, breast cancer, urethral cancer, head and neck cancer, gastrointestinal cancer, stomach cancer, oesophageal cancer, ovarian cancer, renal cancer, melanoma, prostate cancer and thyroid cancer.



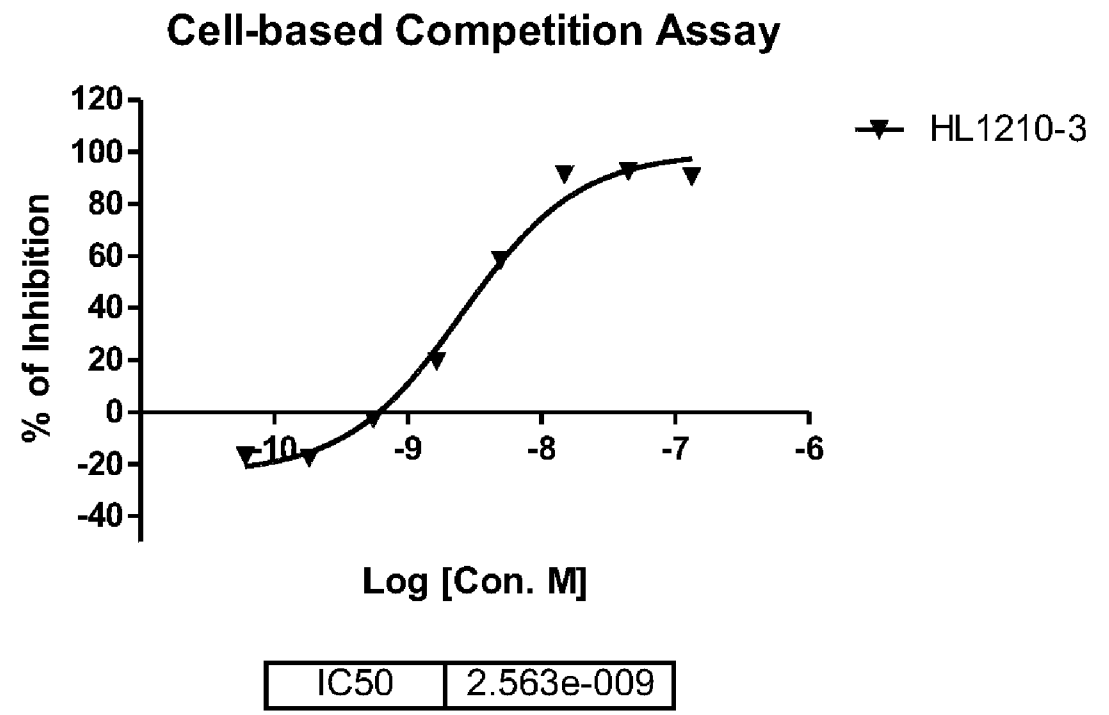
EC50	5.539

FIG. 1



IC50	0.7835

FIG. 2



IC50	2.563e-009

FIG. 3

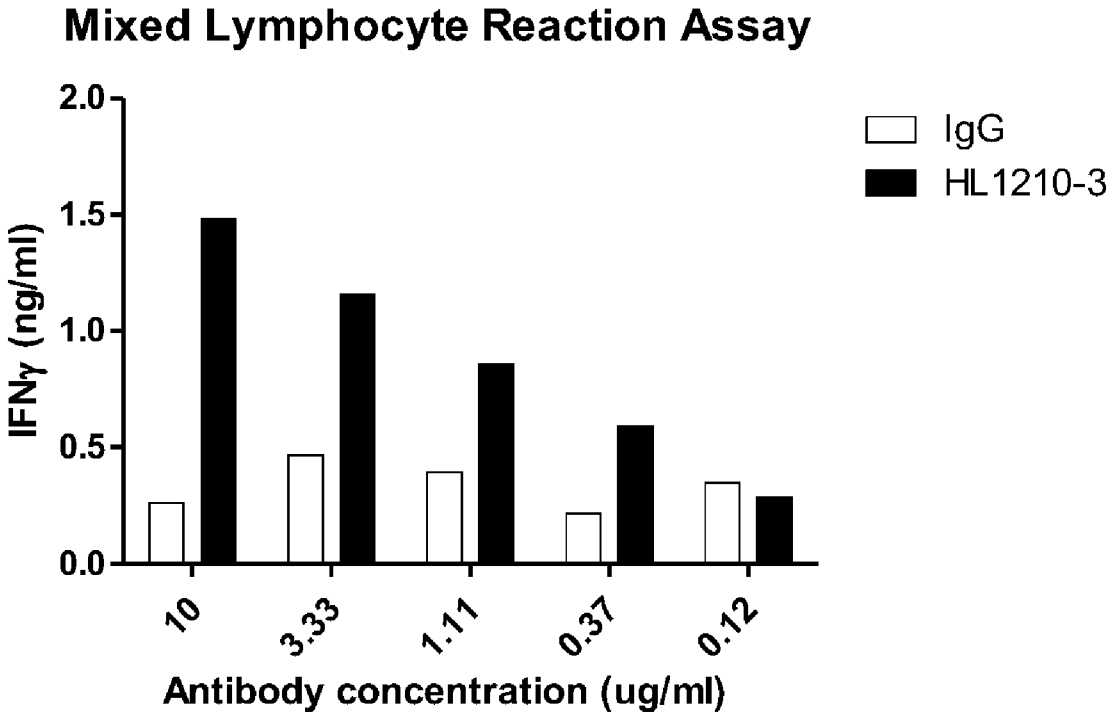


FIG. 4

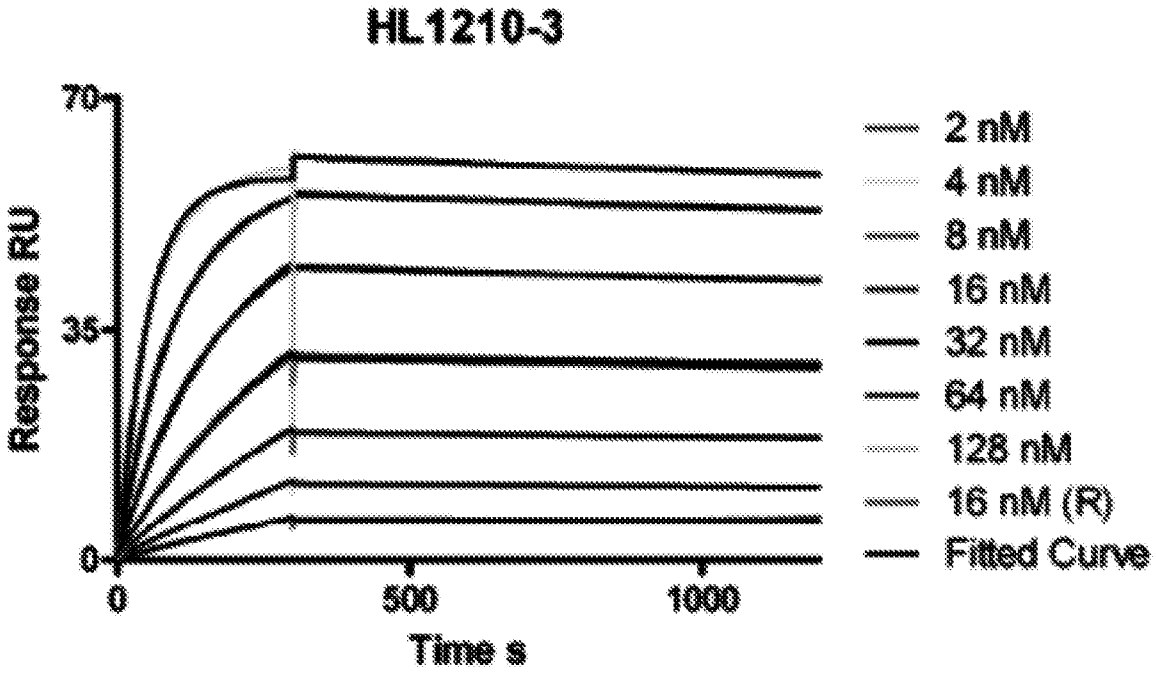
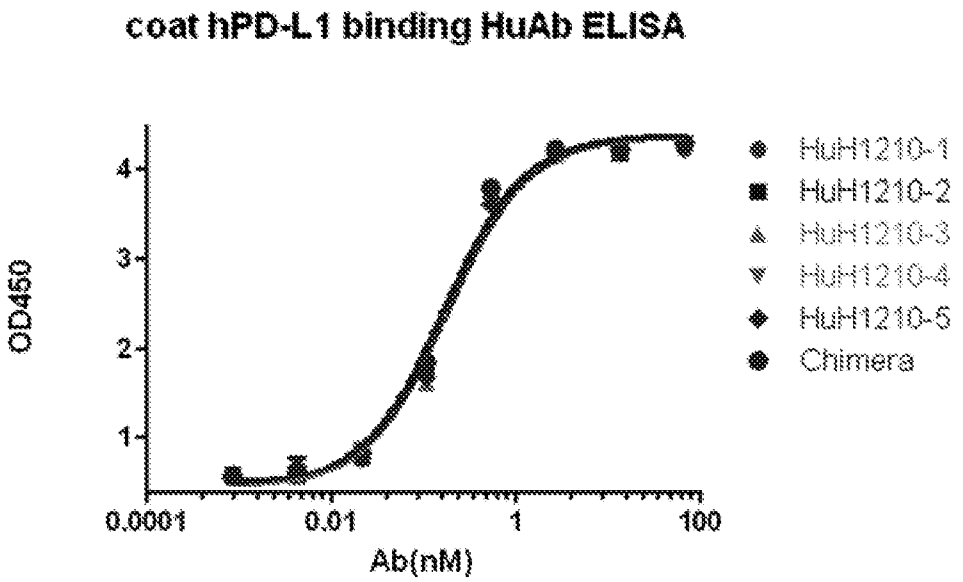
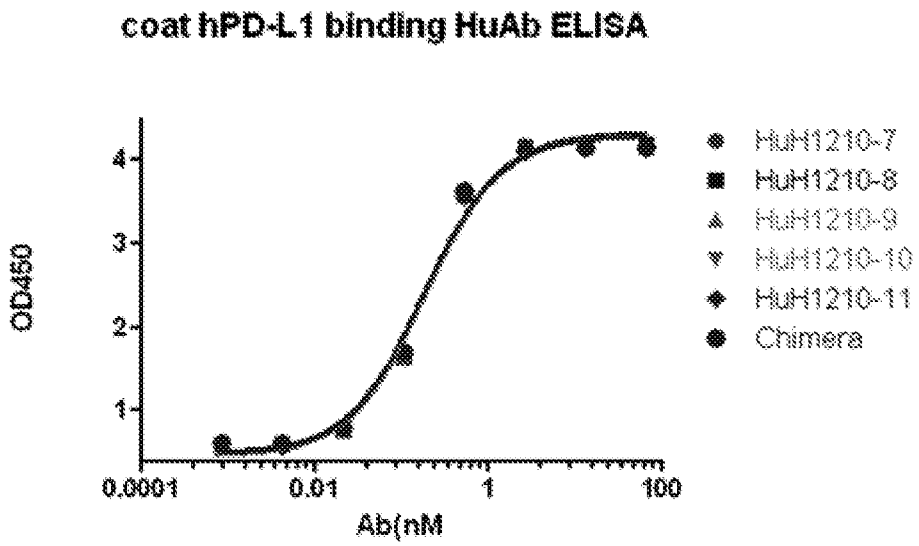

HL1210-3
Response RU
70
35
0
0
500
1000
Time s
2 nM
4 nM
8 nM
16 nM
32 nM
64 nM
128 nM
16 nM (R)
Fitted Curve

FIG. 5



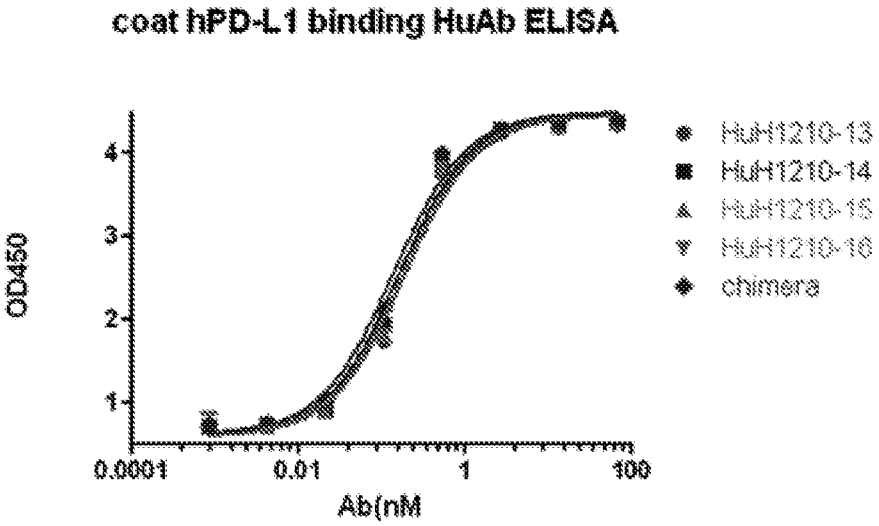
	EC50
HuH1210-1	0.1674
HuH1210-2	0.1682
HuH1210-3	0.1856
HuH1210-4	0.1842
HuH1210-5	0.1928
Chimera	0.1603

FIG. 6A



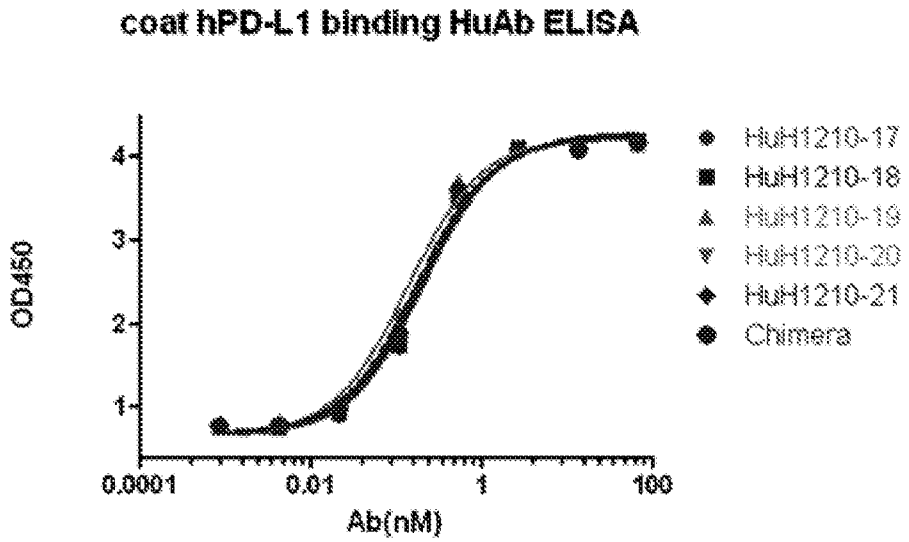
	EC50
HuH1210-7	0.1846
HuH1210-8	0.1865
HuH1210-9	0.1835
HuH1210-10	0.1904
HuH1210-11	0.1764
Chimera	0.1771

FIG. 6B



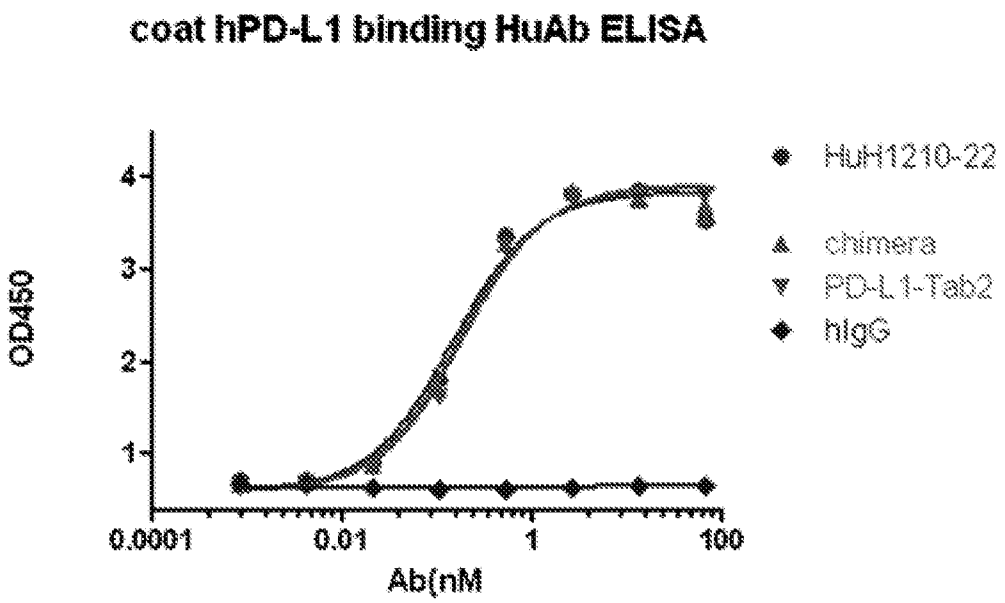
	EC50
HuH1210-13	0.1869
HuH1210-14	0.1629
HuH1210-15	0.1873
HuH1210-16	0.1788
chimera	0.1394

FIG. 6C



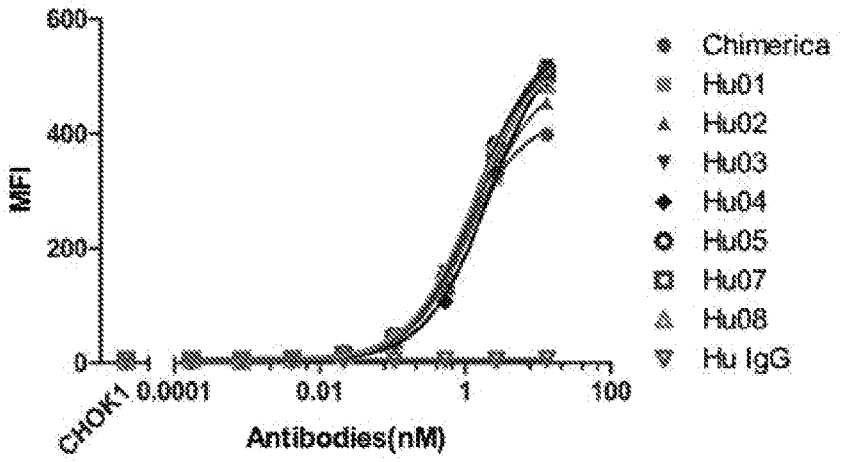
	EC50
HuH1210-17	0.2085
HuH1210-18	0.1951
HuH1210-19	0.1368
HuH1210-20	0.1853
HuH1210-21	0.1916
Chimera	0.1704

FIG. 6D



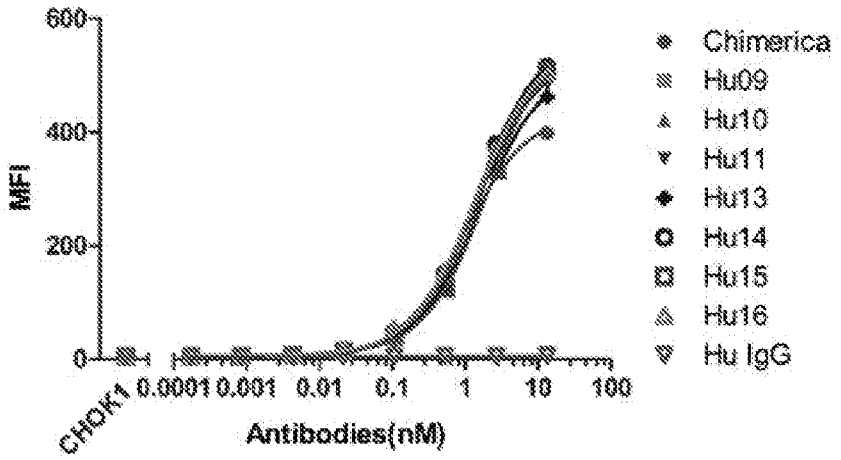
	EC50
HuH1210-22	0.1478
chimera	0.1693
PD-L1-Tab2	0.1876
hIgG	84.82

FIG. 6E



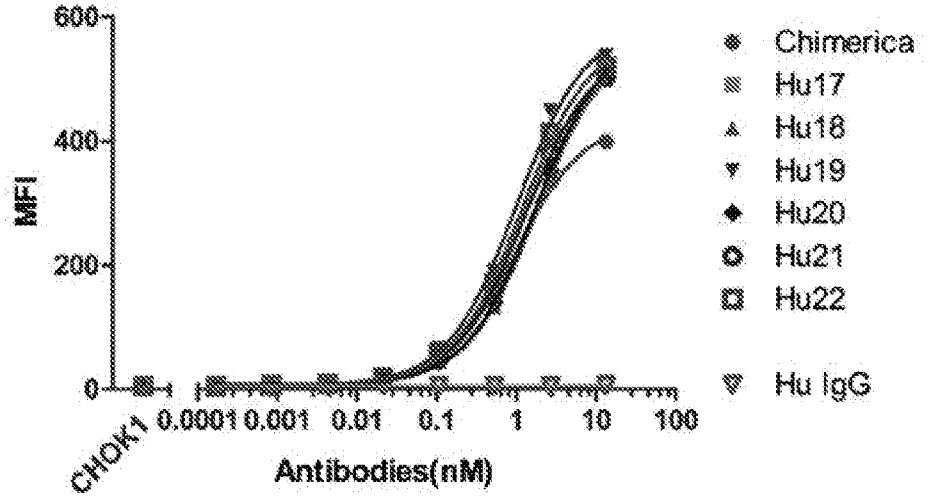
	Chimerica	Hu01	Hu02	Hu03	Hu04	Hu05	Hu07	Hu08	Hu IgG
EC50	0.9504	2.310	1.444	1.374	2.118	1.433	1.612	1.232	~~9662~~

FIG. 7A



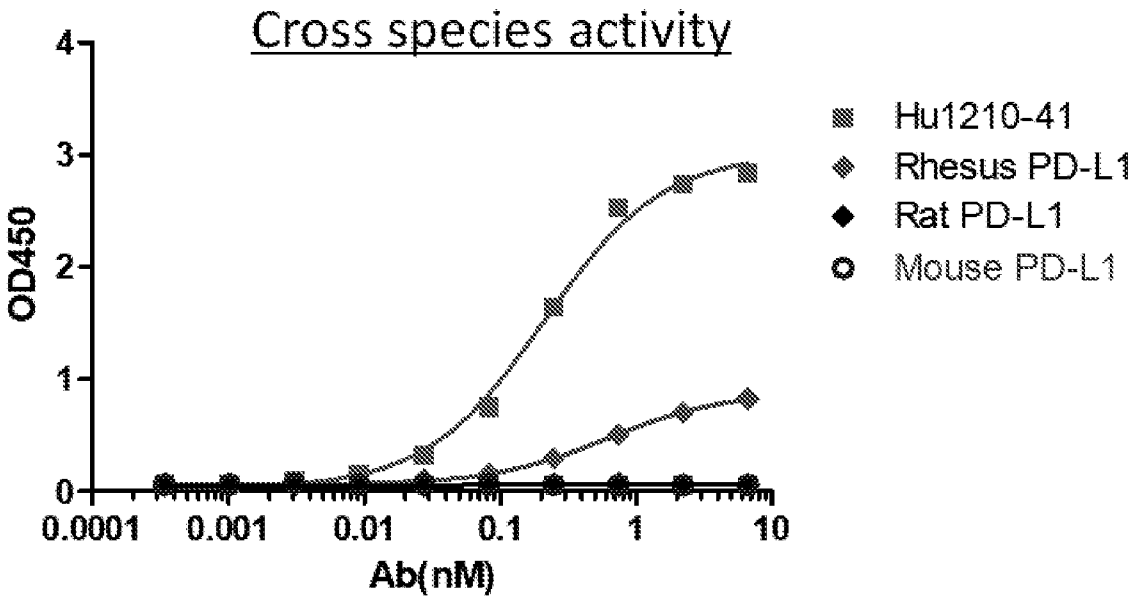
	Chimerica	Hu09	Hu10	Hu11	Hu13	Hu14	Hu15	Hu16	Hu IgG
EC50	0.9504	1.387	1.499	1.404	1.572	1.457	2.042	1.414	~~9662~~

FIG. 7B



	Chimerica	Hu17	Hu18	Hu19	Hu20	Hu21	Hu22	Hu IgG
EC50	0.9504	1.232	1.803	1.011	1.734	1.289	1.168	~~9562~~

FIG. 7C



	EC50
Human PD-L1	0.2105
Rhesus PD-L1	0.6280

FIG. 8

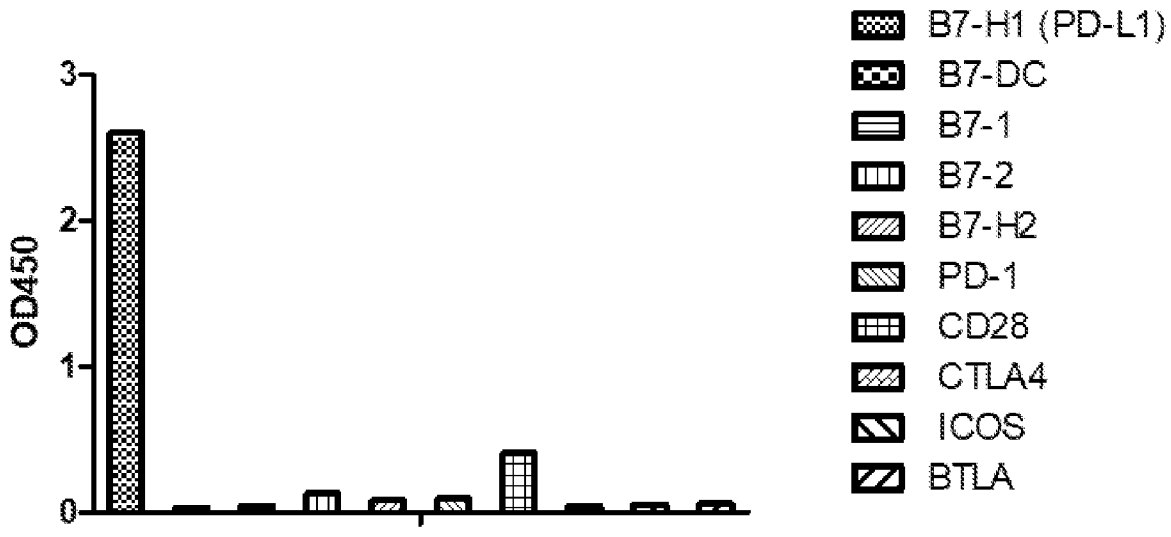


FIG. 9

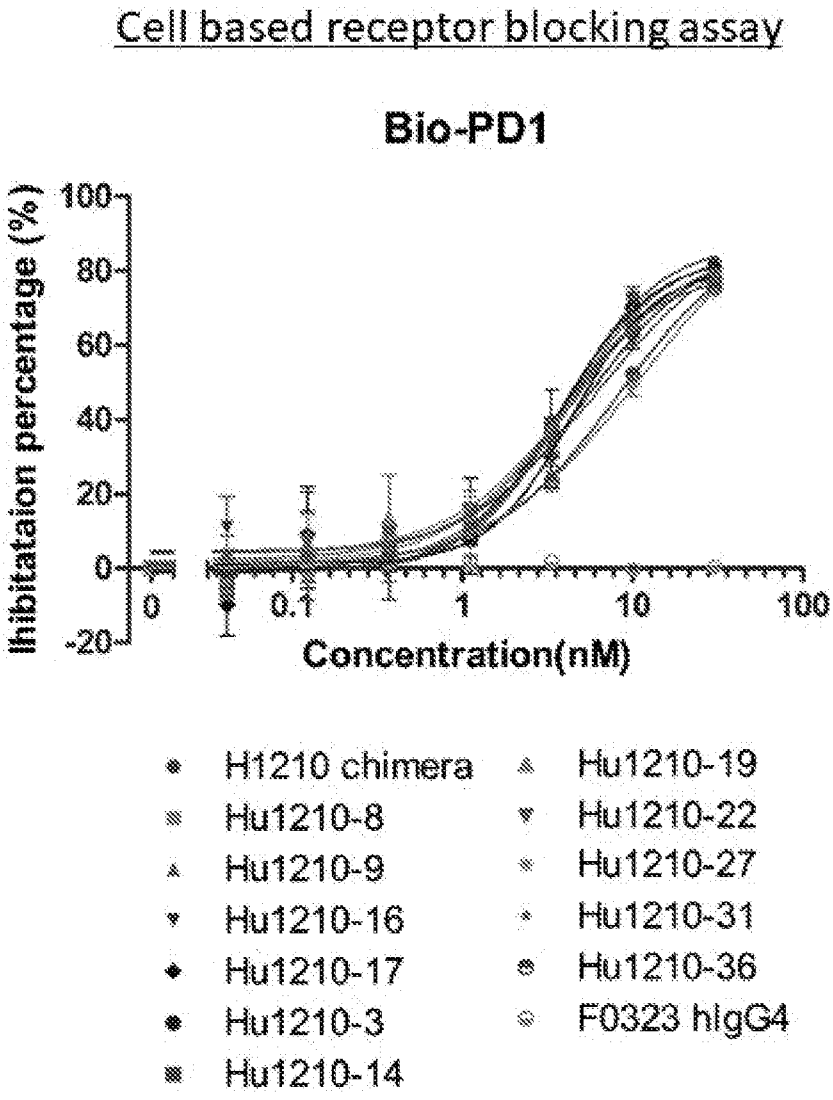

Cell based receptor blocking assay
Bio-PD1
Inhibitataion percentage (%)
100
80
60
40
20
0
-20
0
0.1
1
10
100
Concentration(nM)
H1210 chimera
Hu1210-8
Hu1210-9
Hu1210-16
Hu1210-17
Hu1210-3
Hu1210-14
Hu1210-19
Hu1210-22
Hu1210-27
Hu1210-31
Hu1210-36
F0323 hIgG4

FIG. 10

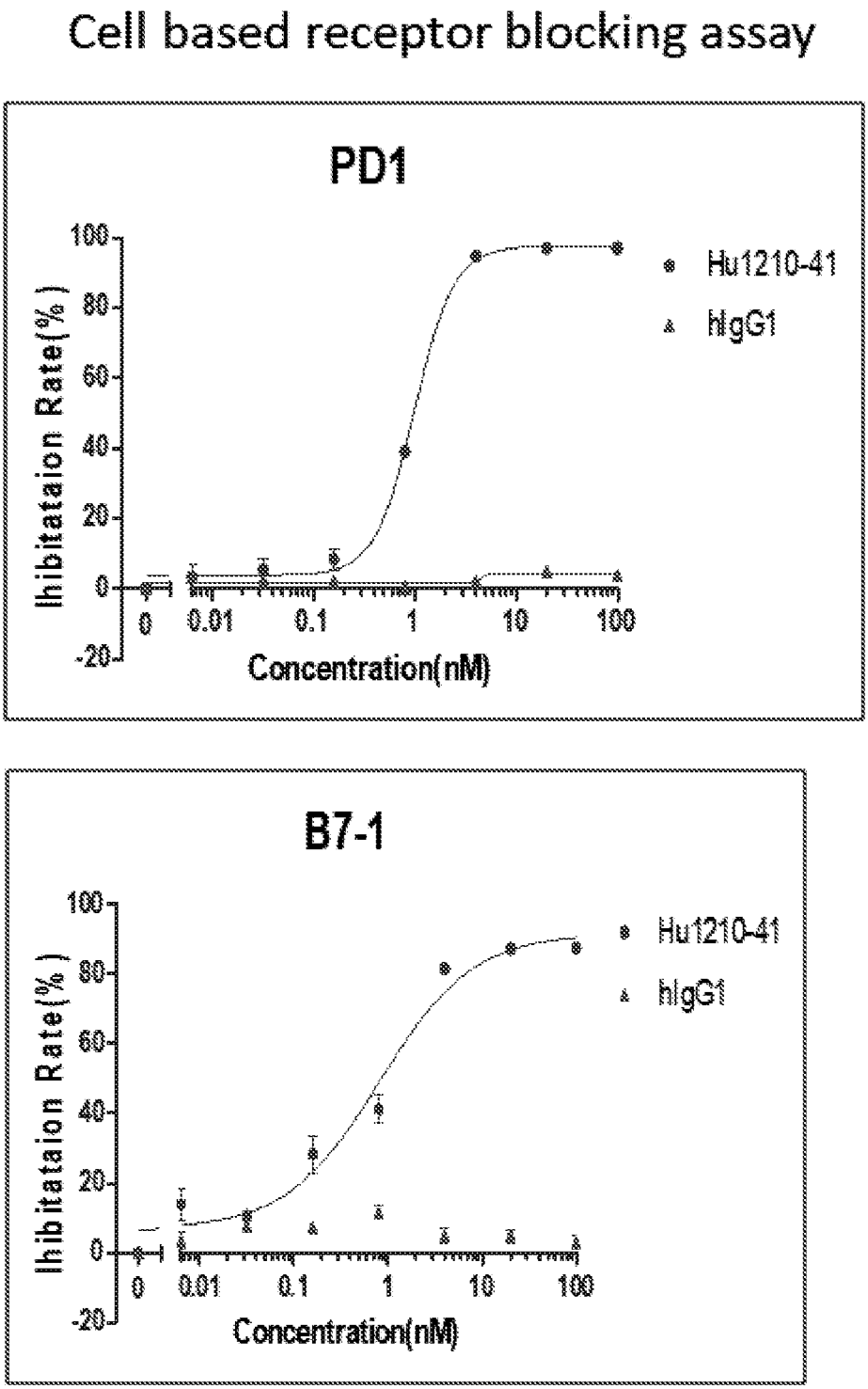

Cell based receptor blocking assay
PD1
Ihibitataion Rate(%)
100
80
60
40
20
0
-20
0
0.01
0.1
1
10
100
Concentration(nM)
Hu1210-41
hIgG1
B7-1
Ihibitataion Rate(%)
100
80
60
40
20
0
-20
0
0.01
0.1
1
10
100
Concentration(nM)
Hu1210-41
hIgG1

FIG. 11

Mixed lymphocyte Reaction assay

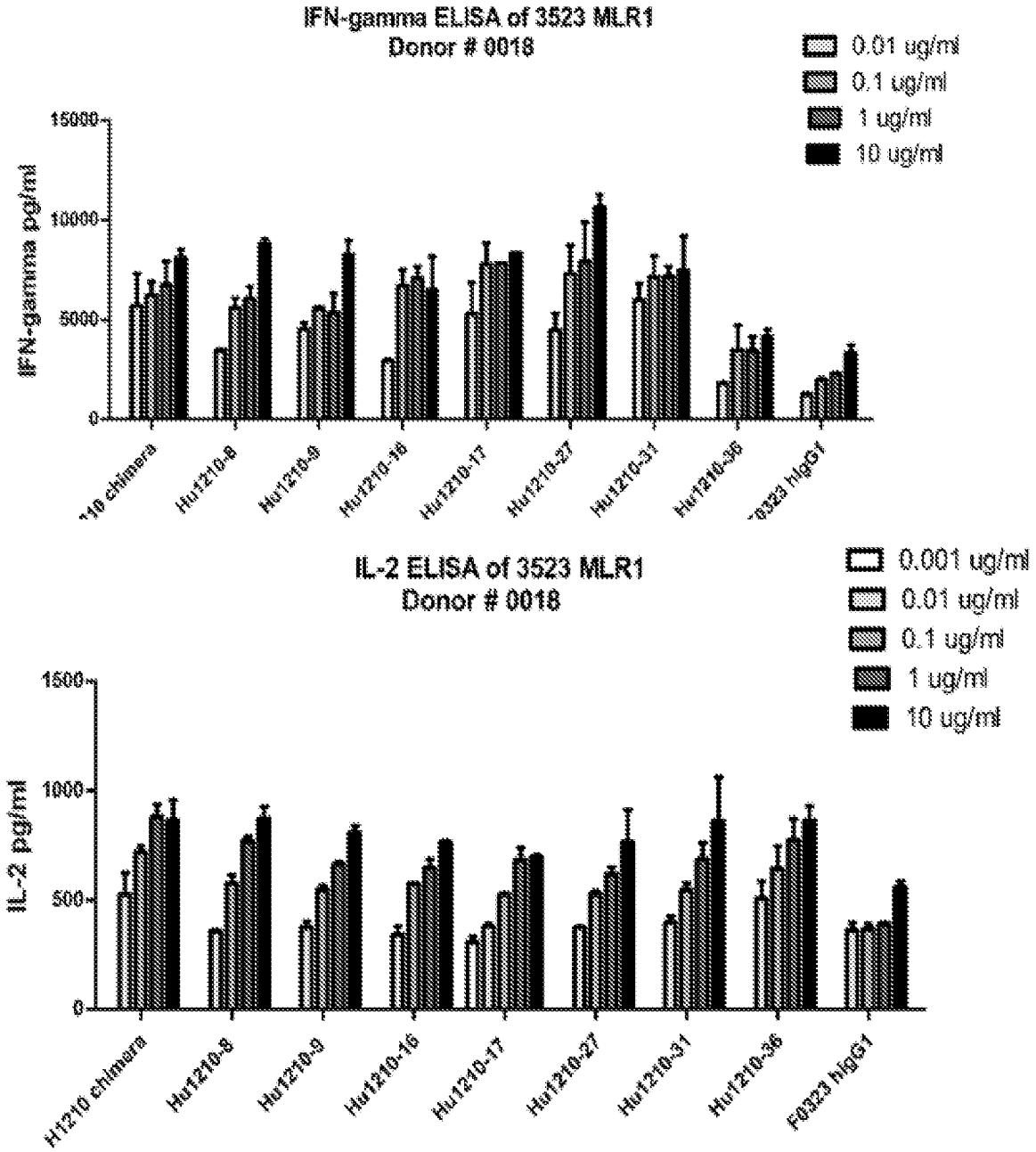


FIG. 12

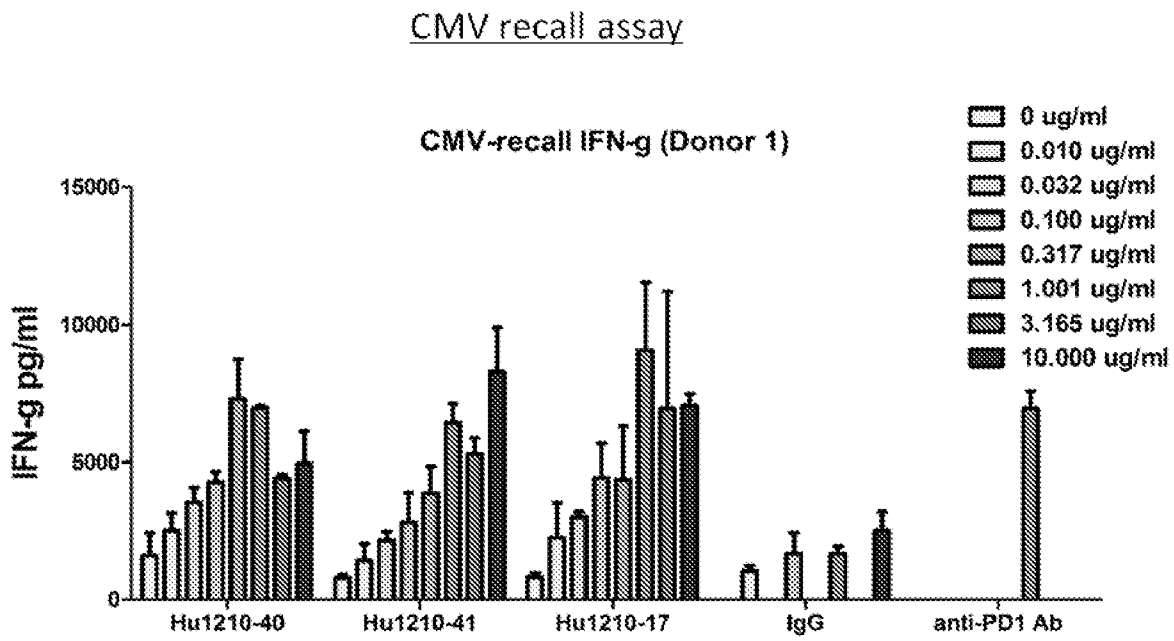

CMV recall assay
CMV-recall IFN-g (Donor 1)
IFN-g pg/ml
15000
10000
5000
0
0 ug/ml
0.010 ug/ml
0.032 ug/ml
0.100 ug/ml
0.317 ug/ml
1.001 ug/ml
3.165 ug/ml
10.000 ug/ml
Hu1210-40
Hu1210-41
Hu1210-17
IgG
anti-PD1 Ab

FIG. 13

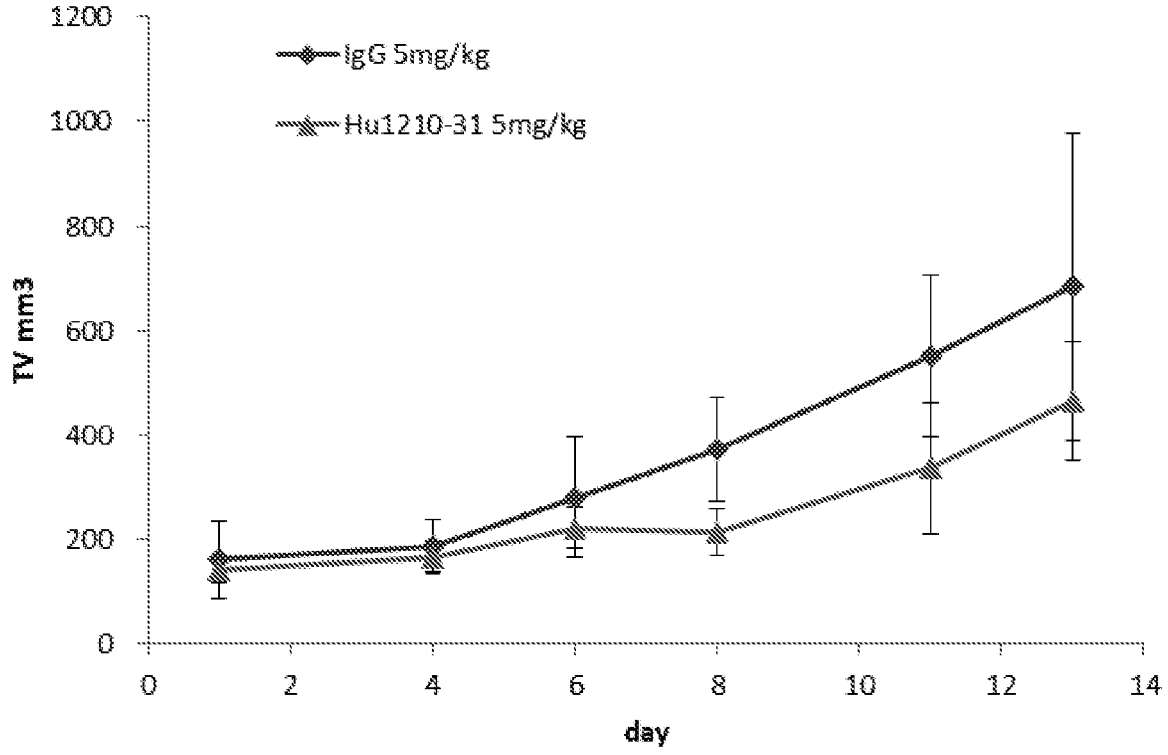
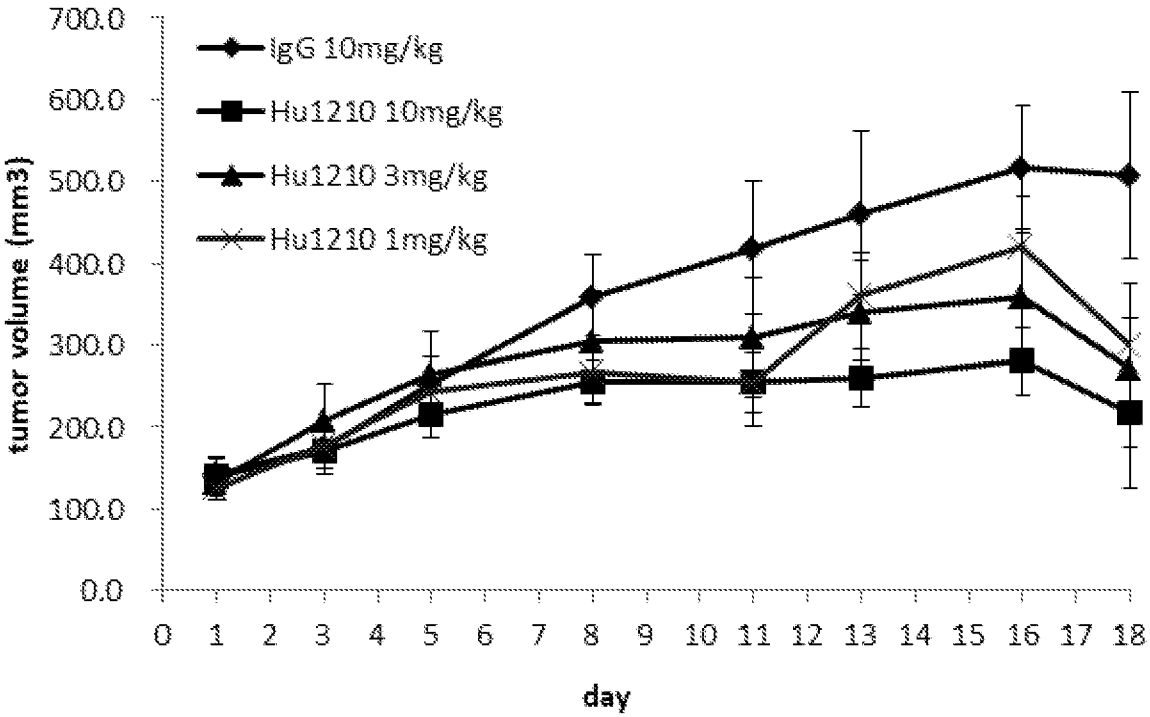

IgG 5mg/kg
Hu1210-31 5mg/kg
TV mm3
1200
1000
800
600
400
200
0
0
2
4
6
8
10
12
14
day

FIG. 14


700.0
600.0
500.0
400.0
300.0
200.0
100.0
0.0
tumor volume (mm3)
IgG 10mg/kg
Hu1210 10mg/kg
Hu1210 3mg/kg
Hu1210 1mg/kg
0 1 2 3 4 5 6 7 8 9 10 11 12 13 14 15 16 17 18
day

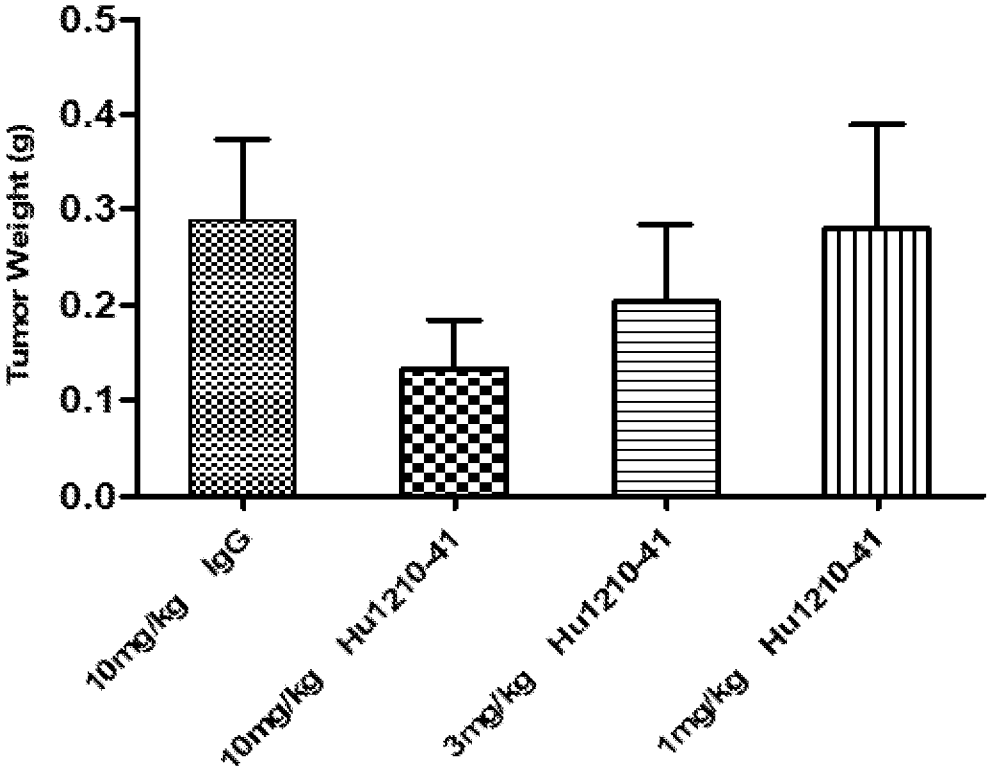

0.5
0.4
0.3
0.2
0.1
0.0
Tumor Weight (g)
10mg/kg IgG
10mg/kg Hu1210-41
3mg/kg Hu1210-41
1mg/kg Hu1210-41

FIG. 15

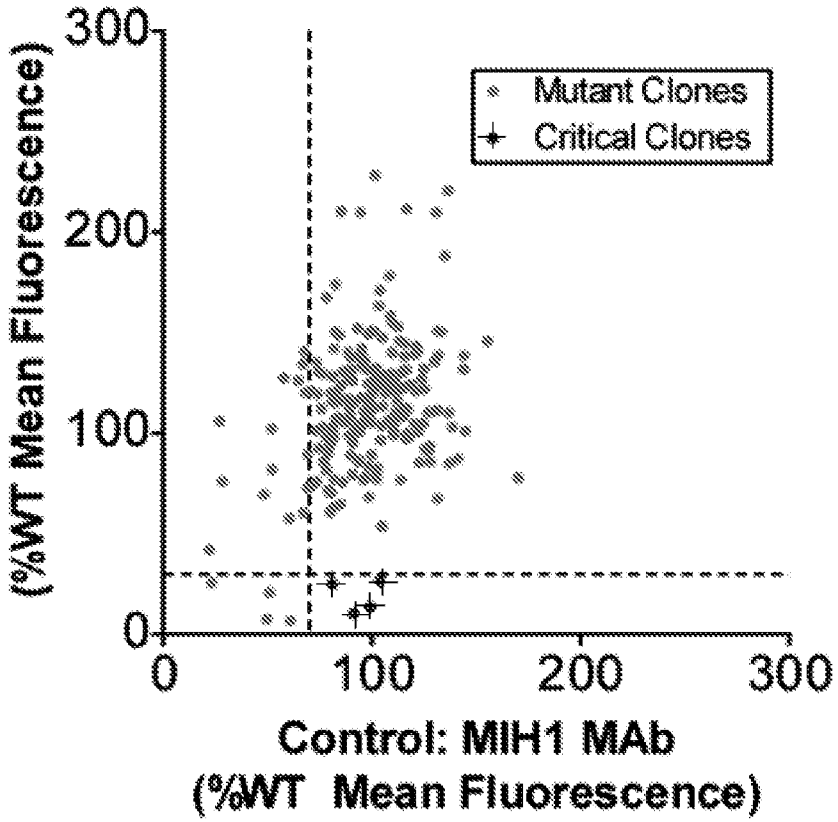

300
200
100
0
(%WT Mean Fluorescence)
Mutant Clones
Critical Clones
0
100
200
300
Control: MIH1 MAb
(%WT Mean Fluorescence)

FIG. 16

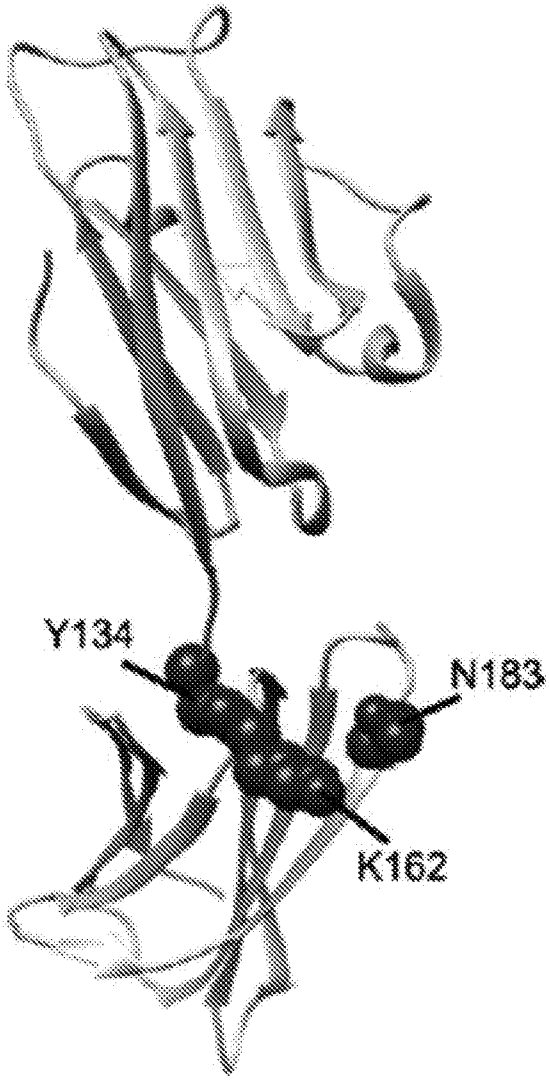


FIG. 17

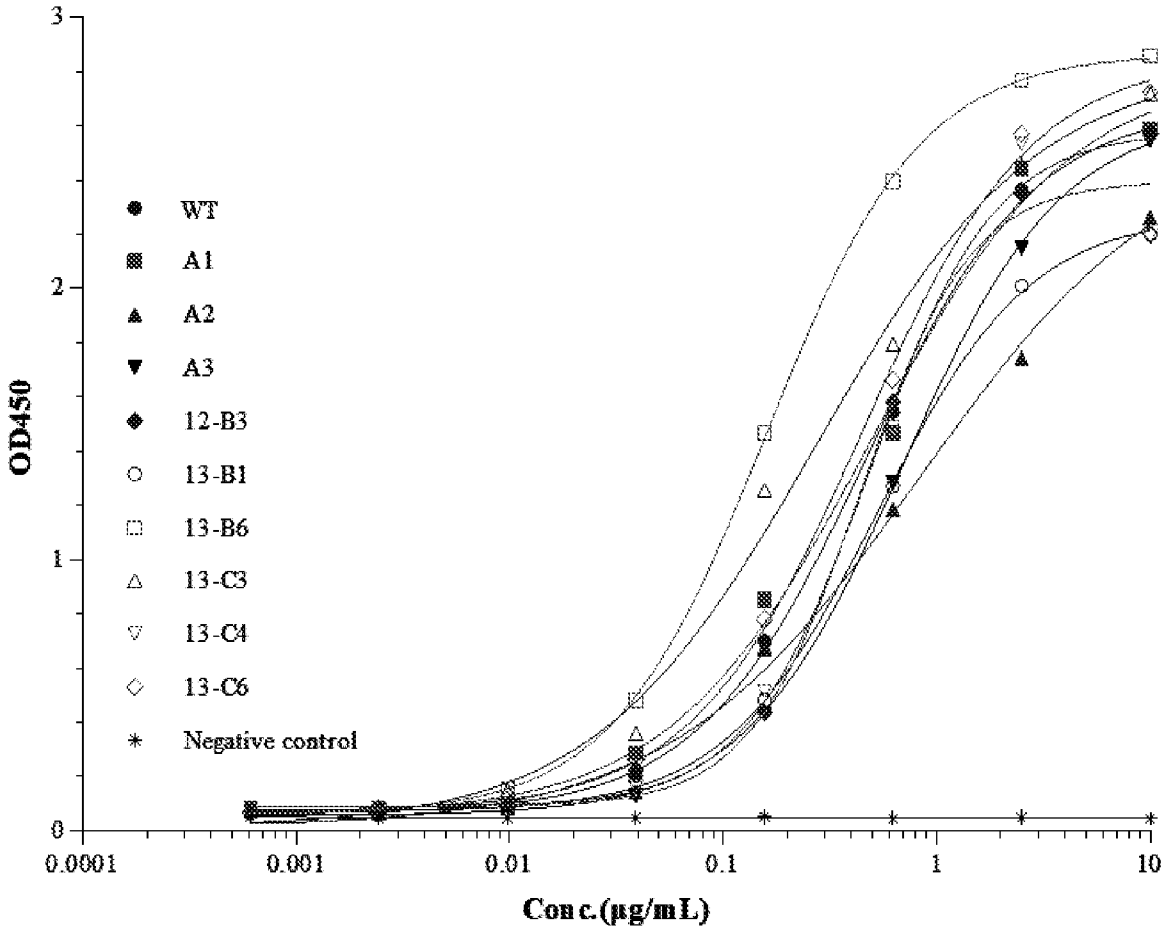
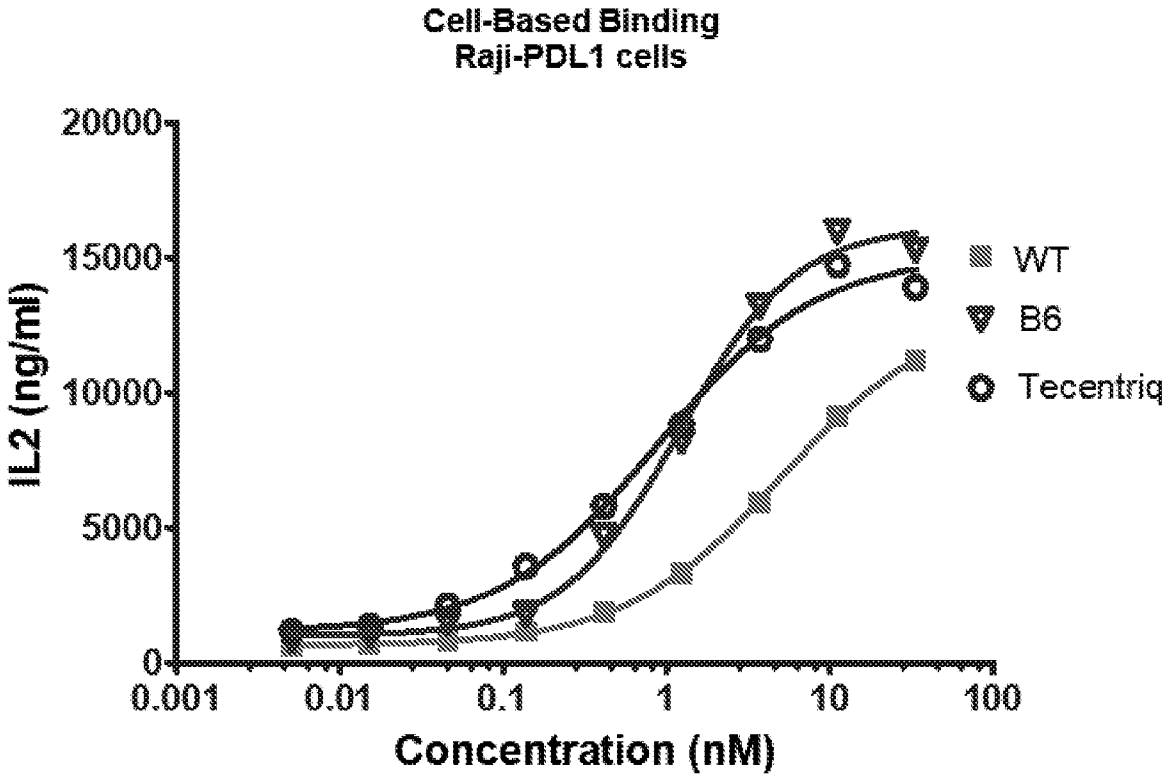
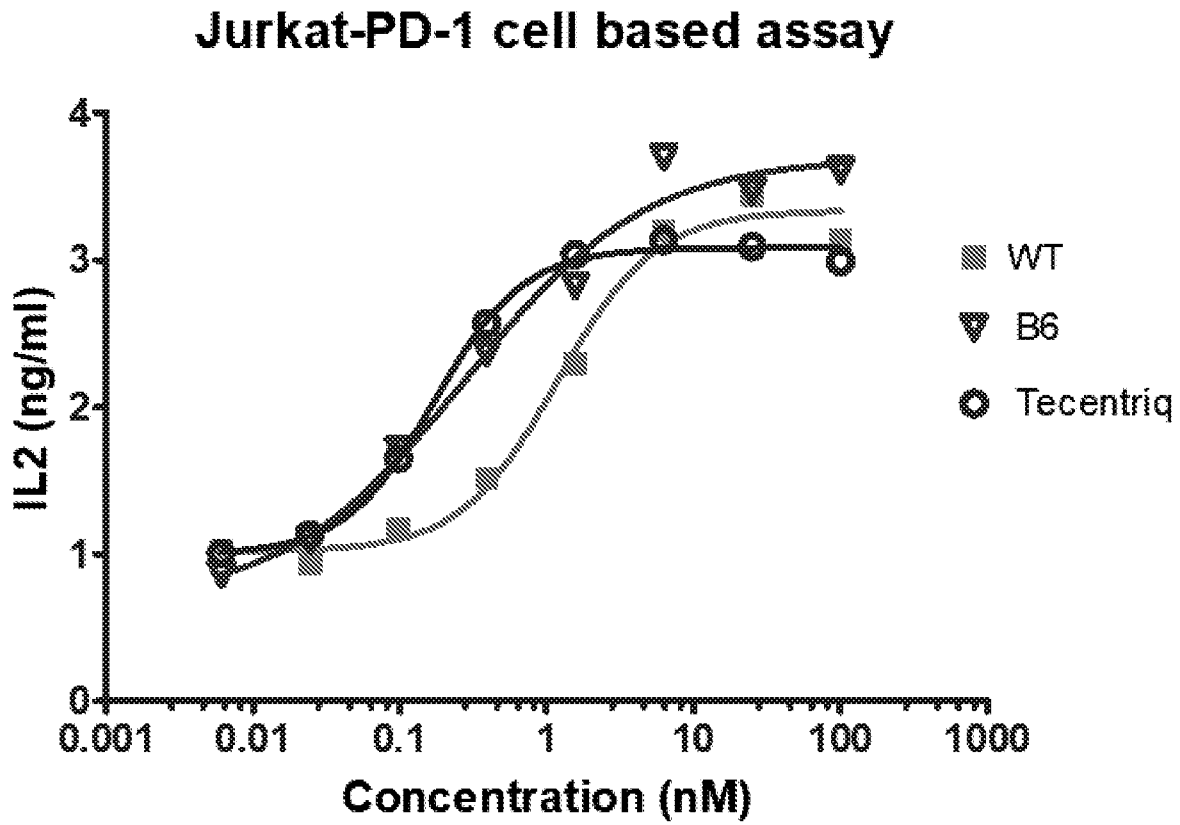

OD450
Conc.(μg/mL)
WT
A1
A2
A3
12-B3
13-B1
13-B6
13-C3
13-C4
13-C6
Negative control
0
1
2
3
0.0001
0.001
0.01
0.1
1
10

FIG. 18



	WT	B6	Tecentriq
EC50	5.043	1.204	0.8905

FIG. 19



	WT	B6	Tecentriq
EC50	1.184	0.2796	0.1715

FIG. 20

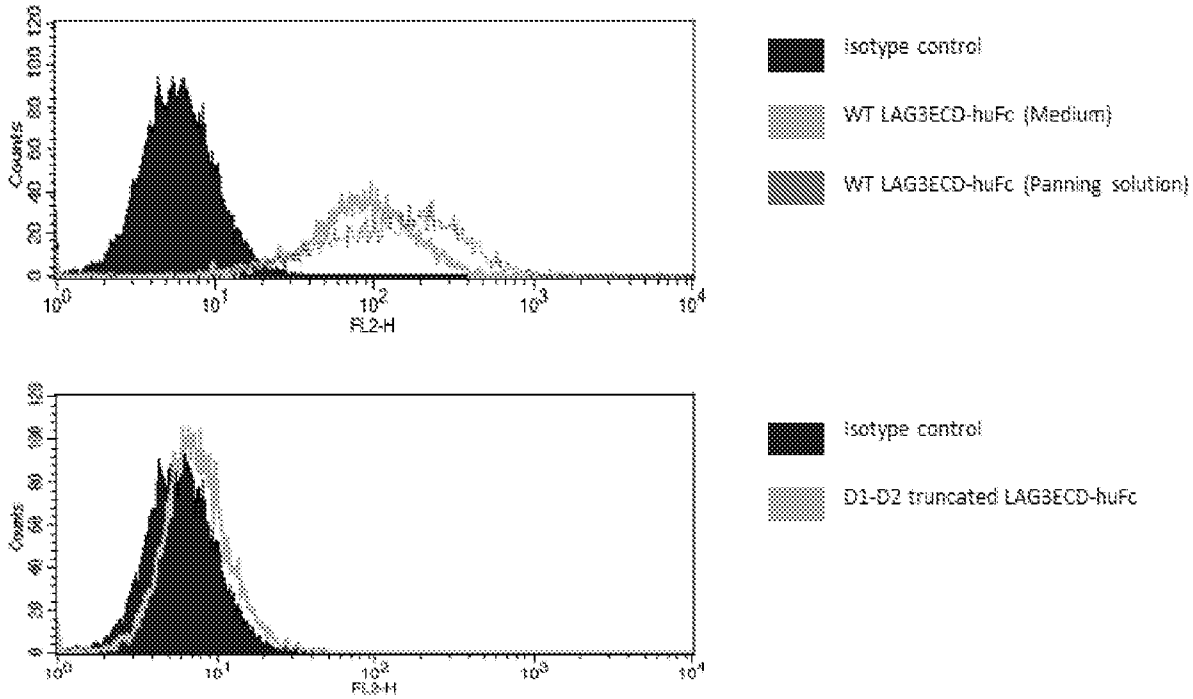
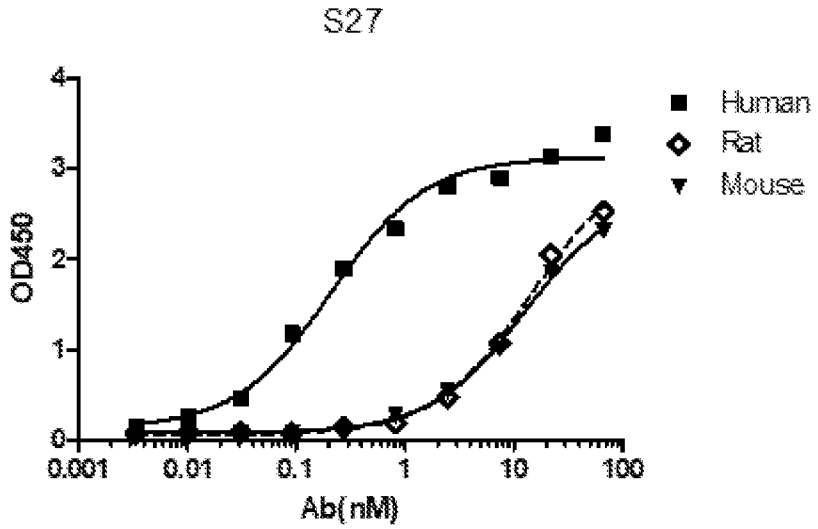
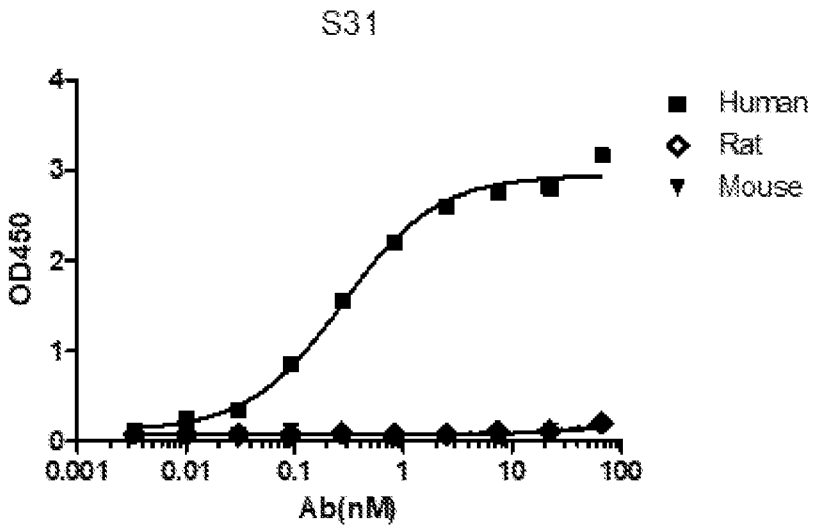

Counts
FL2-H
isotype control
WT LAG3ECD-huFc (Medium)
WT LAG3ECD-huFc (Panning solution)
Counts
FL2-H
isotype control
D1-D2 truncated LAG3ECD-huFc

FIG. 21



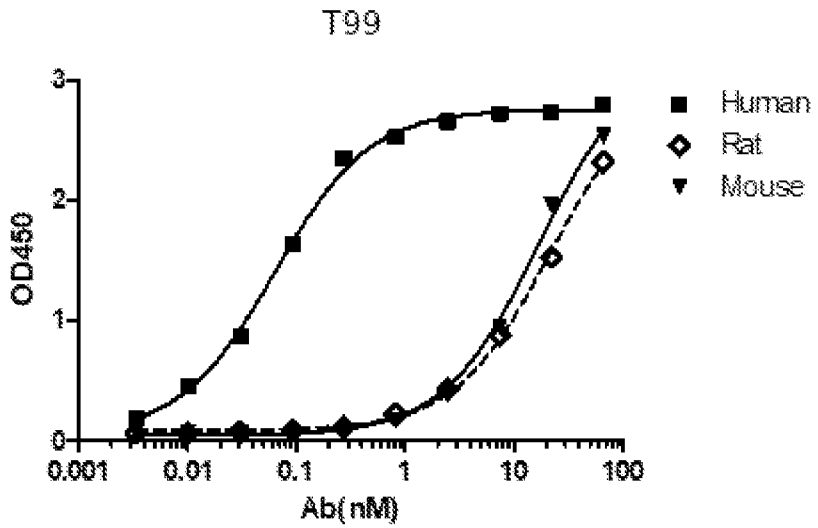
	EC50
Human	0.2096
Rat	13.95
Mouse	12.62

FIG. 22A



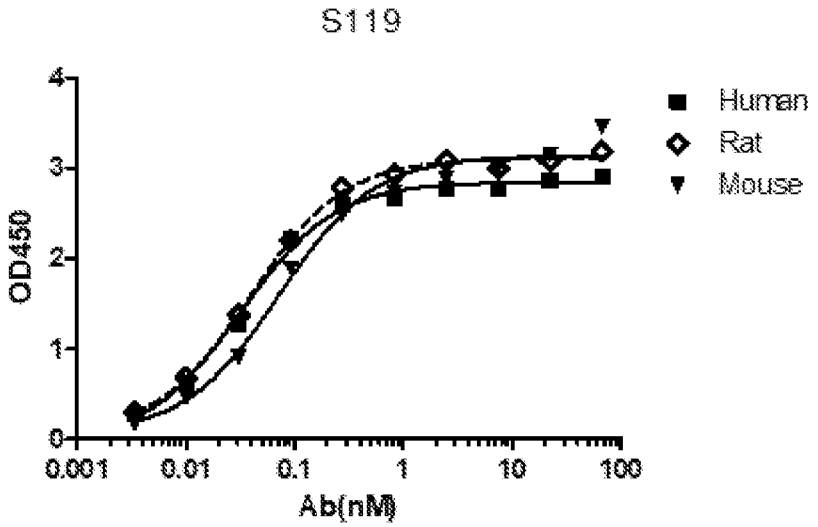
	EC50
Human	0.2789
Rat	~271270
Mouse	119.4

FIG. 22B



	EC50
Human	0.06384
Rat	20.85
Mouse	16.94

FIG. 22C



	EC50
Human	0.03207
Rat	0.03870
Mouse	0.07237

FIG. 22D

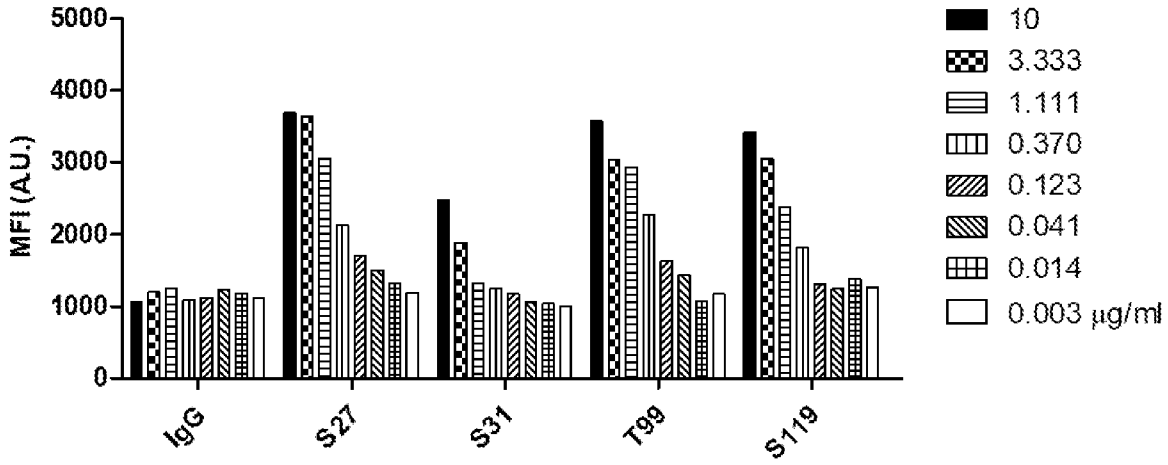

MFI (A.U.)
5000
4000
3000
2000
1000
0
IgG
S27
S31
T99
S119
10
3.333
1.111
0.370
0.123
0.041
0.014
0.003 μg/ml

FIG. 23

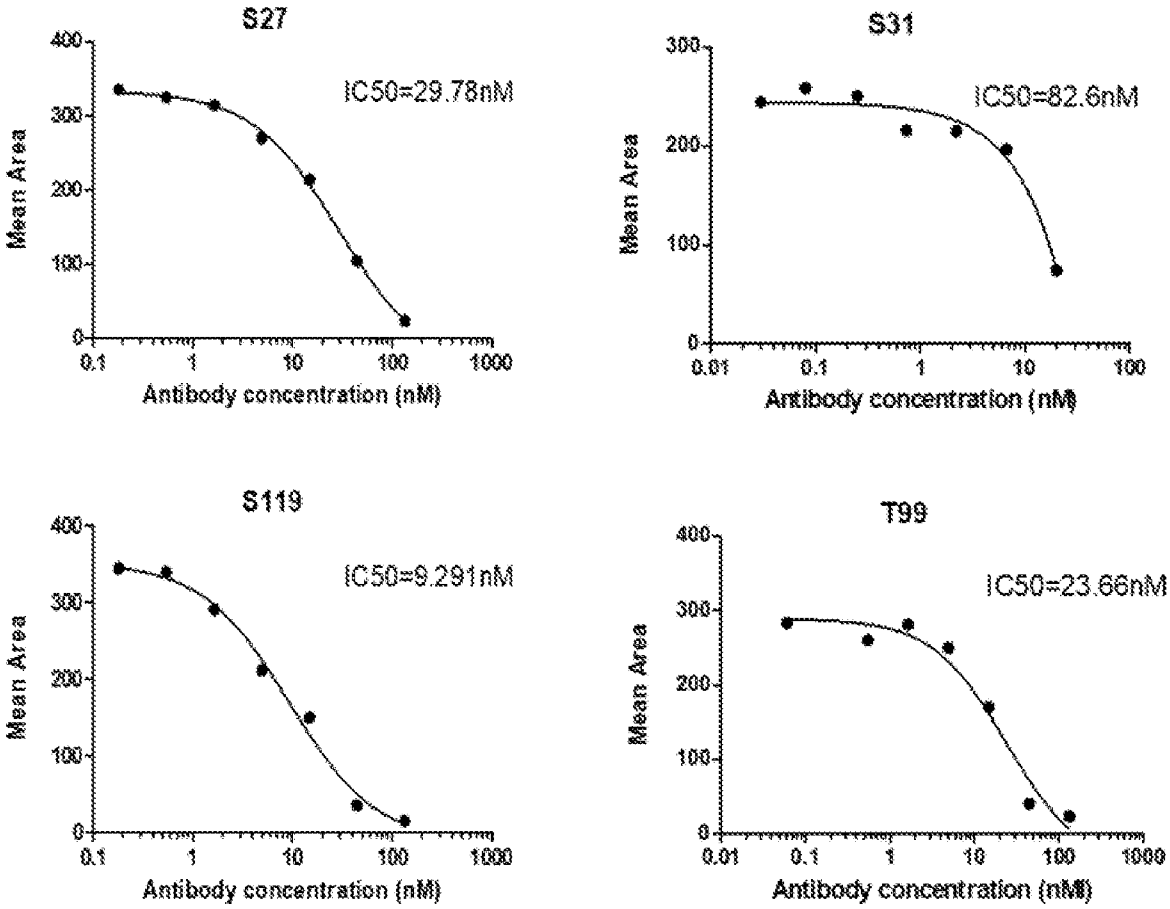

S27
IC50=29.78nM
S31
IC50=82.6nM
S119
IC50=9.291nM
T99
IC50=23.66nM
Mean Area
Antibody concentration (nM)

FIG. 24

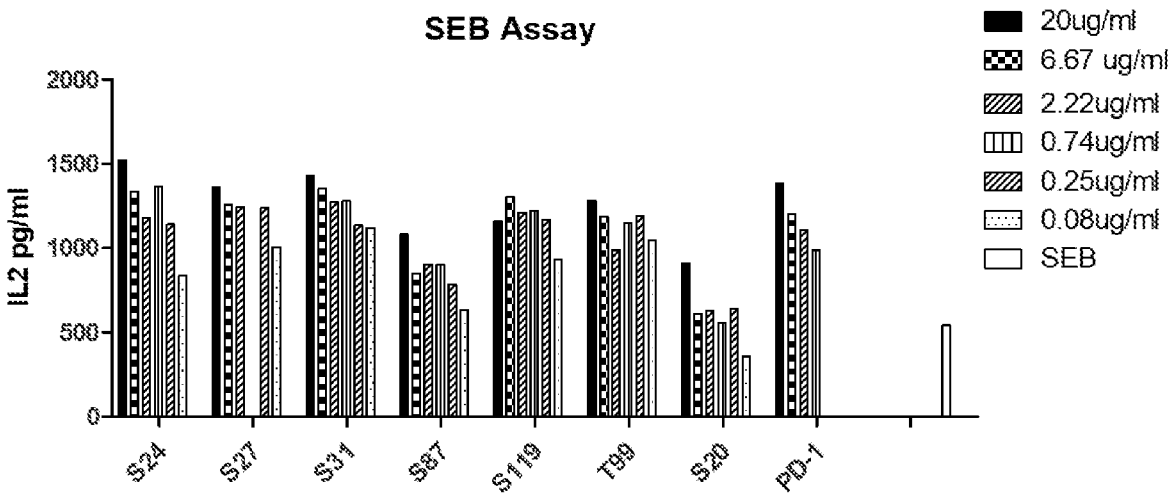

SEB Assay
IL2 pg/ml
2000
1500
1000
500
0
S24
S27
S31
S87
S119
T99
S20
PD-1
20ug/ml
6.67 ug/ml
2.22ug/ml
0.74ug/ml
0.25ug/ml
0.08ug/ml
SEB

FIG. 25

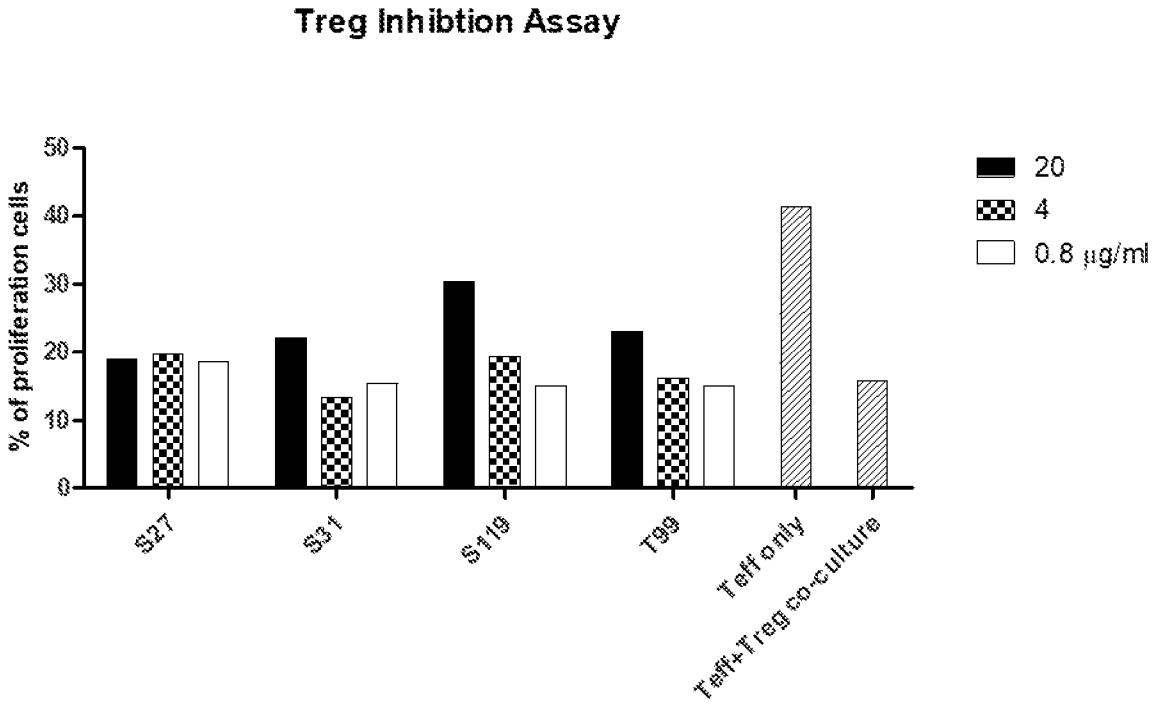
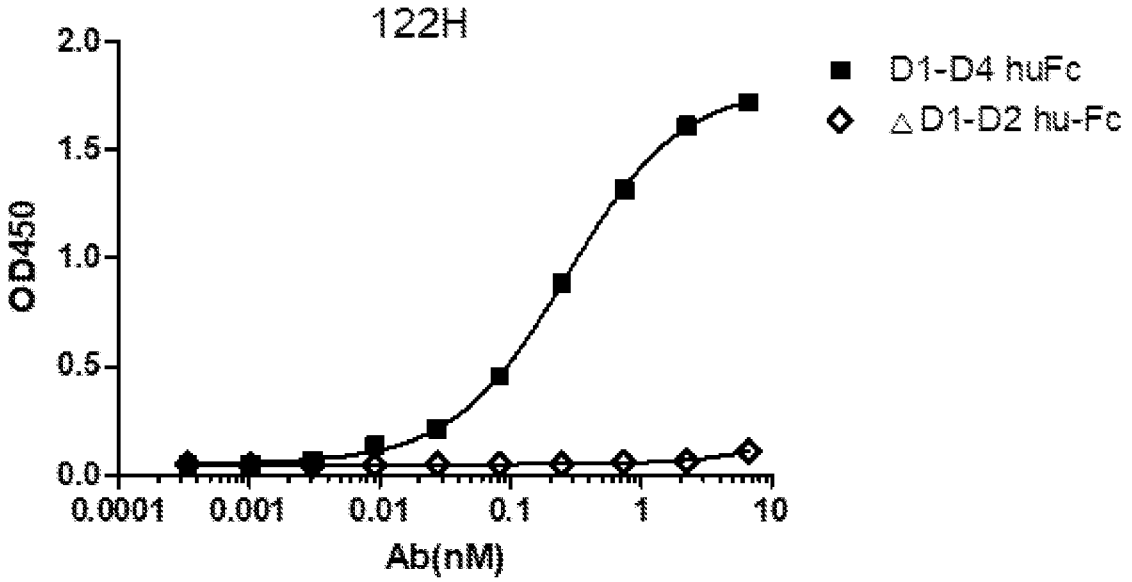
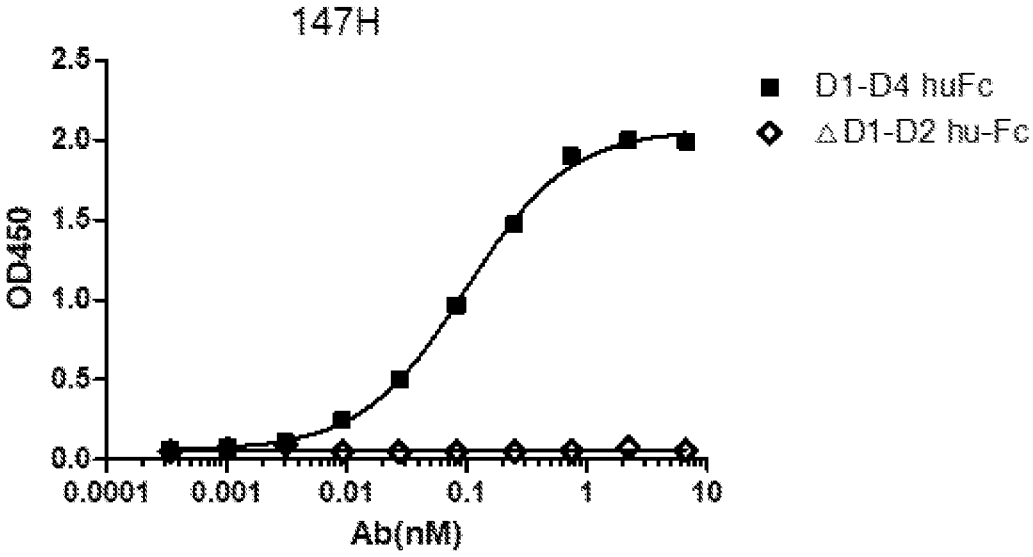

Treg Inhibtion Assay
% of proliferation cells
50
40
30
20
10
0
S27
S31
S119
T99
Teff only
Teff+Treg co-culture
20
4
0.8 μg/ml

FIG. 26



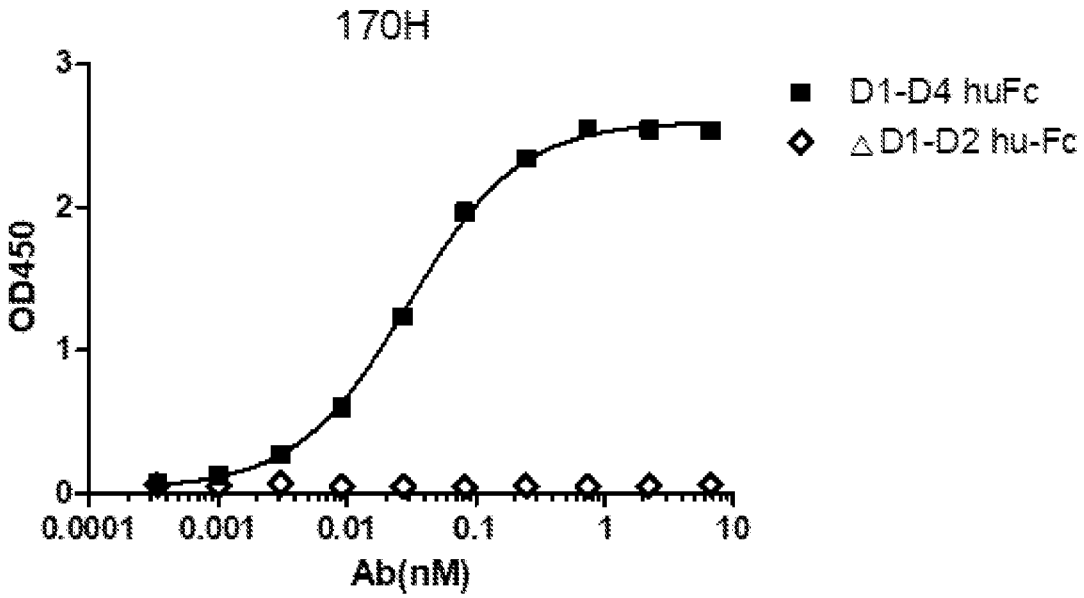
	EC50
D1-D4 huFc	0.2706
△D1-D2 LAG3-Fc	~ 44937

FIG. 27A



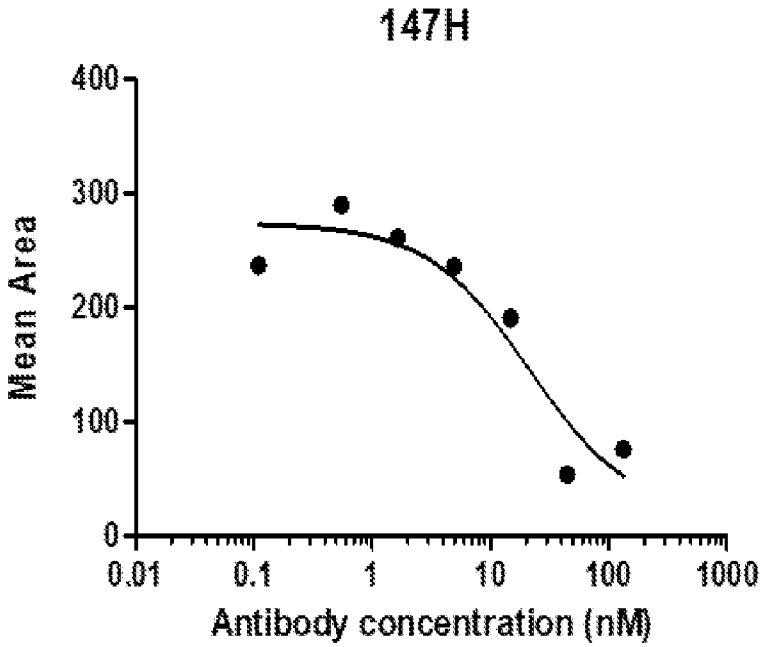
	EC50
D1-D4 huFc	0.09787
△D1-D2 hu-Fc	2.618

FIG. 27B



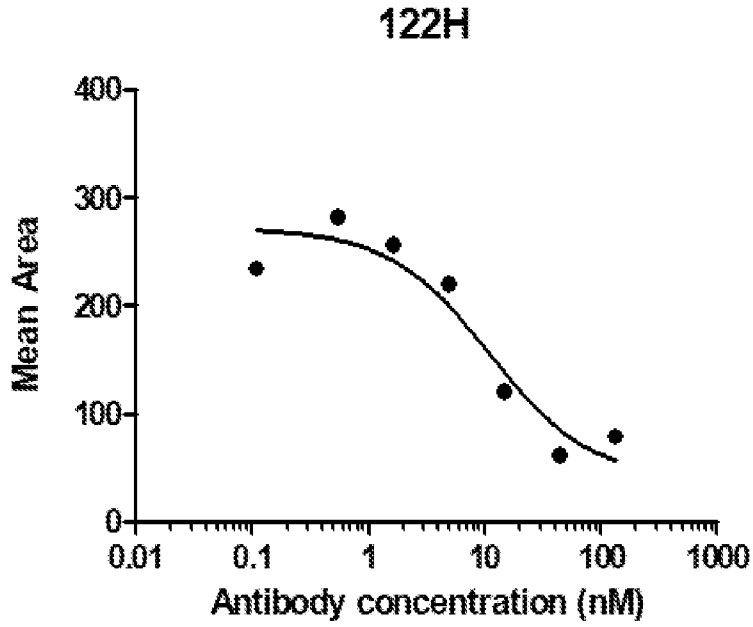
	EC50
D1-D4 huFc	0.02982

FIG. 27C



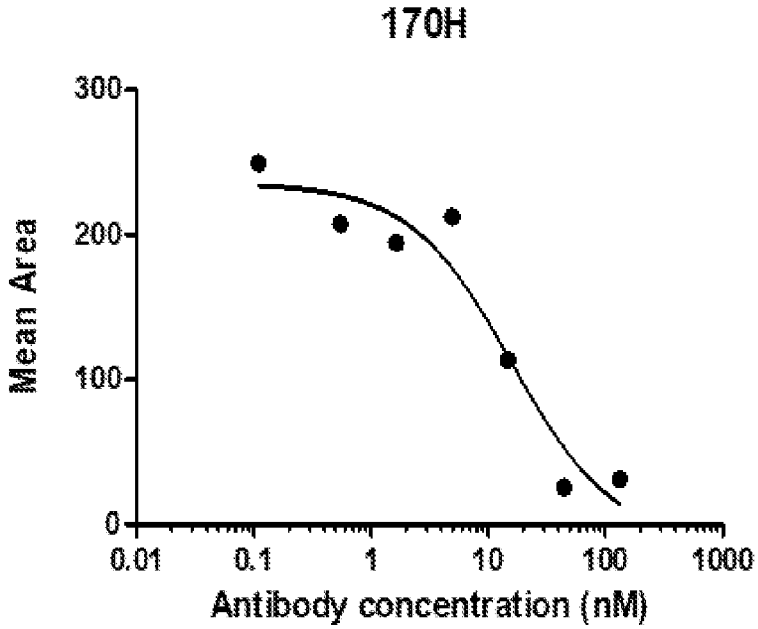
IC50=21.40 nM

FIG. 28A



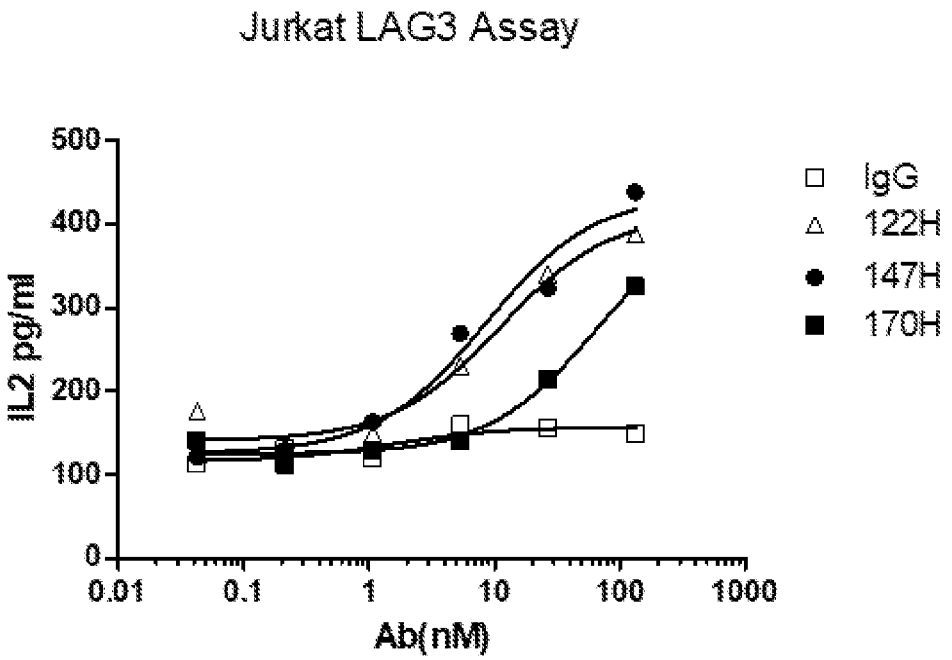
IC50=10.94 nM

FIG. 28B



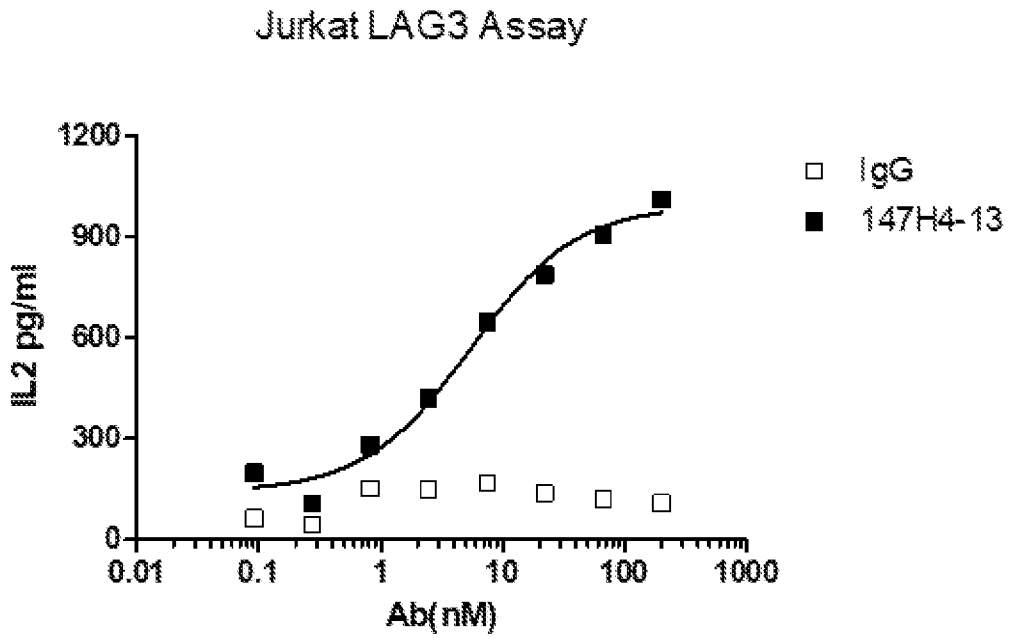
IC50=15.85 nM

FIG. 28C



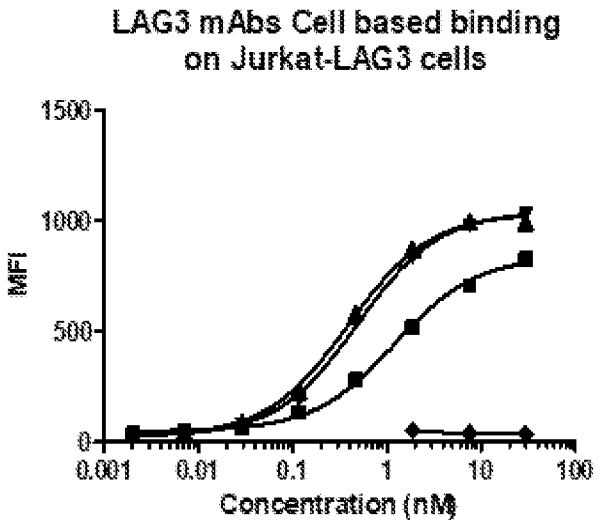
	EC50
IgG	1.471
122H	11.20
147H	8.474
170H	66.50

FIG. 29

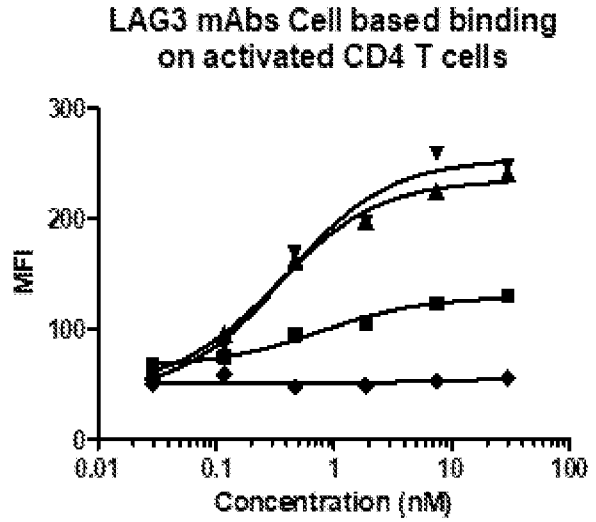


	EC50
147H-13	5.476

FIG. 30



	EC50
147H13	1.225
147H3807b	0.3897
147H3810	0.4925
IgG	0.4632



	EC50
147H13	0.7671
147H3807b	0.3328
147H3810	0.3914
IgG	3.590e+006

FIG. 31

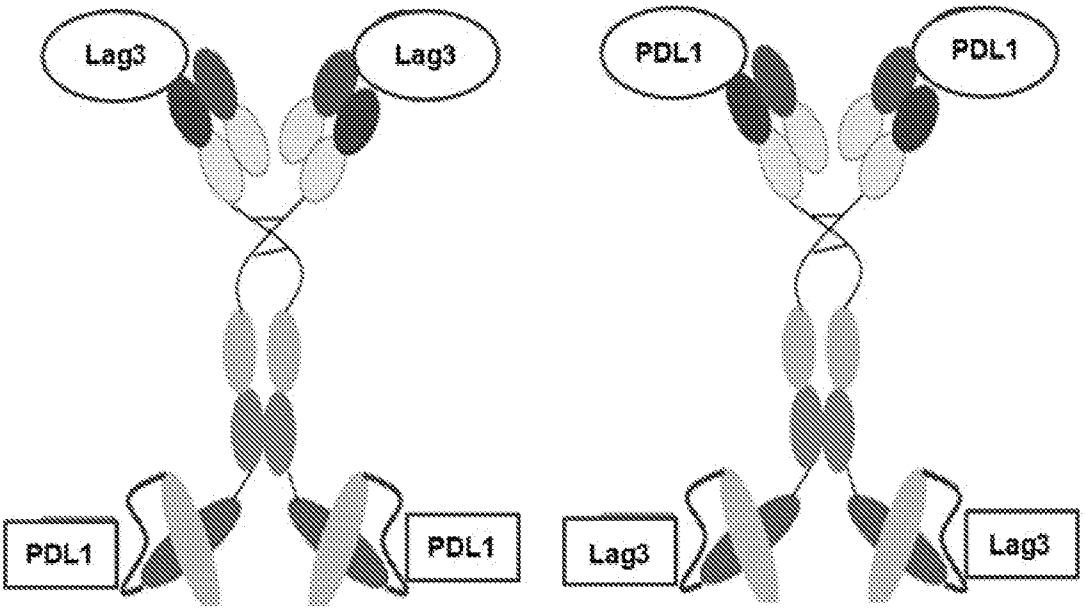


FIG. 32

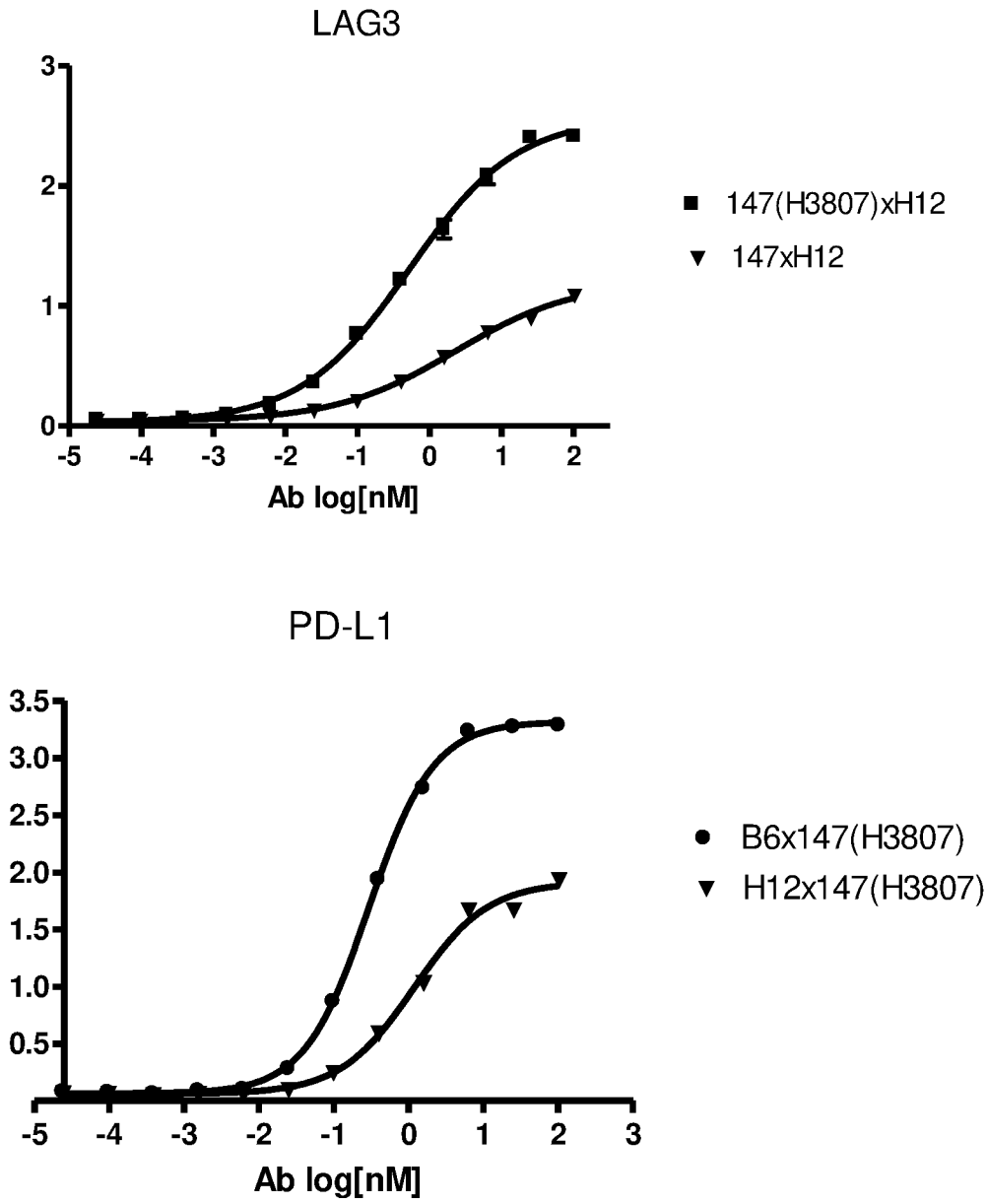

LAG3
3
2
1
0
-5
-4
-3
-2
-1
0
1
2
Ab log[nM]
147(H3807)xH12
147xH12
PD-L1
3.5
3.0
2.5
2.0
1.5
1.0
0.5
-5
-4
-3
-2
-1
0
1
2
3
Ab log[nM]
B6x147(H3807)
H12x147(H3807)

FIG. 33

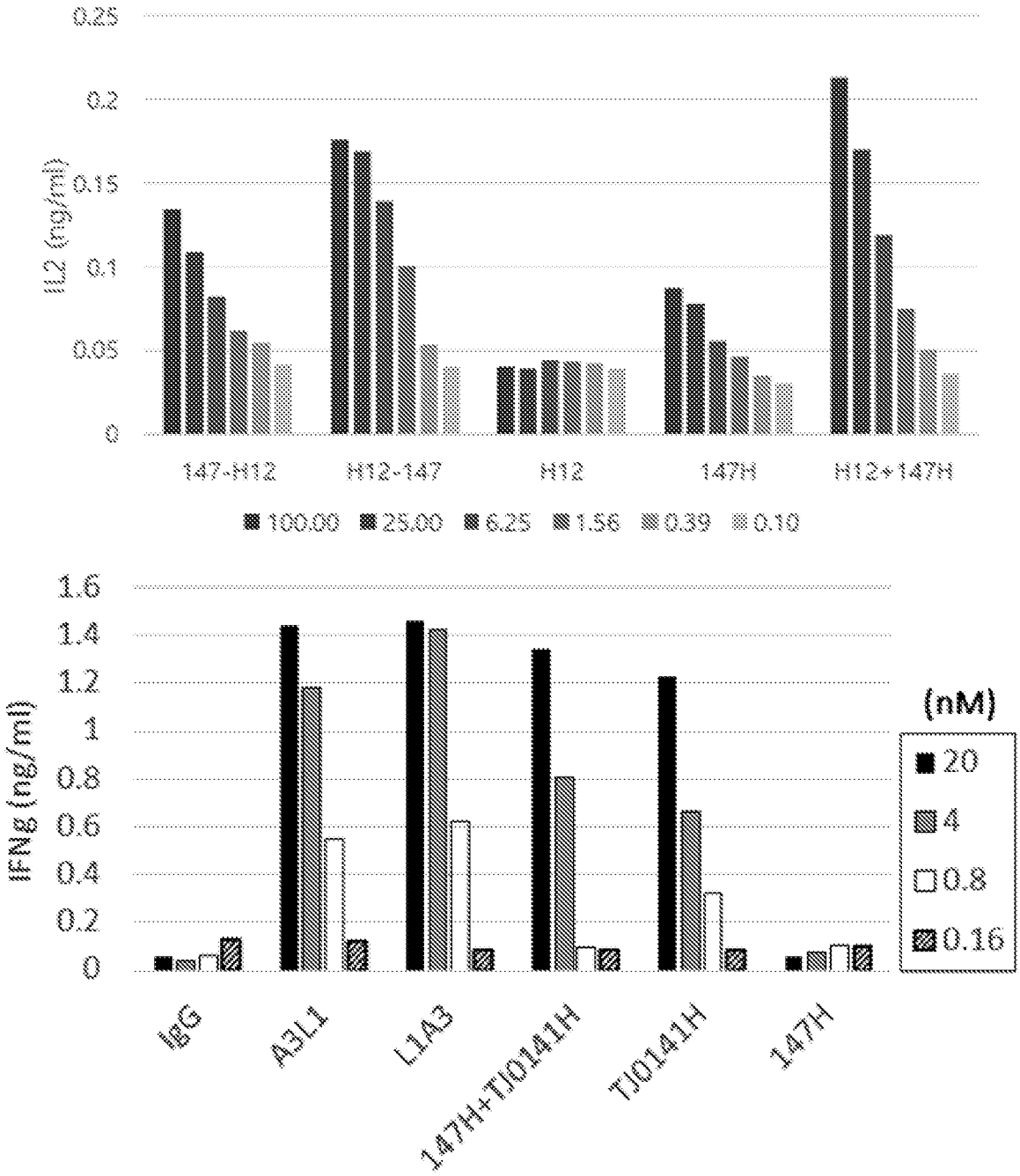

0.25
0.2
0.15
0.1
0.05
0
IL2 (ng/ml)
147-H12
H12-147
H12
147H
H12+147H
100.00
25.00
6.25
1.56
0.39
0.10
1.6
1.4
1.2
1
0.8
0.6
0.4
0.2
0
IFNg (ng/ml)
(nM)
20
4
0.8
0.16
IgG
A3L1
L1A3
147H+TJ0141H
TJ0141H
147H

FIG. 34

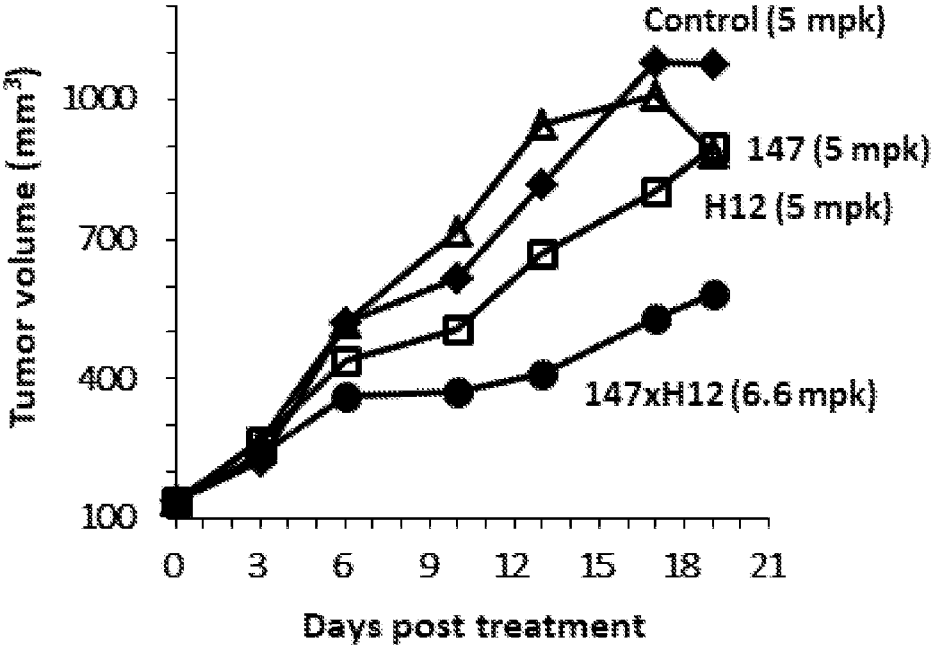

Control (5 mpk)
147 (5 mpk)
H12 (5 mpk)
147xH12 (6.6 mpk)
Tumor volume (mm3)
1000
700
400
100
0
3
6
9
12
15
18
21
Days post treatment

FIG. 35

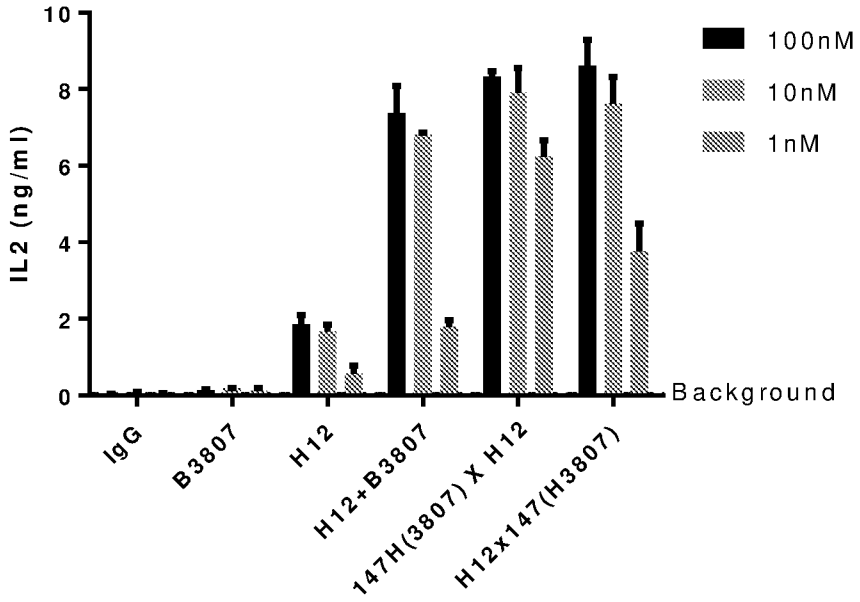

100nM
10nM
1nM
IL2 (ng/ml)
10
8
6
4
2
0
Background
IgG
B3807
H12
H12+B3807
147H(3807) X H12
H12x147(H3807)

FIG. 36

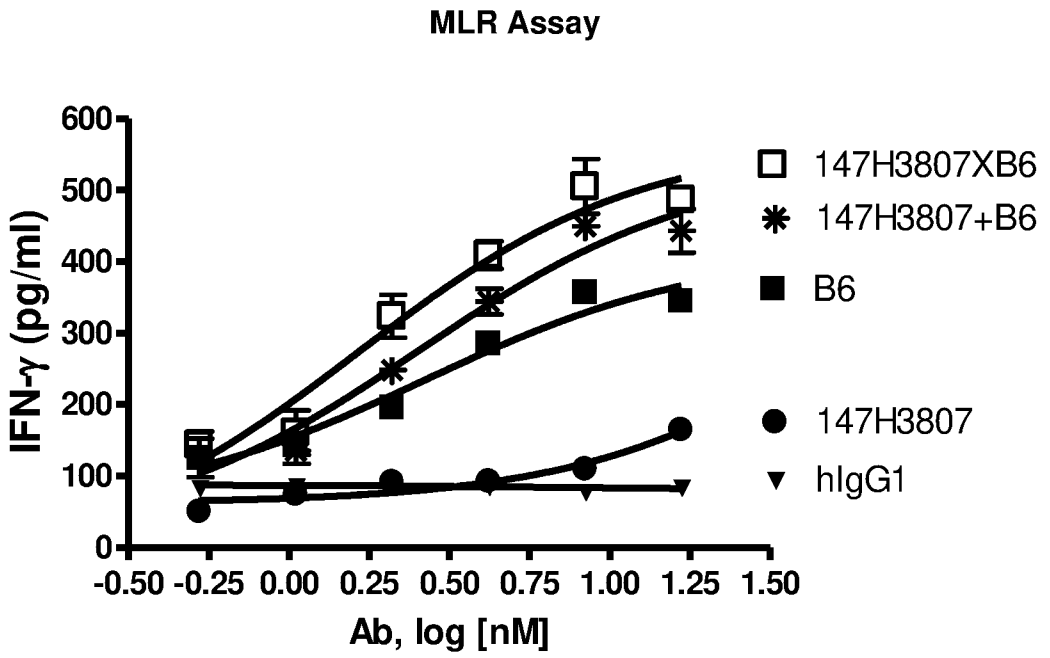
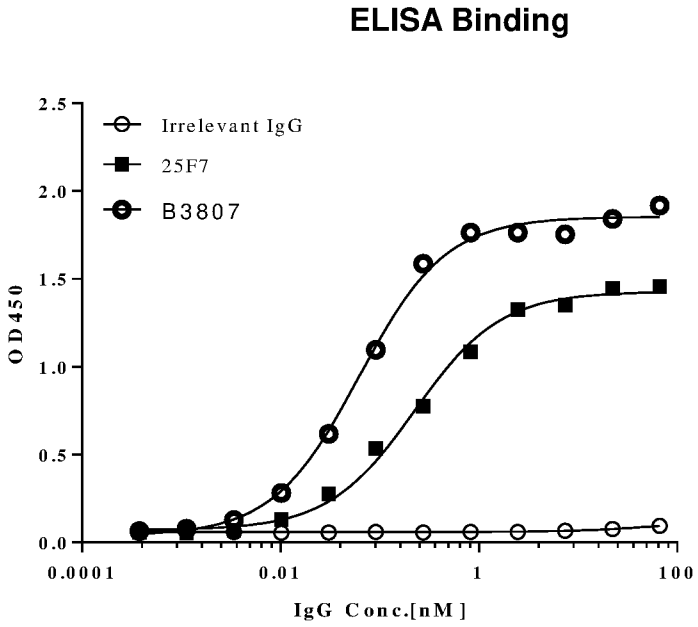


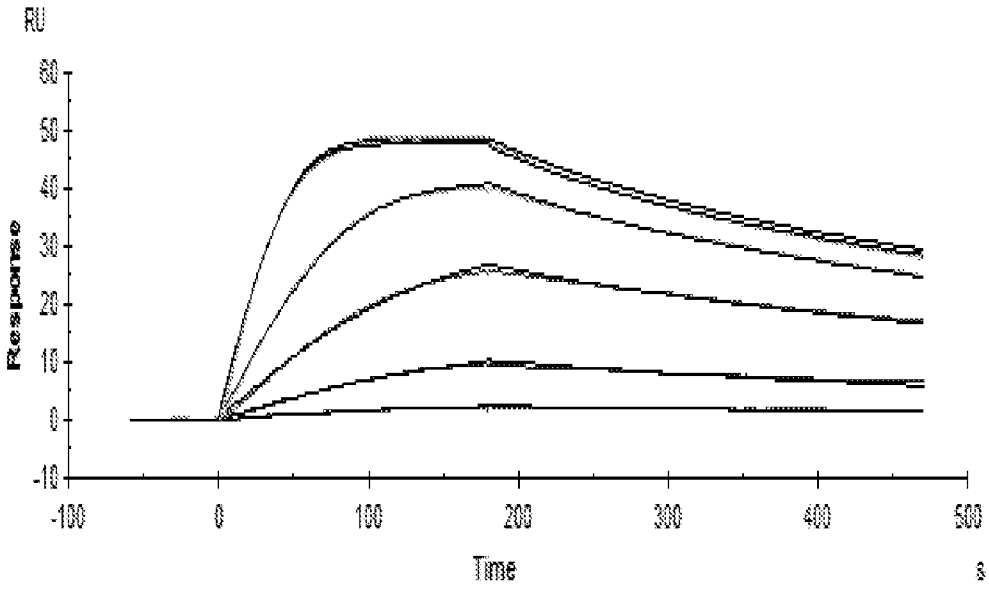
Fig. 37



	25F7	B3807
EC50 (nM)	0.22	0.06

FIG. 38

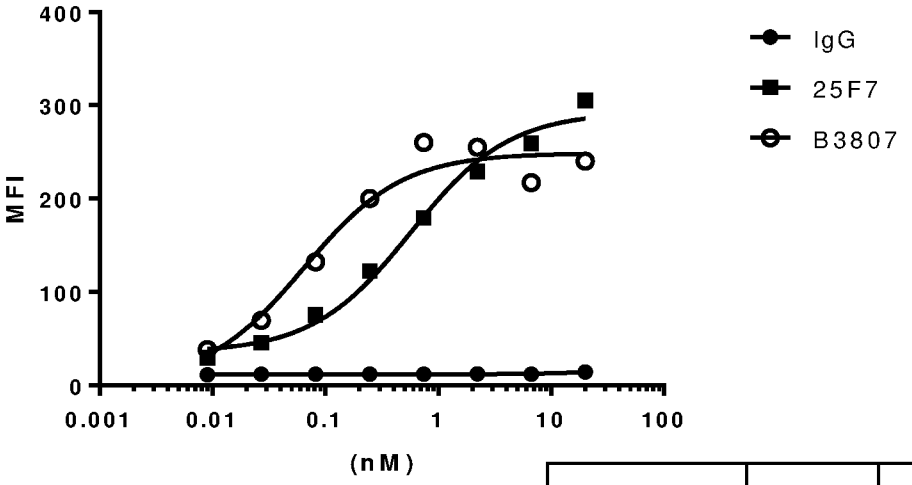
Biocore



	Kon	Koff	KD
B3807	6.08E+06	2.82E-03	4.64E-10

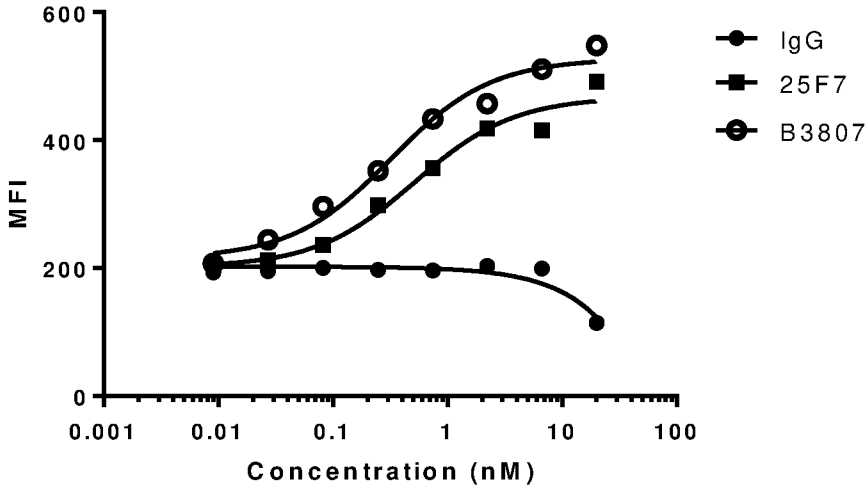
FIG. 39

Jurkat cell-based binding



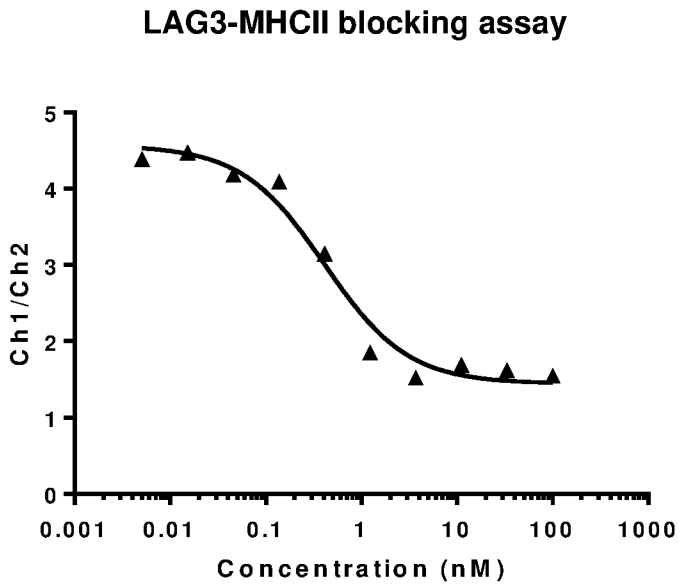
	25F7	B3807
EC50 (nM)	0.57	0.06

Human PBMC-based binding



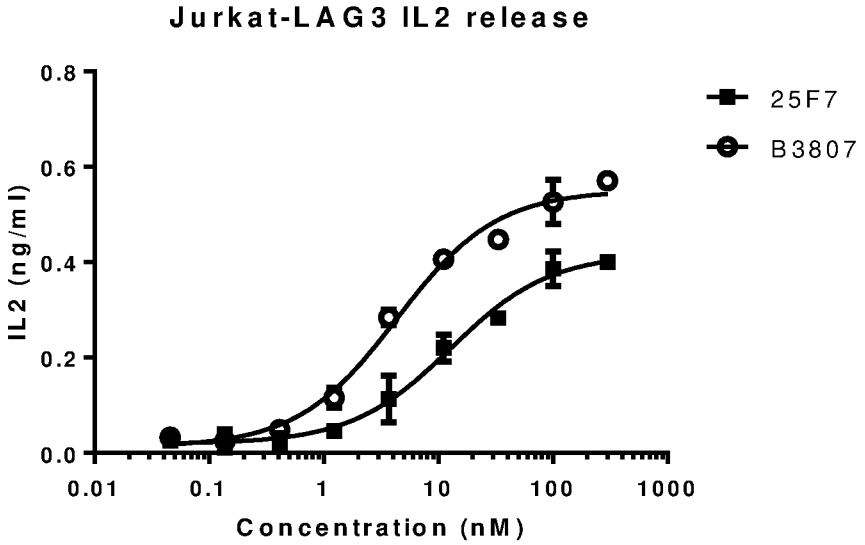
	25F7	B3807
EC50 (nM)	0.52	0.32

FIG. 40



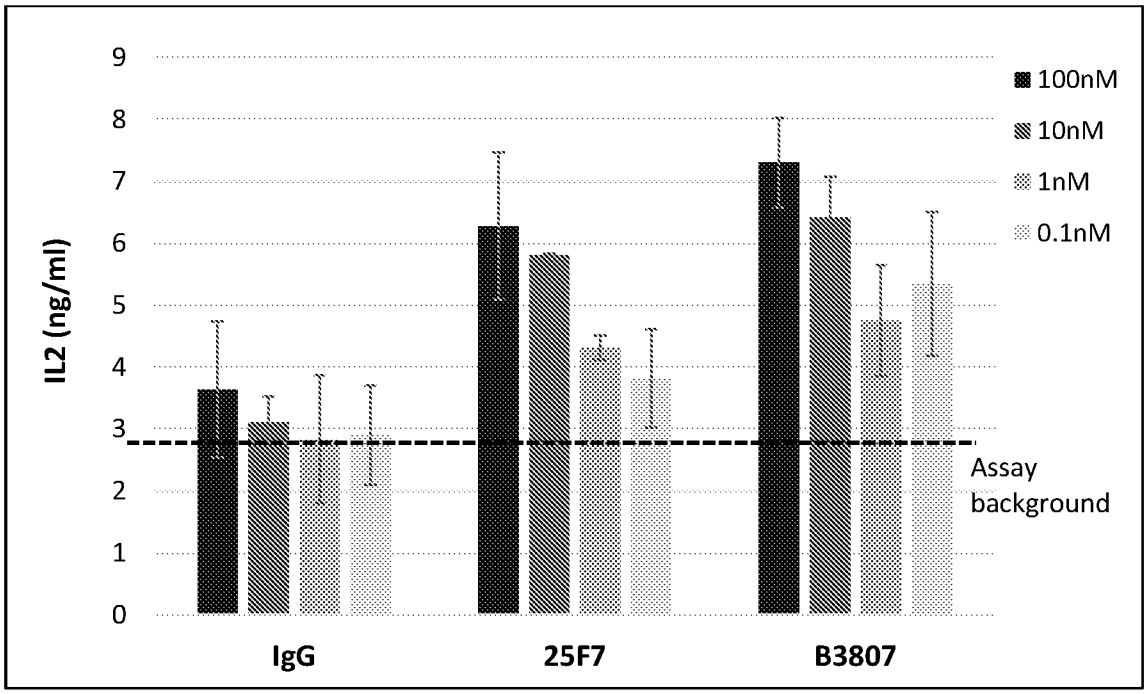
IC50=0.41nM

FIG. 41



	25F7	B3807
EC50 (nM)	12.74	4.35

FIG. 42


IL2 (ng/ml)
9
8
7
6
5
4
3
2
1
0
100nM
10nM
1nM
0.1nM
Assay background
IgG
25F7
B3807

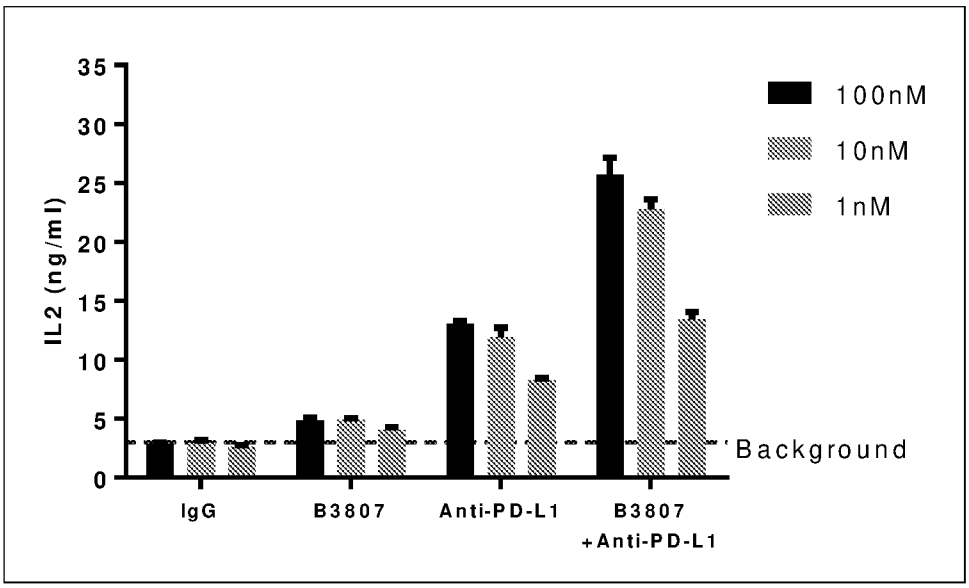
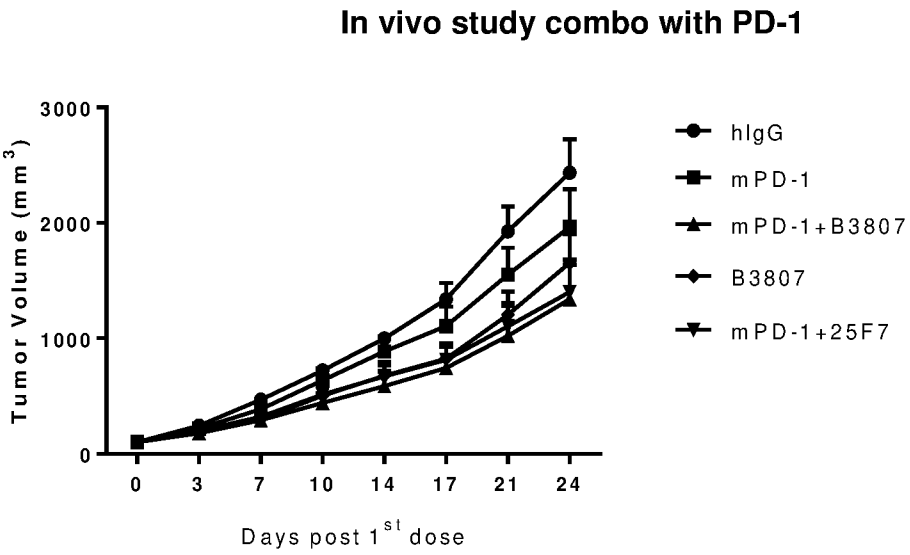

IL2 (ng/ml)
35
30
25
20
15
10
5
0
100nM
10nM
1nM
Background
IgG
B3807
Anti-PD-L1
B3807 +Anti-PD-L1

FIG. 43


In vivo study combo with PD-1
Tumor Volume (mm3)
3000
2000
1000
0
0
3
7
10
14
17
21
24
Days post 1st dose
hIgG
mPD-1
mPD-1+B3807
B3807
mPD-1+25F7

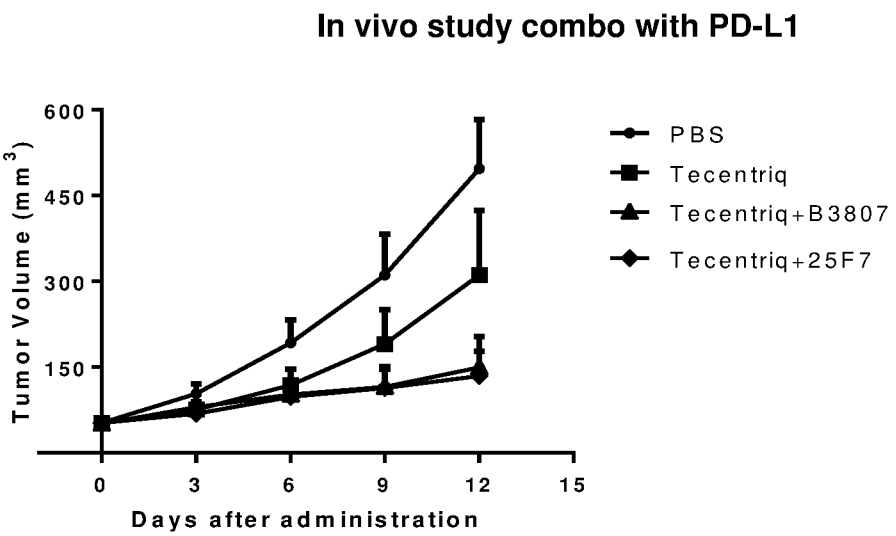

In vivo study combo with PD-L1
Tumor Volume (mm3)
600
450
300
150
0
3
6
9
12
15
Days after administration
PBS
Tecentriq
Tecentriq+B3807
Tecentriq+25F7

FIG. 44

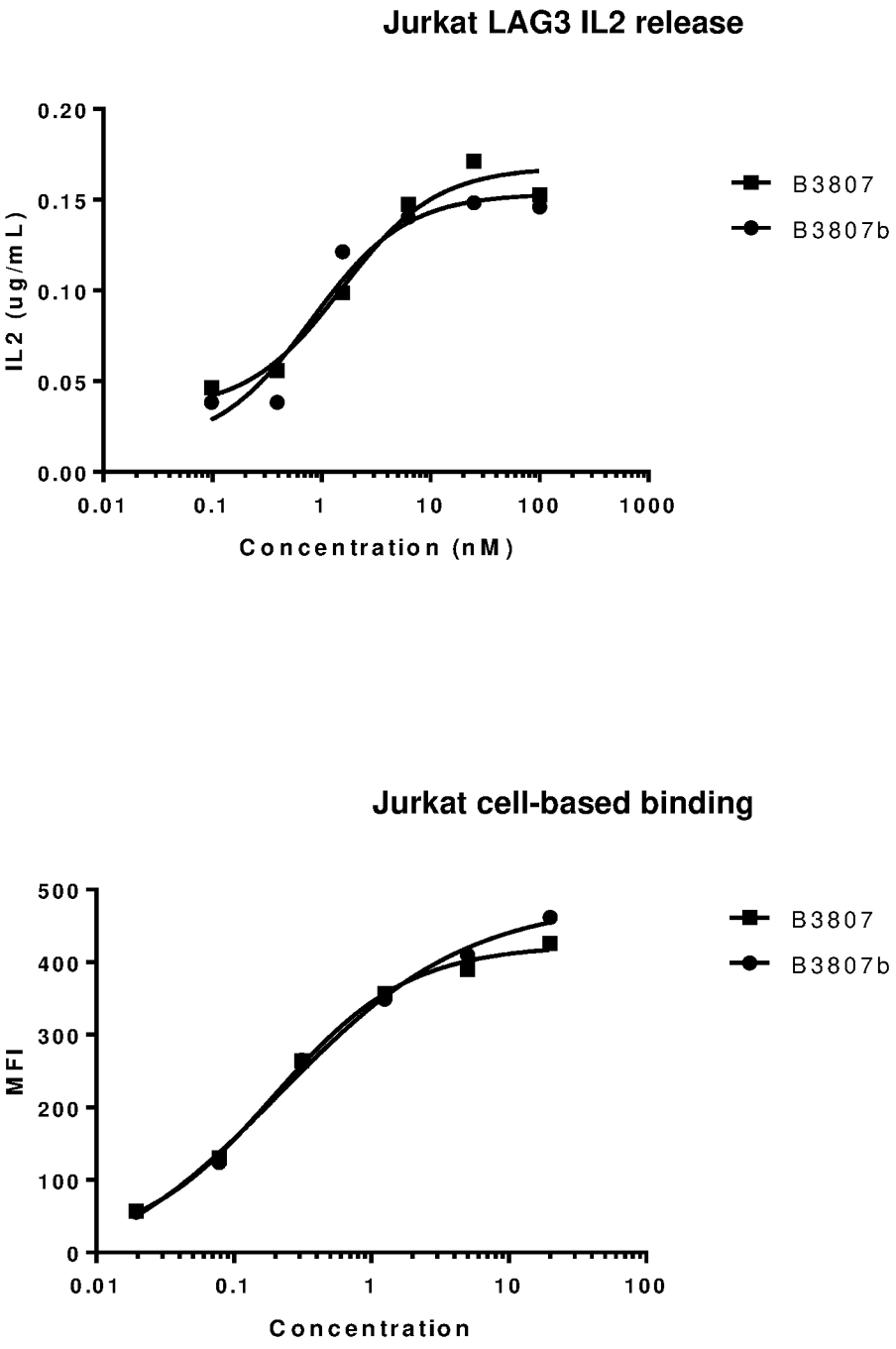
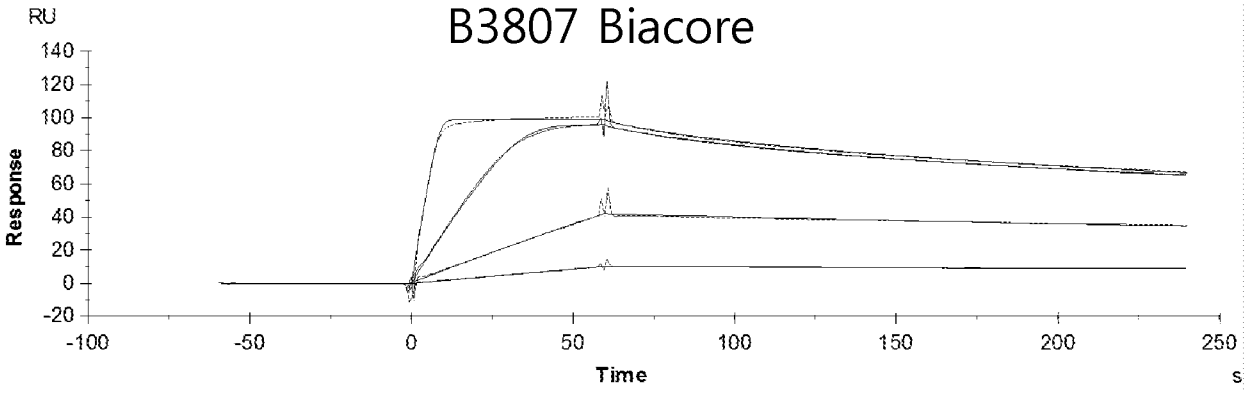
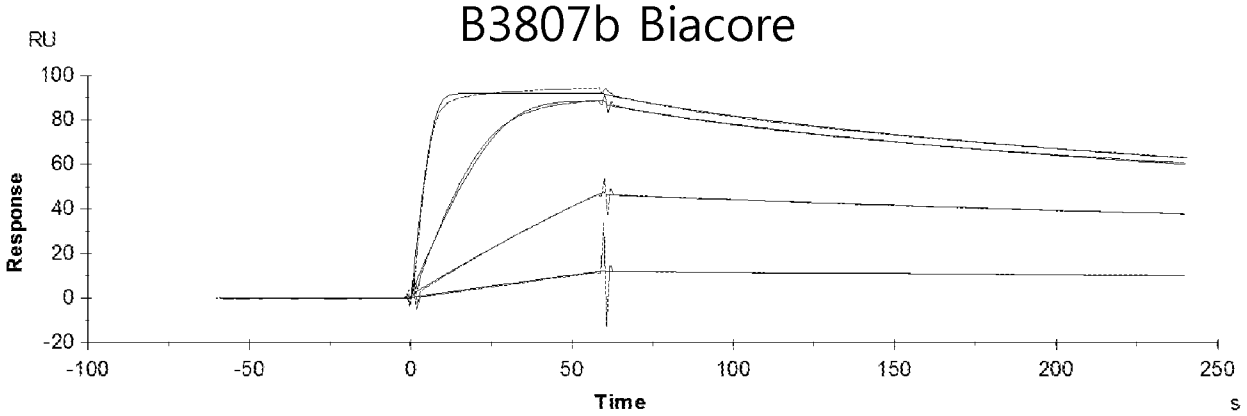

Jurkat LAG3 IL2 release
IL2 (ug/mL)
0.20
0.15
0.10
0.05
0.00
0.01
0.1
1
10
100
1000
Concentration (nM)
B3807
B3807b
Jurkat cell-based binding
MFI
500
400
300
200
100
0
0.01
0.1
1
10
100
Concentration
B3807
B3807b

FIG. 45





	ka (1/Ms)	kd(1/s)	KD (M)
B3807	5.66E+06	5.17E-03	9.15E-10
B3807b	3.46E+06	3.34E-03	9.68E-10

FIG. 46

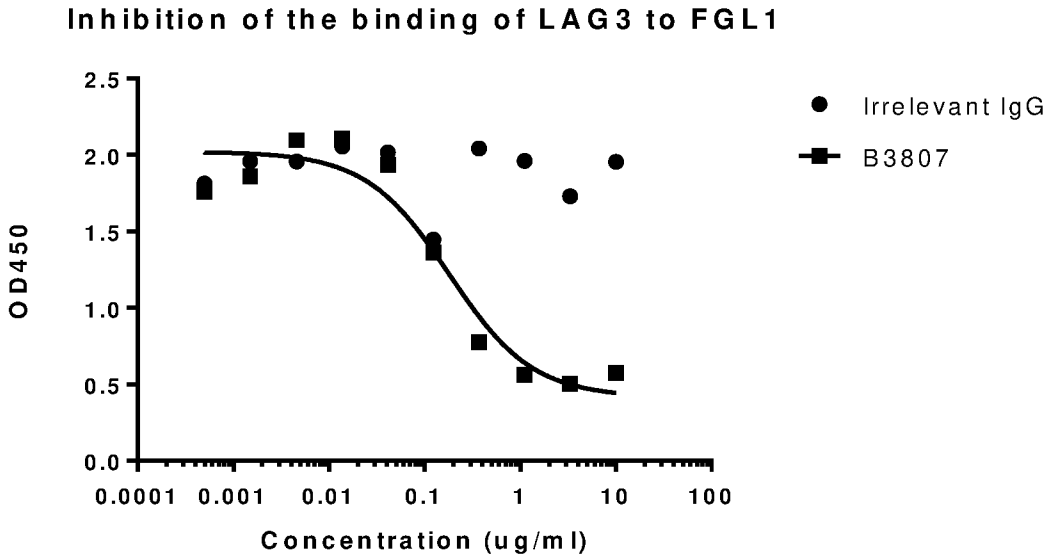

Inhibition of the binding of LAG3 to FGL1
OD450
2.5
2.0
1.5
1.0
0.5
0.0
0.0001
0.001
0.01
0.1
1
10
100
Concentration (ug/ml)
Irrelevant IgG
B3807

FIG. 47

INTERNATIONAL SEARCH REPORT

International application No.
PCT/CN2019/101747

A. CLASSIFICATION OF SUBJECT MATTER

C07K 16/46(2006.01)i; A61P 35/00(2006.01)i; A61K 39/395(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)

C07K; A61P; A61K

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)

CNABS, DWPI, SIPOABS, CNTXT, WOTXT, USTXT, EPTXT, JPTXT, KRTXT, CNKI, WANFANG DATABASE, BAIDU, PubMed, ISI Web of Knowledge, GenBank, EMBL-EBI, Chinese Patent Biological Sequence Retrieval System, STN: I-MAB, ABL BIO, PD-L1, programmed death ligand-1, CD274, cluster of differentiation 274, B7-H1, B7 homolog 1, LAG3, lymphocyte activation gene-3, CD223,bispecific antibody ,bsAb, immunoglobulin C domain, IgC, SEQ ID Nos: 1-555

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	WO 2017215590 A1 (I-MAB) 21 December 2017 (2017-12-21) claims 1-20, 22-24, 47-50, description tables 5, 8 and 13	18, 25-28
A	WO 2017215590 A1 (I-MAB) 21 December 2017 (2017-12-21) claims 1-20, 22-24, 47-50, description tables 5, 8 and 13	1-17
PX	WO 2018153340 A1 (I MAB) 30 August 2018 (2018-08-30) claims 1-35, 40, 42-49, description tables 1-8	19-28
PY	WO 2018153340 A1 (I MAB) 30 August 2018 (2018-08-30) claims 1-35, 40, 42-49, description tables 1-8	1-17
PX	WO 2019185029 A1 (I MAB) 03 October 2019 (2019-10-03) claims 1-78, description page 26 paragraph 2 to page 31 paragraph 1, tables 1, 5, 8 and 13-15	18, 25-28
PY	WO 2019185029 A1 (I MAB) 03 October 2019 (2019-10-03) claims 1-78, description page 26 paragraph 2 to page 31 paragraph 1, tables 1, 5, 8 and 13-15	1-17

☑ 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
05 November 2019	**21 November 2019**

Name and mailing address of the ISA/CN	Authorized officer
National Intellectual Property Administration, PRC **6, Xitucheng Rd., Jimen Bridge, Haidian District, Beijing** **100088** **China**	**YANG,Jiaqian**
Facsimile No. **(86-10)62019451**	Telephone No. **86-(10)-53961939**

INTERNATIONAL SEARCH REPORT

International application No.
PCT/CN2019/101747

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
A	WO 2016061142 A1 (NOVARTIS AG ET AL.) 21 April 2016 (2016-04-21) the whole document	1-28
A	DOODY, J.et al. "A LAG-3/PD-L1 bispecific antibody inhibits tumor growth in two syngeneic colon carcinoma models." *Cancer Immunol. Res.*, Vol. 4, No. 11 Suppl., 30 November 2016 (2016-11-30), the abstract	1-28
A	DAHLÉN, E.et al. "Bispecific antibodies in cancer immunotherapy." *Therapeutic Advances in Vaccines and Immunotherapy.*, Vol. 6, No. 1, 28 March 2018 (2018-03-28), pages 3-17	1-28

INTERNATIONAL SEARCH REPORT

International application No.
PCT/CN2019/101747

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
Information on patent family members

International application No.
PCT/CN2019/101747

Patent document cited in search report	Publication date (day/month/year)	Patent family member(s)	Publication date (day/month/year)
WO 2017215590 A1	21 December 2017	KR 101996019 B1	03 July 2019
		US 2018346574 A1	06 December 2018
		KR 20180039182 A	17 April 2018
		US 2018346573 A1	06 December 2018
		US 2018208659 A1	26 July 2018
		IL 263509 D0	31 January 2019
		SG 11201811003P A	30 January 2019
		CL 2018003583 A1	17 May 2019
		BR 112018075737 A2	26 March 2019
		KR 20190079713 A	05 July 2019
		AU 2017284632 A1	03 January 2019
		CA 3027209 A1	21 December 2017
		EP 3325513 A1	30 May 2018
		US 10059769 B2	28 August 2018
		US 10208119 B2	19 February 2019
		EP 3325513 A4	19 December 2018
		CO 2018013500 A2	19 February 2019
		CN 108350082 A	31 July 2018
		PE 20190510 A1	10 April 2019
WO 2018153340 A1	30 August 2018	KR 20180113195 A	15 October 2018
		CN 109475617 A	15 March 2019
		US 2019225687 A1	25 July 2019
		EP 3389702 A4	14 August 2019
		CA 3053989 A1	30 August 2018
		EP 3389702 A1	24 October 2018
		AU 2018226298 A1	19 September 2019
WO 2019185029 A1	03 October 2019	None	
WO 2016061142 A1	21 April 2016	AU 2015333687 A1	27 April 2017
		TW 201620940 A	16 June 2016
		BR 112017007379 A2	19 December 2017
		US 2018186882 A1	05 July 2018
		CR 20170143 A	19 June 2017
		PE 10672017 A1	24 July 2017
		KR 20170069257 A	20 June 2017
		CN 107001478 A	01 August 2017
		US 9988452 B2	05 June 2018
		TN 2017000129 A1	19 October 2018
		SG 11201702401R A	27 April 2017
		PH 12017500692 A1	09 October 2017
		CA 2964367 A1	21 April 2016
		GT 201700081 A	27 November 2018
		EP 3206711 A1	23 August 2017
		UY 36351 A	01 June 2016
		EA 201790834 A1	31 January 2018
		US 2016108123 A1	21 April 2016
		IL 251563 D0	29 May 2017
		CU 20170052 A7	07 November 2017
		MX 2017004810 A	16 October 2017
		JP 2017536099 A	07 December 2017
		CL 2017000888 A1	18 August 2017

INTERNATIONAL SEARCH REPORT
Information on patent family members

International application No.
PCT/CN2019/101747

Patent document cited in search report	Publication date (day/month/year)	Patent family member(s)	Publication date (day/month/year)