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(71) Applicant *(for all designated States except CN)*: **WUXI BIOLOGICS IRELAND LIMITED** [IE/IE]; One Spencer Dock, North Wall Quay, Dublin 1 (IE).

(71) Applicant *(for CN only)*: **GUANGZHOU GOLIA BIOSCIENCES CO., LTD** [CN/CN]; Room 4, Unit 204, No. 2 Building, Ruihe Road, Science City, High-Tech Development Zone, Guangzhou, Guangdong 510663 (CN).

(72) Inventors: **ZHENG, Yong**; No. 299 Fute Zhong Road, Pudong New District, Shanghai 200131 (CN). **WU, Qiong**; No. 299 Fute Zhong Road, Pudong New District, Shanghai 200131 (CN). **LI, Jing**; No. 299 Fute Zhong Road, Pudong New District, Shanghai 200131 (CN).

(74) Agent: **CCPIT PATENT AND TRADEMARK LAW OFFICE**; 8th Floor, Vantone New World Plaza, 2 Fuchengmenwai Street, Xicheng District, Beijing 100037 (CN).

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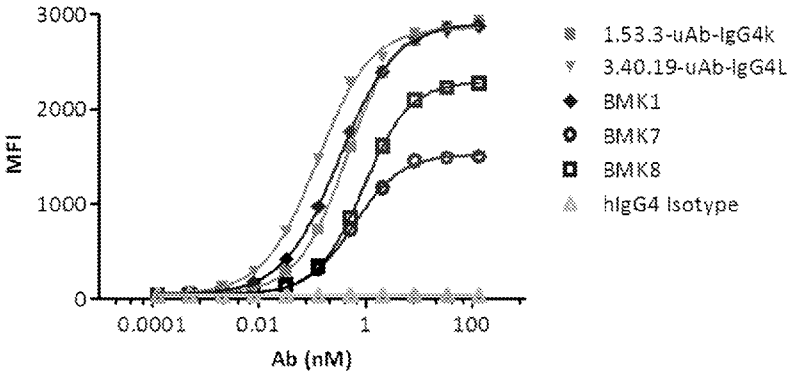


Figure 1

(57) Abstract: Provided are novel fully human monoclonal antibodies that bind to human LAG-3 for the treatment of multiple cancers via modulating human immune function, the methods of hybridoma generation using humanized rats, the nucleic acid molecules encoding the anti-LAG-3 antibodies, expression vectors and host cells used for the expression of anti-LAG-3 antibodies, and methods for validating the function of antibodies in vitro.

WO 2019/165982 A1

MONOCLONAL ANTIBODY AGAINST HUMAN LAG-3, METHOD FOR PREPARING SAME, AND USE THEREOF

SEQUENCE LISTING

The instant application contains a sequence listing and is hereby incorporated by reference in its entirety.

FIELD OF THE INVENTION

This application generally relates to antibodies. More specifically, the application relates to fully human monoclonal antibodies that bind to human LAG-3, a method for preparing the same, and the use thereof.

BACKGROUND OF THE INVENTION

Lymphocyte-activation gene 3 (CD223), also known as LAG-3, is a type I transmembrane protein that is a member of the immune-globulin superfamily (IgSF).

LAG-3 is a cell surface molecule expressed on activated T cells, NK cells, B cells and plasmacytoid dendritic cells, but not on resting T cells. LAG-3 shares approximately 20% amino acid sequence homology with CD4, but binds to MHC class II and a major LAG-3 functional ligand independent from MHC-II, i.e., fibrinogen-like protein 1 (FGL1) like molecule, with higher affinity, providing negative regulation of T cell receptor signaling.

Blockade of LAG-3 in vitro augments T cell proliferation and cytokine production, and LAG-3-deficient mice have a defect in the downregulation of T cell responses induced by the superantigen staphylococcal enterotoxin B, by peptides or by Sendai virus infection. LAG-3 is expressed on both activated natural Treg (nTreg) and induced $CD4^+FoxP3^+$ Treg (iTreg) cells, where expression levels are higher than that observed on activated effector $CD4^+$ T cells. Blockade of LAG-3 on Treg cells abrogates Treg cell suppressor function whereas ectopic expression of LAG-3 in non-Treg $CD4^+$ T cells confers suppressive activity. On the basis of the immunomodulatory role of LAG-3 on T cell function in chronic infection and cancer, the predicted

mechanism of action for LAG-3-specific monoclonal antibodies is to inhibit the negative regulation of tumor-specific effector T cells.

There are only three potential antagonist antibodies that regulate LAG-3 function and anti-tumor immune responses in early clinical developments for the treatment of advanced solid tumors currently. These antibodies are described in patents US 20110150892 A1, US 20170101472 A1 and WO 2015138920 A1, and referred hereinafter as BMK1, BMK7 and BMK5 respectively. BMK8, as described herein, is humanized version of chimeric antibody BMK5. BMK1, BMK7 and BMK8 serve as benchmark antibodies in the context of the application. Accordingly, there remains a need for anti-human LAG-3 antibodies with improved efficacy, such as high binding affinity, low cross-family reactions and good stability. In this application, the inventors have generated a series of antibodies and fully human antibodies against LAG-3 utilizing humanized rats. The antibodies of the instant application have high binding affinity, specifically binding to human LAG-3 protein without cross-family reactions, and are potent to modulate immune responses.

SUMMARY OF THE INVENTION

These and other objectives are provided for by the present invention which, in a broad sense, is directed to novel compounds, methods, compositions and articles of manufacture that provide antibodies with improved efficacy. The benefits provided by the present invention are broadly applicable in the field of antibody therapeutics and diagnostics and may be used in conjunction with antibodies that react with a variety of targets. The present invention provides antibodies, preferably fully human monoclonal antibodies that bind to human LAG-3. It also provides methods of hybridoma generation using humanized rats, nucleic acid molecules encoding the anti-LAG-3 antibodies, expression vectors and host cells used for the expression of anti-LAG-3 antibodies. The invention further provides the methods for validating the function of antibodies in vitro. The antibodies of the invention provide a potent agent for the treatment of multiple diseases via modulating human immune function.

In some aspects, the invention comprises an isolated antibody, or an antigen-binding portion thereof.

In some embodiments, the isolated antibody or the antigen-binding portion thereof has one or more of the following properties:

(a) binds to human LAG-3 with a K_D of 2×10^{-10} M or less;

(b) inhibits binding of LAG-3 to major histocompatibility (MHC) class II molecules;
(c) inhibits binding of LAG-3 to fibrinogen-like protein 1 (FGL1) ligand molecules;
(d) inhibits binding of LAG-3 to LSECtin and/or Galectin-3;
(e) binds to human LAG-3 without cross-family reactions; or
(f) has no cross-reactivity to human CD4.

In some embodiments, the isolated antibody or the antigen-binding portion thereof comprises:

A) one or more heavy chain CDRs (CDRHs) selected from at least one of the group consisting of: (i) a CDRH1 with at least 90% sequence identity to a CDRH1 as set forth in one of the sequences selected from the group consisting of SEQ ID NOs: 1 and 7; (ii) a CDRH2 with at least 90% sequence identity to a CDRH2 as set forth in one of the sequences selected from the group consisting of SEQ ID NOs: 2 and 8; and (iii) a CDRH3 with at least 90% sequence identity to a CDRH3 as set forth in one of the sequences selected from the group consisting of SEQ ID NOs: 3 and 9;

B) one or more light chain CDRs (CDRLs) selected from at least one of the group consisting of: (i) a CDRL1 with at least 90% sequence identity to a CDRL1 as set forth in one of the sequences selected from the group consisting of SEQ ID NOs: 4 and 10; (ii) a CDRL2 with at least 90% sequence identity to a CDRL2 as set forth in one of the sequences selected from the group consisting of SEQ ID NOs: 5 and 11; and (iii) a CDRL3 with at least 90% sequence identity to a CDRL3 as set forth in one of the sequences selected from the group consisting of SEQ ID NOs: 6 and 12; or

C) one or more CDRHs of A) and one or more CDRLs of B).

In some embodiments, the isolated antibody or the antigen-binding portion thereof comprises:

A) one or more (such as 1, 2 or 3) heavy chain CDRs (CDRHs) selected from at least one of the group consisting of: (i) a CDRH1 selected from the group consisting of SEQ ID NOs: 1 and 7or a CDRH1 that differs in amino acid sequence from the CDRH1 by an amino acid addition, deletion or substitution of not more than 2 amino acids; (ii) a CDRH2 selected from the group consisting of SEQ ID NOs: 2 and 8 or a CDRH2 that differs in amino acid sequence from the CDRH2 by an amino acid addition, deletion or substitution of not more than 2 amino acids; and (iii) a CDRH3 selected from the group consisting of SEQ ID NOs: 3 and 9 or a CDRH3 that differs in amino acid sequence from the CDRH3 by an amino acid addition, deletion or substitution of not more than 2 amino acids;

B) one or more (such as 1, 2 or 3) light chain CDRs (CDRLs) selected from at least one of the group consisting of: (i) a CDRL1 selected from the group consisting of SEQ ID NOs: 4 and 10 or a CDRL1 that differs in amino acid sequence from the CDRL1 by an amino acid addition, deletion or substitution of not more than 2 amino acids; (ii) a CDRL2 selected from the group consisting of SEQ ID NOs: 5 and 11 or a CDRL2 that differs in amino acid sequence from the CDRL2 by an amino acid addition, deletion or substitution of not more than 2 amino acids; (iii) a CDRL3 selected from the group consisting of SEQ ID NOs: 6 and 12 or a CDRL3 that differs in amino acid sequence from the CDRL3 by an amino acid addition, deletion or substitution of not more than 2 amino acids; or

C) one or more CDRHs of A) and one or more CDRLs of B).

In some embodiments, the isolated antibody or the antigen-binding portion thereof comprises:

A) a CDRH3 comprising SEQ ID NO: 3 or 9; or

B) a CDRH3 with at least 90% sequence identity to a CDRH3 as set forth in one of the sequences selected from the group consisting of SEQ ID NOs: 3 and 9; or

C) a CDRH3 that differs in amino acid sequence from the CDRH3 of A) by an amino acid addition, deletion or substitution of not more than 2 amino acids,

and wherein the isolated antibody or the antigen-binding portion thereof binds human LAG-3 with a K_D of 2×10^{-10} M or less.

In some embodiments, the isolated antibody or the antigen-binding portion thereof comprises:

(a) a CDRH1 comprising or consisting of SEQ ID NO: 1;

(b) a CDRH2 comprising or consisting of SEQ ID NO: 2;

(c) a CDRH3 comprising or consisting of SEQ ID NO: 3;

(d) a CDRL1 comprising or consisting of SEQ ID NO: 4;

(e) a CDRL2 comprising or consisting of SEQ ID NO: 5; and

(f) a CDRL3 comprising or consisting of SEQ ID NO: 6.

In some embodiments, the isolated antibody or the antigen-binding portion thereof comprises:

(a) a CDRH1 comprising or consisting of SEQ ID NO: 7;

(b) a CDRH2 comprising or consisting of SEQ ID NO: 8;

(c) a CDRH3 comprising or consisting of SEQ ID NO: 9;

(d) a CDRL1 comprising or consisting of SEQ ID NO: 10;

(e) a CDRL2 comprising or consisting of SEQ ID NO: 11; and

(f) a CDRL3 comprising or consisting of SEQ ID NO: 12.

In some embodiments, the isolated antibody or the antigen-binding portion thereof comprises:

(A) a heavy chain variable region:

(i) comprising the amino acid sequence of SEQ ID NO: 13;

(ii) comprising an amino acid sequence at least 85%, at least 90%, or at least 95% identical to SEQ ID NO: 13;

(iii) comprising an amino acid sequence with addition, deletion and/or substitution of one or more (such as 1-10, 1-5, 1-3, 1, 2, 3, 4, or 5) amino acids compared with SEQ ID NO: 13; and/or

(B) a light chain variable region:

(i) comprising the amino acid sequence of SEQ ID NO: 14;

(ii) comprising an amino acid sequence at least 85%, at least 90%, or at least 95% identical to SEQ ID NO: 14; or

(iii) comprising an amino acid sequence with addition, deletion and/or substitution of one or more (such as 1-10, 1-5, 1-3, 1, 2, 3, 4, or 5) amino acids compared with SEQ ID NO: 14.

In some embodiments, the isolated antibody or the antigen-binding portion thereof comprises:

(A) a heavy chain variable region:

(i) comprising the amino acid sequence of SEQ ID NO: 15;

(ii) comprising an amino acid sequence at least 85%, at least 90%, or at least 95% identical to SEQ ID NO: 15; or

(iii) comprising an amino acid sequence with addition, deletion and/or substitution of one or more (such as 1-10, 1-5, 1-3, 1, 2, 3, 4, or 5) amino acids compared with SEQ ID NO: 15; and/or

(B) a light chain variable region:

(i) comprising the amino acid sequence of SEQ ID NO: 16;

(ii) comprising an amino acid sequence at least 85%, at least 90%, or at least 95% identical to SEQ ID NO: 16; or

(iii) comprising an amino acid sequence with addition, deletion and/or substitution of one or more (such as 1-10, 1-5, 1-3, 1, 2, 3, 4, or 5) amino acids compared with SEQ ID NO: 16.

In some aspects, the invention is directed to an isolated nucleic acid molecule, comprising a nucleic acid sequence encoding the heavy chain variable region and/or the light chain variable region of the isolated antibody as disclosed herein.

In some aspects, the invention is directed to an expression vector comprising the nucleic acid molecule encoding the antibody or antigen -binding portion thereof as disclosed herein.

In some aspects, the invention is directed to a host cell comprising the expression vector as disclosed herein.

In some aspects, the invention is directed to a pharmaceutical composition comprising at least one antibody or antigen-binding portion thereof as disclosed herein and a pharmaceutically acceptable carrier.

In some aspects, the invention is directed to a method for preparing an anti-LAG-3 antibody or antigen-binding portion thereof which comprises expressing the antibody or antigen-binding portion thereof in the host cell and isolating the antibody or antigen-binding portion thereof from the host cell.

In some aspects, the invention is directed to a method of modulating an antigen-specific T cell response comprising administering to the subject the antibody or antigen-binding portion thereof as disclosed herein such that an antigen- specific T cell response is modulated in the subject.

In some aspects, the invention is directed to a method of modulating an immune response in a subject comprising administering the antibody or antigen-binding portion thereof as disclosed herein to the subject such that an immune response in the subject is modulated.

In some aspects, the invention is directed to a method for inhibiting or blocking the binding of LAG-3 to MHC class II molecules or FGL1 like molecules comprising contacting said MHC class II molecules or FGL1 like molecules with the antibody the antibody or antigen-binding portion thereof as disclosed herein.

In some aspects, the invention is directed to a method for inhibiting or blocking the binding of LAG-3 to LSECtin and/or Galectin-3 comprising contacting said LSECtin and/or Galectin-3 with the antibody the antibody or antigen-binding portion thereof as disclosed herein.

In some aspects, the invention is directed to a method for inhibiting growth of tumor cells in a subject comprising administering to the subject the antibody or antigen-binding portion thereof as disclosed herein such that growth of the tumor is inhibited in the subject.

In some aspects, the invention is directed to a method for treating viral infection in a subject comprising administering to the subject the antibody the antibody or antigen-binding portion thereof as disclosed herein such that the viral infection is treated in the subject.

In some aspects, the invention is directed to a method for treating or preventing proliferative disorders such as cancers in a subject comprising administering an effective amount of the antibody or antigen-binding portion thereof as disclosed herein to the subject.

In some aspects, the invention is directed to the use of the antibody or antigen-binding portion thereof as disclosed herein in the manufacture of a medicament for treating or preventing proliferative disorders such as cancers.

In some aspects, the invention is directed to the use of the antibody or antigen-binding portion thereof as disclosed herein in the manufacture of a diagnostic agent for diagnosing proliferative disorders such as cancers.

In some aspects, the invention is directed to the antibody or antigen-binding portion thereof as disclosed herein for use in treating or preventing proliferative disorders such as cancers.

In some aspects, the invention is directed to kits or devices and associated methods that employ the antibody or antigen-binding portion thereof as disclosed herein, and pharmaceutical compositions as disclosed herein, which are useful for the treatment of proliferative disorders such as cancer. To this end the present invention preferably provides an article of manufacture useful for treating such disorders comprising a receptacle containing the antibody or antigen-binding portion thereof as disclosed herein and instructional materials for using the antibody or antigen-binding portion thereof as disclosed herein to treat, ameliorate or prevent a proliferative disorder or progression or recurrence thereof. In selected embodiments, the devices and associated methods will comprise the step of contacting at least one circulating tumor cell with the antibody or antigen-binding portion thereof as disclosed herein.

The foregoing is a summary and thus contains, by necessity, simplifications, generalizations, and omissions of detail; consequently, those skilled in the art will appreciate that the summary is illustrative only and is not intended to be in any way limiting. Other aspects, features, and advantages of the methods, compositions and/or devices and/or other subject matter described herein will become apparent in the teachings set forth herein. The summary is provided to introduce a selection of concepts in a simplified form that are further described below in the Detailed Description. This summary is not intended to identify key features or essential features of the claimed subject matter, nor is it intended to be used as an aid in determining the scope of the claimed subject matter. Further, the contents of all references, patents and published patent applications cited throughout this application are incorporated herein in entirety by reference.

BRIEF DESCRIPTION OF THE FIGURES

Figure 1 shows the binding of LAG-3 antibodies to cell surface human LAG-3, expressed by MFI (Mean Fluorescence Intensity) and measured by BD FACSCanto II.

Figure 2 shows the blocking of LAG-3 protein binding to MHC-II expressed on Raji cells.

Figure 3 shows the blocking of LAG-3 protein binding to LSECtin.

Figure 4 shows the blocking of LAG-3 protein binding to Galectin-3.

Figure 5 shows cross-reactivity to cynomolgus LAG-3 as measured by FACS.

Figure 6 shows cross-reactivity to murine LAG-3 as measured by FACS.

Figure 7 shows cross-reactivity to human CD4 as measured by ELISA.

Figure 8A-E shows epitope binning against benchmark antibodies BMK1, BMK7 and BMK5.

Figure 9A-B shows the result of epitope mapping.

Figure 10 shows the effects of human LAG-3 antibodies in reporter gene assay.

Figure 11 shows the effects of human LAG-3 antibodies on human allogeneic mixed lymphocyte reaction, as measured by ELISA and reflected by the level of IFN-γ (ng/mL).

Figure 12 shows the effects of human LAG-3 antibodies on human allogeneic mixed lymphocyte reaction, as measured by ^{3}H-thymidine incorporation and reflected by proliferative

responses which are expressed as the CPM (counts per minute) of triplicate wells.

Figure 13A-B shows the results of CDC test and ADCC test, by determining target cell lysis.

Figure 14A-B shows the results of serum stability test, as measured by FACS and expressed by MFI of the cells.

DETAILED DESCRIPTION OF THE INVENTION

While the present invention may be embodied in many different forms, disclosed herein are specific illustrative embodiments thereof that exemplify the principles of the invention. It should be emphasized that the present invention is not limited to the specific embodiments illustrated. Moreover, any section headings used herein are for organizational purposes only and are not to be construed as limiting the subject matter described.

Unless otherwise defined herein, scientific and technical terms used in connection with the present invention shall have the meanings that are commonly understood by those of ordinary skill in the art. Further, unless otherwise required by context, singular terms shall include pluralities and plural terms shall include the singular. More specifically, as used in this specification and the appended claims, the singular forms "a," "an" and "the" include plural referents unless the context clearly dictates otherwise. Thus, for example, reference to "a protein" includes a plurality of proteins; reference to "a cell" includes mixtures of cells, and the like. In this application, the use of "or" means "and/or" unless stated otherwise. Furthermore, the use of the term "comprising," as well as other forms, such as "comprises" and "comprised", is not limiting. In addition, ranges provided in the specification and appended claims include both end points and all points between the end points.

Generally, nomenclature used in connection with, and techniques of, cell and tissue culture, molecular biology, immunology, microbiology, genetics and protein and nucleic acid chemistry and hybridization described herein are those well known and commonly used in the art. The methods and techniques of the present invention are generally performed according to conventional methods well known in the art and as described in various general and more specific references that are cited and discussed throughout the present specification unless otherwise indicated. See, e.g., Abbas *et al.*, Cellular and Molecular Immunology, 6th ed., W.B. Saunders Company (2010); Sambrook J. & Russell D. *Molecular Cloning: A Laboratory Manual*, 3rd ed., Cold Spring Harbor Laboratory Press, Cold Spring Harbor, N.Y. (2000); Ausubel *et al.*, *Short Protocols in Molecular*

Biology: A Compendium of Methods from Current Protocols in Molecular Biology, Wiley, John & Sons, Inc. (2002); Harlow and Lane *Using Antibodies: A Laboratory Manual*, Cold Spring Harbor Laboratory Press, Cold Spring Harbor, N.Y. (1998); and Coligan *et al.*, *Short Protocols in Protein Science*, Wiley, John & Sons, Inc. (2003). The nomenclature used in connection with, and the laboratory procedures and techniques of, analytical chemistry, synthetic organic chemistry, and medicinal and pharmaceutical chemistry described herein are those well known and commonly used in the art. Moreover, any section headings used herein are for organizational purposes only and are not to be construed as limiting the subject matter described.

Definitions

In order to better understand the invention, the definitions and explanations of the relevant terms are provided as follows.

The term "antibody" or "Ab", as used herein, generally refers to a Y-shaped tetrameric protein comprising two heavy (H) and two light (L) polypeptide chains held together by covalent disulfide bonds and non-covalent interactions. Light chains of an antibody may be classified into κ and λ light chain. Heavy chains may be classified into μ, δ, γ, α and ε, which define isotypes of an antibody as IgM, IgD, IgG, IgA and IgE, respectively. In a light chain and a - heavy chain, a variable region is linked to a constant region via a "J" region of about 12 or more amino acids, and a heavy chain further comprises a "D" region of about 3 or more amino acids. Each heavy chain consists of a heavy chain variable region (V_H) and a heavy chain constant region (C_H). A heavy chain constant region consists of 3 domains (C_H1, C_H2 and C_H3). Each light chain consists of a light chain variable region (V_L) and a light chain constant region (C_L). V_H and V_L region can further be divided into hypervariable regions (called complementary determining regions (CDR)), which are interspaced by relatively conservative regions (called framework region (FR)). Each V_H and V_L consists of 3 CDRs and 4 FRs in the following order: FR1, CDR1, FR2, CDR2, FR3, CDR3, FR4 from N-terminal to C-terminal. The variable region (V_H and V_L) of each heavy/light chain pair forms antigen binding sites, respectively. Distribution of amino acids in various regions or domains follows the definition in Kabat Sequences of Proteins of Immunological Interest (National Institutes of Health, Bethesda, Md. (1987 and 1991)), or Chothia & Lesk (1987) J. Mol. Biol. 196:901-917; Chothia et al., (1989) Nature 342:878-883. Antibodies may be of different antibody isotypes, for example, IgG (e.g., IgG1, IgG2, IgG3 or IgG4 subtype), IgA1, IgA2, IgD, IgE or IgM antibody.

The term "antigen-binding portion" or "antigen-binding fragment" of an antibody, which can be interchangeably used in the context of the application, refers to polypeptides comprising fragments of a full-length antibody, which retain the ability of specifically binding to an antigen that the full-length antibody specifically binds to, and/or compete with the full-length antibody for binding to the same antigen. Generally, see Fundamental Immunology, Ch. 7 (Paul, W., ed., the second edition, Raven Press, N.Y. (1989), which is incorporated herein by reference for all purposes. Antigen binding fragments of an antibody may be produced by recombinant DNA techniques or by enzymatic or chemical cleavage of an intact antibody. Under some conditions, antigen binding fragments include Fab, Fab', $F(ab')_2$, Fd, Fv, dAb and complementary determining region (CDR) fragments, single chain antibody (e.g. scFv), chimeric antibody, diabody and such polypeptides that comprise at least part of antibody sufficient to confer the specific antigen binding ability on the polypeptides. Antigen binding fragments of an antibody may be obtained from a given antibody (e.g., the monoclonal anti-human LAG-3 antibody provided in the instant application) by conventional techniques known by a person skilled in the art (e.g., recombinant DNA technique or enzymatic or chemical cleavage methods), and may be screened for specificity in the same manner by which intact antibodies are screened.

The term "monoclonal antibody" or "mAb", as used herein, refer to a preparation of antibody molecules of single molecular composition. A monoclonal antibody displays a single binding specificity and affinity for a particular epitope.

The term "human antibody" or "fully human antibody", as used herein, is intended to include antibodies having variable regions in which both the framework and CDR regions are derived from human germline immunoglobulin sequences. Furthermore, if the antibody contains a constant region, the constant region also is derived from human germline immunoglobulin sequences. The human antibodies of the invention can include amino acid residues not encoded by human germline immunoglobulin sequences (e.g., mutations introduced by random or site- specific mutagenesis in vitro or by somatic mutation in vivo). However, the term "human antibody", as used herein, is not intended to include antibodies in which CDR sequences derived from the germline of another mammalian species, such as a mouse, have been grafted onto human framework sequences.

The term "human monoclonal antibody", as used herein, refers to antibodies displaying a single binding specificity, which have variable regions in which both the framework and CDR regions are derived from human germline immunoglobulin sequences.

The term "humanized antibody" is intended to refer to antibodies in which CDR sequences derived from the germline of another mammalian species, such as a mouse, have been grafted onto human framework sequences. Additional framework region modifications may be made within the human framework sequences.

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

The term "LAG-3", as used herein, refers to Lymphocyte Activation Gene-3. The term "LAG-3" includes variants, isoforms, homologs, orthologs and paralogs.

The term "human LAG-3", as used herein, refers to human sequence LAG-3, such as the complete amino acid sequence of human LAG-3 having Genbank Accession No. NP_002277. The human LAG-3 sequence may differ from human LAG-3 of Genbank Accession No. NP_002277 by having, e.g., conserved mutations or mutations in non-conserved regions and the LAG-3 has substantially the same biological function as the human LAG-3 of Genbank Accession No. NP_002277. For example, a biological function of human LAG-3 is having an epitope in the extracellular domain of LAG-3 that is specifically bound by an antibody of the instant disclosure or a biological function of human LAG-3 is binding to MHC Class II molecules or FGL1 like molecules.

The term "mouse LAG-3", as used herein, refers to mouse sequence LAG-3, such as the complete amino acid sequence of mouse LAG-3 having Genbank Accession No. NP_032505.

The term "cynomolgus LAG-3", as used herein, refers to cynomolgus sequence LAG-3, such as the complete amino acid sequence of cynomolgus LAG-3 having Genbank Accession No. XP_005570011.1.

The term "Ka", as used herein, is intended to refer to the association rate of a particular antibody-antigen interaction, whereas the term "Kd" as used herein, is intended to refer to the dissociation rate of a particular antibody-antigen interaction. Kd values for antibodies can be determined using methods well established in the art. The term "K_D" as used herein, is intended to refer to the dissociation constant of a particular antibody-antigen interaction, which is obtained from the ratio of Kd to Ka (i.e., Kd/Ka) and is expressed as a molar concentration (M). A preferred method for determining the Kd of an antibody is by using surface plasmon resonance, preferably using a biosensor system such as a Biacore® system.

The term "high affinity" for an IgG antibody, as used herein, refers to an antibody having a K_D of 1×10^{-7} M or less, more preferably 5×10^{-8} M or less, even more preferably 1×10^{-8} M or less, even more preferably 5×10^{-9} M or less and even more preferably 1×10^{-9} M or less for a target antigen.

The term "EC_{50}", as used herein, which is also termed as "half maximal effective concentration" refers to the concentration of a drug, antibody or toxicant which induces a response halfway between the baseline and maximum after a specified exposure time. In the context of the application, EC_{50} is expressed in the unit of "nM".

The ability of "inhibit binding" or "compete for the same epitopes" in this application refers to the ability of an antibody or antigen-binding fragment thereof to inhibit the binding of two molecules (eg, human LAG-3 and human anti-LAG-3 antibody) to any detectable level. In certain embodiments, the binding of the two molecules can be inhibited at least 50% by the antibody or antigen-binding fragment thereof. In certain embodiments, such an inhibitory effect may be greater than 60%, greater than 70%, greater than 80%, or greater than 90%.

The term "epitope", as used herein, refers to a portion on antigen that an immunoglobulin or antibody specifically binds to. "Epitope" is also known as "antigenic determinant". Epitope or antigenic determinant generally consists of chemically active surface groups of a molecule such as amino acids, carbohydrates or sugar side chains, and generally has a specific three-dimensional structure and a specific charge characteristic. For example, an epitope generally comprises at least 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14 or 15 consecutive or non-consecutive amino acids in a unique steric conformation, which may be "linear" or "conformational". See, for example, Epitope Mapping Protocols in Methods in Molecular Biology, Vol. 66, G. E. Morris, Ed. (1996). In a linear epitope, all the interaction sites between a protein and an interaction molecule (e.g., an antibody) are present linearly along the primary amino acid sequence of the protein. In a conformational epitope, the interaction sites span over amino acid residues that are separate from each other in a protein. Antibodies may be screened depending on competitiveness of binding to the same epitope by conventional techniques known by a person skilled in the art. For example, study on competition or cross-competition may be conducted to obtain antibodies that compete or cross-compete with each other for binding to antigens (e.g. RSV fusion protein). High-throughput methods for obtaining antibodies binding to the same epitope, which are based on their cross-competition, are described in an international patent application WO 03/48731.

The term "isolated", as used herein, refers to a state obtained from natural state by artificial means. If a certain "isolated" substance or component is present in nature, it is possible because its natural environment changes, or the substance is isolated from natural environment, or both. For example, a certain un-isolated polynucleotide or polypeptide naturally exists in a certain living animal body, and the same polynucleotide or polypeptide with a high purity isolated from such a natural state is called isolated polynucleotide or polypeptide. The term "isolated" excludes neither the mixed artificial or synthesized substance nor other unpure substances that do not affect the activity of the isolated substance.

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

The term "vector", as used herein, refers to a nucleic acid vehicle which can have a polynucleotide inserted therein. When the vector allows for the expression of the protein encoded by the polynucleotide inserted therein, the vector is called an expression vector. The vector can have the carried genetic material elements expressed in a host cell by transformation, transduction, or transfection into the host cell. Vectors are well known by a person skilled in the art, including, but not limited to plasmids, phages, cosmids, artificial chromosome such as yeast artificial chromosome (YAC), bacterial artificial chromosome (BAC) or P1-derived artificial chromosome (PAC); phage such as λ phage or M13 phage and animal virus. The animal viruses that can be used as vectors, include, but are not limited to, retrovirus (including lentivirus), adenovirus, adeno-associated virus, herpes virus (such as herpes simplex virus), pox virus, baculovirus, papillomavirus, papova virus (such as SV40). A vector may comprise multiple elements for controlling expression, including, but not limited to, a promoter sequence, a transcription initiation sequence, an enhancer sequence, a selection element and a reporter gene. In addition, a vector may comprise origin of replication.

The term "host cell", as used herein, refers to a cell into which a vector can be introduced, including, but not limited to, prokaryotic cell such as *E. coli* or *Bacillus subtilis*, fungal cell such as yeast cell or *Aspergillus*, insect cell such as S2 Drosophila cell or Sf9, and animal cell such as fibroblast, CHO cell, COS cell, NSO cell, HeLa cell, BHK cell, HEK 293 cell or human cell.

The term "identity", as used herein, refers to a relationship between the sequences of two or more polypeptide molecules or two or more nucleic acid molecules, as determined by aligning and comparing the sequences. "Percent identity" means the percent of identical residues between the amino acids or nucleotides in the compared molecules and is calculated based on the size of the smallest of the molecules being compared. For these calculations, gaps in alignments (if any) are preferably addressed by a particular mathematical model or computer program (i.e., an "algorithm"). Methods that can be used to calculate the identity of the aligned nucleic acids or polypeptides include those described in Computational Molecular Biology, (Lesk, A. M., ed.), 1988, New York: Oxford University Press; Biocomputing Informatics and Genome Projects, (Smith, D. W., ed.), 1993, New York: Academic Press; Computer Analysis of Sequence Data, Part I, (Griffin, A. M., and Griffin, H. G., eds.), 1994, New Jersey: Humana Press; von Heinje, G., 1987, Sequence Analysis in Molecular Biology, New York: Academic Press; Sequence Analysis Primer, (Gribskov, M. and Devereux, J., eds.), 1991, New York: M. Stockton Press; and Carillo et al, 1988, SIAMJ. Applied Math. 48:1073.

The term "immunogenicity", as used herein, refers to ability of stimulating the formation of specific antibodies or sensitized lymphocytes in organisms. It not only refers to the property of an antigen to stimulate a specific immunocyte to activate, proliferate and differentiate so as to finally generate immunologic effector substance such as antibody and sensitized lymphocyte, but also refers to the specific immune response that antibody or sensitized T lymphocyte can be formed in immune system of an organism after stimulating the organism with an antigen. Immunogenicity is the most important property of an antigen. Whether an antigen can successfully induce the generation of an immune response in a host depends on three factors, properties of an antigen, reactivity of a host, and immunization means.

The term "transfection", as used herein, refers to the process by which nucleic acids are introduced into eukaryotic cells, particularly mammalian cells. Protocols and techniques for transfection include but not limited to lipid transfection and chemical and physical methods such as electroporation. A number of transfection techniques are well known in the art and are disclosed herein. See, e.g., Graham et al., 1973, Virology 52:456; Sambrook et al., 2001, Molecular Cloning: A Laboratory Manual, supra; Davis et al., 1986, Basic Methods in Molecular Biology, Elsevier; Chu et al, 1981, Gene 13:197. In a specific embodiment of the invention, human LAG-3 gene was transfected into 293F cells.

The term "hybridoma" and the term "hybridoma cell line", as used herein, may be used interchangeably. When the term "hybridoma" and the term "hybridoma cell line" are mentioned, they also include subclone and progeny cell of hybridoma.

The term "SPR" or "surface plasmon resonance", as used herein, refers to and includes an optical phenomenon that allows for the analysis of real-time biospecific interactions by detection of alterations in protein concentrations within a biosensor matrix, for example using the BIAcore system (Pharmacia Biosensor AB, Uppsala, Sweden and Piscataway, N.J.). For further descriptions, see Example 5 and Jönsson, U., et al. (1993) *Ann. Biol. Clin.* 51:19-26; Jönsson, U., et al. (1991) *Biotechniques* 11:620-627; Johnsson, B., et al. (1995) *J. Mol. Recognit.* 8:125-131; and Johnnson, B., et al. (1991) *Anal. Biochem.* 198:268-277.

The term "fluorescence-activated cell sorting" or "FACS", as used herein, refers to a specialized type of flow cytometry. It provides a method for sorting a heterogeneous mixture of biological cells into two or more containers, one cell at a time, based upon the specific light scattering and fluorescent characteristics of each cell (FlowMetric. "Sorting Out Fluorescence Activated Cell Sorting". Retrieved 2017-11-09.). Instruments for carrying out FACS are known to those of skill in the art and are commercially available to the public. Examples of such instruments include FACS Star Plus, FACScan and FACSort instruments from Becton Dickinson (Foster City, Calif.) Epics C from Coulter Epics Division (Hialeah, Fla.) and MoFlo from Cytomation (Colorado Springs, Colo.).

The term "antibody-dependent cell-mediated cytotoxicity" or "ADCC", as used herein, refers to a form of cytotoxicity in which secreted Ig bound onto Fc receptors (FcRs) present on certain cytotoxic cells (e.g. Natural Killer (NK) cells, neutrophils, and macrophages) enable these cytotoxic effector cells to bind specifically to an antigen-bearing target cell and subsequently kill the target cell with cytotoxins. The antibodies "arm" the cytotoxic cells and are absolutely required for such killing. The primary cells for mediating ADCC, NK cells, express FcγRIII only, whereas monocytes express FcγRI, FcγRII and FcγRIII. FcR expression on hematopoietic cells is summarized in Table 3 on page 464 of Ravetch and Kinet, Annu. Rev. Immunol 9:457-92 (1991). To assess ADCC activity of a molecule of interest, an in vitro ADCC assay, such as that described in US Patent No. 5,500,362 or 5,821,337 may be performed. Useful effector cells for such assays include peripheral blood mononuclear cells (PBMC) and Natural Killer (NK) cells. Alternatively, or additionally, ADCC activity of the molecule of interest may be assessed in vivo, e.g., in an animal model such as that disclosed in Clynes et al. PNAS (USA) 95:652-656 (1998).

The term "complement dependent cytotoxicity" or "CDC" refers to the lysis of a target cell in the presence of complement. Activation of the classical complement pathway is initiated by the binding of the first component of the complement system (Clq) to antibodies (of the appropriate subclass) which are bound to their cognate antigen. To assess complement activation, a CDC assay, e.g. as described in Gazzano-Santoro et al., J. Immunol. Methods 202: 163 (1996), may be performed.

The term "subject" includes any human or nonhuman animal, preferably humans.

The term "cancer", as used herein, refers to any or a tumor or a malignant cell growth, proliferation or metastasis-mediated, solid tumors and non-solid tumors such as leukemia and initiate a medical condition.

The term "treatment", "treating" or "treated", as used herein in the context of treating a condition, pertains generally to treatment and therapy, whether of a human or an animal, in which some desired therapeutic effect is achieved, for example, the inhibition of the progress of the condition, and includes a reduction in the rate of progress, a halt in the rate of progress, regression of the condition, amelioration of the condition, and cure of the condition. Treatment as a prophylactic measure (i.e., prophylaxis, prevention) is also included. For cancer, "treating" may refer to dampen or slow the tumor or malignant cell growth, proliferation, or metastasis, or some combination thereof. For tumors, "treatment" includes removal of all or part of the tumor, inhibiting or slowing tumor growth and metastasis, preventing or delaying the development of a tumor, or some combination thereof.

The term "therapeutically-effective amount," as used herein, pertains to that amount of an active compound, or a material, composition or dosage from comprising an active compound, which is effective for producing some desired therapeutic effect, commensurate with a reasonable benefit/risk ratio, when administered in accordance with a desired treatment regimen. Specifically, the "therapeutically-effective amount," refers to an antibody or antigen-binding portion thereof in an amount or concentration effective to treat the human LAG-3-related diseases or conditions.

The present invention in a "host cell", as used herein, refers to a cell with the introduction of exogenous polynucleotides.

The term "pharmaceutically acceptable", as used herein, means that the vehicle, diluent, excipient and/or salts thereof, are chemically and/or physically is compatible with other ingredients in the formulation, and the physiologically compatible with the recipient.

As used herein, the term "a pharmaceutically acceptable carrier and/or excipient" refers to a carrier and/or excipient pharmacologically and/or physiologically compatible with a subject and an active agent, which is well known in the art (see, e.g., Remington's Pharmaceutical Sciences. Edited by Gennaro AR, 19th ed. Pennsylvania: Mack Publishing Company, 1995), and includes, but is not limited to pH adjuster, surfactant, adjuvant and ionic strength enhancer. For example, the pH adjuster includes, but is not limited to, phosphate buffer; the surfactant includes, but is not limited to, cationic, anionic, or non-ionic surfactant, e.g., Tween-80; the ionic strength enhancer includes, but is not limited to, sodium chloride.

As used herein, the term "adjuvant" refers to a non-specific immunopotentiator, which can enhance immune response to an antigen or change the type of immune response in an organism when it is delivered together with the antigen to the organism or is delivered to the organism in advance. There are a variety of adjuvants, including, but not limited to, aluminium adjuvants (for example, aluminum hydroxide), Freund's adjuvants (for example, Freund's complete adjuvant and Freund's incomplete adjuvant), coryne bacterium parvum, lipopolysaccharide, cytokines, and the like. Freund's adjuvant is the most commonly used adjuvant in animal experiments now. Aluminum hydroxide adjuvant is more commonly used in clinical trials.

Anti-LAG-3 Antibodies

In some aspects, the invention comprises an isolated antibody or an antigen-binding portion thereof.

In the context of the application, the "antibody" may include polyclonal antibodies, multiclonal antibodies, monoclonal antibodies, chimeric antibodies, humanized and primatized antibodies, CDR grafted antibodies, human antibodies, recombinantly produced antibodies, intrabodies, multispecific antibodies, bispecific antibodies, monovalent antibodies, multivalent antibodies, anti-idiotypic antibodies, synthetic antibodies, including muteins and variants thereof,; and derivatives thereof including Fc fusions and other modifications, and any other immunoreactive molecule so long as it exhibits preferential association or binding with a LAG-3 protein. Moreover, unless dictated otherwise by contextual constraints the term further comprises all classes of antibodies (i.e. IgA, IgD, IgE, IgG, and IgM) and all subclasses (i.e., IgG1, IgG2, IgG3, IgG4, IgA1, and IgA2). In a preferred embodiment, the antibody is a monoclonal antibody. In a more preferred embodiment, the antibody is a human monoclonal antibody.

Human antibodies can be produced using various techniques known in the art. One technique is phage display in which a library of (preferably human) antibodies is synthesized on phages, the library is screened with the antigen of interest or an antibody-binding portion thereof, and the phage that binds the antigen is isolated, from which one may obtain the immunoreactive fragments. Methods for preparing and screening such libraries are well known in the art and kits for generating phage display libraries are commercially available (e.g., the Pharmacia Recombinant Phage Antibody System, catalog no. 27-9400-01; and the Stratagene SurfZAP™ phage display kit, catalog no. 240612). There also are other methods and reagents that can be used in generating and screening antibody display libraries (see, e.g., Barbas *et al.*, *Proc. Natl. Acad. Sci. USA* 88:7978-7982 (1991)).

Human antibodies can also be made by introducing human immunoglobulin loci into transgenic animals, e.g., mice in which the endogenous immunoglobulin genes have been partially or completely inactivated and human immunoglobulin genes have been introduced. Upon challenge, human antibody production is observed, which closely resembles that seen in humans in all respects, including gene rearrangement, assembly, and antibody repertoire. This approach is described, for example, in U.S.P.Ns. 5,545,807; 5,545,806; 5,569,825; 5,625,126; 5,633,425; 5,661,016, and U.S.P.Ns. 6,075,181 and 6,150,584 regarding XenoMouse® technology; and Lonberg and Huszar, *Intern. Rev. Immunol.* 13:65-93 (1995). Alternatively, the human antibody may be prepared via immortalization of human B lymphocytes producing an antibody directed against a target antigen (such B lymphocytes may be recovered from an individual suffering from a neoplastic disorder or may have been immunized *in vitro*). See, e.g., Cole *et al.*, *Monoclonal Antibodies and Cancer Therapy*, Alan R. Liss, p. 77 (1985); Boerner *et al.*, *J. Immunol*, 147 (l):86-95 (1991); and U.S.P.N. 5,750,373.

Monoclonal antibodies can be prepared using a wide variety of techniques known in the art including hybridoma techniques, recombinant techniques, phage display technologies, transgenic animals (e.g., a XenoMouse®) or some combination thereof. For example, monoclonal antibodies can be produced using hybridoma and art-recognized biochemical and genetic engineering techniques such as described in more detail in An, Zhigiang (ed.) *Therapeutic Monoclonal Antibodies: From Bench to Clinic*, John Wiley and Sons, 1st ed. 2009; Shire et. al. (eds.) *Current Trends in Monoclonal Antibody Development and Manufacturing*, Springer Science + Business Media LLC, 1st ed. 2010; Harlow *et al.*, *Antibodies: A Laboratory Manual*, Cold Spring Harbor Laboratory Press, 2nd ed. 1988; Hammerling, *et al.*, in: *Monoclonal Antibodies and T-Cell Hybridomas* 563-681 (Elsevier, N.Y., 1981) each of which is incorporated herein in its entirety by reference. It should be understood that a selected binding sequence can be further altered, for

example, to improve affinity for the target, to humanize the target binding sequence, to improve its production in cell culture, to reduce its immunogenicity in vivo, to create a multispecific antibody, etc., and that an antibody comprising the altered target binding sequence is also an antibody of this invention. In a preferred embodiment, the anti-human LAG-3 monoclonal antibody is prepared by using hybridoma.

Generation of Hybridomas Producing Human Monoclonal Antibodies of the Invention

To generate hybridomas producing the antibodies of the invention, for instance, human monoclonal antibodies of the invention, splenocytes and/or lymph node cells from immunized mice can be isolated and fused to an appropriate immortalized cell line, such as a mouse myeloma cell line. The resulting hybridomas can be screened for the production of antigen- specific antibodies. Generation of hybridomas is well-known in the art. See, e.g., Harlow and Lane (1988) Antibodies, A Laboratory Manual, Cold Spring Harbor Publications, New York.

Generation of Transfectomas Producing Monoclonal Antibodies of the Invention

Antibodies of the invention also can be produced in a host cell transfectoma using, for example, a combination of recombinant DNA techniques and gene transfection methods as is well known in the art (e.g., Morrison, S. (1985) Science 229:1202). In one embodiment, DNA encoding partial or full-length light and heavy chains obtained by standard molecular biology techniques is inserted into one or more expression vectors such that the genes are operatively linked to transcriptional and translational regulatory sequences. In this context, the term "operatively linked" is intended to mean that an antibody gene is ligated into a vector such that transcriptional and translational control sequences within the vector serve their intended function of regulating the transcription and translation of the antibody gene.

The term “regulatory sequence” is intended to include promoters, enhancers and other expression control elements (e.g., polyadenylation signals) that control the transcription or translation of the antibody chain genes. Such regulatory sequences are described, e.g., in Goeddel (Gene Expression Technology. Methods in Enzymology 185, Academic Press, San Diego, CA (1990)). Exemplary regulatory sequences for mammalian host cell expression include viral elements that direct high levels of protein expression in mammalian cells, such as promoters and/or enhancers derived from cytomegalovirus (CMV), Simian Virus 40 (SV40), adenovirus, (e.g., the adenovirus major late promoter (AdMLP) and polyoma. Alternatively, nonviral regulatory sequences can be used, such as the ubiquitin promoter or β-globin promoter. Still further, regulatory

elements composed of sequences from different sources, such as the SRa promoter system, which contains sequences from the SV40 early promoter and the long terminal repeat of human T cell leukemia virus type 1 (Takebe et al. (1988) MoI. Cell. Biol. 8:466-472). The expression vector and expression control sequences are chosen to be compatible with the expression host cell used.

The antibody light chain gene and the antibody heavy chain gene can be inserted into the same or separate expression vectors. In some embodiments, the variable regions are used to create full-length antibody genes of any antibody isotype by inserting them into expression vectors already encoding heavy chain constant and light chain constant regions of the desired isotype such that the VH segment is operatively linked to the CH segment(s) within the vector and the VL segment is operatively linked to the CL segment within the vector. Additionally or alternatively, the recombinant expression vector can encode a signal peptide that facilitates secretion of the antibody chain from a host cell. The antibody chain gene can be cloned into the vector such that the signal peptide is linked in-frame to the amino terminus of the antibody chain gene. The signal peptide can be an immunoglobulin signal peptide or a heterologous signal peptide (i.e., a signal peptide from a non-immunoglobulin protein).

In addition to the antibody chain genes and regulatory sequences, the recombinant expression vectors of the invention can carry additional sequences, such as sequences that regulate replication of the vector in host cells (e.g., origins of replication) and selectable marker genes. The selectable marker gene facilitates selection of host cells into which the vector has been introduced (see, e.g., U.S. Pat. Nos. 4,399,216; 4,634,665 and 5,179,017). For example, typically the selectable marker gene confers resistance to drugs, such as G418, hygromycin or methotrexate, on a host cell into which the vector has been introduced. Selectable marker genes may include the dihydrofolate reductase (DHFR) gene (for use in dhfr-host cells with methotrexate selection/amplification) and the neo gene (for G418 selection).

For expression of the light and heavy chains, the expression vector(s) encoding the heavy and light chains is transfected into a host cell by standard techniques. The various forms of the term "transfection" are intended to encompass a wide variety of techniques commonly used for the introduction of exogenous DNA into a prokaryotic or eukaryotic host cell, e.g., electroporation, calcium-phosphate precipitation, DEAE-dextran transfection and the like. It is possible to express the antibodies of the invention in either prokaryotic or eukaryotic host cells, for example, mammalian host cells, which can assemble and secrete a properly folded and immunologically active antibody.

Mammalian host cells for expressing the recombinant antibodies of the invention include Chinese Hamster Ovary (CHO cells) (including dhfr" CHO cells, described in Urlaub and Chasin, (1980) Proc. Natl. Acad. ScL USA 77:4216-4220, used with a DHFR selectable marker, e.g., as described in R. J. Kaufman and P. A. Sharp (1982) J. MoI. Biol. 159:601-621), NSO myeloma cells, COS cells and SP2 cells. In particular, for use with NSO myeloma cells, another expression system is the GS gene expression system disclosed in WO 87/04462, WO 89/01036 and EP 338,841. When recombinant expression vectors encoding antibody genes are introduced into mammalian host cells, the antibodies are produced by culturing the host cells for a period of time sufficient to allow for expression of the antibody in the host cells or, secretion of the antibody into the culture medium in which the host cells are grown. Antibodies can be recovered from the culture medium using standard protein purification methods.

Anti-LAG-3 antibodies with certain properties

The antibodies of the invention are characterized by particular functional features or properties of the antibodies. In some embodiments, the isolated antibody or the antigen-binding portion thereof has one or more of the following properties:

(a) binds to human LAG-3 with a K_D of 2×10^{-10} M or less;

(b) inhibits binding of LAG-3 to major histocompatibility (MHC) class II molecules;

(c) inhibits binding of LAG-3 to fibrinogen-like protein 1 (FGL1) ligand molecules;

(d) inhibits binding of LAG-3 to LSECtin and/or Galectin-3; or

(e) binds to human LAG-3 without cross-family reactions.

The antibody of the invention binds to human LAG-3 with high affinity. The binding of an antibody of the invention to LAG-3 can be assessed using one or more techniques well established in the art, for instance, ELISA. The binding specificity of an antibody of the invention can also be determined by monitoring binding of the antibody to cells expressing a LAG-3 protein, e.g., flow cytometry. For example, 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 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., Kd value) can be tested in BIAcore binding assays. Still other suitable binding assays include ELISA assays, for example using a recombinant LAG-3 protein. For instance, an antibody of the invention binds to a human LAG-3 protein with a K_D of 5×10^{-8} M or less, binds to a human LAG-3 protein with a K_D of 2×10^{-8} M or less, binds to a human LAG-3 protein with a K_D of 5×10^{-9} M or less, binds to a human LAG-3 protein with a K_D of 4×10^{-9} M or less, binds to a human LAG-3 protein

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

The ability of the antibody to modulate 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) production in an antigen-specific T cell response. In certain embodiments, an antibody of the invention binds to human LAG-3 and exhibits an ability to stimulate an antigen-specific T cell response. Means by which to evaluate the ability of the antibody to stimulate an immune response may 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.

The isolated antibody or the antigen-binding portion thereof as disclosed herein inhibits binding of LAG-3 to major histocompatibility (MHC) class II molecules, FGL1 like molecules, LSECtin and/or Galectin-3. LAG-3 negatively regulates T cell signaling and functions. Ligands for LAG-3 includes, e.g., major histocompatibility (MHC) Class II molecules, LSECtin and Galectin-3. LAG-3 can interact with MHC class II molecules on the cell surface (Baixeras et al. (1992) J. Exp. Med. 176:327-337; Huard et al. (1996) Eur. J. Immunol. 26: 1180-1186). It has been suggested that the direct binding of LAG-3 to MHC class II plays a role in down-regulating antigen-dependent stimulation of $CD4^+$ T lymphocytes (Huard et al. (1994) Eur. J. Immunol. 24:3216-3221). Recently, Chen Lieping et al. have further demonstrated, through *in vitro* experiments, that FGL1 is a major immune inhibitory ligand of LAG-3, and thus, they have proposed a novel tumor immune evasion pathway FGL1-LAG-3, and blocking the FGL1-LAG-3 interaction may increase anti-tumor effect (Cell. 2019 Jan 10;176(1-2):334-347.e12.).

Galectin-3 is a 31 kD lectin that modulates T cell responses through several mechanisms including apoptosis, TCR cross linking, and TCR down regulation. Galectin-3 binds to LAG-3, and LAG-3 expression is necessary for galectin-3 mediated suppression of $CD8^+$ T cells in vitro. (Kouo et al. (2015) Cancer Immunol. Res. 10.1158: 2326-6066). Anti-LSECtin has been shown to inhibit B16 melanoma cell growth (Xu et al. (2014) Cancer Res. 74(13):3418-3428).

Anti-LAG-3 antibodies comprising CDRs with sequence identity to specific sequences

In some embodiments, the isolated antibody or the antigen-binding portion thereof comprises:

A) one or more heavy chain CDRs (CDRHs) selected from at least one of the group consisting of: (i) a CDRH1 with at least 90% sequence identity to a CDRH1 as set forth in one of

the sequences selected from the group consisting of SEQ ID NOs: 1 and 7; (ii) a CDRH2 with at least 90% sequence identity to a CDRH2 as set forth in one of the sequences selected from the group consisting of SEQ ID NOs: 2 and 8; and (iii) a CDRH3 with at least 90% sequence identity to a CDRH3 as set forth in one of the sequences selected from the group consisting of SEQ ID NOs: 3 and 9;

B) one or more light chain CDRs (CDRLs) selected from at least one of the group consisting of: (i) a CDRL1 with at least 90% sequence identity to a CDRL1 as set forth in one of the sequences selected from the group consisting of SEQ ID NOs: 4 and 10; (ii) a CDRL2 with at least 90% sequence identity to a CDRL2 as set forth in one of the sequences selected from the group consisting of SEQ ID NOs: 5 and 11; and (iii) a CDRL3 with at least 90% sequence identity to a CDRL3 as set forth in one of the sequences selected from the group consisting of SEQ ID NOs: 6 and 12; or

C) one or more CDRHs of A) and one or more CDRLs of B).

The assignment of amino acids to each CDR may be in accordance with one of the numbering schemes provided by Kabat *et al.* (1991) *Sequences of Proteins of Immunological Interest* (5th Ed.), US Dept. of Health and Human Services, PHS, NIH, NIH Publication no. 91-3242; Chothia *et al.*, 1987, PMID: 3681981; Chothia *et al.*, 1989, PMID: 2687698; MacCallum *et al.*,1996, PMID: 8876650; or Dubel, Ed. (2007) *Handbook of Therapeutic Antibodies,* 3rd Ed., Wily-VCH Verlag GmbH and Co. unless otherwise noted.

Variable regions and CDRs in an antibody sequence can be identified according to general rules that have been developed in the art (as set out above, such as, for example, the Kabat numbering system) or by aligning the sequences against a database of known variable regions. Methods for identifying these regions are described in Kontermann and Dubel, eds., Antibody Engineering, Springer, New York, NY, 2001 and Dinarello *et al.*, Current Protocols in Immunology, John Wiley and Sons Inc., Hoboken, NJ, 2000. Exemplary databases of antibody sequences are described in, and can be accessed through, the "Abysis" website at www.bioinf.org.uk/abs (maintained by A.C. Martin in the Department of Biochemistry & Molecular Biology University College London, London, England) and the VBASE2 website at www.vbase2.org, as described in Retter *et al.*, Nucl. Acids Res., 33 (Database issue): D671 -D674 (2005). Preferably sequences are analyzed using the Abysis database, which integrates sequence data from Kabat, IMGT and the Protein Data Bank (PDB) with structural data from the PDB. See Dr. Andrew C. R. Martin's book chapter *Protein Sequence and Structure Analysis of Antibody Variable Domains*. In: *Antibody Engineering Lab Manual* (Ed.: Duebel, S. and Kontermann, R., Springer-Verlag, Heidelberg,

ISBN-13: 978-3540413547, also available on the website bioinforg.uk/abs). The Abysis database website further includes general rules that have been developed for identifying CDRs which can be used in accordance with the teachings herein. Unless otherwise indicated, all CDRs set forth herein are derived according to the Abysis database website as per Kabat.

The percent identity between two amino acid sequences can be determined using the algorithm of E. Meyers and W. Miller (Comput. Appl. Biosci., 4:11-17 (1988)) which has been incorporated into the ALIGN program (version 2.0), using a PAM120 weight residue table, a gap length penalty of 12 and a gap penalty of 4. In addition, the percentage of identity between two amino acid sequences can be determined by the algorithm of Needleman and Wunsch (J. Mol. Biol. 48:444-453 (1970)) which has been incorporated into the GAP program in the GCG software package (available at http://www.gcg.com), using either a Blossum 62 matrix or a PAM250 matrix, and a gap weight of 16, 14, 12, 10, 8, 6, or 4 and a length weight of 1, 2, 3, 4, 5, or 6.

Additionally or alternatively, the protein sequences of the present invention can further be used as a "query sequence" to perform a search against public databases to, for example, identify related sequences. Such searches can be performed using the XBLAST program (version 2.0) of Altschul, et al. (1990) J. MoI. Biol. 215:403-10. BLAST protein searches can be performed with the XBLAST program, score = 50, wordlength = 3 to obtain amino acid sequences homologous to the antibody molecules of the invention. To obtain gapped alignments for comparison purposes, Gapped BLAST can be utilized as described in Altschul et al, (1997) Nucleic Acids Res. 25(17):3389-3402. When utilizing BLAST and Gapped BLAST programs, the default parameters of the respective programs {e.g., XBLAST and NBLAST) can be used. See www.ncbi.nlm.nih.gov.

In other embodiments, the CDR amino acid sequences can be at least 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98% or 99% identical to the respective sequences set forth above. As an illustrative example, the antibody may comprise a CDRH1 with at least 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98% or 99% sequence identity to a CDRH1 as set forth in one of the sequences selected from the group consisting of SEQ ID NOs: 1 and 7.

Anti-LAG-3 antibodies comprising CDRs with amino acid addition, deletion or substitution

In some embodiments, the isolated antibody or the antigen-binding portion thereof comprises:

A) one or more heavy chain CDRs (CDRHs) selected from at least one of the group consisting of: (i) a CDRH1 selected from the group consisting of SEQ ID NOs: 1 and 7 or a CDRH1

that differs in amino acid sequence from the CDRH1 by an amino acid addition, deletion or substitution of not more than 2 amino acids; (ii) a CDRH2 selected from the group consisting of SEQ ID NOs: 2 and 8 or a CDRH2 that differs in amino acid sequence from the CDRH2 by an amino acid addition, deletion or substitution of not more than 2 amino acids; and (iii) a CDRH3 selected from the group consisting of SEQ ID NOs: 3 and 9 or a CDRH3 that differs in amino acid sequence from the CDRH3 by an amino acid addition, deletion or substitution of not more than 2 amino acids;

B) one or more light chain CDRs (CDRLs) selected from at least one of the group consisting of: (i) a CDRL1 selected from the group consisting of SEQ ID NOs: 4 and 10 or a CDRL1 that differs in amino acid sequence from the CDRL1 by an amino acid addition, deletion or substitution of not more than 2 amino acids; (ii) a CDRL2 selected from the group consisting of SEQ ID NOs: 5 and 11 or a CDRL2 that differs in amino acid sequence from the CDRL2 by an amino acid addition, deletion or substitution of not more than 2 amino acids; and (iii) a CDRL3 selected from the group consisting of SEQ ID NOs: 6 and 12 or a CDRL3 that differs in amino acid sequence from the CDRL3 by an amino acid addition, deletion or substitution of not more than 2 amino acids; or

C) one or more CDRHs of A) and one or more CDRLs of B).

Preferably, the CDRs of the isolated antibody or the antigen-binding portion thereof contain a conservative substitution of not more than 2 amino acids, or not more than 1 amino acid. The term “conservative substitution”, as used herein, refers to amino acid substitutions which would not disadvantageously affect or change the essential properties of a protein/polypeptide comprising the amino acid sequence. For example, a conservative substitution may be introduced by standard techniques known in the art such as site-directed mutagenesis and PCR-mediated mutagenesis. Conservative amino acid substitutions include substitutions wherein an amino acid residue is substituted with another amino acid residue having a similar side chain, for example, a residue physically or functionally similar (such as, having similar size, shape, charge, chemical property including the capability of forming covalent bond or hydrogen bond, etc.) to the corresponding amino acid residue. The families of amino acid residues having similar side chains have been defined in the art. These families include amino acids having alkaline side chains (for example, lysine, arginine and histidine), amino acids having acidic side chains (for example, aspartic acid and glutamic acid), amino acids having uncharged polar side chains (for example, glycine, asparagine, glutamine, serine, threonine, tyrosine, cysteine, tryptophan), amino acids having nonpolar side chains (for example, alanine, valine, leucine, isoleucine, proline, phenylalanine, methionine), amino

acids having β-branched side chains (such as threonine, valine, isoleucine) and amino acids having aromatic side chains (for example, tyrosine, phenylalanine, tryptophan, histidine). Therefore, a corresponding amino acid residue is preferably substituted with another amino acid residue from the same side-chain family. Methods for identifying amino acid conservative substitutions are well known in the art (see, for example, Brummell et al., Biochem. 32: 1180-1187 (1993); Kobayashi et al., Protein Eng. 12(10): 879-884 (1999); and Burks et al., Proc. Natl. Acad. Sci. USA 94: 412-417 (1997), which are incorporated herein by reference).

Anti-LAG-3 antibodies comprising CDRs

In some embodiments, the isolated antibody or the antigen-binding portion thereof comprises:

(a) a CDRH1 comprising SEQ ID NO: 1;

(b) a CDRH2 comprising SEQ ID NO: 2;

(c) a CDRH3 comprising SEQ ID NO: 3;

(d) a CDRL1 comprising SEQ ID NO: 4;

(e) a CDRL2 comprising SEQ ID NO: 5; and

(f) a CDRL3 comprising SEQ ID NO: 6.

In a specific embodiment, the isolated antibody or the antigen-binding portion thereof comprises:

(a) a CDRH1 consisting of SEQ ID NO: 1;

(b) a CDRH2 consisting of SEQ ID NO: 2;

(c) a CDRH3 consisting of SEQ ID NO: 3;

(d) a CDRL1 consisting of SEQ ID NO: 4;

(e) a CDRL2 consisting of SEQ ID NO: 5; and

(f) a CDRL3 consisting of SEQ ID NO: 6.

In some embodiments, the isolated antibody or the antigen-binding portion thereof comprises:

(a) a CDRH1 comprising SEQ ID NO: 7;

(b) a CDRH2 comprising SEQ ID NO: 8;

(c) a CDRH3 comprising SEQ ID NO: 9;

(d) a CDRL1 comprising SEQ ID NO: 10;

(e) a CDRL2 comprising SEQ ID NO: 11; and

(f) a CDRL3 comprising SEQ ID NO: 12.

In a specific embodiment, the isolated antibody or the antigen-binding portion thereof comprises:

(a) a CDRH1 consisting of SEQ ID NO: 7;

(b) a CDRH2 consisting of SEQ ID NO: 8;

(c) a CDRH3 consisting of SEQ ID NO: 9;

(d) a CDRL1 consisting of SEQ ID NO: 10;

(e) a CDRL2 consisting of SEQ ID NO: 11; and

(f) a CDRL3 consisting of SEQ ID NO: 12.

Anti-LAG-3 antibodies comprising a heavy chain variable region and a light chain variable region

In some embodiments, the isolated antibody or the antigen-binding portion thereof comprises:

(A) a heavy chain variable region:

(i) comprising the amino acid sequence of SEQ ID NO: 13;

(ii) comprising an amino acid sequence at least 85%, at least 90%, or at least 95% identical to SEQ ID NO: 13; or

(iii) comprising an amino acid sequence with addition, deletion and/or substitution of one or more amino acids compared with SEQ ID NO: 13; and/or

(B) a light chain variable region:

(i) comprising the amino acid sequence of SEQ ID NO: 14;

(ii) comprising an amino acid sequence at least 85%, at least 90%, or at least 95% identical to SEQ ID NO: 14;

(iii) comprising an amino acid sequence with addition, deletion and/or substitution of one or more amino acids compared with SEQ ID NO: 14.

In a specific embodiment, the isolated antibody or the antigen-binding portion thereof comprises:

(a) a heavy chain variable region consisting of the amino acid sequence of SEQ ID NO: 13; and/or

(b) a light chain variable region comprising the amino acid sequence of SEQ ID NO: 14.

In some embodiments, the isolated antibody or the antigen-binding portion thereof comprises:

(A) a heavy chain variable region:

(i) comprising the amino acid sequence of SEQ ID NO: 15;

(ii) comprising an amino acid sequence at least 85%, at least 90%, or at least 95% identical to SEQ ID NO: 15; or

(iii) comprising an amino acid sequence with addition, deletion and/or substitution of one or more amino acids compared with SEQ ID NO: 15; and/or

(B) a light chain variable region:

(i) comprising the amino acid sequence of SEQ ID NO: 16;

(ii) comprising an amino acid sequence at least 85%, at least 90%, or at least 95% identical to SEQ ID NO: 16; or

(iii) comprising an amino acid sequence with addition, deletion and/or substitution of one or more amino acids compared with SEQ ID NO: 16.

In a specific embodiment, the isolated antibody or the antigen-binding portion thereof comprises:

(a) a heavy chain variable region consisting of the amino acid sequence of SEQ ID NO: 15; and/or

(b) a light chain variable region comprising the amino acid sequence of SEQ ID NO: 16.

In other embodiments, the amino acid sequences of the heavy chain variable region and/or the light chain variable region can be at least 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98% or 99% identical to the respective sequences set forth above. As an illustrative example, the antibody may comprise a heavy chain variable region with at least 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98% or 99% sequence identity to a heavy chain variable region consisting of the amino acid sequence of SEQ ID NO: 15.

In some further embodiments, the isolated antibody or the antigen-binding portion thereof may contain conservative substitution or modification of amino acids in the variable regions of the heavy chain and/or light chain. It is understood in the art that certain conservative sequence modification can be made which do not remove antigen binding. See, e.g., Brummell et al. (1993) Biochem 32:1180-8; de Wildt et al. (1997) Prot. Eng. 10:835-41; Komissarov et al. (1997) J. Biol. Chem. 272:26864- 26870; Hall et al. (1992) J. Immunol. 149:1605-12; Kelley and O' Connell (1993) Biochem. 32:6862-35; Adib-Conquy et al. (1998) Int. Immunol. 10:341-6 and Beers et al. (2000) Clin. Can. Res. 6:2835-43.

As described above, the term "conservative substitution", as used herein, refers to amino acid substitutions which would not disadvantageously affect or change the essential properties of a protein/polypeptide comprising the amino acid sequence. For example, a conservative substitution may be introduced by standard techniques known in the art such as site-directed mutagenesis and PCR-mediated mutagenesis. Conservative amino acid substitutions include substitutions wherein an amino acid residue is substituted with another amino acid residue having a similar side chain, for example, a residue physically or functionally similar (such as, having similar size, shape, charge, chemical property including the capability of forming covalent bond or hydrogen bond, etc.) to the corresponding amino acid residue. The families of amino acid residues having similar side chains have been defined in the art. These families include amino acids having alkaline side chains (for example, lysine, arginine and histidine), amino acids having acidic side chains (for example, aspartic acid and glutamic acid), amino acids having uncharged polar side chains (for example, glycine, asparagine, glutamine, serine, threonine, tyrosine, cysteine, tryptophan), amino acids having nonpolar side chains (for example, alanine, valine, leucine, isoleucine, proline, phenylalanine, methionine), amino acids having β-branched side chains (such as threonine, valine, isoleucine)

and amino acids having aromatic side chains (for example, tyrosine, phenylalanine, tryptophan, histidine). Therefore, a corresponding amino acid residue is preferably substituted with another amino acid residue from the same side-chain family. Methods for identifying amino acid conservative substitutions are well known in the art (see, for example, Brummell et al., Biochem. 32: 1180-1187 (1993); Kobayashi et al., Protein Eng. 12(10): 879-884 (1999); and Burks et al., Proc. Natl. Acad. Sci. USA 94: 412-417 (1997), which are incorporated herein by reference).

Binning and epitope mapping

It will further be appreciated the disclosed antibodies will associate with, or bind to, discrete epitopes or immunogenic determinants presented by the selected target or fragment thereof. In certain embodiments, epitope or immunogenic determinants include chemically active surface groupings of molecules such as amino acids, sugar side chains, phosphoryl groups, or sulfonyl groups, and, in certain embodiments, may have specific three-dimensional structural characteristics, and/or specific charge characteristics. Thus, as used herein the term "epitope" includes any protein determinant capable of specific binding to an immunoglobulin or T-cell receptor or otherwise interacting with a molecule. In certain embodiments, an antibody is said to specifically bind (or immunospecifically bind or react) an antigen when it preferentially recognizes its target antigen in a complex mixture of proteins and/or macromolecules. In some embodiments, an antibody is said to specifically bind an antigen when the equilibrium dissociation constant (K_D) is less than or equal to 10^{-6}M or less than or equal to 10^{-7}M, more preferably when the e K_D is less than or equal to 10^{-8}M, and even more preferably when the K_D is less than or equal to 10^{-9}M.

Epitopes formed from contiguous amino acids (sometimes referred to as "linear" or "continuous" epitopes) are typically retained upon protein denaturing, whereas epitopes formed by tertiary folding are typically lost upon protein denaturing. In any event an antibody epitope typically includes at least 3, and more usually, at least 5 or 8-10 amino acids in a unique spatial conformation.

In this respect, it will be appreciated that, in certain embodiments, an epitope may be associated with, or reside in, one or more regions, domains or motifs of, for example, the LAG-3 protein. Similarly, the art-recognized term "motif" will be used in accordance with its common meaning and shall generally refer to a short, conserved region of a protein that is typically ten to twenty contiguous amino acid residues.

In any event once a desired epitope on an antigen is determined, it is possible to generate antibodies to that epitope, e.g., by immunizing with a peptide comprising the epitope using techniques described in the present invention. Alternatively, during the discovery process, the generation and characterization of antibodies may elucidate information about desirable epitopes located in specific domains or motifs. From this information, it is then possible to competitively screen antibodies for binding to the same epitope. An approach to achieve this is to conduct competition studies to find antibodies that competitively bind with one another, i.e. the antibodies compete for binding to the antigen. A high throughput process for binning antibodies based upon their cross-competition is described in WO 03/48731. Other methods of binning or domain level or epitope mapping comprising antibody competition or antigen fragment expression on yeast are well known in the art.

As used herein, the term "binning" refers to methods used to group or classify antibodies based on their antigen binding characteristics and competition. While the techniques are useful for defining and categorizing the antibodies of the instant invention, the bins do not always directly correlate with epitopes and such initial determinations of epitope binding may be further refined and confirmed by other art-recognized methodology in the art and as described herein. However, it will be appreciated that empirical assignment of the antibodies to individual bins provides information that may be indicative of the therapeutic potential of the disclosed antibodies.

More specifically, one can determine whether a selected reference antibody (or fragment thereof) binds to the same epitope or cross competes for binding with a second test antibody (i.e., is in the same bin) by using methods known in the art and set forth in the Examples herein.

Other compatible epitope mapping techniques include alanine scanning mutants, peptide blots (Reineke (2004) Methods Mol Biol 248:443-63) (herein specifically incorporated by reference in its entirety), or peptide cleavage analysis. In addition, methods such as epitope excision, epitope extraction and chemical modification of antigens can be employed (Tomer (2000) Protein Science 9: 487-496) (herein specifically incorporated by reference in its entirety).

Nucleic Acid Molecules Encoding Antibodies of the Invention

In some aspects, the invention is directed to an isolated nucleic acid molecule, comprising a nucleic acid sequence encoding the heavy chain variable region and/or the light chain variable region of the isolated antibody as disclosed herein.

Nucleic acids of the invention can be obtained using standard molecular biology techniques. For antibodies expressed by hybridomas (e.g., hybridomas prepared from transgenic mice carrying human immunoglobulin genes as described further below), cDNAs encoding the light and heavy chains of the antibody made by the hybridoma can be obtained by standard PCR amplification or cDNA cloning techniques. For antibodies obtained from an immunoglobulin gene library (e.g., using phage display techniques), a nucleic acid encoding such antibodies can be recovered from the gene library.

The isolated nucleic acid encoding the VH region can be converted to a full-length heavy chain gene by operatively linking the VH-encoding nucleic acid to another DNA molecule encoding heavy chain constant regions (CH1, CH2 and CH3). The sequences of human heavy chain constant region genes are known in the art (see e.g., Kabat et al. (1991), supra) and DNA fragments encompassing these regions can be obtained by standard PCR amplification. The heavy chain constant region can be an IgG1, IgG2, IgG3, IgG4, IgA, IgE, IgM or IgD constant region, but more preferably is an IgG1 or IgG4 constant region, and most preferably is an IgG4 constant region.

The isolated nucleic acid encoding the VL region can be converted to a full-length light chain gene (as well as a Fab light chain gene) by operatively linking the VL-encoding DNA to another DNA molecule encoding the light chain constant region, CL. The sequences of human light chain constant region genes are known in the art (see e.g., Kabat et al., supra) and DNA fragments encompassing these regions can be obtained by standard PCR amplification. In preferred embodiments, the light chain constant region can be a kappa or lambda constant region.

Once DNA fragments encoding VH and VL segments are obtained, these DNA fragments can be further manipulated by standard recombinant DNA techniques, for example to convert the variable region genes to full-length antibody chain genes, to Fab fragment genes or to a scFv gene. In these manipulations, a VL- or VH-encoding DNA fragment is operatively linked to another DNA fragment encoding another protein, such as an antibody constant region or a flexible linker. The term "operatively linked", as used in this context, is intended to mean that the two DNA fragments are joined such that the amino acid sequences encoded by the two DNA fragments remain in-frame.

Preferred nucleic acids molecules of the invention are those encoding the VH and VL sequences of 1.53.3-uAb-IgG4k and 3.40.19-uAb-IgG4L monoclonal antibodies. DNA sequences encoding the VH sequences of 1.53.3-uAb-IgG4k and 3.40.19-uAb-IgG4L are shown in SEQ ID NOs: 17 and 19, respectively. DNA sequences encoding the VL sequences of 1.53.3-uAb-IgG4k and 3.40.19-uAb-IgG4L are shown in SEQ ID NOs: 18 and 20, respectively. In some embodiments,

the nucleic acids share an at least 80% (e.g. at least 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, or 99%) sequence identity to SEQ ID NOs: 17-20, respectively. In some embodiments, the percentage of identity is derived from the degeneracy of the genetic code, and the encoded protein sequences remain unchanged.

Pharmaceutical Compositions

In some aspects, the invention is directed to a pharmaceutical composition comprising at least one antibody or antigen-binding portion thereof as disclosed herein and a pharmaceutically acceptable carrier.

Components of the compositions

The pharmaceutical composition may optionally contain one or more additional pharmaceutically active ingredients, such as another antibody or a drug. The pharmaceutical compositions of the invention also can be administered in a combination therapy with, for example, another immune-stimulatory agent, anti-cancer agent, an antiviral agent, or a vaccine, such that the anti-LAG-3 antibody enhances the immune response against the vaccine. A pharmaceutically acceptable carrier can include, for example, a pharmaceutically acceptable liquid, gel or solid carriers, an aqueous medium, a non-aqueous medium, an anti-microbial agent, isotonic agents, buffers, antioxidants, anesthetics, suspending/dispersing agent, a chelating agent, a diluent, adjuvant, excipient or a nontoxic auxiliary substance, other known in the art various combinations of components or more.

Suitable components may include, for example, antioxidants, fillers, binders, disintegrating agents, buffers, preservatives, lubricants, flavorings, thickening agents, coloring agents, emulsifiers or stabilizers such as sugars and cyclodextrin. Suitable anti-oxidants may include, for example, methionine, ascorbic acid, EDTA, sodium thiosulfate, platinum, catalase, citric acid, cysteine, mercapto glycerol, thioglycolic acid, Mercapto sorbitol, butyl methyl anisole, butylated hydroxy toluene and/or propylgalacte. As disclosed in the present invention, in a solvent containing an antibody or an antigen-binding fragment of the present invention discloses compositions include one or more anti-oxidants such as methionine, reducing antibody or antigen binding fragment thereof may be oxidized. The oxidation reduction may prevent or reduce a decrease in binding affinity, thereby enhancing antibody stability and extended shelf life. Thus, in some embodiments, the present invention provides a composition comprising one or more antibodies or antigen binding fragment thereof and one or more anti-oxidants such as methionine. The present invention further provides a variety of methods, wherein an antibody or antigen binding fragment thereof is mixed

with one or more anti-oxidants, such as methionine, so that the antibody or antigen binding fragment thereof can be prevented from oxidation, to extend their shelf life and/or increased activity.

Administration, Formulation and Dosage

The pharmaceutical composition of the invention may be administered *in vivo,* to a subject in need thereof, by various routes, including, but not limited to, oral, intravenous, intra-arterial, subcutaneous, parenteral, intranasal, intramuscular, intracranial, intracardiac, intraventricular, intratracheal, buccal, rectal, intraperitoneal, intradermal, topical, transdermal, and intrathecal, or otherwise by implantation or inhalation. The subject compositions may be formulated into preparations in solid, semi-solid, liquid, or gaseous forms; including, but not limited to, tablets, capsules, powders, granules, ointments, solutions, suppositories, enemas, injections, inhalants, and aerosols. The appropriate formulation and route of administration may be selected according to the intended application and therapeutic regimen.

Suitable formulations for enteral administration include hard or soft gelatin capsules, pills, tablets, including coated tablets, elixirs, suspensions, syrups or inhalations and controlled release forms thereof.

Formulations suitable for parenteral administration (e.g., by injection), include aqueous or non-aqueous, isotonic, pyrogen-free, sterile liquids (e.g., solutions, suspensions), in which the active ingredient is dissolved, suspended, or otherwise provided (e.g., in a liposome or other microparticulate). Such liquids may additional contain other pharmaceutically acceptable ingredients, such as anti-oxidants, buffers, preservatives, stabilisers, bacteriostats, suspending agents, thickening agents, and solutes which render the formulation isotonic with the blood (or other relevant bodily fluid) of the intended recipient. Examples of excipients include, for example, water, alcohols, polyols, glycerol, vegetable oils, and the like. Examples of suitable isotonic carriers for use in such formulations include Sodium Chloride Injection, Ringer's Solution, or Lactated Ringer's Injection. Similarly, the particular dosage regimen, i.e., dose, timing and repetition, will depend on the particular individual and that individual's medical history, as well as empirical considerations such as pharmacokinetics (e.g., half-life, clearance rate, etc.).

Frequency of administration may be determined and adjusted over the course of therapy, and is based on reducing the number of proliferative or tumorigenic cells, maintaining the reduction of such neoplastic cells, reducing the proliferation of neoplastic cells, or delaying the development of metastasis. In some embodiments, the dosage administered may be adjusted or attenuated to

manage potential side effects and/or toxicity. Alternatively, sustained continuous release formulations of a subject therapeutic composition may be appropriate.

It will be appreciated by one of skill in the art that appropriate dosages can vary from patient to patient. Determining the optimal dosage will generally involve the balancing of the level of therapeutic benefit against any risk or deleterious side effects. The selected dosage level will depend on a variety of factors including, but not limited to, the activity of the particular compound, the route of administration, the time of administration, the rate of excretion of the compound, the duration of the treatment, other drugs, compounds, and/or materials used in combination, the severity of the condition, and the species, sex, age, weight, condition, general health, and prior medical history of the patient. The amount of compound and route of administration will ultimately be at the discretion of the physician, veterinarian, or clinician, although generally the dosage will be selected to achieve local concentrations at the site of action that achieve the desired effect without causing substantial harmful or deleterious side-effects.

In general, the antibody or the antigen binding portion thereof of the invention may be administered in various ranges. These include about 5 μg/kg body weight to about 100 mg/kg body weight per dose; about 50 μg/kg body weight to about 5 mg/kg body weight per dose; about 100 μg/kg body weight to about 10 mg/kg body weight per dose. Other ranges include about 100 μg/kg body weight to about 20 mg/kg body weight per dose and about 0.5 mg/kg body weight to about 20 mg/kg body weight per dose. In certain embodiments, the dosage is at least about 100 μg/kg body weight, at least about 250 μg/kg body weight, at least about 750 μg/kg body weight, at least about 3 mg/kg body weight, at least about 5 mg/kg body weight, at least about 10 mg/kg body weight.

In any event, the antibody or the antigen binding portion thereof of the invention is preferably administered as needed to subjects in need thereof. Determination of the frequency of administration may be made by persons skilled in the art, such as an attending physician based on considerations of the condition being treated, age of the subject being treated, severity of the condition being treated, general state of health of the subject being treated and the like.

In certain preferred embodiments, the course of treatment involving the antibody or the antigen-binding portion thereof of the instant invention will comprise multiple doses of the selected drug product over a period of weeks or months. More specifically, the antibody or the antigen-binding portion thereof of the instant invention may be administered once every day, every two days, every four days, every week, every ten days, every two weeks, every three weeks, every month, every six weeks, every two months, every ten weeks or every three months. In this regard,

it will be appreciated that the dosages may be altered or the interval may be adjusted based on patient response and clinical practices.

Dosages and regimens may also be determined empirically for the disclosed therapeutic compositions in individuals who have been given one or more administration(s). For example, individuals may be given incremental dosages of a therapeutic composition produced as described herein. In selected embodiments, the dosage may be gradually increased or reduced or attenuated based respectively on empirically determined or observed side effects or toxicity. To assess efficacy of the selected composition, a marker of the specific disease, disorder or condition can be followed as described previously. For cancer, these include direct measurements of tumor size via palpation or visual observation, indirect measurement of tumor size by x-ray or other imaging techniques; an improvement as assessed by direct tumor biopsy and microscopic examination of the tumor sample; the measurement of an indirect tumor marker (e.g., PSA for prostate cancer) or a tumorigenic antigen identified according to the methods described herein, a decrease in pain or paralysis; improved speech, vision, breathing or other disability associated with the tumor; increased appetite; or an increase in quality of life as measured by accepted tests or prolongation of survival. It will be apparent to one of skill in the art that the dosage will vary depending on the individual, the type of neoplastic condition, the stage of neoplastic condition, whether the neoplastic condition has begun to metastasize to other location in the individual, and the past and concurrent treatments being used.

Compatible formulations for parenteral administration (e.g., intravenous injection) will comprise the antibody or antigen-binding portion thereof in concentrations of from about 10 μg/mL to about 100 mg/mL. In certain selected embodiments, the concentrations of the antibody or the antigen binding portion thereof will comprise 20 μg/mL, 40 μg/mL, 60 μg/mL, 80 μg/mL, 100 μg/mL, 200 μg/mL, 300, μg/mL, 400 μg/mL, 500 μg/mL, 600 μg/mL, 700 μg/mL, 800 μg/mL, 900 μg/mL or 1 mg/mL. In other preferred embodiments ADC concentrations will comprise 2 mg/mL, 3 mg/mL, 4 mg/mL, 5 mg/mL, 6 mg/mL, 8 mg/mL, 10 mg/mL, 12 mg/mL, 14 mg/mL, 16 mg/mL, 18 mg/mL, 20 mg/mL, 25 mg/mL, 30 mg/mL, 35 mg/mL, 40 mg/mL, 45 mg/mL, 50 mg/mL, 60 mg/mL, 70 mg/mL, 80 mg/mL, 90 mg/mL or 100 mg/mL.

Applications of the Invention

The antibodies, antibody compositions and methods of the present invention have numerous in vitro and in vivo utilities involving, for example, detection of LAG-3 or enhancement of immune response by blockade of LAG-3. For example, these molecules can be administered to cells in

culture, in vitro or ex vivo, or to human subjects, e.g., in vivo, to enhance immunity in a variety of situations. The immune response can be modulated, for instance, augmented, stimulated or up-regulated.

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

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

Given the ability of anti-LAG-3 antibodies of the invention to inhibit the binding of LAG-3 to MHC Class II molecules or FGL1 like molecules and to stimulate antigen- specific T cell responses, the invention also provides in vitro and in vivo methods of using the antibodies of the invention to stimulate, enhance or upregulate antigen- specific T cell responses. For example, the invention provides a method of stimulating an antigen- specific T cell response comprising administering to the subject the antibody of the invention or an antigen binding portion thereof such that an antigen- specific T cell response is stimulated. Any suitable indicator of an antigen-specific T cell response can be used to measure the antigen- specific T cell response.

Treatment of cancers

Non-limiting examples of such suitable indicators include increased T cell proliferation in the presence of the antibody and/or increase cytokine production in the presence of the antibody.

In a preferred embodiment, interleukin-2 production by the antigen- specific T cell is stimulated. The invention also provides a method of stimulating an immune response (e.g., an antigen- specific T cell response) in a subject comprising administering an antibody or an antigen binding portion thereof of the invention to the subject such that an immune response (e.g., an antigen- specific T cell response) in the subject is stimulated. In a preferred embodiment, the subject is a cancer-bearing subject and an immune response against the tumor is stimulated. Cancer Blockade of LAG-3 by antibodies can enhance the immune response to cancerous cells in the patient. An anti-LAG-3 antibody can be used alone to or used in conjunction with other immunogenic agents, standard cancer treatments, or other antibodies.

Examples of cancers that can be treated using the methods of the invention include bone cancer, pancreatic cancer, skin cancer, cancer of the head or neck, cutaneous or intraocular malignant melanoma, uterine cancer, ovarian cancer, rectal cancer, cancer of the anal region, stomach cancer, testicular cancer, carcinoma of the fallopian tubes, carcinoma of the endometrium, carcinoma of the cervix, carcinoma of the vagina, carcinoma of the vulva, Hodgkin's Disease, non-Hodgkin's lymphoma, cancer of the esophagus, cancer of the small intestine, cancer of the endocrine system, cancer of the thyroid gland, cancer of the parathyroid gland, cancer of the adrenal gland, sarcoma of soft tissue, cancer of the urethra, cancer of the penis, chronic or acute leukemias including acute myeloid leukemia, chronic myeloid leukemia, acute lymphoblastic leukemia, chronic lymphocytic leukemia, solid tumors of childhood, lymphocytic lymphoma, cancer of the bladder, cancer of the kidney or ureter, carcinoma of the renal pelvis, neoplasm of the central nervous system (CNS), primary CNS lymphoma, tumor angiogenesis, spinal axis tumor, brain stem glioma, pituitary adenoma, Kaposi's sarcoma, epidermoid cancer, squamous cell cancer, T-cell lymphoma, environmentally induced cancers including those induced by asbestos, and combinations of said cancers.

The antibody or the antigen-binding portion thereof may be used in combination with chemical therapies or radiotherapies.

Combined use with chemotherapies

The antibody or the antigen-binding portion thereof may be used in combination with an anti-cancer agent, a cytotoxic agent or chemotherapeutic agent.

The term "anti-cancer agent" or "anti-proliferative agent" means any agent that can be used to treat a cell proliferative disorder such as cancer, and includes, but is not limited to, cytotoxic agents, cytostatic agents, anti-angiogenic agents, debulking agents, chemotherapeutic agents,

radiotherapy and radiotherapeutic agents, targeted anti-cancer agents, BRMs, therapeutic antibodies, cancer vaccines, cytokines, hormone therapies, radiation therapy and anti-metastatic agents and immunotherapeutic agents. It will be appreciated that, in selected embodiments as discussed above, such anti-cancer agents may comprise conjugates and may be associated with the disclosed site-specific antibodies prior to administration. More specifically, in certain embodiments selected anti-cancer agents will be linked to the unpaired cysteines of the engineered antibodies to provide engineered conjugates as set forth herein. Accordingly, such engineered conjugates are expressly contemplated as being within the scope of the instant invention. In other embodiments, the disclosed anti-cancer agents will be given in combination with site-specific conjugates comprising a different therapeutic agent as set forth above.

As used herein the term "cytotoxic agent" means a substance that is toxic to the cells and decreases or inhibits the function of cells and/or causes destruction of cells. In certain embodiments, the substance is a naturally occurring molecule derived from a living organism. Examples of cytotoxic agents include, but are not limited to, small molecule toxins or enzymatically active toxins of bacteria (e.g., Diptheria toxin, Pseudomonas endotoxin and exotoxin, Staphylococcal enterotoxin A), fungal (e.g., α-sarcin, restrictocin), plants (e.g., abrin, ricin, modeccin, viscumin, pokeweed anti-viral protein, saporin, gelonin, momoridin, trichosanthin, barley toxin, Aleurites fordii proteins, dianthin proteins, Phytolacca mericana proteins (PAPI, PAPII, and PAP-S), Momordica charantia inhibitor, curcin, crotin, saponaria officinalis inhibitor, gelonin, mitegellin, restrictocin, phenomycin, neomycin, and the tricothecenes) or animals, (e.g., cytotoxic RNases, such as extracellular pancreatic RNases; DNase I, including fragments and/or variants thereof).

For the purposes of the instant invention a "chemotherapeutic agent" comprises a chemical compound that non-specifically decreases or inhibits the growth, proliferation, and/or survival of cancer cells (e.g., cytotoxic or cytostatic agents). Such chemical agents are often directed to intracellular processes necessary for cell growth or division, and are thus particularly effective against cancerous cells, which generally grow and divide rapidly. For example, vincristine depolymerizes microtubules, and thus inhibits cells from entering mitosis. In general, chemotherapeutic agents can include any chemical agent that inhibits, or is designed to inhibit, a cancerous cell or a cell likely to become cancerous or generate tumorigenic progeny (e.g., TIC). Such agents are often administered, and are often most effective, in combination, e.g., in regimens such as CHOP or FOLFIRI.

Examples of anti-cancer agents that may be used in combination with the site-specific constructs of the present invention (either as a component of a site specific conjugate or in an unconjugated state) include, but are not limited to, alkylating agents, alkyl sulfonates, aziridines,

ethylenimines and methylamelamines, acetogenins, a camptothecin, bryostatin, callystatin, CC-1065, cryptophycins, dolastatin, duocarmycin, eleutherobin, pancratistatin, a sarcodictyin, spongistatin, nitrogen mustards, antibiotics, enediyne antibiotics, dynemicin, bisphosphonates, esperamicin, chromoprotein enediyne antiobiotic chromophores, aclacinomysins, actinomycin, authramycin, azaserine, bleomycins, cactinomycin, carabicin, carminomycin, carzinophilin, chromomycinis, dactinomycin, daunorubicin, detorubicin, 6-diazo-5-oxo-L-norleucine, ADRIAMYCIN® doxorubicin, epirubicin, esorubicin, idarubicin, marcellomycin, mitomycins, mycophenolic acid, nogalamycin, olivomycins, peplomycin, potfiromycin, puromycin, quelamycin, rodorubicin, streptonigrin, streptozocin, tubercidin, ubenimex, zinostatin, zorubicin; anti-metabolites, erlotinib, vemurafenib, crizotinib,sorafenib, ibrutinib, enzalutamide, folic acid analogues, purine analogs, androgens, anti-adrenals, folic acid replenisher such as frolinic acid, aceglatone, aldophosphamide glycoside, aminolevulinic acid, eniluracil, amsacrine, bestrabucil, bisantrene, edatraxate, defofamine, demecolcine, diaziquone, elfornithine, elliptinium acetate, an epothilone, etoglucid, gallium nitrate, hydroxyurea, lentinan, lonidainine, maytansinoids, mitoguazone, mitoxantrone, mopidanmol, nitraerine, pentostatin, phenamet, pirarubicin, losoxantrone, podophyllinic acid, 2- ethylhydrazide, procarbazine, PSK® polysaccharide complex (JHS Natural Products, Eugene, OR), razoxane; rhizoxin; sizofiran; spirogermanium; tenuazonic acid; triaziquone; 2,2',2"-trichlorotriethylamine; trichothecenes (especially T-2 toxin, verracurin A, roridin A and anguidine); urethan; vindesine; dacarbazine; mannomustine; mitobronitol; mitolactol; pipobroman; gacytosine; arabinoside ("Ara-C"); cyclophosphamide; thiotepa; taxoids, chloranbucil; GEMZAR® gemcitabine; 6-thioguanine; mercaptopurine; methotrexate; platinum analogs, vinblastine; platinum; etoposide (VP-16); ifosfamide; mitoxantrone; vincristine; NAVELBINE® vinorelbine; novantrone; teniposide; edatrexate; daunomycin; aminopterin; xeloda; ibandronate; irinotecan (Camptosar, CPT-11), topoisomerase inhibitor RFS 2000; difluorometlhylornithine; retinoids; capecitabine; combretastatin; leucovorin; oxaliplatin; inhibitors of PKC-alpha, Raf, H-Ras, EGFR and VEGF-A that reduce cell proliferation and pharmaceutically acceptable salts, acids or derivatives of any of the above. Also included in this definition are anti-hormonal agents that act to regulate or inhibit hormone action on tumors such as anti-estrogens and selective estrogen receptor modulators, aromatase inhibitors that inhibit the enzyme aromatase, which regulates estrogen production in the adrenal glands, and anti-androgens; as well as troxacitabine (a 1,3- dioxolane nucleoside cytosine analog); antisense oligonucleotides, ribozymes such as a VEGF expression inhibitor and a HER2 expression inhibitor; vaccines, PROLEUKIN® rIL-2; LURTOTECAN® topoisomerase 1 inhibitor; ABARELIX® rmRH; Vinorelbine and Esperamicins and pharmaceutically acceptable salts, acids or derivatives of any of the above.

Combined use with radiotherapies

The present invention also provides for the combination of the antibody or the antigen-binding portion thereof with radiotherapy (i.e., any mechanism for inducing DNA damage locally within tumor cells such as gamma-irradiation, X-rays, UV-irradiation, microwaves, electronic emissions and the like). Combination therapy using the directed delivery of radioisotopes to tumor cells is also contemplated, and the disclosed conjugates may be used in connection with a targeted anti-cancer agent or other targeting means. Typically, radiation therapy is administered in pulses over a period of time from about 1 to about 2 weeks. The radiation therapy may be administered to subjects having head and neck cancer for about 6 to 7 weeks. Optionally, the radiation therapy may be administered as a single dose or as multiple, sequential doses.

Diagnosis

The invention provides *in vitro* and *in vivo* methods for detecting, diagnosing or monitoring proliferative disorders and methods of screening cells from a patient to identify tumor cells including tumorigenic cells. Such methods include identifying an individual having cancer for treatment or monitoring progression of a cancer, comprising contacting the patient or a sample obtained from a patient (either *in vivo* or *in vitro*) with an antibody as described herein and detecting presence or absence, or level of association, of the antibody to bound or free target molecules in the sample. In some embodiments, the antibody will comprise a detectable label or reporter molecule as described herein.

In some embodiments, the association of the antibody with particular cells in the sample can denote that the sample may contain tumorigenic cells, thereby indicating that the individual having cancer may be effectively treated with an antibody as described herein.

Samples can be analyzed by numerous assays, for example, radioimmunoassays, enzyme immunoassays (e.g. ELISA), competitive-binding assays, fluorescent immunoassays, immunoblot assays, Western Blot analysis and flow cytometry assays. Compatible *in vivo* theragnostic or diagnostic assays can comprise art recognized imaging or monitoring techniques, for example, magnetic resonance imaging, computerized tomography (e.g. CAT scan), positron tomography (e.g., PET scan), radiography, ultrasound, etc., as would be known by those skilled in the art.

Pharmaceutical packs and kits

Pharmaceutical packs and kits comprising one or more containers, comprising one or more doses of the antibody or the antigen-binding portion thereof are also provided. In certain embodiments, a unit dosage is provided wherein the unit dosage contains a predetermined amount of a composition comprising, for example, the antibody or the antigen-binding portion thereof, with or without one or more additional agents. For other embodiments, such a unit dosage is supplied in single-use prefilled syringe for injection. In still other embodiments, the composition contained in the unit dosage may comprise saline, sucrose, or the like; a buffer, such as phosphate, or the like; and/or be formulated within a stable and effective pH range. Alternatively, in certain embodiments, the conjugate composition may be provided as a lyophilized powder that may be reconstituted upon addition of an appropriate liquid, for example, sterile water or saline solution. In certain preferred embodiments, the composition comprises one or more substances that inhibit protein aggregation, including, but not limited to, sucrose and arginine. Any label on, or associated with, the container(s) indicates that the enclosed conjugate composition is used for treating the neoplastic disease condition of choice.

The present invention also provides kits for producing single-dose or multi-dose administration units of site-specific conjugates and, optionally, one or more anti-cancer agents. The kit comprises a container and a label or package insert on or associated with the container. Suitable containers include, for example, bottles, vials, syringes, etc. The containers may be formed from a variety of materials such as glass or plastic and contain a pharmaceutically effective amount of the disclosed conjugates in a conjugated or unconjugated form. In other preferred embodiments, the container(s) comprise a sterile access port (for example the container may be an intravenous solution bag or a vial having a stopper pierceable by a hypodermic injection needle). Such kits will generally contain in a suitable container a pharmaceutically acceptable formulation of the engineered conjugate and, optionally, one or more anti-cancer agents in the same or different containers. The kits may also contain other pharmaceutically acceptable formulations, either for diagnosis or combined therapy. For example, in addition to the antibody or the antigen-binding portion thereof of the invention such kits may contain any one or more of a range of anti-cancer agents such as chemotherapeutic or radiotherapeutic drugs; anti-angiogenic agents; anti-metastatic agents; targeted anti-cancer agents; cytotoxic agents; and/or other anti-cancer agents.

More specifically the kits may have a single container that contains the disclosed the antibody or the antigen-binding portion thereof, with or without additional components, or they may have distinct containers for each desired agent. Where combined therapeutics are provided for conjugation, a single solution may be pre-mixed, either in a molar equivalent combination, or with one component in excess of the other. Alternatively, the conjugates and any optional anti-

cancer agent of the kit may be maintained separately within distinct containers prior to administration to a patient. The kits may also comprise a second/third container means for containing a sterile, pharmaceutically acceptable buffer or other diluent such as bacteriostatic water for injection (BWFI), phosphate-buffered saline (PBS), Ringer's solution and dextrose solution.

When the components of the kit are provided in one or more liquid solutions, the liquid solution is preferably an aqueous solution, with a sterile aqueous or saline solution being particularly preferred. However, the components of the kit may be provided as dried powder(s). When reagents or components are provided as a dry powder, the powder can be reconstituted by the addition of a suitable solvent. It is envisioned that the solvent may also be provided in another container.

As indicated briefly above the kits may also contain a means by which to administer the antibody or the antigen-binding portion thereof and any optional components to a patient, e.g., one or more needles, I.V. bags or syringes, or even an eye dropper, pipette, or other such like apparatus, from which the formulation may be injected or introduced into the animal or applied to a diseased area of the body. The kits of the present invention will also typically include a means for containing the vials, or such like, and other component in close confinement for commercial sale, such as, e.g., injection or blow-molded plastic containers into which the desired vials and other apparatus are placed and retained.

Sequence Listing Summary

Appended to the instant application is a sequence listing comprising a number of nucleic acid and amino acid sequences. The following table provides a summary of the included sequences.

SEQ ID NO.	Description
1	CDRH1 of 1.53.3-uAb-IgG4k
2	CDRH2 of 1.53.3-uAb-IgG4k
3	CDRH3 of 1.53.3-uAb-IgG4k
4	CDRL1 of 1.53.3-uAb-IgG4k

5	CDRL2 of 1.53.3-uAb-IgG4k
6	CDRL3 of 1.53.3-uAb-IgG4k
7	CDRH1 of 3.40.19-uAb-IgG4L
8	CDRH2 of 3.40.19-uAb-IgG4L
9	CDRH3 of 3.40.19-uAb-IgG4L
10	CDRL1 of 3.40.19-uAb-IgG4L
11	CDRL2 of 3.40.19-uAb-IgG4L
12	CDRL3 of 3.40.19-uAb-IgG4L
13	VH of 1.53.3-uAb-IgG4k
14	VL of 1.53.3-uAb-IgG4k
15	VH of 3.40.19-uAb-IgG4L
16	VL of 3.40.19-uAb-IgG4L
17	DNA sequence encoding VH of 1.53.3-uAb-IgG4k
18	DNA sequence encoding VL of 1.53.3-uAb-IgG4k
19	DNA sequence encoding VH of 3.40.19-uAb-IgG4L
20	DNA sequence encoding VL of 3.40.19-uAb-IgG4L
21	Amino acid sequence of human LAG-3 ECD
22	DNA sequence encoding human LAG-3 ECD
23	Amino acid sequence of full-length human LAG-3
24	DNA sequence encoding full-length human LAG-3

25	Amino acid sequence of full-length mouse LAG-3
26	DNA sequence encoding full-length mouse LAG-3
27	Amino acid sequence of full-length cynomolgus LAG-3
28	DNA sequence encoding full-length cynomolgus LAG-3

EXAMPLES

The present invention, thus generally described, will be understood more readily by reference to the following Examples, which are provided by way of illustration and are not intended to be limiting of the instant invention. The Examples are not intended to represent that the experiments below are all or the only experiments performed.

EXAMPLE 1

Preparation of Materials

1. Immunogen generation

Nucleic acid encoding human LAG-3 ECD (extracellular domain, ECD) with SEQ ID NO: 22 or full-length human LAG-3 with SEQ ID NO: 24 was synthesized by Sangon Biotech. The amino acid sequence of LAG-3 ECD and the DNA sequence encoding the same are shown in SEQ ID NOs: 21 and 22, and the amino acid sequence of full-length LAG-3 and the DNA sequence encoding the same are shown in SEQ ID NOs: 23 and 24, respectively. LAG-3 gene fragments were amplified from the synthesized nucleic acid and inserted into the expression vector pcDNA3.3 (ThermoFisher). The inserted LAG-3 gene fragment was further confirmed by DNA sequencing. Fusion proteins containing human LAG-3 ECD with various tags, including human Fc, mouse Fc and His tags, were obtained by transfection of human LAG-3 gene into 293F cells (ThermoFisher). The cells were cultured in a FreeStyle 293 Expression Medium (ThermoFisher) at 37 °C, 5% CO_2. After 5 days of culture, supernatants harvested from the culture of transiently transfected cells were used for protein purification. The fusion proteins were purified by nickel, protein A and/or SEC column. An untagged LAG-3 ECD protein was generated by cleavage of ECD-hFc fusion protein

with a cut site using Factor Xa protease (New England Biolabs). Purified proteins were used for immunization, screening and characterization.

2. Production of Benchmark Antibodies

Gene sequences of anti-human LAG-3 benchmark antibodies (BMK1 and BMK7) were synthesized based on the information disclosed in patent applications US20110150892 A1 and US 20170101472 A1 (BMK1 was referred to as "25F7" in US20110150892 A1) and (BMK7 was referred to as "H4sH15482P" in US 20170101472 A1), respectively. Benchmark antibody BMK8 is the humanized version of chimeric antibody BMK5 which was described in WO2015138920 A1 and referred to as "BAP050-chi". BMK8 was referred to as "BAP050-hum01" in WO2015138920 A1. The synthesized gene sequences were incorporated into plasmids pcDNA3.3, as described in above section 1. The plasmids were transiently transfected into 293F cells. The cells were cultured in the same way as described in section 1. After 5 days of culture, supernatants harvested from the culture of transiently transfected cells were used for protein purification. The benchmark antibodies were purified from the supernatants.

3. Establishment of Stable Cell Lines

Human, mouse and cynomolgus LAG-3 transfectant cell lines were generated. Briefly, Flp-In-293, Flp-In-CHO or 293F cells were transfected with pcDNA3.3 expression vector containing full-length of human, mouse and cynomolgus LAG-3 using Lipofectamine 2000 transfection kit according to manufacturer's protocol, respectively. At 48-72 hours post transfection, the transfected cells were cultured in medium containing blasticidin for selection and tested for LAG-3 expression. Human LAG-3-expressing cell lines, cynomolgus monkey LAG-3-expressing cell lines, and mouse LAG-3 expressing cell lines were obtained by limiting dilution.

EXAMPLE 2

Antibody Hybridoma Generation

1. Immunization and cell fusion

OMT rats (transgenic rats having recombinant immunoglobulin loci, as described and produced in US8,907,157 B2), 24 weeks of age, were immunized with 12.5 μg of hFc-tagged human LAG-3 ECD protein and 12.5 μg of His-tagged mouse LAG-3 in adjuvant alternately, producing antibodies in which both the framework and CDR regions are derived from human

germline immunoglobulin sequences. The immunized rats were bled every two weeks for serum collection and titers against human LAG-3 in the serum were measured by ELISA. The 96-well plates coated with human LAG-3.ECD.hFc were co-incubated with diluted rat serum (first 1:100, then 3-fold dilution in 2% BSA) for two hours. Goat anti rat-IgG-Fc-HRP was used as secondary antibody. The color was developed by dispensing 100 μL of TMB substrate, and then stopped by 100 μL of 2N HCl. The absorbance was read at 450 nM using a microplate reader. Once the antibody titer reached sufficiently high, rats were given a final boost with 40 μg of human LAG-3 ECD protein in DPBS without adjuvant. On the day of fusion, lymph nodes and spleen were removed from immunized rats under sterile condition, and prepared into single cell suspension. The isolated cells were then mixed with myeloma cell SP2/0 at a ratio of 1:1. Electro cell fusion was performed using BTX 2000 Electro cell manipulator. The cells were then seeded in 96-well plates at the density of 1×10^4 cells/well, and cultured at 37 °C, 5% CO_2, until ready for screening.

2. Primary and confirmatory screening of hybridoma supernatants

ELISA assay was used as the first screening method to test the binding of hybridoma supernatants to LAG-3 protein. Plates in section 1 of this Example were coated with human LAG-3 ECD.hFc at 1 μg/mL overnight at 4 °C. After blocking and washing, the hybridoma supernatants were transferred to the coated plates and incubated at room temperature for 1 h. The plates were then washed and subsequently incubated with secondary antibody goat anti-rat IgG HRP for 1 h. After washing, TMB substrate was added and the color reaction was stopped by 2M HCl. The absorbance at 450 nm was read using a microplate reader.

In order to confirm the native binding of LAG-3 antibodies to conformational LAG-3 molecules expressed on cell membrane, flow cytometry analysis was performed on LAG-3 transfected CHO-K1 cell line. CHO-K1 cells expressing human LAG-3 were transferred into 96-well U-bottom plates at a density of 1×10^5 cells/well. The hybridoma supernatants were then transferred to the plates and incubated for 1 h at 4 °C. After washing with 1×PBS/1%BSA, the secondary antibody goat anti-rat IgG Alexa647 was added and incubated with cells at 4 °C in the dark for 0.5 h. The cells were then washed and resuspended in 1×PBS/1%BSA before being analyzed by flow cytometry. The binding of antibodies to parental CHO-K1 cell line was performed in parallel as negative controls.

The blocking activities of antibodies were used as confirmatory screening to select potential antibody hits. Selected antibodies were tested for the ability to block the binding of LAG-3 protein to human MHC-II expressing cell line Raji by FACS analysis. Raji cells were transferred into 96-well U-bottom plates at the density of 1×10^5 cells/well. The supernatant was incubated with mFc-

tagged LAG-3 protein at 4°C for 30 min. The mixture was transferred into the 96-well plates seeded with Raji cells. The secondary antibody, PE-labeled goat anti-mouse-IgG antibody (no cross-reactivity to rat IgG Fc, Jackson Immunoresearch Lab) was incubated with cells at 4 °C in the dark for 0.5 h. The cells were then washed and resuspended in 1×PBS/1%BSA and analyzed by flow cytometry.

3. Hybridoma sub-cloning:

Once specific binding was verified through primary and confirmatory screening, the positive hybridoma cell lines were sub-cloned to get monoclonal anti-hLAG-3 antibodies by using semi-solid medium approach. In the semi-solid medium approach, for each hybridoma cell line, cells were diluted in semi-solid cloning medium (STEMCELL Technologies) and seeded in 6-well plates. The cells were cultured for 8-10 days in an incubator (37℃, 5% CO_2) until monoclones were visible in semi-solid medium. Clones were picked and transferred to 96-well plate in HAT medium (hypoxanthine-aminopterin-thymidine medium) with 10% FBS. The positive clones were confirmed by binding ELISA and FACS against human LAG-3 as described above.

EXAMPLE 3

Hybridoma sequencing and fully human antibody molecules construction

1. Hybridoma sequencing

Total RNA was extracted from hybridoma cells by using RNeasy Plus Mini Kit (Qiagen) and first strand cDNA was prepared as shown in Table 1 and Table 2. Antibody VH and VL genes were amplified from cDNA as shown in Table 3 and Table 4 by using 3'-constant region degenerated primer and 5'-degenerated primer sets, which are complementary to the upstream signal sequence-coding region of Ig variable sequences. Reagent information including the manufactures is shown in Table 5.

The PCR product (10 μL) was ligated into pMD18-T vector and 10 μL of the ligation product was transformed into Top10 competent cells. Transformed cells were plated on 2-YT+Cab plates and incubated overnight at 37°C. Positive clones were randomly picked for sequencing at Shanghai Biosune Biotech Co., Ltd.

Table 1. cDNA amplification reaction (20 μL)

Component	Amount
Up to 5 μg total RNA	5 μL
Primer (50 μM oligo(dT)$_{20}$/50 ng/μL random hexamers)	1 μL/1 μL
Annealing Buffer	1 μL
RNase/DNase-free water	to 8 μL
65°C for 5 min, then immediately place on ice for at least 1 minute	
2×First-Strand Reaction Mix	10 μL
SuperScript™ III/RNaseOUT™ Enzyme Mix	2 μL

Table 2. cDNA amplification reaction condition

	Step 1	Step 2	Step 3	Step 4
Temperature (℃)	25	50	85	4
Time	10 min	50 min	5 min	∞

Table 3. PCR Reaction system (50 μL)

Component	Amount
cDNA	2.0 μL
Premix Ex Taq	25 μL
5'-degenerated primer sets (10 pM)	2.5 μL
3'-constant region degenerated primer (10 pM)	1 μL
ddH_2O	19.5 μL

Table 4. PCR Reaction condition

	Step 1	Step 2	Step 3	Step 4	Step 5
Temperature (℃)	95	94	58	72	72
Time	4 min	45 sec	45 sec	1 min	10 min
Cycles	NA	30		NA	NA

Table 5. Reagent information

Reagent	Manufacturers
RNeasy Plus Mini Kit	QIAGEN
SuperScript III First-Strand Synthesis SuperMix	Invitrogen
Premix Ex Taq hot start	TaKaRa
DNA Gel Extraction Kit	Axygen
pMD 18-T vector	TaKaRa

Two lead antibodies are named as "1.53.3-uAb-IgG4k" and "3.40.19-uAb-IgG4L", respectively.

The sequences of CDRs of 1.53.3-uAb-IgG4k were determined as follows:

Description	SEQ ID NO.	Sequence Information
CDRH1	1	GGSFSGYYWS
CDRH2	2	EINHRGNTNYNPSLKS
CDRH3	3	GEDYSDYDYYGDF
CDRL1	4	RASQSISSYLA
CDRL2	5	AASNRAT
CDRL3	6	QQRSNWPLT

The sequences of heavy chain and light chain variable regions of 1.53.3-uAb-IgG4k are as follows:

Description	SEQ ID NO.	Sequence Information
VH	13	QVQLQQWGAGLLKPSETLSLTCGVYGGSFSGYYW SWIRQPPGMGLEWIGEINHRGNTNYNPSLKSRVTIS EDTSKNQFSLRLSSVTAADTAVYFCTRGEDYSDYD YYGDFWGQGTLVTVSS
VL	14	EIVLTQSPATLSLSQGERATLSCRASQSISSYLAWY QQKPGQAPRLLIYAASNRATGIPARFSGSGSGTDFT LTISSLEPEDFAIYYCQQRSNWPLTFGGGTKVEIK

The sequences of CDRs of 3.40.19-uAb-IgG4L are as follows:

Description	SEQ ID NO.	Sequence Information
CDRH1	7	GDSISSTSYYWG
CDRH2	8	SFYYSGSTYYNPSLKS
CDRH3	9	MQLWSYDVDV
CDRL1	10	TGTSSDVGGYDYVA
CDRL2	11	DVSERPS
CDRL3	12	SSYTSTTTLVV

The sequences of heavy chain and light chain variable regions of 3.40.19-uAb-IgG4L are as follows:

Description	SEQ ID NO.	Sequence Information
VH	15	QLQLQESGPGLVKPSETLSLTCTVSGDSISSTSYY WGWIRQPPGKGLEWIGSFYYSGSTYYNPSLKSR VTISVDTSKNQFSLKLNSVTAADTAVYYCARMQ LWSYDVDVWGQGTTVTVSS
VL	16	QSALTQPASVSGSPGQSITISCTGTSSDVGGYDY VAWYQQHPGKVPKLMIYDVSERPSGVSNRFSGS KSGNTASLTISGLQAEDEADYYCSSYTSTTTLVV FGGGTKLSVL

2. Fully human antibody molecule construction

VH and VL genes were re-amplified with cloning primers containing appropriate restriction sites and cloned into expression vectors to create corresponding clones of chimeric antibodies.

EXAMPLE 4

Binding of LAG-3 antibodies to cell surface human LAG-3

Various concentrations of testing antibodies, positive and negative controls were added to human LAG-3 transfectant cells, and then the binding of antibodies onto the surface of the cells was detected by corresponding PE-labeled secondary antibodies. The data was shown in Figure 1 and EC_{50} was shown in Table 6.

Table 6

Ab	**EC_{50} (nM)**
1.53.3-uAb-IgG4k	0.43
3.40.19-uAb-IgG4L	0.13
BMK1	0.32
BMK7	0.61
BMK8	0.90

As shown in Figure 1 and Table 6, surprisingly, the EC_{50} of 3.40.19-uAb-IgG4L (0.13) for binding to cell surface LAG-3 is significantly lower than that of all the three benchmark antibodies BMK1(0.32), BMK7 (0.61) and BMK8 (0.90). Further, the EC_{50} of 1.53.3-uAb-IgG4k (0.43) for binding to cell surface LAG-3 is much lower that of BMK7 (0.61) and BMK8 (0.90). These results indicate that 1.53.3-uAb-IgG4k and 3.40.19-uAb-IgG4L are effective in binding to human LAG-3, and the binding effects are superior over or comparable to the benchmark antibodies.

EXAMPLE 5

Blocking of LAG-3 protein binding to MHC-II expressed on Raji cells

Antibodies were serially diluted in 1% BSA-PBS and incubated with mFc-tagged LAG-3 protein at 4°C for 30 min. The mixture was transferred into the 96-well plates seeded with Raji cells. Goat anti-mouse IgG Fc-PE antibody was used to detect the binding of LAG-3 protein to Raji cells. The MFI was evaluated by flow cytometry and analyzed by the software FlowJo (version 7.6.1). The data was shown in Figure 2 and EC_{50} was shown in Table 7.

Table 7

Ab	EC_{50} (nM)
1.53.3-uAb-IgG4k	0.80
3.40.19-uAb-IgG4L	0.67
BMK1	0.76
BMK7	1.25
BMK8	0.88

As shown in Figure 2 and Table 7, surprisingly, the EC_{50} of 3.40.19-uAb-IgG4L (0.67) for binding to MHC-II expressed on Raji cells is significantly lower than that of all the three benchmark antibodies BMK1(0.76), BMK7 (1.25) and BMK8 (0.88). Further, the EC_{50} of 1.53.3-uAb-IgG4k (0.80) for binding to MHC-II expressed on Raji cells is lower that of BMK7 (1.25) and BMK8 (0.88). These results indicate that 1.53.3-uAb-IgG4k and 3.40.19-uAb-IgG4L are effective in blockage of the binding to MHC-II expressed on Raji cells, and the blocking effects are superior over or comparable to the benchmark antibodies.

EXAMPLE 6

Blocking of LAG-3 protein binding to LSECtin and Galectin-3

96-well plates were coated with human LSECtin or Galectin-3 at 0.5 μg/mL overnight at 4 °C, respectively. Antibodies were serially diluted in 1% BSA-PBS and mixed with mFc-tagged LAG-3 protein. After blocking and washing, the mixture was transferred to the plates and incubated at room temperature for 1 h. The plates were then washed and subsequently incubated with corresponding secondary antibody for 60 min. After washing, TMB substrate was added and the color reaction was stopped by 2M HCl. The absorbance at 450 nm was read using a microplate reader. The data was shown in Figures 3 and 4. EC_{50} was shown in Table 8.

Table 8

Ab	EC_{50} (nM)	
	LSECtin blocking	Galectin-3 blocking
1.53.3-uAb-IgG4k	0.71	0.74
3.40.19-uAb-IgG4L	0.51	0.56
BMK1	0.68	0.63
BMK7	0.59	0.79
BMK8	1.06	1.07

As shown in Figure 3 and 4 as well as Table 7, both 1.53.3-uAb-IgG4k and 3.40.19-uAb-IgG4L are effective in blocking the binding of LAG-3 to LSECtin or Galectin-3, and the blocking effects are superior over or comparable to the benchmark antibodies.

EXAMPLE 7

Full kinetic binding affinity test

Full kinetic binding affinity tested by surface plasmon resonance (SPR):

Antibodies were characterized for affinity and binding kinetics to human LAG-3 by SPR assay using Biacore 8K. Goat anti-human Fc was pre-immobilized to a sensor chip (CM5), and anti-LAG-3 antibodies were captured when injected to the chip. Various concentrations of human LAG-3 protein and running buffer were flowed through the sensor chip at a flow rate of 30 μL/min for an association phase of 300 s, followed by 3600 s dissociation. The association and dissociation curve was fit by 1:1 Langmuir binding model using Biacore 8K Evaluation Software. The data was shown in Table 9.

Table 9

Ab	ka (1/Ms)	kd (1/s)	KD (M)
1.53.3-uAb-IgG4k	6.60E+05	3.33E-05	5.05E-11
3.40.19-uAb-IgG4L	1.05E+06	1.11E-05	1.06E-11
BMK1	4.87E+05	3.34E-04	6.85E-10
BMK7	2.13E+05	1.06E-04	4.97E-10
BMK8	8.46E+04	6.74E-06	7.97E-11

Binding affinity of LAG-3 antibodies to cell surface LAG-3 molecules tested by fluorescence-activated cell sorting (FACS)

Antibody binding affinity to cell surface LAG-3 was measured by FACS analysis. Flp-In-293 cells expressing human LAG-3 were transferred in to 96-well U-bottom plates at a density of 5×10^5 cells/mL. Tested antibodies were serially diluted in wash buffer (1×PBS/1%BSA) and incubated with cells at 4 °C for 1 h. The secondary antibody goat anti-human IgG Fc FITC (3.5 moles FITC per mole IgG) was added and incubated at 4 °C in the dark for 0.5 h. The cells were then washed once and resuspended in 1×PBS/1%BSA, and analyzed by flow cytometry. Fluorescence intensity will be converted to bound molecules/cell based on the quantitative beads (QuantumTM MESF Kits, Bangs Laboratories, Inc.). Affinity was calculated using Graphpad Prism 5. The data was shown in table 10.

Table 10

Ab	KD (M)
1.53.3-uAb-IgG4k	1.60E-10
3.40.19-uAb-IgG4L	5.30E-11
BMK1	2.70E-10
BMK7	5.80E-10
BMK8	9.40E-10

As tested by SPR and FACS, the antibodies of the invention, as represented by 1.53.3-uAb-IgG4k and 3.40.19-uAb-IgG4L, are effective in binding to human LAG-3, and the binding effects are superior over or comparable to the benchmark antibodies.

EXAMPLE 8

Orthologue (cross-species) and homologue (cross-family) binding

Cross-reactivity to cynomolgus LAG-3 and murine LAG-3

Cross-reactivity to cynomolgus and murine LAG-3 was measured by FACS. Murine LAG-3-expressing Flp-In-CHO cells or cynomolgus LAG-3-expressing 293F cells were transferred into 96-well U-bottom plates at a density of 1×10^5 cells/well. Testing antibodies were serially diluted in wash buffer (1×PBS/1%BSA) and incubated with cells at 4 °C for 1 h. After washing with 1×PBS/1%BSA, corresponding secondary antibody was applied and incubated with cells at 4 °C in the dark for 1 h. The cells were then washed and resuspended in 1×PBS/1%BSA and then analyzed by flow cytometry. The data was shown in Figure 5 and Figure 6. EC_{50} was shown in Table 11.

Table 11

Ab	EC_{50} (nM)
1.53.3-uAb-IgG4k	4.01
3.40.19-uAb-IgG4L	3.92

BMK1	86.0
BMK7	2.65
BMK8	3.05

As demonstrated in Figure 5, LAG-3 antibodies of the invention "1.53.3-uAb-IgG4k" and "3.40.19-uAb-IgG4L" bound to cell surface cynomolgus LAG-3. And as demonstrated in Figure 6, LAG-3 antibodies of the invention "1.53.3-uAb-IgG4k" and "3.40.19-uAb-IgG4L" did not bind to cell surface mouse LAG-3.

Cross-reactivity to human CD4

Cross-reactivity to human CD4 was measured by ELISA. Plates were coated with human CD4 at 1 μg/mL overnight at 4 °C. After blocking and washing, 1 μg/mL of LAG-3 antibodies were added to the plates and incubated at room temperature for 1 h. The plates were then washed and subsequently incubated with corresponding secondary antibody for 45 min. After washing, TMB substrate was added and the color reaction was stopped by 2M HCl. The absorbance at 450 nm was read using a microplate reader. The data was shown in Figure 7.

These results indicate that LAG-3 antibodies of the invention "1.53.3-uAb-IgG4k" and "3.40.19-uAb-IgG4L" did not bind to human CD4 protein.

EXAMPLE 9

Epitope binning against BMK1, BMK7 and BMK5

The binding epitope of LAG-3 antibodies was binned against benchmark antibodies BMK1, BMK7 and BMK5 by FACS assay. Flp-In-293 cells expressing human LAG-3 at the cell surface were incubated with biotinylated benchmark antibodies at concentration of 1 μg/mL for 1 hour, followed by the adding of serially diluted LAG-3 antibodies. Streptavidin-PE antibody (Jackson Immunoresearch Lab) was used to detect the binding of benchmark antibodies to cells. The MFI was evaluated by flow cytometry and analyzed by FlowJo. The data was shown in Figure 8A-E.

It was found that 1.53.3-uAb-IgG4k of the invention competed with BMKs, but 3.40.19-uAb-IgG4L did not compete with BMKs. 1.53.3-uAb-IgG4k shares close epitope with BMK1 and BMK7, but not BMK5. Surprisingly, 3.40.19-uAb-IgG4L has different epitope with all of BMK1, BMK7 and BMK5.

EXAMPLE 10

Domain mapping and Epitope mapping

1. Domain mapping

LAG-3 has an extracellular domain of 421 aa (P30-L450) including four extracellular immunoglobulin superfamily (IgSF)-like domains, namely Domain 1 ("D1," aa. 37-167), Domain 2 ("D2," aa 168-252), Domain 3 ("D3," aa. 265-343) and Domain 4 ("D4," aa. 348-419). 10 variants as follows were constructed by replacing the following residues of the extracellular domain of human LAG-3 with corresponding mouse LAG-3 amino acid (also referred to as "aa" in the context of the disclosure).

(1) Variant 1: xPro1.FL-x1: Human LAG-3 aa 168 to 419 replaced with the mouse counterparts

(2) Variant 2: xPro1.FL-x2: Human LAG-3 aa 37 to 167, and aa 265 to 419 replaced with the mouse counterparts

(3) Variant 3: xPro1.FL-x3: Human LAG-3 aa 37 to 252, and aa 348-419 replaced with the mouse counterparts

(4) Variant 4: xPro1.FL-x4: Human LAG-3 aa 37 to 343 replaced with the mouse counterparts

(5) Variant 5: xPro1.FL-x5: Human LAG-3 aa 265 to 419 replaced with the mouse counterparts

(6) Variant 6: xPro1.FL-x6: Human LAG-3 aa 37 to 167, and aa 348 to 419 replaced with the mouse counterparts

(7) Variant 7: xPro1.FL-x7: Human LAG-3 aa 37 to 252 replaced with the mouse counterparts

(8) Variant 8: xPro1.FL-x8: Human LAG-3 aa 168 to 343 replaced with the mouse counterparts

(9) Variant 9: xPro1.FL-x9: Human LAG-3 aa 348 to 419 replaced with the mouse counterparts

(10) Variant 10: xPro1.FL-x10: Human LAG-3 aa 37 to 167 replaced with the mouse counterparts

The 10 variants were cloned into pcDNA3 vector and used for 293F cell transfection. Briefly, 293F cells were diluted to a density of 1×10^6 cells/mL with FreeStyle 293F medium and aliquots of 3 mL/well were added to 24-well plate. Transfections were performed using 293fectin reagent (Life Technologies). For each transfection, 3 μg of DNA were diluted in 150 μL Opti-MEM I-reduced serum medium (Life Technologies), and then combined with 6 μL 293fectin reagent pre-diluted in 150 μL Opti-MEM I reduced serum medium. The DNA/Lipofectamine mixture was allowed to stand at 25℃ for 20 min before being added to the culture. The transfected cells were analyzed by flow cytometry at 48 h post-transfection.

Binding of antibodies to chimeric LAG-3 variants or full length human/mouse LAG-3 was analyzed by flow cytometry. Briefly, 1 μg/mL antibodies were incubated with chimeric LAG-3-expressing transfected 293F cells for 1 hour at 4℃, and then incubated with 3 μg/mL goat anti-human IgG Fc R-PE (Jackson) for 40 min at 4℃. Cells were analyzed with flow cytometer.

The binding abilities of the antibodies 1.53.3-uAb-IgG4k and 3.40.19-uAb-hIgG4L to the 10 variants were tested and the results were shown in Table 12 below.

Table 12. The binding MFI value of LAG-3 antibodies on 10 variants

	1.53.3-uAb-IgG4k		3.40.19-uAb-hIgG4L	
Antigen variant	**MFI**	**PE+ %**	**MFI**	**PE+ %**
Variant 1	8582	97	9428	89.4
Variant 2	45	1.77	40.6	0.305
Variant 3	41.7	2.03	54	0.426
Variant 4	41	1.42	41.7	0.658
Variant 5	6260	97.1	7070	75.9
Variant 6	59.9	1.86	44.1	0.674
Variant 7	39.3	1.52	44.3	0.859
Variant 8	7432	97.6	5236	70.1
Variant 9	7030	97.3	8317	87.3

Variant 10	42.3	1.98	68.5	0.988
hPro1.FL (SEQ ID NO: 23)	6539	96.8	6517	83.5
mPro1.FL (SEQ ID NO: 25)	43.9	1.1	34.5	0.252
Blank	29.4	0	29.4	0
Negative control	30.6	0.656	29.4	0.1

According to the FACS binding activity of antibodies, both two lead antibodies "1.53.3-uAb-IgG4k" and "3.40.19-uAb-hIgG4L" bound to Domain 1 (i.e. aa. 37-167). So further epitope mapping of Domain 1 (G37-Q167, 131 aa) was performed by alanine scanning experiments.

2. Epitope mapping

Alanine scanning experiments on human LAG-3 were conducted for epitope mapping. Alanine residues on human LAG-3 were mutated to glycine codons, and all other residues (except for cysteine residues) were mutated to alanine codons. For each residue of the human LAG-3 extracellular domain (ECD), site amino acid substitutions were made using two sequential PCR steps. A pcDNA3.3-LAG-3-D12.mFc plasmid that encodes ECD domain 1 and domain 2 of human LAG-3 and a C-terminal mFc-tag was used as template, and a set of mutagenic primers was used for first step PCR using the QuikChange lightning multisite-directed mutagenesis kit (Agilent technologies, Palo Alto, CA). *Dpn* I endonuclease was used to digest the parental template after mutant strand synthesis reaction. In the second-step PCR, linear DNA expression cassette which composed of a CMV promoter, extracellular domain 1 and domain 2 (D1 and D2) of LAG-3, a mFc-tag and a herpes simplex virus thymidine kinase (TK) polyadenylation was amplified and transiently expressed in Expi293 cells at 37℃ (Life Technologies, Gaithersburg, MD), quantified by Protein A-HPLC and mFc-ELISA quantification Kit (Bethyl, USA).

For ELISA binding assay, the antibody 1.53.3-uAb-IgG4k or 3.40.19-uAb-hIgG4L (2 μg/mL) was coated in plates. After interacting with the supernatant that contains quantified LAG-3 mutants or human LAG-3-ECD.D12.mFc protein, HRP conjugated anti-mFc antibody (1:5000; Bethyl, USA) was added as a detection antibody. Absorbance was normalized according to the average absorbance of control mutants. After setting an additional cutoff to the binding fold change (< 0.75),

the final determined epitope residues were identified. The hotspots of the antibodies 1.53.3-uAb-IgG4k and 3.40.19-uAb-hIgG4L were shown in Table 13 and Table 14.

Table 13. Hotspots of 1.53.3-uAb-IgG4k antibody

Residue		Fold Change
G	37	0.558
L	42	0.624
A	59	0.718
V	61	0.637
W	63	0.585
H	65	0.627
P	72	0.743
W	92	0.554
V	101	0.504
L	122	0.607
A	139	0.708
D	143	0.671
G	145	0.692
E	146	0.701
Y	147	0.637
L	153	0.749
L	164	0.586

Table 14. Hotspots of 3.40.19-uAb-IgG4L antibody

Residue		Fold Change
G	37	0.284
A	40	0.608
L	42	0.306
P	43	0.494
A	59	0.542
V	61	0.514
T	62	0.727
W	63	0.171
H	65	0.489
T	100	0.738
V	101	0.275

L	122	0.391
G	130	0.102
D	131	0.045
L	134	0.040
W	135	0.049
L	136	0.043
R	137	0.101
P	138	0.106
A	139	0.368
D	143	0.529
G	145	0.466
E	146	0.582
Y	147	0.190
V	151	0.043
L	153	0.632
D	155	0.701
R	163	0.655
L	164	0.278

As no existed LAG-3 structure, the structure of the LAG-3 (aa: 31-431) was modeled based on the known structure of the myelin-associated glycoprotein (PDB: 5FLU, sequence identity 18%). Based on alanine scanning results, hotspots of two antibodies were identified and shown in Figure 9A and Figure 9B.

Based on the results, it can be seen that the 1.53.5-uAb-IgG4k antibody bound to W92 site which belongs to the extra loop (G70-Y99), while the 3.40.19-uAb-IgG4L antibody bound to L134-P138 region.

EXAMPLE 11

In vitro function of LAG-3 antibodies tested by cell-based assays

Effects of human LAG-3 antibodies in reporter gene assay

Jurkat cells expressing human LAG-3 along with stably integrated IL-2 luciferase reporter gene were seeded in 96-well plates along with Raji cells in the presence of SEE. Testing antibodies

were added to the cells. The plates were incubated for overnight at 37°C, 5% CO_2. After incubation, reconstituted luciferase substrate was added and the luciferase intensity was measured by a microplate spectrophotometer. The data was shown in Figure 10 and EC_{50} was shown in Table 15.

Table 15

Ab	EC_{50} (nM)
1.53.3-uAb-IgG4k	1.07
3.40.19-uAb-IgG4L	0.21
BMK1	0.59
BMK7	2.65
BMK8	65.3

As demonstrated in Figure 10, LAG-3 antibodies enhanced IL-2 pathway of Jurkat in reporter gene assay. Further, as shown in Table 15, the EC_{50} of 3.40.19-uAb-IgG4L in this assay is significantly lower than all of the three benchmark antibodies.

Effects of human LAG-3 antibodies on human allogeneic mixed lymphocyte reaction

Human peripheral blood mononuclear cells (PBMCs) were freshly isolated from healthy donors using Ficoll-Paque PLUS gradient centrifugation. Monocytes were isolated using human monocyte enrichment kit according to the manufacturer's instructions. Cells were cultured in medium containing GM-CSF and IL-4 for 5 to 7 days to generate dendritic cells (DC). Human $CD4^+$ T cells were isolated using human $CD4^+$ T cell enrichment kit according to the manufacturer's protocol. Purified $CD4^+$ T cells were co-cultured with allogeneic immature DCs (iDCs) and various concentrations of LAG-3 antibodies in 96-well plates. On Day 5, the culture supernatants were harvested for IFN-γ test and T cell proliferation test. Human IFN-γ was measured by ELISA using matched antibody pairs. The plates were pre-coated with capture antibody specific for human IFN-γ (Pierce-M700A). The biotin-conjugated anti-IFN-γ antibody (Pierce-M701B) was used as detecting antibody. During the last 16 h, ^{3}H-thymidine was added at 1 μ Ci/well. ^{3}H-thymidine incorporation was measured by scintillation counting and proliferative responses were expressed as the CPM (counts per minute) of triplicate wells. The data was shown in Figures 11 and 12.

As demonstrated in Figure 11, LAG-3 antibodies of the invention "1.53.3-uAb-IgG4k" and "3.40.19-uAb-IgG4L" enhanced IFN-γ secretion in mixed lymphocyte reaction. Further, as shown

in Figure 12, LAG-3 antibodies of the invention "1.53.3-uAb-IgG4k" and "3.40.19-uAb-IgG4L" enhanced T cell proliferation in mixed lymphocyte reaction.

Example 12

ADCC and CDC tests

In order to assess their ability to trigger Fc effector function, the anti-LAG-3 antibodies were evaluated whether they could mediate antibody-dependent cellular cell-mediated cytotoxicity (ADCC) and complement-dependent cytotoxicity (CDC) activity.

ADCC test

Human LAG-3-expressing Flp-In-293 cells and various concentrations of LAG-3 antibodies were pre-incubated in 96-well round-bottom plate for 30 minutes; and then PBMCs, as effector, were added with the effector/target ratio of 50:1. The plate was kept at 37 °C, 5% CO_2 for 4 hours. Target cell lysis was determined by LDH-based Cytotoxicity Detection Kit. The absorbance at 492 nm was read using a microplate reader. Herceptin and HER2-expressing cell line SK-Br-3 were used as positive control.

CDC test

Human LAG-3-expressing Flp-In-293, as target, and various concentrations of LAG-3 antibodies were mixed in 96-well round-bottom plate. Human complement was added at a final dilution of 1:50. The plate was kept at 37 °C, 5% CO_2 for 2 hours. Target cell lysis was determined by CellTiter-Glo. The luminescence was read using a microplate reader. Rituximab and CD20-expressing cell line Raji was used as positive control.

The data for ADCC test and CDC test were shown in Figures 13A and 13B. It is demonstrated that, LAG-3 antibodies of the invention, as represented by 1.53.3-uAb-IgG4k and 3.40.19-uAb-IgG4L, did not mediate ADCC (Figures 13A) and CDC (Figures 13B) effects.

Example 13

Serum Stability Test

The lead Abs were incubated in freshly isolated human serum (serum content > 95%) at 37°C.

On indicated time points, an aliquot of serum treated sample was removed from the incubator and snap frozen in liquid N_2, and then stored at -80°C until ready for test. The samples were quickly thawed immediately prior to the stability test. Human LAG-3 transfectant cells were incubated with various concentrations of lead antibodies at 4°C for 1 hour. PE-labeled goat anti-human IgG was used to detect the binding of lead antibodies onto the cells. MFI of the cells was measured by a flow cytometer (BD FACSCanto II) and analyzed by FlowJo. The data was shown in Figures 14A and 14B.

It is demonstrated that the LAG-3 antibodies of the invention, as represented by 1.53.3-uAb-IgG4k and 3.40.19-uAb-IgG4L, were stable in fresh human serum for up to 14 days.

Those skilled in the art will further appreciate that the present invention may be embodied in other specific forms without departing from the spirit or central attributes thereof. In that the foregoing description of the present invention discloses only exemplary embodiments thereof, it is to be understood that other variations are contemplated as being within the scope of the present invention. Accordingly, the present invention is not limited to the particular embodiments that have been described in detail herein. Rather, reference should be made to the appended claims as indicative of the scope and content of the invention.

CLAIMS

1. An isolated antibody or the antigen-binding portion thereof, wherein the isolated antibody or the antigen-binding portion thereof comprises:

A) one or more heavy chain CDRs (CDRHs) selected from at least one of the group consisting of:

(i) a CDRH1 with at least 90%, sequence identity to a CDRH1 as set forth in one of the sequences selected from the group consisting of SEQ ID NOs: 1 and 7;

(ii) a CDRH2 with at least 90%, sequence identity to a CDRH2 as set forth in one of the sequences selected from the group consisting of SEQ ID NOs: 2 and 8; and

(iii) a CDRH3 with at least 90%, sequence identity to a CDRH3 as set forth in one of the sequences selected from the group consisting of SEQ ID NOs: 3 and 9;

B) one or more light chain CDRs (CDRLs) selected from at least one of the group consisting of:

(i) a CDRL1 with at least 90%, sequence identity to a CDRL1 as set forth in one of the sequences selected from the group consisting of SEQ ID NOs: 4 and 10;

(ii) a CDRL2 with at least 90%, sequence identity to a CDRL2 as set forth in one of the sequences selected from the group consisting of SEQ ID NOs: 5 and 11; and

(iii) a CDRL3 with at least 90%, sequence identity to a CDRL3 as set forth in one of the sequences selected from the group consisting of SEQ ID NOs: 6 and 12; or

C) one or more CDRHs of A) and one or more CDRLs of B).

2. The isolated antibody or the antigen-binding portion thereof of claim 1, wherein the isolated antibody or the antigen-binding portion thereof comprises:

A) one or more heavy chain CDRs (CDRHs) selected from at least one of the group consisting of:

(i) a CDRH1 selected from the group consisting of SEQ ID NOs: 1 and 7 or a CDRH1 that differs in amino acid sequence from the CDRH1 by an amino acid addition, deletion or substitution of not more than 2 amino acids;

(ii) a CDRH2 selected from the group consisting of SEQ ID NOs: 2 and 8 or a CDRH2 that differs in amino acid sequence from the CDRH2 by an amino acid addition, deletion or substitution of not more than 2 amino acids; and

(iii) a CDRH3 selected from the group consisting of SEQ ID NOs: 3 and 9 or a CDRH3 that differs in amino acid sequence from the CDRH3 by an amino acid addition, deletion or substitution of not more than 2 amino acids;

B) one or more light chain CDRs (CDRLs) selected from at least one of the group consisting of:

(i) a CDRL1 selected from the group consisting of SEQ ID NOs: 4 and 10 or a CDRL1 that differs in amino acid sequence from the CDRL1 by an amino acid addition, deletion or substitution of not more than 2 amino acids;

(ii) a CDRL2 selected from the group consisting of SEQ ID NOs: 5 and 11 or a CDRL2 that differs in amino acid sequence from the CDRL2 by an amino acid addition, deletion or substitution of not more than 2 amino acids; and

(iii) a CDRL3 selected from the group consisting of SEQ ID NOs: 6 and 12 or a CDRL3 that differs in amino acid sequence from the CDRL3 by an amino acid addition, deletion or substitution of not more than 2 amino acids; or

C) one or more CDRHs of A) and one or more CDRLs of B).

3. The isolated antibody or the antigen-binding portion thereof of claim 1, wherein the isolated antibody or the antigen-binding portion thereof comprises:

(a) a CDRH1 comprising or consisting of SEQ ID NO: 1;

(b) a CDRH2 comprising or consisting of SEQ ID NO: 2;

(c) a CDRH3 comprising or consisting of SEQ ID NO: 3;

(d) a CDRL1 comprising or consisting of SEQ ID NO: 4;

(e) a CDRL2 comprising or consisting of SEQ ID NO: 5; and

(f) a CDRL3 comprising or consisting of SEQ ID NO: 6.

4. The isolated antibody or the antigen-binding portion thereof of claim 1, wherein the isolated antibody or the antigen-binding portion thereof comprises:

(a) a CDRH1 comprising or consisting of SEQ ID NO: 7;

(b) a CDRH2 comprising or consisting of SEQ ID NO: 8;

(c) a CDRH3 comprising or consisting of SEQ ID NO: 9;

(d) a CDRL1 comprising or consisting of SEQ ID NO: 10;

(e) a CDRL2 comprising or consisting of SEQ ID NO: 11; and

(f) a CDRL3 comprising or consisting of SEQ ID NO: 12.

5. The isolated antibody or the antigen-binding portion thereof of claim 1, wherein the isolated antibody or the antigen-binding portion thereof comprises:

(A) a heavy chain variable region:

(i) comprising the amino acid sequence of SEQ ID NO: 13;

(ii) comprising an amino acid sequence at least 85%, 90%, or 95% identical to SEQ ID NO: 13; or

(iii) comprising an amino acid sequence with addition, deletion and/or substitution of one or more amino acids compared with SEQ ID NO: 13; and/or

(B) a light chain variable region:

(i) comprising the amino acid sequence of SEQ ID NO: 14;

(ii) comprising an amino acid sequence at least 85%, at least 90%, or at least 95% identical to SEQ ID NO: 14; or

(iii) comprising an amino acid sequence with addition, deletion and/or substitution of one or more amino acids compared with SEQ ID NO: 14.

6. The isolated antibody or the antigen-binding portion thereof of claim 1, wherein the isolated antibody or the antigen-binding portion thereof comprises:

(A) a heavy chain variable region:

(i) comprising the amino acid sequence of SEQ ID NO: 15;

(ii) comprising an amino acid sequence at least 85%, at least 90%, or at least 95% identical to SEQ ID NO: 15; or

(iii) comprising an amino acid sequence with addition, deletion and/or substitution of one or more amino acids compared with SEQ ID NO: 15; and/or

(B) a light chain variable region:

(i) comprising the amino acid sequence of SEQ ID NO: 16;

(ii) comprising an amino acid sequence at least 85%, at least 90%, or at least 95% identical to SEQ ID NO: 16; or

(iii) comprising an amino acid sequence with addition, deletion and/or substitution of one or more amino acids compared with SEQ ID NO: 16.

7. The isolated antibody or the antigen-binding portion thereof of any of claims 1-6, having one or more of the following properties:

(a) binds to human LAG-3 with a K_D of 2×10^{-10} M or less;

(b) inhibits binding of LAG-3 to major histocompatibility (MHC) class II molecules;

(c) inhibits binding of LAG-3 to fibrinogen-like protein 1 (FGL1) ligand molecules;

(d) inhibits binding of LAG-3 to LSECtin and/or Galectin-3;

(e) binds to human LAG-3 without cross-family reactions; or

(f) has no cross-reactivity to human CD4.

8. The isolated antibody or the antigen-binding portion thereof of any one of claims 1-7, wherein the antibody is a monoclonal antibody, for example, a fully human monoclonal antibody, for example, a fully human monoclonal antibody produced by a transgenic mammal, preferably a transgenic rat, more preferably a transgenic rat with recombinant immunoglobulin loci.

9. An isolated nucleic acid molecule, comprising a nucleic acid sequence encoding the heavy chain variable region and/or the light chain variable region of the isolated antibody as defined in any of claims 1-8, for example, a nucleic acid sequence as shown in SEQ ID NOs: 17-20.

10. An expression vector comprising the nucleic acid molecule of claim 9.

11. A host cell comprising the expression vector of claim 10.

12. A pharmaceutical composition comprising at least one antibody or antigen-binding portion thereof as defined in any of claims 1-8 and a pharmaceutically acceptable carrier.

13. A method for preparing antibody or antigen-binding portion thereof as defined in any of claims 1-8 comprising the steps of:

- expressing the antibody or antigen-binding portion thereof as defined in any of claims 1-8 in the host cell of claim 11; and

- isolating the antibody or antigen-binding portion thereof from the host cell.

14. A method of modulating an antigen-specific T cell response or modulating an immune response in a subject, comprising administering to the subject the antibody or antigen-binding portion thereof as defined in any of claims 1-8 such that an antigen- specific T cell response or an immune response is modulated in the subject.

15. A method for inhibiting or blocking the binding of LAG-3 to MHC class II molecules, FGL1 like molecules, LSECtin and/or Galectin-3, comprising contacting said MHC class II molecules, FGL1 like molecules, LSECtin and/or Galectin-3 with the antibody or antigen-binding portion thereof as defined in any of claims 1-8.

16. A method for inhibiting growth of tumor cells in a subject, comprising administering to the subject the antibody or antigen-binding portion thereof as defined in any of claims 1-8 such that growth of the tumor is inhibited in the subject.

17. A method for treating or preventing proliferative disorders such as cancers in a subject, comprising administering an effective amount of the antibody or antigen-binding portion thereof as defined in any of claims 1-8 to the subject.

18. Use of the antibody or antigen-binding portion thereof as defined in any of claims 1-8 in the manufacture of a medicament for treating or preventing proliferative disorders such as cancers.

19. Use of the antibody or antigen-binding portion thereof as defined in any of claims 1-8 in the manufacture of a diagnostic agent for diagnosing proliferative disorders such as cancers.

20. Antibody or antigen-binding portion thereof as defined in any of claims 1-8 for use in treating or preventing proliferative disorders such as cancers.

21. Antibody or antigen-binding portion thereof as defined in any of claims 1-8 for use in diagnosing proliferative disorders such as cancers.

22. A kit for treating or diagnosing proliferative disorders such as cancers, comprising a container comprising at least one antibody or antigen-binding portion thereof as defined in any of claims 1-8.

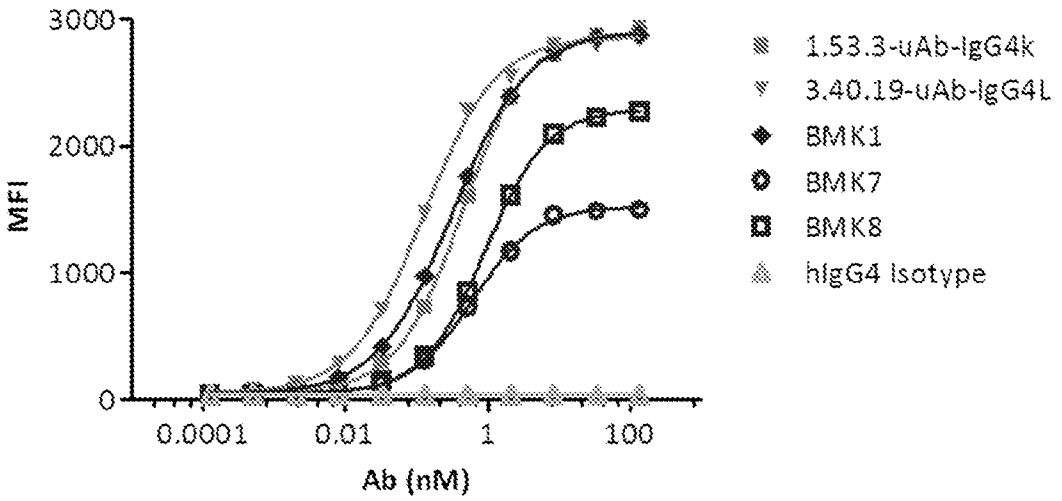


Figure 1

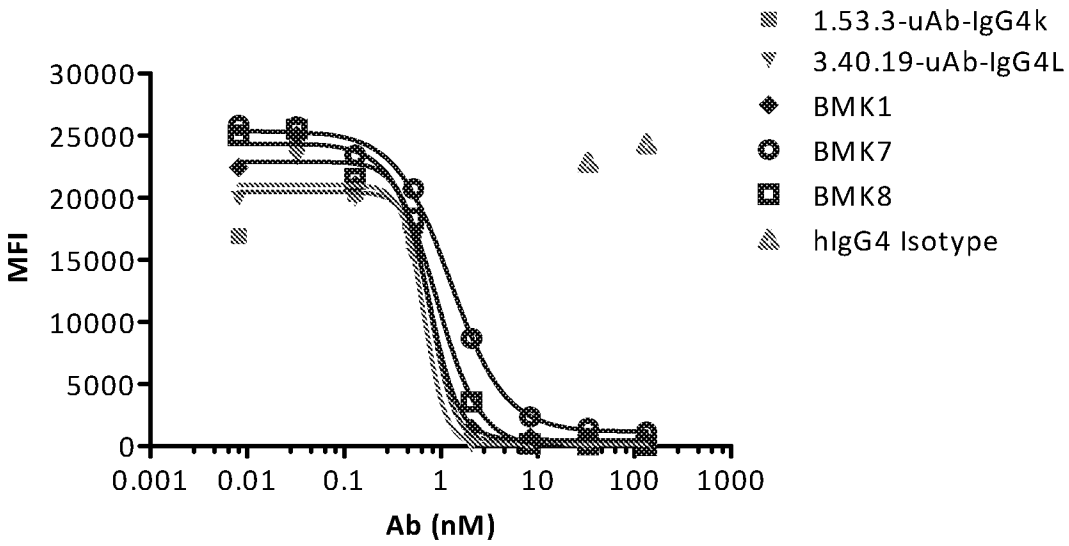


Figure 2

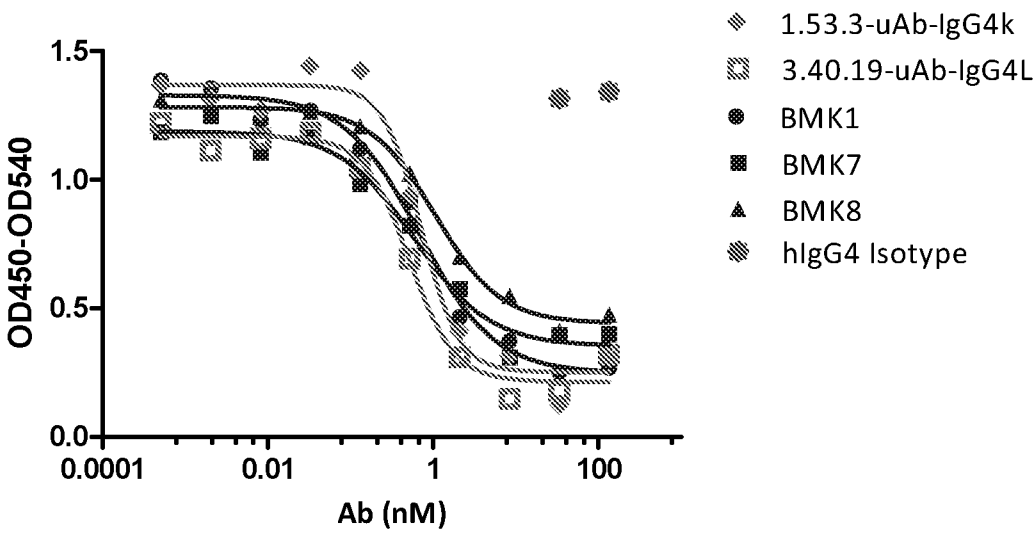


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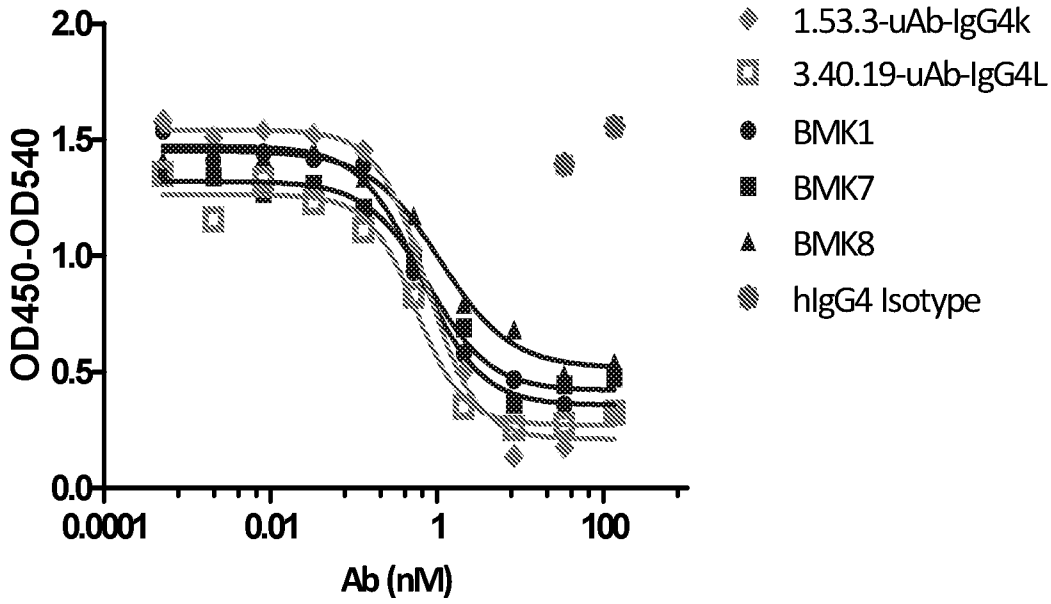


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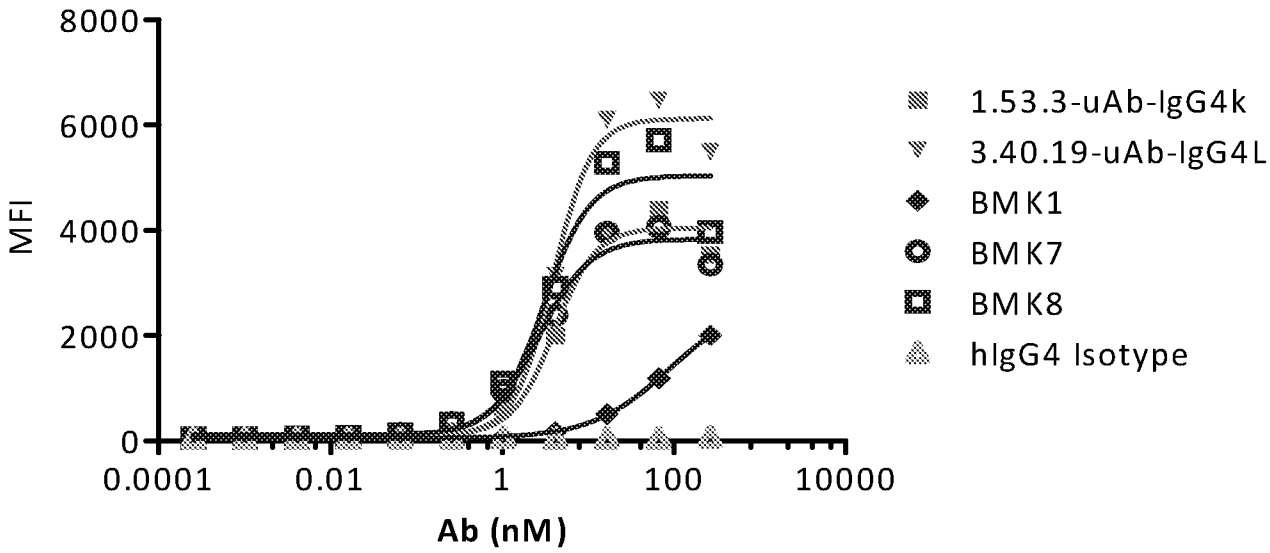


Figure 5

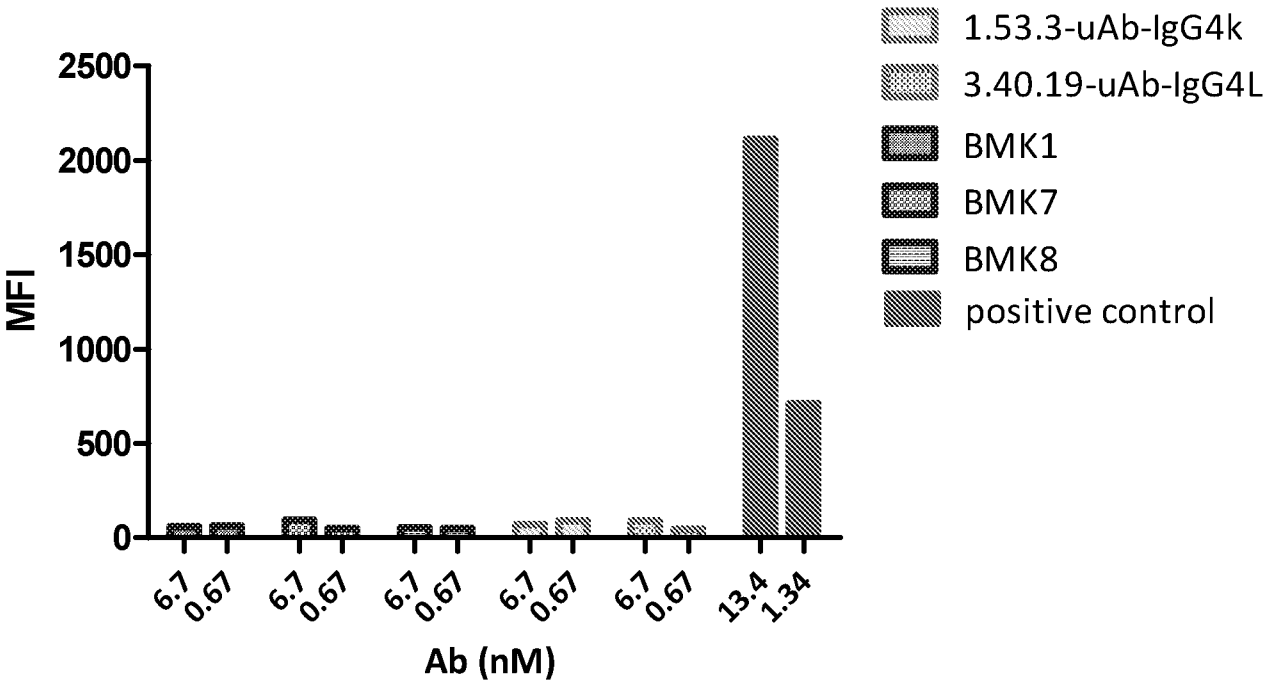


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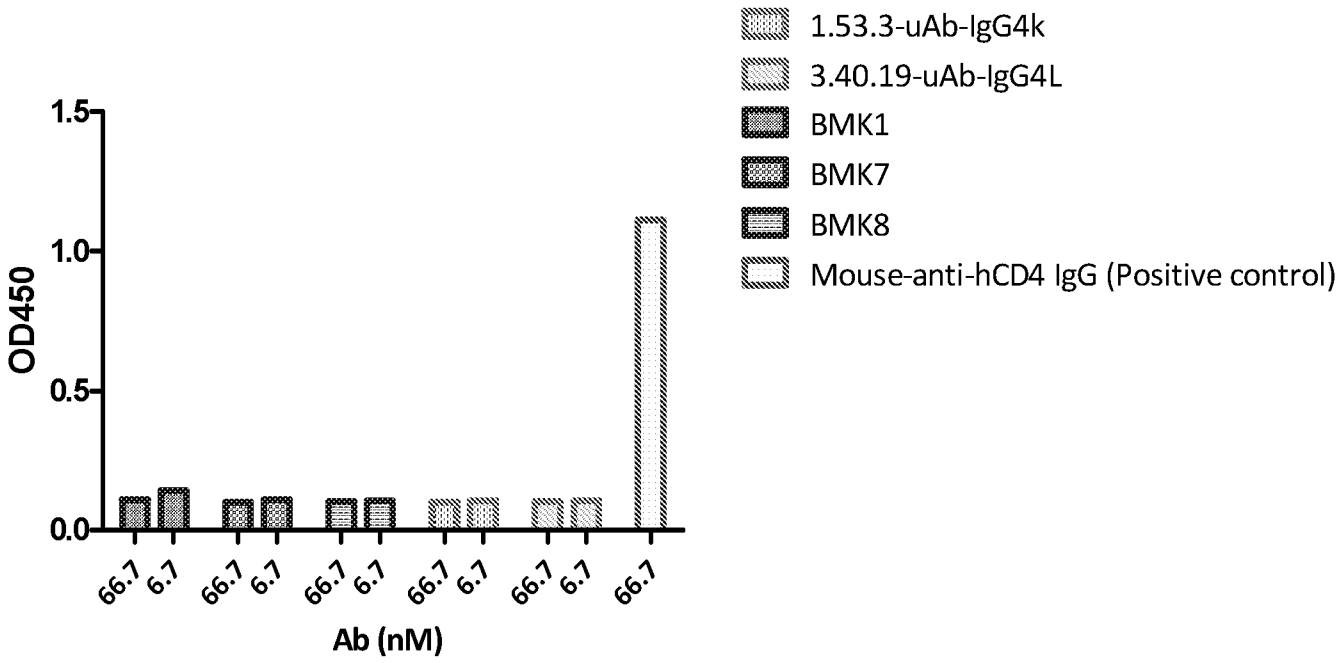

1.53.3-uAb-IgG4k
3.40.19-uAb-IgG4L
BMK1
BMK7
BMK8
Mouse-anti-hCD4 IgG (Positive control)
OD450
1.5
1.0
0.5
0.0
66.7
6.7
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6.7
66.7
6.7
66.7
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66.7
6.7
66.7
Ab (nM)

Figure 7

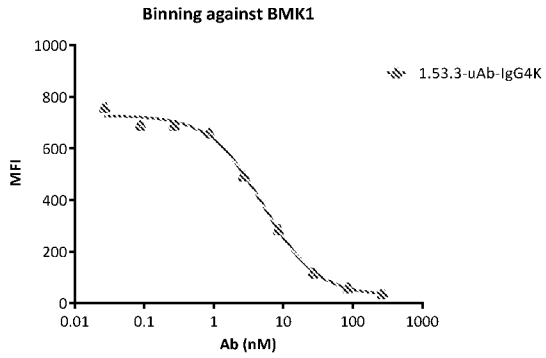


Figure 8A

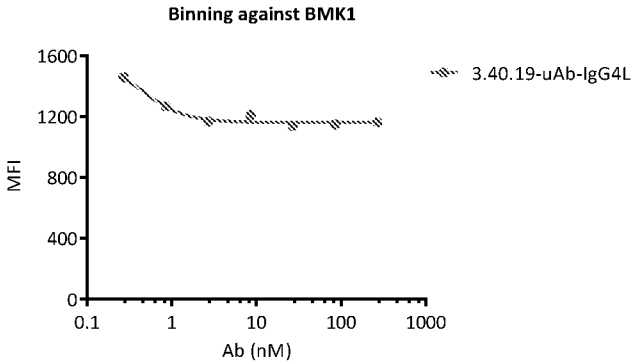


Figure 8B

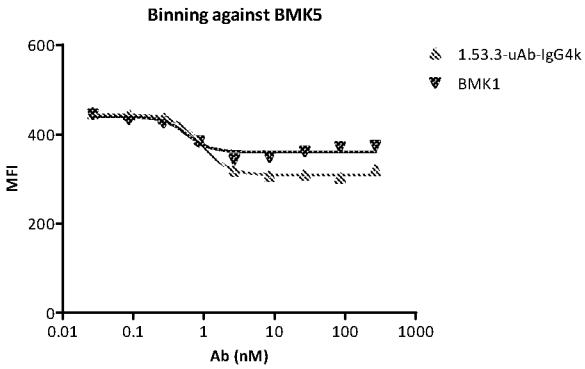


Figure 8C

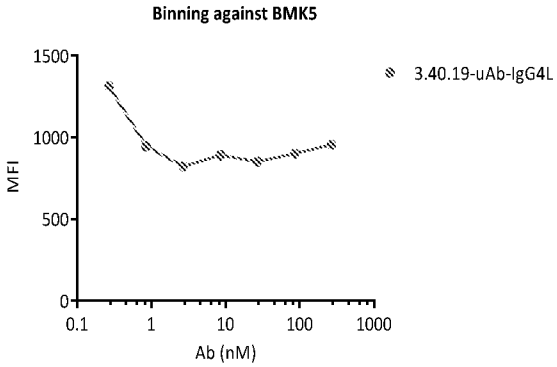


Figure 8D

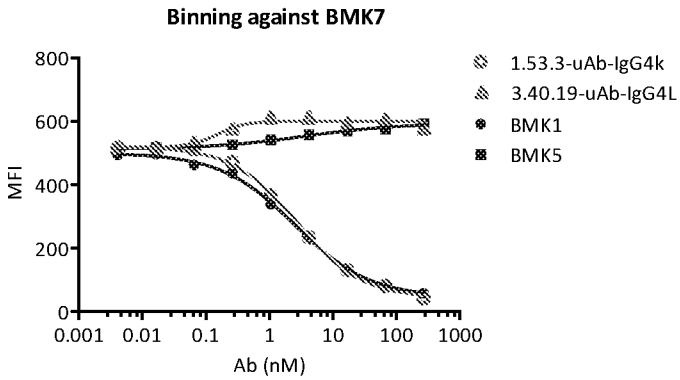


Figure 8E

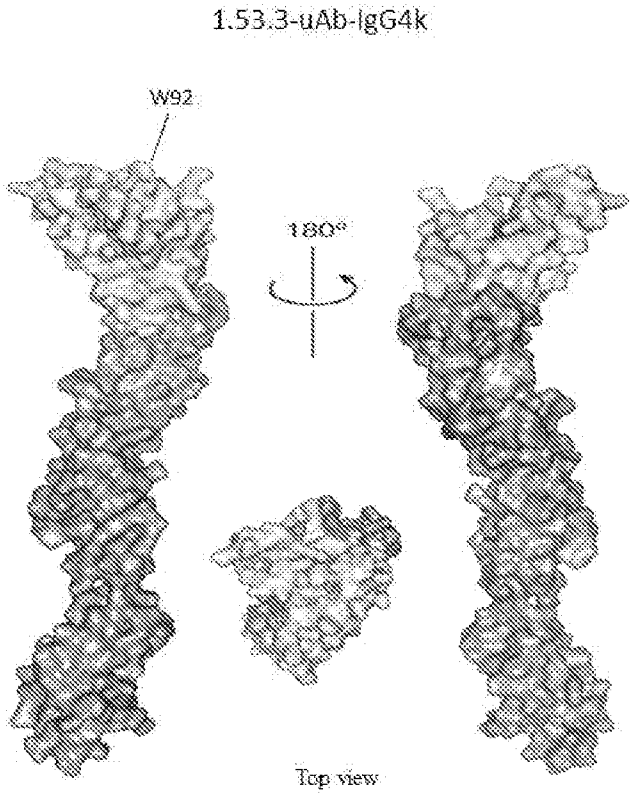


Figure 9A

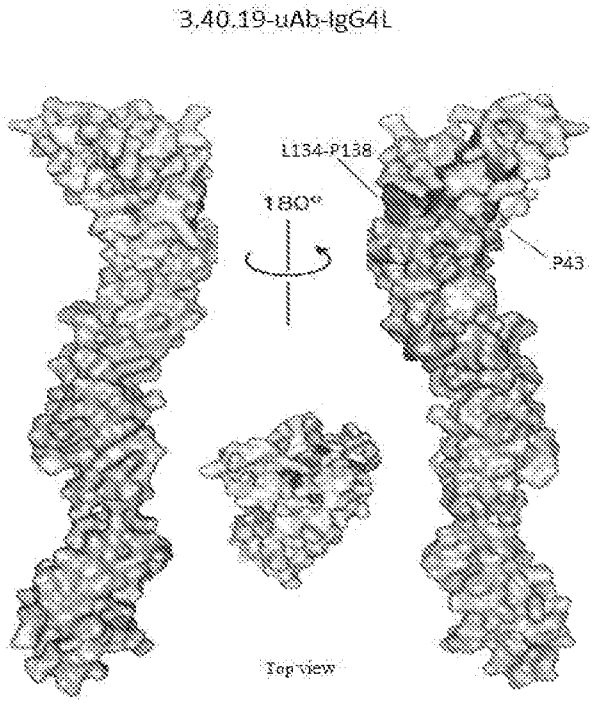


Figure 9B

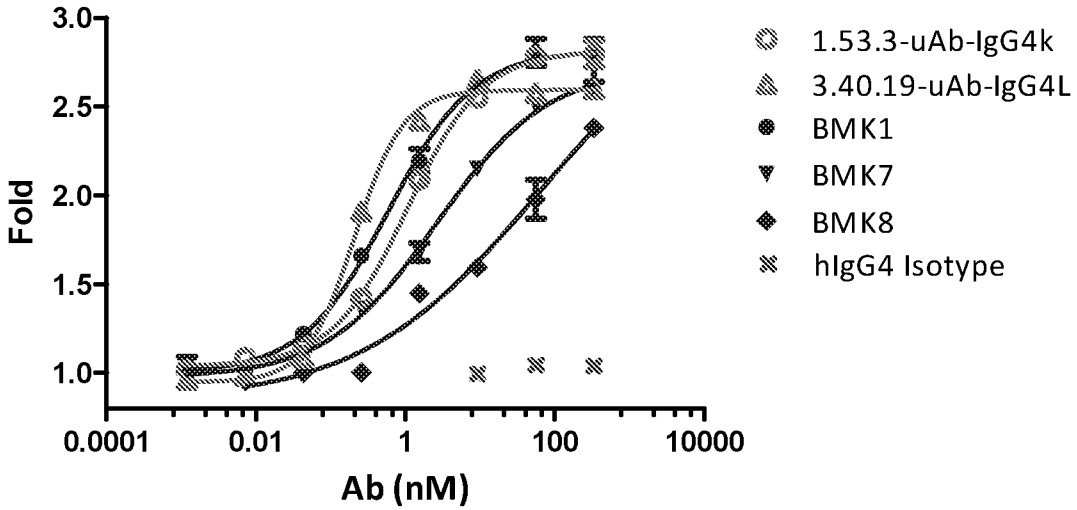


Figure 10

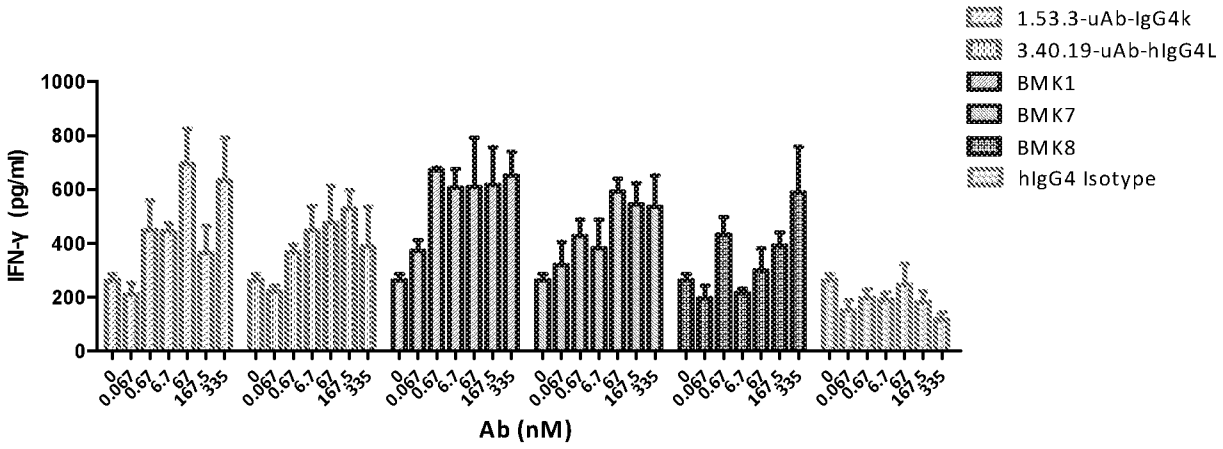


Figure 11

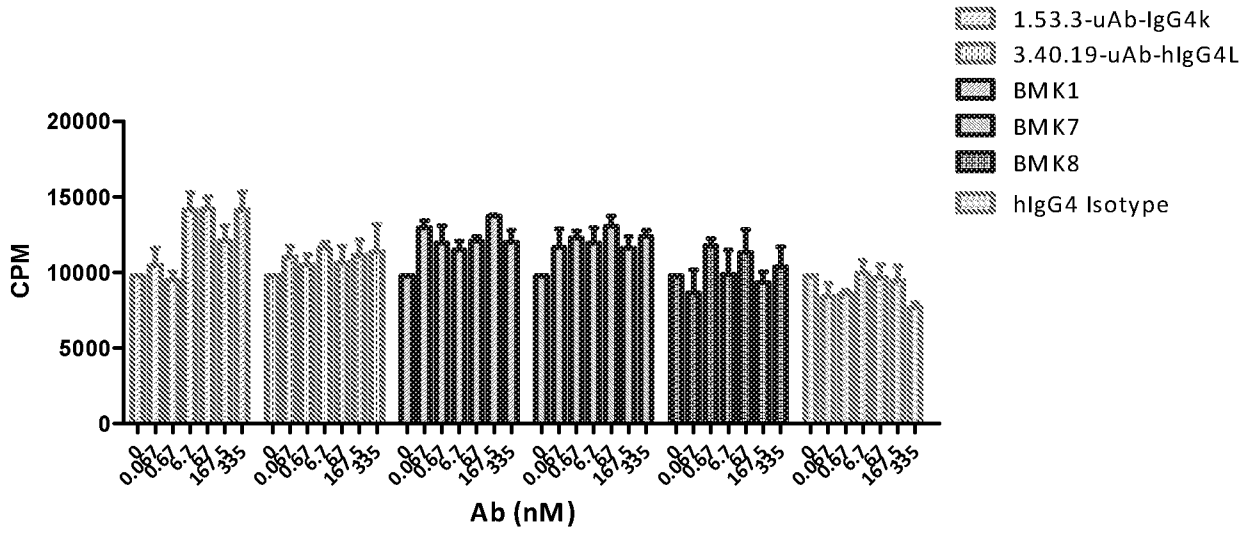


Figure 12

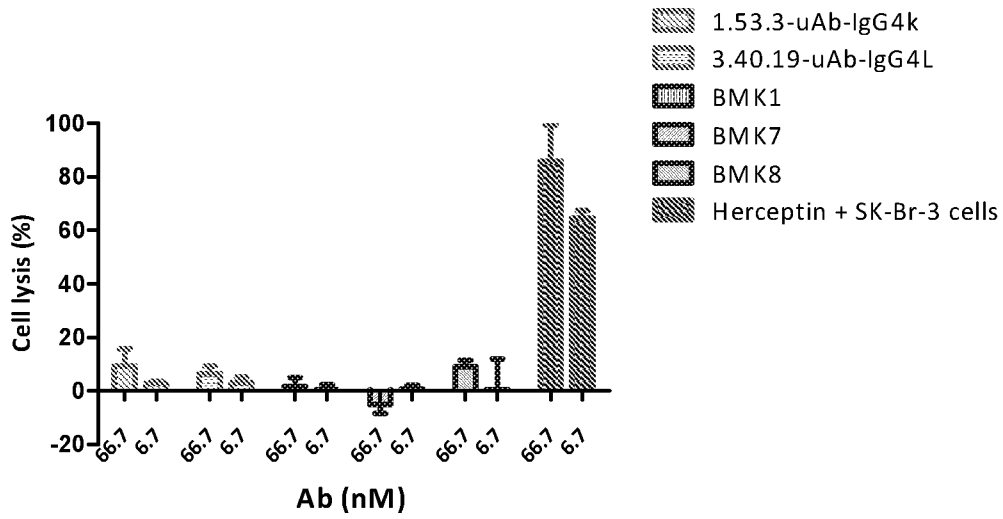


Figure 13A

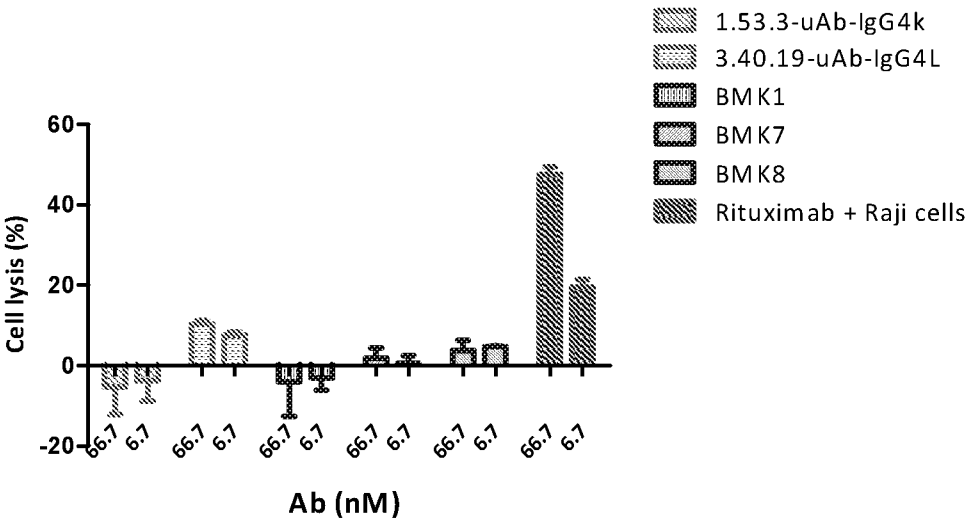


Figure 13B

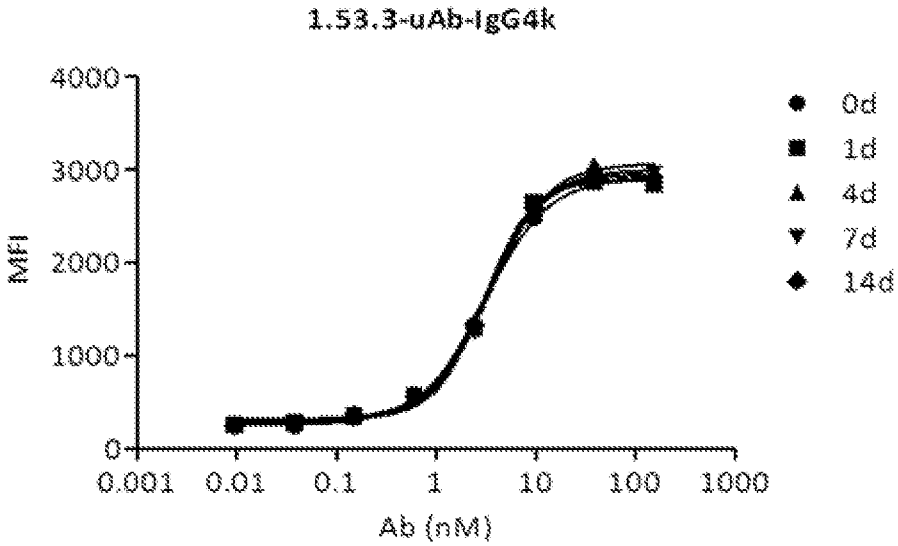


Figure 14A

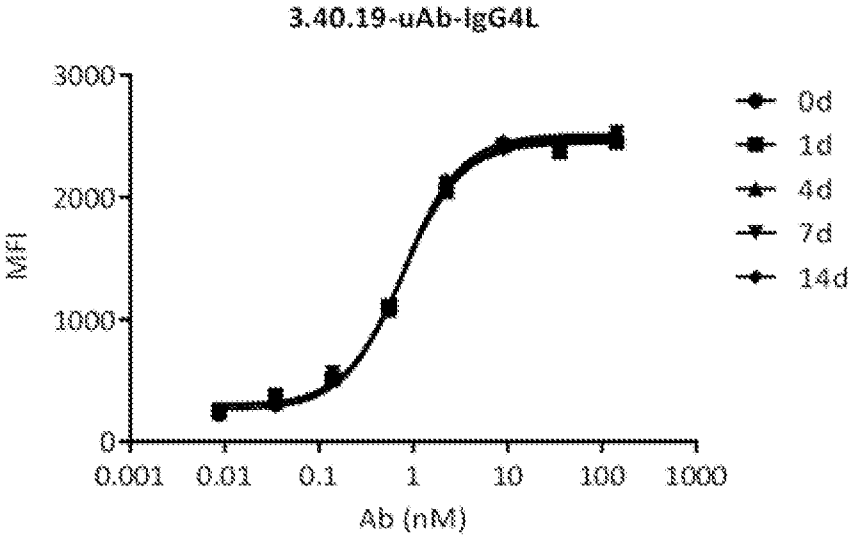


Figure 14B

INTERNATIONAL SEARCH REPORT

International application No.
PCT/CN2019/076356

A. CLASSIFICATION OF SUBJECT MATTER

A61K 39/395(2006.01)i; C07K 16/28(2006.01)i; A61P 35/00(2006.01)i

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

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)

A61K, C07K, A61P

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

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

DWPI, SIPOABS, CPRSABS, CPEA, TWABS, CNABS, JPABS, CNTXT, USTXT, WOTXT, EPTXT, CNKI, WANFANG, ISI Web of Science, Elsevier Science, GenBank: LAG-3, lymphocyte activation gene 3, antibody, CDR, heavy chain, light chain, HC, LC, cancer, tumor, carcinoma, immune response, kit, search on SEQ ID NOs: 1-20

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	CN 107405397 A (AGENCY FOR SCIENCE, TECHNILOGY AND RESEARCH) 28 November 2017 (2017-11-28) see claims 56, 78 and 90	1, 2, 8, 12, 14, 16-22
X	CN 107530420 A (AGENCY FOR SCIENCE, TECHNILOGY AND RESEARCH) 02 January 2018 (2018-01-02) see claims 1, 43 and 55	1, 2, 8, 12, 14, 16-22
X	CN 106336460 A (HANGZHOU BEIYI PHARMACEUTICAL CO LTD) 18 January 2017 (2017-01-18) see claims 1 and 9	1, 2, 8, 12, 14, 16-22
X	CN 102174105 A (U3 PHARMA GMBH, ETC.) 07 September 2011 (2011-09-07) see claims 1 and 30-36	1, 2, 8, 12, 14, 16-22
X	CN 102666589 A (KALOBIOS PHARMACEUTICALS INC) 12 September 2012 (2012-09-12) see claims 16 and 49	1, 2, 8, 12, 14, 16-22
X	CN 101326196 A (GENENTECH INC) 17 December 2008 (2008-12-17) see claims 12 and 21	1, 2, 8, 12, 14, 16-22

☑ 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
25 May 2019	**31 May 2019**
Name and mailing address of the ISA/CN **National Intellectual Property Administration, PRC** **6, Xitucheng Rd., Jimen Bridge, Haidian District, Beijing 100088** **China** Facsimile No. **(86-10)62019451**	Authorized officer **LI,Yang** Telephone No. **62411031**

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	NZ 579734 A (IMCLONE LLC) 12 January 2012 (2012-01-12) see the description, paragraphs [0008], [0011] and [0014]	1, 2, 8, 12, 14, 16-22
A	WO 2018034227 A1 (UNIV HOKKAIDO NAT UNIV CORP) 22 February 2018 (2018-02-22) see the whole document	1-22
A	WO 2017220569 A1 (F-STAR DELTA LTD) 28 December 2017 (2017-12-28) see the whole document	1-22

INTERNATIONAL SEARCH REPORT

International application No.
PCT/CN2019/076356

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

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

1. ☑ Claims Nos.: **14, 16 and 17**
because they relate to subject matter not required to be searched by this Authority, namely:

[1] The subject matter of claims 14, 16 and 17 is the case set forth in PCT Regulation 39.1 (iv). The subject matter of claims 14, 16 and 17 might be reasonably expected to be directed to use of the antibody or antigen-binding portion thereof as defined in any of claims 1-8 in the manufacture of a medicament for modulating an antigen-specific T cell response or modulating an immune response, for inhibiting growth of tumor cells in a subject, and for treating or preventing proliferative disorders in a subject, respectively. The search report about claims 14, 16 and 17 is carried out on the basis of said condition.

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

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

INTERNATIONAL SEARCH REPORT
Information on patent family members

International application No.
PCT/CN2019/076356

Patent document cited in search report			Publication date (day/month/year)	Patent family member(s)			Publication date (day/month/year)
CN	107405397	A	28 November 2017	EP	3212231	A4	28 March 2018
				AU	2015340056	A1	25 May 2017
				CA	2965960	A1	06 May 2016
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INTERNATIONAL SEARCH REPORT
Information on patent family members

International application No.
PCT/CN2019/076356

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INTERNATIONAL SEARCH REPORT
Information on patent family members

International application No.
PCT/CN2019/076356

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