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APPLICATION NUMBER: ***62/172,277***
FILING DATE: ***June 08, 2015***

THE COUNTRY CODE AND NUMBER OF YOUR PRIORITY APPLICATION, TO BE USED FOR FILING ABROAD UNDER THE PARIS CONVENTION, IS ***US62/172,277***

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Electronic Acknowledgement Receipt	
EFS ID:	22558560
Application Number:	62172277
International Application Number:	
Confirmation Number:	1005
Title of Invention:	LAG-3-Binding Molecules and Methods of Use Thereof
First Named Inventor/Applicant Name:	Leslie S. Johnson
Customer Number:	66522
Filer:	Lisa Karen Kreppel
Filer Authorized By:	
Attorney Docket Number:	1301.0121P
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1		1301_0121P_Specification.pdf	590256 [illegible]	yes	112

Multipart Description/PDF files in .zip description

Document Description	Start	End
Specification	1	105
Claims	106	111
Abstract	112	112

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2	Drawings-only black and white line drawings	1301_0121P_Figures.pdf	3320626 [illegible]	no	19

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3	Application Data Sheet	aia0014.pdf	1894809 [illegible]	no	8

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PROVISIONAL APPLICATION FOR PATENT COVER SHEET – Page 1 of 2

This is a request for filing a PROVISIONAL APPLICATION FOR PATENT under 37 CFR 1.53(c).

Express Mail Label No. _____

INVENTOR(S)		
Given Name (first and middle [if any])	Family Name or Surname	Residence (City and either State or Foreign Country)
Leslie S.	Johnson	Darnestown, MD
Paul A.	Moore	Bethesda, MD
Doug	Smith	San Mateo, CA
Ross	La Motte-Mohs	Gaithersburg, MD
Kalpana	Shah	Boyds, MD

Additional inventors are being named on the _____ separately numbered sheets attached hereto.

TITLE OF THE INVENTION (500 characters max):

LAG-3-Binding Molecules and Methods of Use Thereof

Direct all correspondence to: **CORRESPONDENCE ADDRESS**

☑ The address corresponding to Customer Number: 66522

OR

☐ Firm or Individual Name

Address

City	State	Zip
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ENCLOSED APPLICATION PARTS (*check all that apply*)

☑ Application Data Sheet. See 37 CFR 1.76.
☐ CD(s), Number of CDs _____
☑ Drawing(s) *Number of Sheets* 19
☐ Other (specify) _____
☑ Specification (e.g., description of the invention) *Number of Pages* 112

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PROVISIONAL APPLICATION FOR PATENT COVER SHEET -- Page 2 of 2

The invention was made by an agency of the United States Government or under a contract with an agency of the United States Government.

- [x] No.
- [ ] Yes, the invention was made by an agency of the U.S. Government. The U.S. Government agency name is: _____
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SIGNATURE /Lisa Kreppel/ DATE June 8, 2015

TYPED OR PRINTED NAME Lisa Kreppel REGISTRATION NO. 56970 (*if appropriate*)

TELEPHONE 301-963-0717 DOCKET NUMBER 1301.0121P

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7. A record from this system of records may be disclosed, as a routine use, to the Administrator, General Services, or his/her designee, during an inspection of records conducted by GSA as part of that agency's responsibility to recommend improvements in records management practices and programs, under authority of 44 U.S.C. 2904 and 2906. Such disclosure shall be made in accordance with the GSA regulations governing inspection of records for this purpose, and any other relevant (*i.e.*, GSA or Commerce) directive. Such disclosure shall not be used to make determinations about individuals.
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9. A record from this system of records may be disclosed, as a routine use, to a Federal, State, or local law enforcement agency, if the USPTO becomes aware of a violation or potential violation of law or regulation.

Title of the Invention:

LAG-3-Binding Molecules and Methods of Use Thereof

Reference to Sequence Listing:

[0001] This application includes one or more Sequence Listings pursuant to 37 C.F.R. 1.821 et seq., which are disclosed in both paper and computer-readable media, and which paper and computer-readable disclosures are herein incorporated by reference in their entirety.

Background of the Invention:

Field of the Invention:

[0002] The present invention is directed to LAG-3 binding molecules that comprise the LAG-3-binding domain of the anti-LAG-3 antibodies: LAG-3 mAb 1, LAG-3 mAb 2, LAG-3 mAb 3, LAG-3 mAb 4, LAG-3 mAb 5, or LAG-3 mAb 6. The invention particularly concerns LAG-3 binding molecules that are humanized or chimeric versions of such antibodies, or that comprise LAG-3 binding-fragments of such anti-LAG-3 antibodies (especially immunocongugates, diabodies, BiTEs, bispecific antibodies, *etc.*). The invention particularly concerns such LAG-3-binding molecules that are additionally capable of binding an epitope of a molecule involved in regulating an immune check point that is present on the surface of an immune cell. The present invention also pertains to methods of detecting LAG-3, as well as methods of using such LAG-3-binding molecules to stimulate an immune response. The present invention also pertains to methods of combination therapy in which a LAG-3-binding molecule is administered in combination with one or more additional molecules that are effective in stimulating an immune response to thereby further enhance, stimulate or upregulate such immune response in a subject.

Description of Related Art:

I. Cell Mediated Immune Responses

[0003] The immune system of humans and other mammals is responsible for providing protection against infection and disease. Such protection is provided both by

a humoral immune response and by a cell mediated immune response. The humoral response results in the production of antibodies and other biomolecules that are capable of recognizing and neutralizing foreign targets (antigens). In contrast, the cell mediated immune response involves the activation of macrophages, Natural Killer cells (NK), and antigen specific cytotoxic T-lymphocytes by T-cells, and the release of various cytokines in response to the recognition of an antigen (Dong, C. *et al.* (2003) "*Immune Regulation by Novel Costimulatory Molecules*," Immunolog. Res. 28(1):39-48).

[0004] The ability of T-cells to optimally mediate an immune response against an antigen requires two distinct signaling interactions (Viglietta, V. *et al.* (2007) "*Modulating Co-Stimulation*," Neurotherapeutics 4:666-675; Korman, A.J. *et al.* (2007) "*Checkpoint Blockade in Cancer Immunotherapy*," Adv. Immunol. 90:297-339). First, antigen that has been arrayed on the surface of Antigen-Presenting Cells (**APC**) must be presented to an antigen specific naive $CD4^+$ T-cell. Such presentation delivers a signal via the T-Cell Receptor (**TCR**) that directs the T-cell to initiate an immune response that will be specific to the presented antigen. Second, a series of costimulatory and inhibitory signals, mediated through interactions between the APC and distinct T-cell surface molecules, triggers first the activation and proliferation of the T-cells and ultimately their inhibition. Thus, the first signal confers specificity to the immune response whereas the second signal serves to determine the nature, magnitude and duration of the response.

[0005] The immune system is tightly controlled by costimulatory and co-inhibitory ligands and receptors. These molecules provide the second signal for T-cell activation and provide a balanced network of positive and negative signals to maximize immune responses against infection while limiting immunity to self (Wang, L. *et al.* (March 7, 2011) "*VISTA, A Novel Mouse Ig Superfamily Ligand That Negatively Regulates T-Cell Responses*," J. Exp. Med. 10.1084/jem.20100619:1-16; Lepenies, B. *et al.* (2008) "*The Role Of Negative Costimulators During Parasitic Infections*," Endocrine, Metabolic & Immune Disorders - Drug Targets 8:279-288). Of particular importance is binding between the B7.1 (CD80) and B7.2 (CD86) ligands of the Antigen-Presenting Cell and the CD28 and CTLA-4 receptors of the $CD4^+$ T lymphocyte (Sharpe, A.H. *et al.* (2002) "*The B7-CD28 Superfamily*," Nature Rev. Immunol. 2:116-126; Dong, C. *et al.* (2003)

"*Immune Regulation by Novel Costimulatory Molecules*," Immunolog. Res. 28(1):39-48; Lindley, P.S. *et al.* (2009) "*The Clinical Utility Of Inhibiting CD28-Mediated Costimulation*," Immunol. Rev. 229:307-321). Binding of B7.1 or of B7.2 to CD28 stimulates T-cell activation; binding of B7.1 or B7.2 to CTLA-4 inhibits such activation (Dong, C. *et al.* (2003) "*Immune Regulation by Novel Costimulatory Molecules*," Immunolog. Res. 28(1):39-48; Lindley, P.S. *et al.* (2009) "*The Clinical Utility Of Inhibiting CD28-Mediated Costimulation*," Immunol. Rev. 229:307-321; Greenwald, R.J. *et al.* (2005) "*The B7 Family Revisited*," Ann. Rev. Immunol. 23:515-548). CD28 is constitutively expressed on the surface of T-cells (Gross, J., *et al.* (1992) "*Identification And Distribution Of The Costimulatory Receptor CD28 In The Mouse*," J. Immunol. 149:380–388), whereas CTLA4 expression is rapidly upregulated following T-cell activation (Linsley, P. *et al.* (1996) "*Intracellular Trafficking Of CTLA4 And Focal Localization Towards Sites Of TCR Engagement*," Immunity 4:535–543). Since CTLA4 is the higher affinity receptor (Sharpe, A.H. *et al.* (2002) "*The B7-CD28 Superfamily*," Nature Rev. Immunol. 2:116-126), binding first initiates T-cell proliferation (via CD28) and then inhibits it (via nascent expression of CTLA4), thereby dampening the effect when proliferation is no longer needed.

[0006] Further investigations into the ligands of the CD28 receptor have led to the identification and characterization of a set of related B7 molecules (the "B7 Superfamily") (Coyle, A.J. *et al.* (2001) "*The Expanding B7 Superfamily: Increasing Complexity In Costimulatory Signals Regulating T-Cell Function*," Nature Immunol. 2(3):203-209; Sharpe, A.H. *et al.* (2002) "*The B7-CD28 Superfamily*," Nature Rev. Immunol. 2:116-126; Greenwald, R.J. *et al.* (2005) "*The B7 Family Revisited*," Ann. Rev. Immunol. 23:515-548; Collins, M. *et al.* (2005) "*The B7 Family Of Immune-Regulatory Ligands*," Genome Biol. 6:223.1-223.7; Loke, P. *et al.* (2004) "*Emerging Mechanisms Of Immune Regulation: The Extended B7 Family And Regulatory T-Cells*." Arthritis Res. Ther. 6:208-214; Korman, A.J. *et al.* (2007) "*Checkpoint Blockade in Cancer Immunotherapy*," Adv. Immunol. 90:297-339; Flies, D.B. *et al.* (2007) "*The New B7s: Playing a Pivotal Role in Tumor Immunity*," J. Immunother. 30(3):251-260; Agarwal, A. *et al.* (2008) "*The Role Of Positive Costimulatory Molecules In Transplantation And Tolerance*," Curr. Opin. Organ Transplant. 13:366-372; Lenschow, D.J. *et al.* (1996) "*CD28/B7 System of T-Cell Costimulation*," Ann.

Rev. Immunol. 14:233-258; Wang, S. *et al.* (2004) "*Co-Signaling Molecules Of The B7-CD28 Family In Positive And Negative Regulation Of T Lymphocyte Responses*," Microbes Infect. 6:759-766). There are currently several known members of the family: B7.1 (CD80), B7.2 (CD86), the inducible co-stimulator ligand (ICOS-L), the programmed death-1 ligand (PD-L1; B7-H1), the programmed death-2 ligand (PD-L2; B7-DC), B7-H3, B7-H4 and B7-H6 (Collins, M. *et al.* (2005) "*The B7 Family Of Immune-Regulatory Ligands*," Genome Biol. 6:223.1-223.7; Flajnik, M.F. *et al.* (2012) "*Evolution Of The B7 Family: Co-Evolution Of B7H6 And Nkp30, Identification Of A New B7 Family Member, B7H7, And Of B7's Historical Relationship With The MHC*," Immunogenetics epub doi.org/10.1007/s00251-012-0616-2).

II. Lymphocyte Activation Gene-3 ("LAG-3")

[0007] The Lymphocyte Activation Gene 3 encodes a cell surface receptor protein that is referred to as "LAG-3," or CD223 (Triebel, F. *et al.* (1990) "*LAG-3, A Novel Lymphocyte Activation Gene Closely Related To CD4*," J. Exp. Med. 171(5):1393-1405). LAG-3 is expressed by activated CD4+ and CD8+ T-cells and by NK cells, and is constitutively expressed by plasmacytoid dendritic cells. LAG-3 is not expressed by B cells, monocytes or any other cell types tested (Workman, C.J. *et al.* (2009) "*LAG-3 Regulates Plasmacytoid Dendritic Cell Homeostasis*," J. Immunol. 182(4):1885-1891).

[0008] LAG-3 has been found to be closely related to the T-cell co-receptor CD4 (Triebel, F. *et al.* (1990) "*LAG-3, A Novel Lymphocyte Activation Gene Closely Related To CD4*," J. Exp. Med. 171(5):1393-1405; Grosso, J.F. *et al.* (2009) "*Functionally Distinct LAG-3 and PD-1 Subsets on Activated and Chronically Stimulated CD8 T-Cells*," J. Immunol. 182(11):6659-6669; Huang, C.T. *et al.* (2004) "*Role Of LAG-3 In Regulatory T-Cells*," Immunity 21:503-513; Workman, C.J. *et al.* (2009) "*LAG-3 Regulates Plasmacytoid Dendritic Cell Homeostasis*," J. Immunol. 182(4):1885-1891). Like CD4, LAG-3 also binds to MHC class II molecules but does so with significantly higher affinity (Workman, C.J. *et al.* (2002) "*Phenotypic Analysis Of The Murine CD4-Related Glycoprotein, CD223 (LAG-3)*," Eur. J. Immunol. 32:2255-2263; Huard, B. *et al.* (1995) "*CD4/Major Histocompatibility Complex Class II Interaction Analyzed With CD4- And Lymphocyte Activation Gene-3 (LAG-3)-Ig Fusion Proteins*," Eur. J. Immunol. 25:2718-2721; Huard, B. *et al.* (1994) "*Cellular Expression And Tissue*

Distribution Of The Human LAG-3- Encoded Protein, An MHC Class II Ligand," Immunogenetics 39:213-217).

[0009] Studies have shown that LAG-3 plays an important role in negatively regulating T-cell proliferation, function and homeostasis (Workman, C.J. *et al.* (2009) "*LAG-3 Regulates Plasmacytoid Dendritic Cell Homeostasis*," J. Immunol. 182(4):1885-1891; Workman, C.J. *et al.* (2002) "*Cutting Edge: Molecular Analysis Of The Negative Regulatory Function Of Lymphocyte Activation Gene-3*," J. Immunol. 169:5392-5395; Workman, C.J. *et al.* (2003) "*The CD4-Related Molecule, LAG-3 (CD223), Regulates The Expansion Of Activated T-Cells*," Eur. J. Immunol. 33:970-979; Workman, C.J. (2005) "*Negative Regulation Of T-Cell Homeostasis By Lymphocyte Activation Gene-3 (CD223)*," J. Immunol. 174:688-695; Hannier, S. *et al.* (1998) "*CD3/TCR Complex-Associated Lymphocyte Activation Gene-3 Molecules Inhibit CD3/TCR Signaling*," J. Immunol. 161:4058-4065; Huard, B. *et al.* (1994) "*Lymphocyte-Activation Gene 3/Major Histocompatibility Complex Class II Interaction Modulates The Antigenic Response Of CD4+ T Lymphocytes*," Eur. J. Immunol. 24:3216-3221).

[0010] Studies have suggested that inhibiting LAG-3 function through antibody blockade can reverse LAG-3-mediated immune system inhibition and partially restore effector function (Grosso, J.F. *et al.* (2009) "*Functionally Distinct LAG-3 and PD-1 Subsets on Activated and Chronically Stimulated CD8 T-Cells*," J. Immunol. 182(11):6659-6669; Grosso, J.F. *et al.* (2007) "*LAG-3 Regulates CD8+ T-Cell Accumulation And Effector Function During Self And Tumor Tolerance*," J. Clin. Invest. 117:3383-3392). LAG-3 has been found to negatively regulate T-cell expansion via inhibition of TCR-induced calcium fluxes, and controls the size of the memory T-cell pool (Matsuzaki, J. *et al.* (2010) "*Tumor-Infiltrating NY-ESO-1-Specific CD8+ T-Cells Are Negatively Regulated By LAG-3 and PD-1 In Human Ovarian Cancer*," Proc. Natl. Acad. Sci. (U.S.A.) 107(17):7875-7880; Workman C.J., *et al.* (2004) "*Lymphocyte Activation Gene-3 (CD223) Regulates The Size Of The Expanding T-Cell Population Following Antigen Activation in vivo*," J. Immunol. 172:5450-5455).

[0011] However, despite all such prior advances, a need remains for improved compositions capable of more vigorously directing the body's immune system to attack cancer cells or pathogen-infected cells, especially at lower therapeutic concentrations. For although the adaptive immune system can be a potent defense mechanism against cancer and disease, it is often hampered by immune suppressive mechanisms in the tumor microenvironment, such as the expression of LAG-3. Furthermore, co-inhibitory molecules expressed by tumor cells, immune cells, and stromal cells in the tumor milieu can dominantly attenuate T-cell responses against cancer cells. Thus, a need remains for potent LAG-3-binding molecules. In particular, a need exists for LAG-3-binding molecules that a desirable binding kinetic profile, bind different LAG-3 epitopes and/or exhibit single agent activity that could provide improved therapeutic value to patients suffering from cancer or other diseases and conditions. The present invention is directed to these and other goals.

Summary of the Invention:

[0012] The present invention is directed to LAG-3 binding molecules that comprise the LAG-3-binding domain of the anti-LAG-3 antibodies: LAG-3 mAb 1, LAG-3 mAb 2, LAG-3 mAb 3, LAG-3 mAb 4, LAG-3 mAb 5, or LAG-3 mAb 6. The invention particularly concerns LAG-3 binding molecules that are humanized or chimeric versions of such antibodies, or that comprise LAG-3 binding-fragments of such anti-LAG-3 antibodies (especially immunoconjugates, diabodies, BiTEs, bispecific antibodies, *etc.*). The invention particularly concerns such LAG-3-binding molecules that are additionally capable of binding an epitope of a molecule involved in regulating an immune check point that is present on the surface of an immune cell. The present invention also pertains to methods of detecting LAG-3, as well as methods of using such LAG-3-binding molecules to stimulate an immune response. The present invention also pertains to methods of combination therapy in which a LAG-3-binding molecule is administered in combination with one or more additional molecules that are effective in stimulating an immune response to thereby further enhance, stimulate or upregulate such immune response in a subject.

[0013] In detail, the invention provides an anti-human LAG-3-binding molecule that comprises the three Heavy Chain CDR Domains, CDR_H1, CDR_H2 and CDR_H3 and the three Light Chain CDR Domains, CDR_L1, CDR_L2, and CDR_L3, wherein:

(A) (1) the CDR_H1 Domain, CDR_H2 Domain, and CDR_H3 Domain are the Heavy Chain CDRs of LAG-3 mAb 1, and respectively have the amino acid sequences: **SEQ ID NO:4**, **SEQ ID NO:5**, and **SEQ ID NO:6**; and

(2) the CDR_L1 Domain, CDR_L2 Domain, and CDR_L3 Domain are the Light Chain CDRs of LAG-3 mAb 1, and respectively have the amino acid sequences: **SEQ ID NO:9**, **SEQ ID NO:10**, and **SEQ ID NO:11**;

or

(B) (1) the CDR_H1 Domain, CDR_H2 Domain, and CDR_H3 Domain are the Heavy Chain CDRs of LAG-3 mAb 2, and respectively have the amino acid sequences: **SEQ ID NO:14**, **SEQ ID NO:15**, and **SEQ ID NO:16**; and

(2) the CDR_L1 Domain, CDR_L2 Domain, and CDR_L3 Domain are the Light Chain CDRs of LAG-3 mAb 2, and, respectively have the amino acid sequences: **SEQ ID NO:19**, **SEQ ID NO:20**, and **SEQ ID NO:21;**

or

(C) (1) the CDR_H1 Domain, CDR_H2 Domain, and CDR_H3 Domain are the Heavy Chain CDRs of LAG-3 mAb 3, and respectively have the amino acid sequences: **SEQ ID NO:54**, **SEQ ID NO:55**, and **SEQ ID NO:56**; and

(2) the CDR_L1 Domain, CDR_L2 Domain, and CDR_L3 Domain are the Light Chain CDRs of LAG-3 mAb 3, and, respectively have the amino acid sequences: **SEQ ID NO:59**, **SEQ ID NO:60**, and **SEQ ID NO:61**;

or

(D) (1) the CDR_H1 Domain, CDR_H2 Domain, and CDR_H3 Domain are the Heavy Chain CDRs of LAG-3 mAb 4, and respectively have the amino acid sequences: **SEQ ID NO:64**, **SEQ ID NO:65**, and **SEQ ID NO:66**; and

(2) the CDR_L1 Domain, CDR_L2 Domain, and CDR_L3 Domain are the Light Chain CDRs of LAG-3 mAb 4, and, respectively have the amino acid sequences: **SEQ ID NO:69**, **SEQ ID NO:70**, and **SEQ ID NO:71**;
or

(E) (1) the CDR_H1 Domain, CDR_H2 Domain, and CDR_H3 Domain are the Heavy Chain CDRs of LAG-3 mAb 5, and respectively have the amino acid sequences: **SEQ ID NO:74**, **SEQ ID NO:75**, and **SEQ ID NO:76**; and

(2) the CDR_L1 Domain, CDR_L2 Domain, and CDR_L3 Domain are the Light Chain CDRs of LAG-3 mAb 5, and, respectively have the amino acid sequences: **SEQ ID NO:79**, **SEQ ID NO:80**, and **SEQ ID NO:81**;
or

(F) (1) the CDR_H1 Domain, CDR_H2 Domain, and CDR_H3 Domain are the Heavy Chain CDRs of LAG-3 mAb 6 VH1, and respectively have the amino acid sequences: **SEQ ID NO:84**, **SEQ ID NO:85**, and **SEQ ID NO:86**; and

(2) the CDR_L1 Domain, CDR_L2 Domain, and CDR_L3 Domain are the Light Chain CDRs of LAG-3 mAb 6, and, respectively have the amino acid sequences: **SEQ ID NO:89**, **SEQ ID NO:90**, and **SEQ ID NO:91**.

[0014] The invention further concerns the embodiments of such anti-human LAG-3-binding molecules wherein the Heavy Chain Variable Domain has the amino acid sequence of **SEQ ID NO:2**, **SEQ ID NO:12**, **SEQ ID NO:52**, **SEQ ID NO:62**, or **SEQ ID NO:72**.

[0015] The invention further concerns the embodiments of such anti-human LAG-3-binding molecules wherein the Light Chain Variable Domain has the amino acid sequence of **SEQ ID NO:7**, **SEQ ID NO:17**, **SEQ ID NO:57**, **SEQ ID NO:67**, or **SEQ ID NO:77**.

[0016] The invention further concerns the embodiments of such anti-human LAG-3-binding molecules wherein the Heavy Chain Variable Domain has the amino acid sequence of **SEQ ID NO:92** or **SEQ ID NO:94**.

[0017] The invention further concerns the embodiments of such anti-human LAG-3-binding molecules wherein the Light Chain Variable Domain has the amino acid sequence of **SEQ ID NO:96, SEQ ID NO:98, SEQ ID NO:100**, or **SEQ ID NO:102.**

[0018] The invention further concerns the embodiments of all such anti-human LAG-3-binding molecules wherein the molecule is an antibody, and especially wherein the molecule is a chimeric antibody or a humanized antibody.

[0019] The invention further concerns the embodiment wherein the anti-human LAG-3-binding molecule is a bispecific binding molecule, capable of simultaneously binding to human LAG-3 and to a second epitope, and particularly concerns the embodiment wherein the second epitope is an epitope of a molecule involved in regulating an immune check point present on the surface of an immune cell (especially wherein the second epitope is an epitope of B7-H3, B7-H4, BTLA, CD40, CD40L, CD47, CD70, CD80, CD86, CD94, CD137, CD137L, CD226, CTLA-4, Galectin-9, GITR, GITRL, HHLA2, ICOS, ICOSL, KIR, LAG-3, LIGHT, MHC class I or II, NKG2a, NKG2d, OX40, OX40L, PD1H, PD-1, PD-L1, PD-L2, PVR, SIRPa, TCR, TIGIT, TIM-3 or VISTA, and most particularly wherein the second epitope is an epitope of CD137, OX40, PD-1, TIGIT, or TIM-3).

[0020] The invention further concerns the embodiment of such anti-human LAG-3-binding molecules wherein the molecule is a diabody, the diabody being a covalently bonded complex that comprises two, or three, or four polypeptide chains. The invention additionally concerns the embodiment of such anti-human LAG-3-binding molecules wherein the molecule comprises an Fc Region. The invention additionally concerns the embodiment of such anti-human LAG-3-binding molecules wherein the molecule comprises an Albumin-Binding Domain, and especially a deimmunized Albumin-Binding Domain.

[0021] The invention further concerns the embodiments of all such anti-human LAG-3-binding molecules wherein the molecule comprises an Fc Region, and wherein the Fc Region is a variant Fc Region that comprises one or more amino acid modifications that reduces the affinity of the variant Fc Region for an FcγR or stabilize said Fc Region, and more particularly, wherein the modifications comprise at least one

amino acid substitution selected from the group consisting of L234A; L235A; or L234A and L235A, wherein the numbering is that of the EU index as in Kabat.

[0022] The invention further concerns the embodiments in which any of the above-described LAG-3-binding molecules is used to stimulate a T-cell mediate immune response. The invention additionally concerns the embodiments in which any of the above-described LAG-3-binding molecules is used in the treatment of a disease or condition associated with a suppressed immune system, especially cancer or an infection.

[0023] The invention particularly concerns such use in the treatment or diagnosis or prognosis of cancer, wherein the cancer is characterized by the presence of a cancer cell selected from the group consisting of a cell of: an adrenal gland tumor, an AIDS-associated cancer, an alveolar soft part sarcoma, an astrocytic tumor, bladder cancer, bone cancer, a brain and spinal cord cancer, a metastatic brain tumor, a breast cancer, a carotid body tumors, a cervical cancer, a chondrosarcoma, a chordoma, a chromophobe renal cell carcinoma, a clear cell carcinoma, a colon cancer, a colorectal cancer, a cutaneous benign fibrous histiocytoma, a desmoplastic small round cell tumor, an ependymoma, a Ewing's tumor, an extraskeletal myxoid chondrosarcoma, a fibrogenesis imperfecta ossium, a fibrous dysplasia of the bone, a gallbladder or bile duct cancer, gastric cancer, a gestational trophoblastic disease, a germ cell tumor, a head and neck cancer, hepatocellular carcinoma, an islet cell tumor, a Kaposi's Sarcoma, a kidney cancer, a leukemia, a lipoma/benign lipomatous tumor, a liposarcoma/malignant lipomatous tumor, a liver cancer, a lymphoma, a lung cancer, a medulloblastoma, a melanoma, a meningioma, a multiple endocrine neoplasia, a multiple myeloma, a myelodysplastic syndrome, a neuroblastoma, a neuroendocrine tumors, an ovarian cancer, a pancreatic cancer, a papillary thyroid carcinoma, a parathyroid tumor, a pediatric cancer, a peripheral nerve sheath tumor, a phacochromocytoma, a pituitary tumor, a prostate cancer, a posterious uveal melanoma, a rare hematologic disorder, a renal metastatic cancer, a rhabdoid tumor, a rhabdomysarcoma, a sarcoma, a skin cancer, a soft-tissue sarcoma, a squamous cell cancer, a stomach cancer, a synovial sarcoma, a testicular cancer, a thymic carcinoma, a thymoma, a thyroid metastatic cancer, and a uterine cancer.

[0024] The invention particularly concerns such use in the treatment or diagnosis or prognosis of cancer, wherein the cancer is colorectal cancer, hepatocellular carcinoma, glioma, kidney cancer, breast cancer, multiple myeloma, bladder cancer, neuroblastoma; sarcoma, non-Hodgkin's lymphoma, non-small cell lung cancer, ovarian cancer, pancreatic cancer, a rectal cancer, acute myeloid leukemia (AML), chronic myelogenous leukemia (CML), acute B lymphoblastic leukemia (B-ALL), chronic lymphocytic leukemia (CLL), hairy cell leukemia (HCL), blastic plasmacytoid dendritic cell neoplasm (BPDCN), non-Hodgkin's lymphomas (NHL), including mantel cell leukemia (MCL), and small lymphocytic lymphoma (SLL), Hodgkin's lymphoma, systemic mastocytosis, or Burkitt's lymphoma.

[0025] The invention further concerns the embodiments in which any of the above-described LAG-3-binding molecules is detectably labeled and is used in the detection of LAG-3.

Brief Description of the Drawings:

[0026] **Figure 1** provides a schematic of a representative covalently bonded diabody having two epitope binding sites composed of two polypeptide chains, each having an E-coil or K-coil Heterodimer-Promoting Domain. A cysteine residue may be present in a linker and/or in the Heterodimer-Promoting Domain as shown in **Figure 3B**. VL and VH Domains that recognize the same epitope are shown using the same shading.

[0027] **Figure 2** provides a schematic of a representative covalently bonded diabody molecule having two epitope binding sites composed of two polypeptide chains, each having a CH2 and CH3 Domain, such that the associated chains form an Fc Region that comprises all or part of a naturally occurring Fc Region. VL and VH Domains that recognize the same epitope are shown using the same shading.

[0028] **Figures 3A-3C** provide schematics showing representative tetravalent diabodies having four epitope binding sites composed of two pairs of polypeptide chains (*i.e.*, four polypeptide chains in all). One polypeptide of each pair possesses a CH2 and CH3 Domain, such that the associated chains form an Fc Region that comprises all or part of a naturally occurring Fc Region. VL and VH Domains that

recognize the same epitope are shown using the same shading. The two pairs of polypeptide chains may be same. In such embodiments wherein the VL and VH Domains recognize different epitopes (as shown in **Figure 3C**), the resulting molecule is bispecific and bivalent with respect to each bound epitope. In such embodiments wherein the VL and VH Domains recognize the same epitope (*e.g.*, the same VL Domain CDRs and the same VH Domain CDRs are used on both chains) the resulting molecule is monospecific and tetravalent with respect to a single epitope. Alternatively, the two pairs of polypeptides may be different. In such embodiments wherein the VL and VH Domains of each pair of polypeptides recognize different epitopes (as shown in **Figure 3C**), the resulting molecule is tetraspecific and monovalent with respect to each bound epitope. **Figure 3A** shows an Fc diabody which contains a peptide Heterodimer-Promoting Domain comprising a cysteine residue. **Figure 3B** shows an Fc diabody, which contains E-coil and K-coil Heterodimer-Promoting Domains comprising a cysteine residue and a linker (with an optional cysteine residue). **Figure 3C**, shows an Ig diabody, which contains antibody CH1 and CL domains.

[0029] **Figures 4A and 4B** provide schematics of a representative covalently bonded diabody molecule having two epitope binding sites composed of three polypeptide chains. Two of the polypeptide chains possess a CH2 and CH3 Domain, such that the associated chains form an Fc Region that comprises all or part of an Fc Region. The polypeptide chains comprising the VL and VH Domain further comprise a Heterodimer-Promoting Domain. VL and VH Domains that recognize the same epitope are shown using the same shading.

[0030] **Figures 5A-5C** show that LAG-3 mAb 1 and LAG-3 mAb 6 bind an epitope that is distinct from the one bound by the reference antibody LAG 3 mAb A. The binding profiles of labeled LAG-3 mAb 1, LAG-3 mAb 6 and LAG-3 mAb A in the absence of a competitor antibody (**Panel A**), in the presence of excess LAG-3 mAb 1 (**Panel B**), excess LAG-3 mAb 6 (**Panel C**), or LAG-3 mAb A (**Panel D**).

[0031] **Figure 6** shows that LAG-3 mAb 1, LAG-3 mAb 2, LAG-3 mAb 3, LAG-3 mAb 4, and LAG-3 mAb 5, inhibit the binding of soluble human LAG-3 (shLAG-3) to MHC class II as determined in an ELISA assay.

[0032] **Figures 7A-7C** show that the isolated antibodies inhibit the binding of shLAG-3 to the surface of MHC class II expressing Daudi cells. **Panel A** shows the inhibition curves of LAG-3 mAb 1, LAG-3 mAb 2, LAG-3 mAb 3, LAG-3 mAb 4, LAG-3 mAb 5, and the reference antibody LAG-3 mAb A. **Panel B** shows the inhibition curves of LAG-3 mAb 1, LAG-3 mAb 6 and LAG-3 mAb A. **Panel C** shows the inhibition curves of LAG-3 mAb 1 and humanized hLAG-3 1 (1.4), hLAG-3 1 (1.2), hLAG-3 1 (2.2), hLAG-3 1 (1.1). Each panel represents a separate experiment.

[0033] **Figures 8A-8C** show that expression of LAG-3 is upregulated in stimulated CD4+ T-cells. Flow cytometric analysis of LAG-3 expression on unstimulated CD4+ T-cells (**Panel A**) and CD3/CD28 bead-stimulated CD4+ T-cells from two different donors (**Panels B** and **C**) on day 11 and 14, respectively. All cells were co-stained with anti-CD4 antibody.

[0034] **Figure 9A-9F** show that the isolated anti-LAG-3 antibodies bind to stimulated but not unstimulated T-cells. Flow cytometric analysis of CD3/CD28 stimulated CD4+ T-cells (**Panels, A-C**) and unstimulated CD4+ T-cells (**Panels, D-F**) labeled with LAG-3 mAb 1 (**Panels, A** and **D**), LAG-3 mAb 2 (**Panels, B** and **E**), or LAG-3 mAb 3 (**Panels, C** and **F**). All cells were co-stained with anti-CD4 antibody.

[0035] **Figures 10A-10D** show that the isolated anti-LAG-3 antibodies bind to stimulated but not unstimulated T-cells. Flow cytometric analysis of CD3/CD28 stimulated CD4+ T-cells (**Panels, A-B**) and unstimulated CD4+ T-cells (**Panels, C-D**) labeled with LAG-3 mAb 4 (**Panels, A** and **C**), or LAG-3 mAb 5 (**Panels, B** and **D**). All cells were co-stained with anti-CD4 antibody.

[0036] **Figures 11A-11F** show that expression of LAG-3 and PD-1 is upregulated on Staphylococcus aureus enterotoxin type B antigen (SEB)-stimulated peripheral blood mononuclear cells (PBMCs) from a representative donor D:34765. Flow cytometric analysis of SEB-stimulated PBMCs from a representative donor 48 hours after primary stimulation (**Panels, A-B**) labeled with anti-LAG-3/anti-CD3 antibodies (**Panel A**), or anti-PD-1/anti-CD3 antibodies (**Panel B**) and on day five post-SEB-stimulation (**Panels, D-E**) cells treated with SEB alone (**Panel C**) or with isotype

control antibody (**Panel D**) during the secondary stimulation, labeled with anti-LAG-3/anti-PD-1 antibodies.

[0037] **Figure 12A-12F** show that expression of LAG-3 and PD-1 is upregulated on SEB-stimulated PBMCs from another representative donor D:53724. Flow cytometric analysis of SEB-stimulated PBMCs from a representative donor 48 hours after primary stimulation (**Panels, A-B**) labeled with anti-LAG-3/anti-CD3 antibodies (**Panel A**), or anti-PD-1/anti-CD3 antibodies (**Panel B**) and on day five post-SEB-stimulation (**Panels, D-E**) cells treated with SEB alone (**Panel C**) or with isotype control antibody (**Panel D**) labeled with anti-LAG-3/anti-PD-1 antibodies.

[0038] **Figure 13** shows that LAG-3 mAb 1 is able to stimulate cytokine production to levels comparable to treatment with anti-PD-1 antibodies. IFNγ (**Panel A**) and TNFα (**Panel B**), secretion profiles from SEB-stimulated PBMCs treated with anti-LAG-3 or anti-PD-1 antibodies.

[0039] **Figure 14** shows that LAG-3 mAb 1 is able to stimulate cytokine production to levels comparable to treatment with anti-PD-1 antibodies and that treatment with LAG-3 mAb 1 in combination with an anti-PD-1 provided the largest enhancement of cytokine release. IFNγ secretion profiles from SEB-stimulated PBMCs treated with anti-LAG-3 and anti-PD-1 antibodies alone and in combination.

Detailed Description of the Invention:

[0040] The present invention is directed to LAG-3 binding molecules that comprise the LAG-3-binding domain of the anti-LAG-3 antibodies: LAG-3 mAb 1, LAG-3 mAb 2, LAG-3 mAb 3, LAG-3 mAb 4, LAG-3 mAb 5, or LAG-3 mAb 6. The invention particularly concerns LAG-3 binding molecules that are humanized or chimeric versions of such antibodies, or that comprise LAG-3 binding-fragments of such anti-LAG-3 antibodies (especially immunocongugates, diabodies, BiTEs, bispecific antibodies, *etc.*). The invention particularly concerns such LAG-3-binding molecules that are additionally capable of binding an epitope of a molecule involved in regulating an immune check point that is present on the surface of an immune cell. The present invention also pertains to methods of detecting LAG-3, as well as methods of using such LAG-3-binding molecules to stimulate an immune response. The present

invention also pertains to methods of combination therapy in which a LAG-3-binding molecule is administered in combination with one or more additional molecules that are effective in stimulating an immune response to thereby further enhance, stimulate or upregulate such immune response in a subject..

I. Antibodies and Their Binding Domains

[0041] The antibodies of the present invention are immunoglobulin molecules capable of specific binding to a target, such as a carbohydrate, polynucleotide, lipid, polypeptide, *etc.*, through at least one antigen recognition site, located in the Variable Domain of the immunoglobulin molecule. As used herein, the terms "**antibody**" and "**antibodies**" refer to monoclonal antibodies, multispecific antibodies, human antibodies, humanized antibodies, synthetic antibodies, chimeric antibodies, polyclonal antibodies, camelized antibodies, single-chain Fvs (scFv), single-chain antibodies, Fab fragments, F(ab') fragments, disulfide-linked bispecific Fvs (sdFv), intrabodies, and epitope binding fragments of any of the above. In particular, antibodies include immunoglobulin molecules and immunologically active fragments of immunoglobulin molecules, *i.e.*, molecules that contain an antigen-binding site. Immunoglobulin molecules can be of any type (*e.g.*, IgG, IgE, IgM, IgD, IgA and IgY), class (*e.g.*, IgG_1, IgG_2, IgG_3, IgG_4, IgA_1 and IgA_2) or subclass. In addition to their known uses in diagnostics, antibodies have been shown to be useful as therapeutic agents. The last few decades have seen a revival of interest in the therapeutic potential of antibodies, and antibodies have become one of the leading classes of biotechnology-derived drugs (Chan, C.E. *et al.* (2009) "*The Use Of Antibodies In The Treatment Of Infectious Diseases*," Singapore Med. J. 50(7):663-666). Nearly 200 antibody-based drugs have been approved for use or are under development.

[0042] The term "**monoclonal antibody**" refers to a homogeneous antibody population wherein the monoclonal antibody is comprised of amino acids (naturally occurring and non-naturally occurring) that are involved in the selective binding of an antigen. Monoclonal antibodies are highly specific, being directed against a single epitope (or antigenic site). The term "monoclonal antibody" encompasses not only intact monoclonal antibodies and full-length monoclonal antibodies, but also fragments thereof (such as Fab, Fab', $F(ab')_2$ Fv), single-chain (scFv), mutants thereof, fusion

proteins comprising an antibody portion, humanized monoclonal antibodies, chimeric monoclonal antibodies, and any other modified configuration of the immunoglobulin molecule that comprises an antigen recognition site of the required specificity and the ability to bind to an antigen. It is not intended to be limited as regards to the source of the antibody or the manner in which it is made (*e.g.*, by hybridoma, phage selection, recombinant expression, transgenic animals, *etc.*). The term includes whole immunoglobulins as well as the fragments *etc.* described above under the definition of "antibody." Methods of making monoclonal antibodies are known in the art. One method which may be employed is the method of Kohler, G. *et al.* (1975) "*Continuous Cultures Of Fused Cells Secreting Antibody Of Predefined Specificity*," Nature 256:495-497 or a modification thereof. Typically, monoclonal antibodies are developed in mice, rats or rabbits. The antibodies are produced by immunizing an animal with an immunogenic amount of cells, cell extracts, or protein preparations that contain the desired epitope. The immunogen can be, but is not limited to, primary cells, cultured cell lines, cancerous cells, proteins, peptides, nucleic acids, or tissue. Cells used for immunization may be cultured for a period of time (*e.g.*, at least 24 hours) prior to their use as an immunogen. Cells may be used as immunogens by themselves or in combination with a non-denaturing adjuvant, such as Ribi (see, *e.g.*, Jennings, V.M. (1995) "*Review of Selected Adjuvants Used in Antibody Production*," ILAR J. 37(3):119-125). In general, cells should be kept intact and preferably viable when used as immunogens. Intact cells may allow antigens to be better detected than ruptured cells by the immunized animal. Use of denaturing or harsh adjuvants, *e.g.*, Freud's adjuvant, may rupture cells and therefore is discouraged. The immunogen may be administered multiple times at periodic intervals such as, bi weekly, or weekly, or may be administered in such a way as to maintain viability in the animal (*e.g.*, in a tissue recombinant). Alternatively, existing monoclonal antibodies and any other equivalent antibodies that are immunospecific for a desired pathogenic epitope can be sequenced and produced recombinantly by any means known in the art. In one embodiment, such an antibody is sequenced and the polynucleotide sequence is then cloned into a vector for expression or propagation. The sequence encoding the antibody of interest may be maintained in a vector in a host cell and the host cell can then be expanded and frozen for future use. The polynucleotide sequence of such antibodies may be used for genetic

manipulation to generate the multispecific (*e.g.*, bispecific, trispecific and tetraspecific) molecules of the invention as well as an affinity optimized, a chimeric antibody, a humanized antibody, and/or a caninized antibody, to improve the affinity, or other characteristics of the antibody. The general principle in humanizing an antibody involves retaining the basic sequence of the antigen-binding portion of the antibody, while swapping the non-human remainder of the antibody with human antibody sequences.

[0043] Natural antibodies (such as IgG antibodies) are composed of two **Light Chains** complexed with two **Heavy Chains**. Each light chain contains a Variable Domain (**VL**) and a Constant Domain (**CL**). Each heavy chain contains a Variable Domain (**VH**), three Constant Domains (**CH1**, **CH2** and **CH3**), and a hinge domain located between the **CH1** and **CH2** Domains. The basic structural unit of naturally occurring immunoglobulins (*e.g.*, IgG) is thus a tetramer having two light chains and two heavy chains, usually expressed as a glycoprotein of about 150,000 Da. The amino-terminal ("N") portion of each chain includes a Variable Domain of about 100 to 110 or more amino acids primarily responsible for antigen recognition. The carboxy-terminal ("C") portion of each chain defines a constant region, with light chains having a single constant domain and heavy chains usually having three constant domains and a hinge region. Thus, the structure of the light chains of an IgG molecule is n-VL-CL-c and the structure of the IgG heavy chains is n-VH-CH1-H-CH2-CH3-c (where H is the hinge region, and n and c represent, respectively, the N-terminus and the C-terminus of the polypeptide). The Variable Domains of an IgG molecule consist of the complementarity determining regions (**CDR**), which contain the residues in contact with epitope, and non-CDR segments, referred to as framework segments (**FR**), which in general maintain the structure and determine the positioning of the CDR loops so as to permit such contacting (although certain framework residues may also contact antigen). Thus, the V_L and VH Domains have the structure n-FR1-CDR1-FR2-CDR2-FR3-CDR3-FR4-c. Polypeptides that are (or may serve as) the first, second and third CDR of an antibody Light Chain are herein respectively designated **CDR_L1 Domain**, **CDR_L2 Domain**, and **CDR_L3 Domain**. Similarly, polypeptides that are (or may serve as) the first, second and third CDR of an antibody heavy chain are herein respectively designated **CDR_H1 Domain**, **CDR_H2 Domain**, and **CDR_H3 Domain**. Thus, the terms

$CDR_{L}1$ Domain, $CDR_{L}2$ Domain, $CDR_{L}3$ Domain, $CDR_{H}1$ Domain, $CDR_{H}2$ Domain, and $CDR_{H}3$ Domain are directed to polypeptides that when incorporated into a protein cause that protein to be able to bind to an specific epitope regardless of whether such protein is an antibody having light and heavy chains or a diabody or a single-chain binding molecule (*e.g.*, an scFv, a BiTe, *etc.*), or is another type of protein.

[0044] The invention particularly encompasses single-chain Variable Domain fragments ("**scFv**") of the anti-LAG-3 antibodies of this invention and multispecific binding molecules comprising the same. Single-chain Variable Domain fragments are made by linking Light and/or Heavy chain Variable Domain by using a short linking peptide. Bird *et al.* (1988) ("*Single-Chain Antigen-Binding Proteins*," Science 242:423-426) describes example of linking peptides which bridge approximately 3.5 nm between the carboxy terminus of one Variable Domain and the amino terminus of the other Variable Domain. Linkers of other sequences have been designed and used (Bird *et al.* (1988) "*Single-Chain Antigen-Binding Proteins*," Science 242:423-426). Linkers can in turn be modified for additional functions, such as attachment of drugs or attachment to solid supports. The single-chain variants can be produced either recombinantly or synthetically. For synthetic production of scFv, an automated synthesizer can be used. For recombinant production of scFv, a suitable plasmid containing polynucleotide that encodes the scFv can be introduced into a suitable host cell, either eukaryotic, such as yeast, plant, insect or mammalian cells, or prokaryotic, such as E. coli. Polynucleotides encoding the scFv of interest can be made by routine manipulations such as ligation of polynucleotides. The resultant scFv can be isolated using standard protein purification techniques known in the art.

[0045] The invention also particularly encompasses humanized variants of the anti-LAG-3 antibodies of the invention and multispecific binding molecules comprising the same. The term "**humanized**" antibody refers to a chimeric molecule, generally prepared using recombinant techniques, having an antigen-binding site of an immunoglobulin from a non-human species and a remaining immunoglobulin structure of the molecule that is based upon the structure and /or sequence of a human immunoglobulin. The anti-human LAG-3 antibodies of the present invention include humanized, chimeric or caninized variants of antibodies LAG-3 mAb 1, LAG-3 mAb

2, LAG-3 mAb 3, LAG-3 mAb 4, LAG-3 mAb 5, or LAG-3 mAb 6. The polynucleotide sequence of the variable domains of such antibodies may be used for genetic manipulation to generate such derivatives and to improve the affinity, or other characteristics of such antibodies. The general principle in humanizing an antibody involves retaining the basic sequence of the antigen-binding portion of the antibody, while swapping the non-human remainder of the antibody with human antibody sequences. There are four general steps to humanize a monoclonal antibody. These are: (1) determining the nucleotide and predicted amino acid sequence of the starting antibody light and heavy variable domains (2) designing the humanized antibody or caninized antibody, *i.e.*, deciding which antibody framework region to use during the humanizing or canonizing process (3) the actual humanizing or caninizing methodologies/techniques and (4) the transfection and expression of the humanized antibody. See, for example, U.S. Patents Nos. 4,816,567; 5,807,715; 5,866,692; and 6,331,415.

[0046] The antigen-binding site may comprise either complete Variable Domain fused onto constant domains or only the complementarity determining regions (CDRs) grafted onto appropriate framework regions in the variable domains. Antigen-binding sites may be wild-type or modified by one or more amino acid substitutions. This eliminates the constant region as an immunogen in human individuals, but the possibility of an immune response to the foreign variable domain remains (LoBuglio, A.F. *et al.* (1989) "*Mouse/Human Chimeric Monoclonal Antibody In Man: Kinetics And Immune Response*," Proc. Natl. Acad. Sci. (U.S.A.) 86:4220-4224). Another approach focuses not only on providing human-derived constant regions, but modifying the variable domains as well so as to reshape them as closely as possible to human form. It is known that the variable domains of both heavy and light chains contain three complementarity determining regions (CDRs) which vary in response to the antigens in question and determine binding capability, flanked by four framework regions (FRs) which are relatively conserved in a given species and which putatively provide a scaffolding for the CDRs. When non-human antibodies are prepared with respect to a particular antigen, the variable domains can be "reshaped" or "humanized" by grafting CDRs derived from non-human antibody on the FRs present in the human antibody to be modified. Application of this approach to various antibodies has been reported by

Sato, K. *et al.* (1993) Cancer Res 53:851-856. Riechmann, L. *et al.* (1988) "*Reshaping Human Antibodies for Therapy*," Nature 332:323-327; Verhoeyen, M. *et al.* (1988) "*Reshaping Human Antibodies: Grafting An Antilysozyme Activity*," Science 239:1534-1536; Kettleborough, C. A. *et al.* (1991) "*Humanization Of A Mouse Monoclonal Antibody By CDR-Grafting: The Importance Of Framework Residues On Loop Conformation*," Protein Engineering 4:773-3783; Maeda, H. *et al.* (1991) "*Construction Of Reshaped Human Antibodies With HIV-Neutralizing Activity*," Human Antibodies Hybridoma 2:124-134; Gorman, S. D. *et al.* (1991) "*Reshaping A Therapeutic CD4 Antibody*," Proc. Natl. Acad. Sci. (U.S.A.) 88:4181-4185; Tempest, P.R. *et al.* (1991) "*Reshaping A Human Monoclonal Antibody To Inhibit Human Respiratory Syncytial Virus Infection in vivo*," Bio/Technology 9:266-271; Co, M. S. *et al.* (1991) "*Humanized Antibodies For Antiviral Therapy*," Proc. Natl. Acad. Sci. (U.S.A.) 88:2869-2873; Carter, P. *et al.* (1992) "*Humanization Of An Anti-p185her2 Antibody For Human Cancer Therapy*," Proc. Natl. Acad. Sci. (U.S.A.) 89:4285-4289; and Co, M.S. *et al.* (1992) "*Chimeric And Humanized Antibodies With Specificity For The CD33 Antigen*," J. Immunol. 148:1149-1154. In some embodiments, humanized antibodies preserve all CDR sequences (for example, a humanized mouse antibody which contains all six CDRs from the mouse antibodies). In other embodiments, humanized antibodies have one or more CDRs (one, two, three, four, five, or six) which differ in sequence relative to the original antibody.

[0047] A number of "humanized" antibody molecules comprising an antigen-binding site derived from a non-human immunoglobulin have been described, including chimeric antibodies having rodent or modified rodent Variable Domain and their associated complementarity determining regions (CDRs) fused to human constant domains (see, for example, Winter *et al.* (1991) "*Man-made Antibodies*," Nature 349:293-299; Lobuglio *et al.* (1989) "*Mouse/Human Chimeric Monoclonal Antibody In Man: Kinetics And Immune Response*," Proc. Natl. Acad. Sci. (U.S.A.) 86:4220-4224 (1989), Shaw *et al.* (1987) "*Characterization Of A Mouse/Human Chimeric Monoclonal Antibody (17-1A) To A Colon Cancer Tumor-Associated Antigen*," J. Immunol. 138:4534-4538, and Brown *et al.* (1987) "*Tumor-Specific Genetically Engineered Murine/Human Chimeric Monoclonal Antibody*," Cancer Res. 47:3577-3583). Other references describe rodent CDRs grafted into a human supporting

framework region (FR) prior to fusion with an appropriate human antibody constant domain (see, for example, Riechmann, L. *et al.* (1988) "*Reshaping Human Antibodies for Therapy*," Nature 332:323-327; Verhoeyen, M. *et al.* (1988) "*Reshaping Human Antibodies: Grafting An Antilysozyme Activity*," Science 239:1534-1536; and Jones *et al.* (1986) "*Replacing The Complementarity-Determining Regions In A Human Antibody With Those From A Mouse*," Nature 321:522-525). Another reference describes rodent CDRs supported by recombinantly veneered rodent framework regions. See, for example, European Patent Publication No. 519,596. These "humanized" molecules are designed to minimize unwanted immunological response towards rodent anti-human antibody molecules, which limits the duration and effectiveness of therapeutic applications of those moieties in human recipients. Other methods of humanizing antibodies that may also be utilized are disclosed by Daugherty *et al.* (1991) "*Polymerase Chain Reaction Facilitates The Cloning, CDR-Grafting, And Rapid Expression Of A Murine Monoclonal Antibody Directed Against The CD18 Component Of Leukocyte Integrins*," Nucl. Acids Res. 19:2471-2476 and in U.S. Patents Nos. 6,180,377; 6,054,297; 5,997,867; and 5,866,692.

II. Fcγ Receptors (FcγRs)

[0048] The CH2 and CH3 Domains of the two heavy chains interact to form the **Fc Region**, which is a domain that is recognized by cellular **Fc Receptors (FcγRs)**. As used herein, the term "Fc Region" is used to define a C-terminal region of an IgG heavy chain. The amino acid sequence of the CH2-CH3 Domain of an exemplary human IgG1 is (**SEQ ID NO:22**):

```
231       240         250         260         270         280
APELLGGPSV  FLFPPKPKDT  LMISRTPEVT  CVVVDVSHED  PEVKFNWYVD

          290         300         310         320         330
GVEVHNAKTK  PREEQYNSTY  RVVSVLTVLH  QDWLNGKEYK  CKVSNKALPA

          340         350         360         370         380
PIEKTISKAK  GQPREPQVYT  LPPSREEMTK  NQVSLTCLVK  GFYPSDIAVE

          390         400         410         420         430
WESNGQPENN  YKTTPPVLDS  DGSFFLYSKL  TVDKSRWQQG  NVFSCSVMHE

          440       447
ALHNHYTQKS  LSLSPGK
```

[0049] The amino acid sequence of the CH2-CH3 Domain of an exemplary human IgG2 is (**SEQ ID NO:23**):

```
231       240           250           260           270           280
APPVA-GPSV    FLFPPKPKDT    LMISRTPEVT    CVVVDVSHED    PEVQFNWYVD

          290           300           310           320           330
GVEVHNAKTK    PREEQFNSTF    RVVSVLTVVH    QDWLNGKEYK    CKVSNKGLPA

          340           350           360           370           380
PIEKTISKTK    GQPREPQVYT    LPPSREEMTK    NQVSLTCLVK    GFYPSDISVE

          390           400           410           420           430
WESNGQPENN    YKTTPPMLDS    DGSFFLYSKL    TVDKSRWQQG    NVFSCSVMHE

          440       447
ALHNHYTQKS    LSLSPGK
```

[0050] The amino acid sequence of the CH2-CH3 Domain of an exemplary human IgG3 is (**SEQ ID NO:27**):

```
231       240           250           260           270           280
APELLGGPSV    FLFPPKPKDT    LMISRTPEVT    CVVVDVSHED    PEVQFKWYVD

          290           300           310           320           330
GVEVHNAKTK    PREEQYNSTF    RVVSVLTVLH    QDWLNGKEYK    CKVSNKALPA

          340           350           360           370           380
PIEKTISKTK    GQPREPQVYT    LPPSREEMTK    NQVSLTCLVK    GFYPSDIAVE

          390           400           410           420           430
WESSGQPENN    YNTTPPMLDS    DGSFFLYSKL    TVDKSRWQQG    NIFSCSVMHE

          440       447
ALHNRFTQKS    LSLSPGK
```

[0051] The amino acid sequence of the CH2-CH3 Domain of an exemplary human IgG4 is (**SEQ ID NO:24**):

```
231       240           250           260           270           280
APEFLGGPSV    FLFPPKPKDT    LMISRTPEVT    CVVVDVSQED    PEVQFNWYVD

          290           300           310           320           330
GVEVHNAKTK    PREEQFNSTY    RVVSVLTVLH    QDWLNGKEYK    CKVSNKGLPS

          340           350           360           370           380
SIEKTISKAK    GQPREPQVYT    LPPSQEEMTK    NQVSLTCLVK    GFYPSDIAVE
```

```
          390           400           410           420           430
WESNGQPENN    YKTTPPVLDS    DGSFFLYSRL    TVDKSRWQEG    NVFSCSVMHE

          440       447
ALHNHYTQKS    LSLSLGK
```

[0052] Throughout the present specification, the numbering of the residues in an IgG heavy chain is that of the EU index as in Kabat *et al.,* Sequences of Proteins of Immunological Interest, 5th Ed. Public Health Service, NH1, MD (1991), expressly incorporated herein by references. The "EU index as in Kabat" refers to the numbering of the human IgG1 EU antibody. Amino acids from the Variable Domains of the mature heavy and light chains of immunoglobulins are designated by the position of an amino acid in the chain. Kabat described numerous amino acid sequences for antibodies, identified an amino acid consensus sequence for each subgroup, and assigned a residue number to each amino acid. Kabat's numbering scheme is extendible to antibodies not included in his compendium by aligning the antibody in question with one of the consensus sequences in Kabat by reference to conserved amino acids. This method for assigning residue numbers has become standard in the field and readily identifies amino acids at equivalent positions in different antibodies, including chimeric or humanized variants. For example, an amino acid at position 50 of a human antibody light chain occupies the equivalent position to an amino acid at position 50 of a mouse antibody light chain.

[0053] Polymorphisms have been observed at a number of different positions within antibody constant regions (*e.g.*, Fc positions, including but not limited to positions 270, 272, 312, 315, 356, and 358 as numbered by the EU index as set forth in Kabat), and thus slight differences between the presented sequence and sequences in the prior art can exist. Polymorphic forms of human immunoglobulins have been well-characterized. At present, 18 Gm allotypes are known: G1m (1, 2, 3, 17) or G1m (a, x, f, z), G2m (23) or G2m (n), G3m (5, 6, 10, 11, 13, 14, 15, 16, 21, 24, 26, 27, 28) or G3m (b1, c3, b3, b0, b3, b4, s, t, g1, c5, u, v, g5) (Lefranc, *et al.*, "*The Human IgG Subclasses: Molecular Analysis Of Structure, Function And Regulation.*" Pergamon, Oxford, pp. 43-78 (1990); Lefranc, G. *et al.*, 1979, Hum. Genet.: 50, 199-211). It is specifically contemplated that the antibodies of the present invention may be incorporate any allotype, isoallotype, or haplotype of any immunoglobulin gene, and

are not limited to the allotype, isoallotype or haplotype of the sequences provided herein. Furthermore, in some expression systems the C-terminal amino acid residue (bolded above) of the CH3 Domain may be post-translationally removed. Accordingly, the C-terminal residue of the CH3 Domain is an optional amino acid residue in the LAG-3-binding molecules of the invention. Specifically encompassed by the instant invention are LAG-3-binding molecules lacking the C-terminal residue of the CH3 Domain. Also specifically encompassed by the instant invention are such constructs comprising the C-terminal residue of the CH3 Domain.

[0054] Activating and inhibitory signals are transduced through the Fc Receptors (FcγRs) following their ligation to an Fc Region. These diametrically opposing functions result from structural differences among the different receptor isoforms. Two distinct domains within the cytoplasmic signaling domains of the receptor called immunoreceptor tyrosine-based activation motifs (ITAMs) or immunoreceptor tyrosine-based inhibitory motifs (ITIMS) account for the different responses. The recruitment of different cytoplasmic enzymes to these structures dictates the outcome of the FcγR-mediated cellular responses. ITAM-containing FcγR complexes include FcγRI, FcγRIIA, FcγRIIIA, whereas ITIM-containing complexes only include FcγRIIB. Human neutrophils express the FcγRIIA gene. FcγRIIA clustering via immune complexes or specific antibody cross-linking serves to aggregate ITAMs along with receptor-associated kinases which facilitate ITAM phosphorylation. ITAM phosphorylation serves as a docking site for Syk kinase, activation of which results in activation of downstream substrates (*e.g.*, PI_3K). Cellular activation leads to release of proinflammatory mediators. The FcγRIIB gene is expressed on B lymphocytes; its extracellular domain is 96% identical to FcγRIIA and binds IgG complexes in an indistinguishable manner. The presence of an ITIM in the cytoplasmic domain of FcγRIIB defines this inhibitory subclass of FcγR. Recently the molecular basis of this inhibition was established. When co-ligated along with an activating FcγR, the ITIM in FcγRIIB becomes phosphorylated and attracts the SH2 domain of the inositol polyphosphate 5'-phosphatase (SHIP), which hydrolyzes phosphoinositol messengers released as a consequence of ITAM-containing FcγR- mediated tyrosine kinase activation, consequently preventing the influx of intracellular Ca^{++}. Thus cross-linking of FcγRIIB dampens the activating response to FcγR ligation and inhibits cellular

responsiveness. B-cell activation, B-cell proliferation and antibody secretion is thus aborted.

III. Bispecific Antibodies, Multispecific Diabodies and DART® Diabodies

[0055] The ability of an antibody to bind an epitope of an antigen depends upon the presence and amino acid sequence of the antibody's VL and VH Domains. Interaction of an antibody light chain and an antibody heavy chain and, in particular, interaction of its VL and VH Domains forms one of the two epitope binding sites of a natural antibody. Natural antibodies are capable of binding to only one epitope species (*i.e.*, they are monospecific), although they can bind multiple copies of that species (*i.e.*, exhibiting bivalency or multivalency).

[0056] The binding domains of the present invention bind to epitopes in an "**immunospecific**" manner. As used herein, an antibody, diabody or other epitope binding molecule is said to "**immunospecifically**" bind a region of another molecule (*i.e.*, an epitope) if it reacts or associates more frequently, more rapidly, with greater duration and/or with greater affinity with that epitope relative to alternative epitopes. For example, an antibody that immunospecifically binds to a viral epitope is an antibody that binds this viral epitope with greater affinity, avidity, more readily, and /or with greater duration than it immunospecifically binds to other viral epitopes or non-viral epitopes. It is also understood by reading this definition that, for example, an antibody (or moiety or epitope) that immunospecifically binds to a first target may or may not specifically or preferentially bind to a second target. As such, "specific binding" does not necessarily require (although it can include) exclusive binding. Generally, but not necessarily, reference to binding means "specific" binding. Two molecules are said to be capable of binding to one another in a "**physiospecific**" manner, if such binding exhibits the specificity with which receptors bind to their respective ligands.

[0057] The functionality of antibodies can be enhanced by generating multispecific antibody-based molecules that can simultaneously bind two separate and distinct antigens (or different epitopes of the same antigen) and/or by generating antibody-based

molecule having higher valency (*i.e.*, more than two binding sites) for the same epitope and/or antigen.

[0058] In order to provide molecules having greater capability than natural antibodies, a wide variety of recombinant bispecific antibody formats have been developed (see, *e.g.*, PCT Publication Nos. WO 2008/003116, WO 2009/132876, WO 2008/003103, WO 2007/146968, WO 2009/018386, WO 2012/009544, WO 2013/070565), most of which use linker peptides either to fuse a further binding protein (*e.g.*, an scFv, VL, VH, *etc.*) to, or within the antibody core (IgA, IgD, IgE, IgG or IgM), or to fuse multiple antibody binding portions (*e.g.*, two Fab fragments or scFvs). Alternative formats use linker peptides to fuse a binding protein (*e.g.*, an scFv, VL, VH, *etc.*) to an a dimerization domain such as the CH2-CH3 Domain or alternative polypeptides (WO 2005/070966, WO 2006/107786A WO 2006/107617A, WO 2007/046893). Typically, such approaches involve compromises and trade-offs. For example, PCT Publications Nos. WO 2013/174873, WO 2011/133886 and WO 2010/136172 disclose that the use of linkers may cause problems in therapeutic settings, and teaches a trispecific antibody in which the CL and CH1 Domains are switched from their respective natural positions and the VL and VH Domains have been diversified (WO 2008/027236; WO 2010/108127) to allow them to bind to more than one antigen. Thus, the molecules disclosed in these documents trade binding specificity for the ability to bind additional antigen species. PCT Publications Nos. WO 2013/163427 and WO 2013/119903 disclose modifying the CH2 Domain to contain a fusion protein adduct comprising a binding domain. The document notes that the CH2 Domain likely plays only a minimal role in mediating effector function. PCT Publications Nos. WO 2010/028797, WO2010028796 and WO 2010/028795 disclose recombinant antibodies whose Fc Regions have been replaced with additional VL and VH Domains, so as to form trivalent binding molecules. PCT Publications Nos. WO 2003/025018 and WO2003012069 disclose recombinant diabodies whose individual chains contain scFv Domains. PCT Publications No. WO 2013/006544 discloses multivalent Fab molecules that are synthesized as a single polypeptide chain and then subjected to proteolysis to yield heterodimeric structures. Thus, the molecules disclosed in these documents trade all or some of the capability of mediating effector function for the ability to bind additional antigen species. PCT Publications Nos. WO 2014/022540,

WO 2013/003652, WO 2012/162583, WO 2012/156430, WO 2011/086091, WO 2008/024188, WO 2007/024715, WO 2007/075270, WO 1998/002463, WO 1992/022583 and WO 1991/003493 disclose adding additional binding domains or functional groups to an antibody or an antibody portion (*e.g.*, adding a diabody to the antibody's light chain, or adding additional VL and VH Domains to the antibody's light and heavy chains, or adding a heterologous fusion protein or chaining multiple Fab Domains to one another). Thus, the molecules disclosed in these documents trade native antibody structure for the ability to bind additional antigen species.

[0059] The art has additionally noted the capability to produce **diabodies** that differ from such natural antibodies in being capable of binding two or more different epitope species (*i.e.*, exhibiting bispecificity or multispecificity in addition to bivalency or multivalency) (see, *e.g.*, Holliger *et al.* (1993) "*'Diabodies': Small Bivalent And Bispecific Antibody Fragments,*" Proc. Natl. Acad. Sci. (U.S.A.) 90:6444-6448; US 2004/0058400 (Hollinger *et al.*); US 2004/0220388 (Mertens *et al.*); Alt *et al.* (1999) FEBS Lett. 454(1-2):90-94; Lu, D. *et al.* (2005) "*A Fully Human Recombinant IgG-Like Bispecific Antibody To Both The Epidermal Growth Factor Receptor And The Insulin-Like Growth Factor Receptor For Enhanced Antitumor Activity*," J. Biol. Chem. 280(20):19665-19672; WO 02/02781 (Mertens *et al.*); Olafsen, T. *et al.* (2004) "*Covalent Disulfide-Linked Anti-CEA Diabody Allows Site-Specific Conjugation And Radiolabeling For Tumor Targeting Applications*," Protein Eng. Des. Sel. 17(1):21-27; Wu, A. *et al.* (2001) "*Multimerization Of A Chimeric Anti-CD20 Single Chain Fv-Fv Fusion Protein Is Mediated Through Variable Domain Exchange*," Protein Engineering 14(2):1025-1033; Asano *et al.* (2004) "*A Diabody For Cancer Immunotherapy And Its Functional Enhancement By Fusion Of Human Fc Domain*," Abstract 3P-683, J. Biochem. 76(8):992; Takemura, S. *et al.* (2000) "*Construction Of A Diabody (Small Recombinant Bispecific Antibody) Using A Refolding System,*" Protein Eng. 13(8):583-588; Baeuerle, P.A. *et al.* (2009) "*Bispecific T-Cell Engaging Antibodies For Cancer Therapy*," Cancer Res. 69(12):4941-4944).

[0060] The design of a diabody is based on the antibody derivative known as a single-chain Variable Domain fragment (**scFv**). Such molecules are made by linking Light and/ or Heavy chain Variable Domains by using a short linking peptide. Bird *et*

al. (1988) ("*Single-Chain Antigen-Binding Proteins*," Science 242:423-426) describes example of linking peptides which bridge approximately 3.5 nm between the carboxy terminus of one Variable Domain and the amino terminus of the other Variable Domain. Linkers of other sequences have been designed and used (Bird *et al.* (1988) "*Single-Chain Antigen-Binding Proteins*," Science 242:423-426). Linkers can in turn be modified for additional functions, such as attachment of drugs or attachment to solid supports. The single-chain variants can be produced either recombinantly or synthetically. For synthetic production of scFv, an automated synthesizer can be used. For recombinant production of scFv, a suitable plasmid containing polynucleotide that encodes the scFv can be introduced into a suitable host cell, either eukaryotic, such as yeast, plant, insect or mammalian cells, or prokaryotic, such as *E. coli*. Polynucleotides encoding the scFv of interest can be made by routine manipulations such as ligation of polynucleotides. The resultant scFv can be isolated using standard protein purification techniques known in the art.

[0061] The provision of non-monospecific diabodies provides a significant advantage over antibodies, including but not limited to, the capacity to co-ligate and co-localize cells that express different epitopes. Bispecific diabodies thus have wide-ranging applications including therapy and immunodiagnosis. Bispecificity allows for great flexibility in the design and engineering of the diabody in various applications, providing enhanced avidity to multimeric antigens, the cross-linking of differing antigens, and directed targeting to specific cell types relying on the presence of both target antigens. Due to their increased valency, low dissociation rates and rapid clearance from the circulation (for diabodies of small size, at or below ~50 kDa), diabody molecules known in the art have also shown particular use in the field of tumor imaging (Fitzgerald *et al.* (1997) *"Improved Tumour Targeting By Disulphide Stabilized Diabodies Expressed In Pichia pastoris,"* Protein Eng. 10:1221).

[0062] The bispecificity of diabodies has led to their use for co-ligating differing cells, for example, the cross-linking of cytotoxic T-cells to tumor cells (Staerz *et al.* (1985) *"Hybrid Antibodies Can Target Sites For Attack By T Cells,"* Nature 314:628-631, and Holliger *et al.* (1996) *"Specific Killing Of Lymphoma Cells By Cytotoxic T-Cells Mediated By A Bispecific Diabody,"* Protein Eng. 9:299-305; Marvin *et al.* (2005)

"*Recombinant Approaches To IgG-Like Bispecific Antibodies*," Acta Pharmacol. Sin. 26:649-658). Alternatively, or additionally, bispecific diabodies can be used to co-ligate receptors on the surface of different cells or on a single cell. Co-ligation of different cells and/or receptors is useful to modulation effector functions and/or immune cell signaling. Multispecific molecules (*e.g.*, bispecific diabodies) comprising epitope binding domains may be directed to a surface determinant of any immune cell such as B7-H3 (CD276), B7-H4 (VTCN1), BTLA (CD272), CD3, CD8, CD16, CD27, CD32, CD40, CD40L, CD47, CD64, CD70 (CD27L), CD80 (B7-1), CD86 (B7-2), CD94 (KLRD1), CD137 (4-1BB), CD137L (4-1BBL), CD226, CTLA-4 (CD152), Galectin-9, GITR, GITRL, HHLA2, ICOS (CD278), ICOSL (CD275), Killer Activation Receptor (KIR), LAG-3 (CD223), LIGHT (TNFSF14, CD258), MHC class I or II, NKG2a, NKG2d, OX40 (CD134), OX40L (CD134L), PD1H, PD-1 (CD279), PD-L1 (B7-H1, CD274), PD-L2 (B7-CD, CD273), PVR (NECL5, CD155), SIRPa, TCR, TIGIT, TIM-3 (HAVCR2), and/or VISTA (PD-1H), which are expressed on T lymphocytes, Natural Killer (NK) cells, Antigen-presenting cells or other mononuclear cell. In particular, epitope binding domains directed to a cell surface receptor that is involved in regulating an immune checkpoint (or the ligand thereof) are useful in the generation of bispecific or multispecific binding molecules which antagonize or block the inhibitory signaling of immune checkpoint molecules and thereby stimulate, upregulate or enhance, immune responses in a subject. Molecules involved in regulating immune checkpoints include, but are not limited to B7-H3, B7-H4, BTLA, CD40, CD40L, CD47, CD70, CD80, CD86, CD94, CD137, CD137L, CD226, CTLA-4, Galectin-9, GITR, GITRL, HHLA2, ICOS, ICOSL, KIR, LAG-3, LIGHT, MHC class I or II, NKG2a, NKG2d, OX40, OX40L, PD1H, PD-1, PD-L1, PD-L2, PVR, SIRPa, TCR, TIGIT, TIM-3 and/or VISTA.

[0063] However, the above advantages come at a salient cost. The formation of such non-monospecific diabodies requires the successful assembly of two or more distinct and different polypeptides (*i.e.*, such formation requires that the diabodies be formed through the heterodimerization of different polypeptide chain species). This fact is in contrast to monospecific diabodies, which are formed through the homodimerization of identical polypeptide chains. Because at least two dissimilar polypeptides (*i.e.*, two polypeptide species) must be provided in order to form a non-

monospecific diabody, and because homodimerization of such polypeptides leads to inactive molecules (Takemura, S. *et al.* (2000) "*Construction Of A Diabody (Small Recombinant Bispecific Antibody) Using A Refolding System,*" Protein Eng. 13(8):583-588), the production of such polypeptides must be accomplished in such a way as to prevent covalent bonding between polypeptides of the same species (*i.e.*, so as to prevent homodimerization) (Takemura, S. *et al.* (2000) "*Construction Of A Diabody (Small Recombinant Bispecific Antibody) Using A Refolding System,*" Protein Eng. 13(8):583-588). The art has therefore taught the non-covalent association of such polypeptides (see, *e.g.*, Olafsen *et al.* (2004) *"Covalent Disulfide-Linked Anti-CEA Diabody Allows Site-Specific Conjugation And Radiolabeling For Tumor Targeting Applications,"* Prot. Engr. Des. Sel. 17:21-27; Asano *et al.* (2004) "*A Diabody For Cancer Immunotherapy And Its Functional Enhancement By Fusion Of Human Fc Domain,*" Abstract 3P-683, J. Biochem. 76(8):992; Takemura, S. *et al.* (2000) "*Construction Of A Diabody (Small Recombinant Bispecific Antibody) Using A Refolding System,*" Protein Eng. 13(8):583-588; Lu, D. *et al.* (2005) "*A Fully Human Recombinant IgG-Like Bispecific Antibody To Both The Epidermal Growth Factor Receptor And The Insulin-Like Growth Factor Receptor For Enhanced Antitumor Activity,*" J. Biol. Chem. 280(20):19665-19672).

[0064] However, the art has recognized that bispecific diabodies composed of non-covalently associated polypeptides are unstable and readily dissociate into non-functional monomers (see, *e.g.*, Lu, D. *et al.* (2005) "*A Fully Human Recombinant IgG-Like Bispecific Antibody To Both The Epidermal Growth Factor Receptor And The Insulin-Like Growth Factor Receptor For Enhanced Antitumor Activity,*" J. Biol. Chem. 280(20):19665-19672).

[0065] In the face of this challenge, the art has succeeded in developing stable, covalently bonded heterodimeric non-monospecific diabodies, termed **DART®** (**Dual Affinity Re-Targeting Reagents**) diabodies; see, *e.g.*, United States Patent Publications No. 2013-0295121; 2010-0174053 and 2009-0060910; European Patent Publication No. EP 2714079; EP 2601216; EP 2376109; EP 2158221 and PCT Publications No. WO 2012/162068; WO 2012/018687; WO 2010/080538; and Moore, P.A. *et al.* (2011) "*Application Of Dual Affinity Retargeting Molecules To Achieve*

Optimal Redirected T-Cell Killing Of B-Cell Lymphoma," Blood 117(17):4542-4551; Veri, M.C. *et al.* (2010) "*Therapeutic Control Of B Cell Activation Via Recruitment Of Fcgamma Receptor IIb (CD32B) Inhibitory Function With A Novel Bispecific Antibody Scaffold*," Arthritis Rheum. 62(7):1933-1943; Johnson, S. *et al.* (2010) "*Effector Cell Recruitment With Novel Fv-Based Dual-Affinity Re-Targeting Protein Leads To Potent Tumor Cytolysis And in vivo B-Cell Depletion*," J. Mol. Biol. 399(3):436-449). Such diabodies comprise two or more covalently complexed polypeptides and involve engineering one or more cysteine residues into each of the employed polypeptide species that permit disulfide bonds to form and thereby covalently bond two polypeptide chains. For example, the addition of a cysteine residue to the C-terminus of such constructs has been shown to allow disulfide bonding between the polypeptide chains, stabilizing the resulting heterodimer without interfering with the binding characteristics of the bivalent molecule.

[0066] Each of the two polypeptides of the simplest bispecific **DART®** diabody comprises three domains. The first polypeptide comprises (in the N-terminal to C-terminal direction): (i) a First Domain that comprises a binding region of a Light Chain Variable Domain of a first immunoglobulin (VL1), (ii) a Second Domain that comprises a binding region of a Heavy Chain Variable Domain of a second immunoglobulin (VH2), and (iii) a Third Domain that contains a cysteine residue (or a cysteine-containing domain) and a Heterodimer-Promoting Domain that serves to promote heterodimerization with the second polypeptide of the diabody and to covalently bond the diabody's first and second polypeptides to one another. The second polypeptide contains (in the N-terminal to C-terminal direction): (i) a First Domain that comprises a binding region of a Light Chain Variable Domain of the second immunoglobulin (VL2), (ii) a Second Domain that comprises a binding region of a Heavy Chain Variable Domain of the first immunoglobulin (VH1), and (iii) a Third Domain that contains a cysteine residue (or a cysteine-containing domain) anda complementary Heterodimerization-Promoting Domain that complexes with the Heterodimerization-Promoting Domain of the first polypeptide chain in order to promote heterodimerization with the first polypeptide chain. The cysteine residue (or a cysteine-containing domain) of the third domain of the second polypeptide chain serves to promote the covalent bonding of the second polypeptide chain to the first polypeptide chain of the diabody.

Such molecules are stable, potent and have the ability to simultaneously bind two or more antigens. In one embodiment, the Third Domains of the first and second polypeptides each contain a cysteine residue, which serves to bind the polypeptides together via a disulfide bond. **Figure 1** provides a schematic of such a diabody, which utilizes E-coil/K-coil heterodimerization domains and a cysteine containing linker for covalent bonding. As provided in **Figures 2-3**, f one or both of the polypeptides may additionally possesses the sequence of a CH2-CH3 Domain, such that complexing between the two diabody polypeptides forms an Fc Region that is capable of binding to the Fc receptor of cells (such as B lymphocytes, dendritic cells, natural killer cells, macrophages, neutrophils, eosinophils, basophils and mast cells). As provided in more detail below, the CH2 and/or CH3 Domains of such polypeptide chains need not be identical in sequence, and advantageously are modified to foster complexing between the two polypeptide chains.

[0067] Many variations of such molecules have been described (see, *e.g.*, United States Patent Publications No. 2013-0295121; 2010-0174053 and 2009-0060910; European Patent Publication No. EP 2714079; EP 2601216; EP 2376109; EP 2158221 and PCT Publications No. WO 2012/162068; WO 2012/018687; WO 2010/080538). These Fc Region-containing **DART®** diabodies may comprise two pairs of polypeptide chains. The first polypeptide chain comprises (in the N-terminal to C-terminal direction): (i) a First Domain that comprises a binding region of a Light Chain Variable Domain of a first immunoglobulin (VL1), (ii) a Second Domain that comprises a binding region of a Heavy Chain Variable Domain of a second immunoglobulin (VH2), (iii) a Third Domain that contains a cysteine residue (or a cysteine-containing domain) and a serves to promote heterodimerization with the second polypeptide of the diabody and to covalently bond the diabody's first and second polypeptides to one another, and (iv) a CH2-CH3 Domain. The second polypeptide contains (in the N-terminal to C-terminal direction): (i) a First Domain that comprises a binding region of a Light Chain Variable Domain of the second immunoglobulin (VL2), (ii) a Second Domain that comprises a binding region of a Heavy Chain Variable Domain of the first immunoglobulin (VH1), and (iii)) a Third Domain that contains a cysteine residue (or a cysteine-containing domain) and a Heterodimerization-Promoting Domain that promotes heterodimerization with the first polypeptide chain. Here two first

polypeptides complex with each other to form an Fc Region. **Figures 3A-3C** provide schematics of three variations of such diabodies utilizing different Heterodimer-Promoting Domains. Other Fc-Region-containing DART® diabodies may comprise three polypeptide chains. The first polypeptide of such DART® diabodies contains three domains: (i) a VL1-containing Domain, (ii) a VH2-containing Domain and (iii) a Domain containing a CH2-CH3 sequence. The second polypeptide of such DART® diabodies contains: (i) a VL2-containing Domain, (ii) a VH1-containing Domain and (iii) a Domain that promotes heterodimerization and covalent bonding with the diabody's first polypeptide chain. The third polypeptide of such DART® diabodies comprises a CH2-CH3 sequence. Thus, the first and second polypeptide chains of such DART® diabodies associate together to form a VL1/VH1 binding site that is capable of binding to the epitope, as well as a VL2/VH2 binding site that is capable of binding to the second epitope. Such more complex DART™ molecules also possess cysteine-containing domains which function to form a covalently bonded complex. Thus, the first and second polypeptides are bonded to one another through a disulfide bond involving cysteine residues in their respective Third Domains. Notably, the first and third polypeptide chains complex with one another to form an Fc Region that is stabilized via a disulfide bond. **Figures 4A-4B** provide schematics of such diabodies comprising three polypeptide chains.

[0068] Alternative constructs are known in the art for applications where a tetravalent molecule is desirable but an Fc is not required including, but not limited to, tetravalent tandem antibodies, also referred to as "**TandAbs**" (see, *e.g.* United States Patent Publications Nos. 2005-0079170, 2007-0031436, 2010-0099853, 2011-020667 2013-0189263; European Patent Publication Nos. EP 1078004, EP 2371866, EP 2361936 and EP 1293514; PCT Publications Nos. WO 1999/057150, WO 2003/025018, and WO 2013/013700) which are formed by the homo-dimerization of two identical chains each possessing a VH1, VL2, VH2, and VL2 Domain.

IV. The Anti-Human LAG-3-Binding Molecules of the Present Invention

[0069] The preferred LAG-3-binding molecules of the present invention include antibodies, diabodies, BiTEs, *etc.* are capable of binding to a continuous or discontinuous (*e.g.*, conformational) portion (**epitope**) of human LAG-3. The LAG-3-

binding molecules of the present invention will preferably also exhibit the ability to bind to LAG-3 molecules of one or more non-human species, in particular, primate species (and especially a primate species, such as cynomolgus monkey). Human LAG-3 (NCBI Sequence NP_002277.4; including a 22 amino acid residue signal sequence (shown underlined) and the 503 amino acid residue mature protein) has the amino acid sequence (**SEQ ID NO:1**):

MWEAQFLGLL FLQPLWVAPV KPLQPGAEVP VVWAQEGAPA QLPCSPTIPL
QDLSLLRRAG VTWQHQPDSG PPAAAPGHPL APGPHPAAPS SWGPRPRRYT
VLSVGPGGLR SGRLPLQPRV QLDERGRQRG DFSLWLRPAR RADAGEYRAA
VHLRDRALSC RLRLRLGQAS MTASPPGSLR ASDWVTLNCS FSRPDRPASV
HWFRNRGQGR VPVRESPHHH LAESFLFLPQ VSPMDSGPWG CILTYRDGFN
VSIMYNLTVL GLEPPTPLTV YAGAGSRVGL PCRLPAGVGT RSFLTAKWTP
PGGGPDLLVT GDNGDFTLRL EDVSQAQAGT YTCHIHLQEQ QLNATVTLAI
ITVTPKSFGS PGSLGKLLCE VTPVSGQERF VWSSLDTPSQ RSFSGPWLEA
QEAQLLSQPW QCQLYQGERL LGAAVYFTEL SSPGAQRSGR APGALPAGHL
LLFLILGVLS LLLLVTGAFG FHLWRRQWRP RRFSALEQGI HPPQAQSKIE
ELEQEPEPEP EPEPEPEPEP EPEQL

[0070] In certain embodiments the anti-human LAG-3-binding molecules of the invention are characterized by any (one or more) of the following criteria:

(1) specifically binds human LAG-3 as endogenously expressed on the surface of a stimulated human T-cell;

(2) specifically binds human LAG-3 with an equilibrium binding constant (K_D) of 40 nM or less;

(3) specifically binds human LAG-3 with an equilibrium binding constant (K_D) of 0.5 nM or less;

(4) specifically binds non-human primate LAG-3 (*e.g.*, LAG-3 of cynomolgus monkey);

(5) inhibits (*i.e.*, blocks or interferes with) the binding of LAG-3 to MHC class II;

(6) stimulates an immune response;

(7) stimulates antigen specific T-cell response as a single agent;

(8) synergizes with an anti-PD-1 antibody to stimulate an antigen specific T-cell response;

(9) binds the same epitope of LAG-3 as the anti-LAG-3 antibody LAG-3 mAb 1 or LAG-3 mAb 6 and/or

(10) does not compete with the anti-LAG-3 antibody 257F (BMS 986016, Medarex/BMS) for LAG-3 binding (*e.g.*, as measured by Biacore Analysis).

[0071] As used here the term "antigen specific T-cell response" refers to responses by a T-cell that result from stimulation of the T-cell with the antigen for which the T-cell is specific. Non-limiting examples of responses by a T-cell upon antigen specific stimulation include proliferation and cytokine production (*e.g.*, TNF-α, IFN-γ production). The ability of a molecule to stimulate an antigen specific T-cell response may be determined, for example, using the *Staphylococcus aureus* Enterotoxin type B antigen (SEB)-stimulated PBMC assay described herein.

[0072] The preferred anti-human LAG-3-binding molecules of the present invention possess the VH and/or VL Domains of murine anti-human LAG-3 monoclonal antibodies "**LAG-3 mAb 1**," "**LAG-3 mAb 2**," "**LAG-3 mAb 3**," "**LAG-3 mAb 4**," "**LAG-3 mAb 5**," and/or "**LAG-3 mAb 6**" and more preferably possess 1, 2 or all 3 of the CDR_{HS} of the VH Domain and/or 1, 2 or all 3 of the CDR_{LS} of the VL Domain of such anti-human LAG-3 monoclonal antibodies. Such preferred anti-human LAG-3-binding molecules include bispecific (or multispecific) antibodies, chimeric or humanized antibodies, BiTes, diabodies, *etc*, and such binding molecules having variant Fc Regions.

[0073] The invention particularly relates to LAG-3-binding molecules comprising a LAG-3 binding domain that possess:

(A) (1) the three CDR_{HS} of the VH Domain of the anti-human LAG-3 antibody LAG-3 mAb 1;

(2) the three CDR_{LS} of the VL Domain of the anti-human LAG-3 antibody LAG-3 mAb 1;

(3) the three CDR_{HS} of the VH Domain of the anti-human LAG-3 antibody LAG-3 mAb 1 and the three CDR_{LS} of the VL Domain of the anti-human LAG-3 antibody LAG-3 mAb 1;

(4) the VH Domain of the anti-human LAG-3 antibody LAG-3 mAb 1;

(5) the VL Domain of the anti-human LAG-3 antibody LAG-3 mAb 1;

(6) the VH and VL Domains of the anti-human LAG-3 antibody LAG-3 mAb 1;

(B) (1) the three CDR_{HS} of the VH Domain of the anti-human LAG-3 antibody LAG-3 mAb 2;

(2) the three CDR_{LS} of the VL Domain of the anti-human LAG-3 antibody LAG-3 mAb 2;

(3) the three CDR_{HS} of the VH Domain of the anti-human LAG-3 antibody LAG-3 mAb 2 and the three CDR_{LS} of the VL Domain of the anti-human LAG-3 antibody LAG-3 mAb 2;

(4) the VH Domain of the anti-human LAG-3 antibody LAG-3 mAb 2;

(5) the VL Domain of the anti-human LAG-3 antibody LAG-3 mAb 2;

(6) the VH and VL Domains of the anti-human LAG-3 antibody LAG-3 mAb 2;

(C) (1) the three CDR_{HS} of the VH Domain of the anti-human LAG-3 antibody LAG-3 mAb 3;

(2) the three CDR_{LS} of the VL Domain of the anti-human LAG-3 antibody LAG-3 mAb 3;

(3) the three CDR_{HS} of the VH Domain of the anti-human LAG-3 antibody LAG-3 mAb 3 and the three CDR_{LS} of the VL Domain of the anti-human LAG-3 antibody LAG-3 mAb 3;

(4) the VH Domain of the anti-human LAG-3 antibody LAG-3 mAb 3;

(5) the VL Domain of the anti-human LAG-3 antibody LAG-3 mAb 3;

(6) the VH and VL Domains of the anti-human LAG-3 antibody LAG-3 mAb 3;

(D) (1) the three CDR_{HS} of the VH Domain of the anti-human LAG-3 antibody LAG-3 mAb 4;

(2) the three CDR_{LS} of the VL Domain of the anti-human LAG-3 antibody LAG-3 mAb 4;

(3) the three CDR_{HS} of the VH Domain of the anti-human LAG-3 antibody LAG-3 mAb 4 and the three CDR_{LS} of the VL Domain of the anti-human LAG-3 antibody LAG-3 mAb 4;

(4) the VH Domain of the anti-human LAG-3 antibody LAG-3 mAb 4;

(5) the VL Domain of the anti-human LAG-3 antibody LAG-3 mAb 4;

(6) the VH and VL Domains of the anti-human LAG-3 antibody LAG-3 mAb 4;

(E) (1) the three CDR_{HS} of the VH Domain of the anti-human LAG-3 antibody LAG-3 mAb 5;

(2) the three CDR_{LS} of the VL Domain of the anti-human LAG-3 antibody LAG-3 mAb 5;

(3) the three CDR_{HS} of the VH Domain of the anti-human LAG-3 antibody LAG-3 mAb 5 and the three CDR_{LS} of the VL Domain of the anti-human LAG-3 antibody LAG-3 mAb 5;

(4) the VH Domain of the anti-human LAG-3 antibody LAG-3 mAb 5;

(5) the VL Domain of the anti-human LAG-3 antibody LAG-3 mAb 5;

(6) the VH and VL Domains of the anti-human LAG-3 antibody LAG-3 mAb 5;

(F) (1) the three CDR_{HS} of the VH Domain of the anti-human LAG-3 antibody LAG-3 mAb 6;

(2) the three CDR_{LS} of the VL Domain of the anti-human LAG-3 antibody LAG-3 mAb 6;

(3) the three CDR_{HS} of the VH Domain of the anti-human LAG-3 antibody LAG-3 mAb 6 and the three CDR_{LS} of the VL Domain of the anti-human LAG-3 antibody LAG-3 mAb 6;

(4) the VH Domain of the anti-human LAG-3 antibody LAG-3 mAb 6;

(5) the VL Domain of the anti-human LAG-3 antibody LAG-3 mAb 6;

(6) the VH and VL Domains of the anti-human LAG-3 antibody LAG-3 mAb 6,

(G) (1) the three CDR_{LS} of the VL Domain of the anti-human LAG-3 antibody hLAG-3 mAb 1 VL4;

(2) the three CDR_{HS} of the VH Domain of the anti-human LAG-3 antibody LAG-3 mAb 1 and the three CDRs of the VL Domain of the anti-human LAG-3 antibody hLAG-3 mAb 1 VL4;

or

that binds the same epitope as LAG-3 mAb 1, LAG-3 mAb 2, LAG-3 mAb 3, LAG-3 mAb 4, LAG-3 mAb 5 or LAG-3 mAb 6 .

A. The Anti-Human LAG-3 Antibody LAG-3 mAb 1

1. Murine Anti-Human Antibody LAG-3 mAb 1

[0074] The amino acid sequence of the VH Domain of LAG-3 mAb 1 (**SEQ ID NO:2**) is shown below (CDR_H residues are shown underlined).

QIQLVQSGPE LKKPGETVKI SCKASG**YTFR NYGMN**WVKQA PGKVLKWMG**W**
INTYTGESTY ADDFEGRFAF SLGTSASTAY LQINILKNED TATYFCAR**ES**
LYDYYSMDYW GQGTSVTVSS

CDR_H1 of LAG-3 mAb 1 (**SEQ ID NO:4**): **YTFRNYGMN**

CDR_H2 of LAG-3 mAb 1 (**SEQ ID NO:5**): **WINTYTGESTYADDFEG**

CDR_H3 of LAG-3 mAb 1 (**SEQ ID NO:6**): **ESLYDYYSMDY**

[0075] An exemplary polynucleotide that encodes the VH Domain of LAG-3 mAb 1 is **SEQ ID NO:3** (nucleotides encoding the CDR_H residues are shown underlined):

cagatccagt tggtgcagtc tggacctgag ctgaagaagc ctggagagac

agtcaagatc tcctgcaagg cttctgggta taccttcaga aactatggaa
tgaactgggt gaagcaggct ccaggaaagg ttttaaagtg gatgggctgg
ataaacacct acactggaga gtcaacatat gctgatgact tcgagggacg
gtttgccttc tctttgggaa cctctgccag cactgcctat ttgcagatca
acatcctcaa aaatgaggac acggctacat atttctgtgc aagagaatcc
ctctatgatt actattctat ggactactgg ggtcaaggaa cctcagtcac
cgtctcctca

[0076] The amino acid sequence of the VL Domain of LAG-3 mAb 1 (**SEQ ID NO:7**) is shown below (CDR$_L$ residues are shown underlined):

DVVVTQTPLT LSVTIGQPAS ISCKSSQSLL HSDGKTYLNW LLQRPGQSPK
RLIYLVSELD SGVPDRFTGS GSGTDFTLKI SRVEAEDLGV YYCWQGTHFP
YTFGGGTKLE IK

CDR$_L$1 of LAG-3 mAb 1 (**SEQ ID NO:9**): **KSSQSLLHSDGKTYLN**

CDR$_L$2 of LAG-3 mAb 1 (**SEQ ID NO:10**): **LVSELDS**

CDR$_L$3 of LAG-3 mAb 1 (**SEQ ID NO:11**): **WQGTHFPYT**

[0077] An exemplary polynucleotide that encodes the VL Domain of LAG-3 mAb 1 is **SEQ ID NO:8** (nucleotides encoding the CDR$_L$ residues are shown underlined):

gatgttgtgg tgacccagac tccactcact ttgtcggtta ccattggaca
accagcctcc atctcttgca agtcaagtca gagcctctta catagtgatg
gaaagacata tttgaattgg ttgttacaga ggccaggcca gtctccagag
cgcctaatct atctggtgtc tgaactggac tctggagtcc ctgacaggtt
cactggcagt ggatcaggga cagatttcac actgaaaatc agcagagtgg
aggctgagga tttgggagtt tattattgct ggcaaggtac acattttccg
tacacgttcg gaggggggac caagctggaa ataaaa

2. Humanization of the Anti-Human LAG-3 Antibody LAG-3 mAb 1 to Form "hLAG-3 mAb 1"

[0078] The above-described murine anti-human LAG-3 antibody LAG-3mAb 1 was humanized in order to demonstrate the capability of humanizing an anti-human LAG-3 antibody so as to decrease its antigenicity upon administration to a human recipient. The humanization yielded two humanized VH Domains, designated herein as "**hLAG-3 mAb 1 VH-1**," and "**hLAG-3 mAb 1 VH-2**," and four humanized VL Domains designated herein as "**hLAG-3 mAb 1 VL-1**," "**hLAG-3 mAb 1 VL-2**," "**hLAG-3 mAb 1 VL-3**," and "**hLAG-3 mAb 1 VL-4**." Any of the humanized VL Domains may be paired with the humanized VH Domains. Accordingly, any antibody

comprising one of the humanized VL Domains paired with the humanized VH Domain is referred to generically as "**hLAG-3 mAb 1**," and particular combinations of humanized VH/VL Domains are referred to by reference to the specific VH/VL Domains, for example a humanized antibody comprising hLAG-3 mAb 1 VH-1 and hLAG-3 mAb 1 VL-2 is specifically referred to as "**hLAG-3 mAb 1(1.2)**."

[0079] The amino acid sequence of the VH Domain of **hLAG-3 mAb 1 VH-1** (**SEQ ID NO:92**) is shown below (CDR$_H$ residues are shown underlined):

QVQLVQSGAE VKKPGASVKV SCKASG**YTFT NYGMN**WVRQA PGQGLEWMG**W**
INTYTGESTY ADDFEGRFVF SMDTSASTAY LQISSLKAED TAVYYCAR**ES**
LYDYYSMDYW GQGTTVTVSS

[0080] An exemplary polynucleotide that encodes **hLAG-3 mAb 1 VH-1** is **SEQ ID NO:93** (nucleotides encoding the CDR$_H$ residues are shown underlined):

caggtgcaac tggttcaatc cggcgccgag gtgaaaaagc ctggcgcctc
cgtgaaagtg tcctgtaagg catctgggta tacgttcaca aattatggta
tgaactgggt gcgacaggca ccagggcagg gactggaatg gatggggtgg
atcaatactt atacaggcga gagtacttat gctgacgatt tcgagggcag
atttgtcttc tccatggaca ccagcgctag taccgcttat ctccagatta
gttctctcaa ggcggaggac acagctgttt attattgtgc cgcgagagt
ttgtatgact actatagcat ggattactgg ggacaaggta caaccgtgac
agtgagttcc

[0081] The amino acid sequence of the VH Domain of **hLAG-3 mAb 1 VH-2** (**SEQ ID NO:94**) is shown below (CDR$_H$ residues are shown underlined):

QVQLVQSGAE VKKPGASVKV SCKASG**YTFT NYGMN**WVRQA PGQGLEWMG**W**
INTYTGESTY ADDFEGRFVF SMDTSASTAY LQISSLKAED TAVYFCAR**ES**
LYDYYSMDYW GQGTTVTVSS

[0082] An exemplary polynucleotide that encodes **hLAG-3 mAb 1 VH-2** is **SEQ ID NO:95** (nucleotides encoding the CDR$_H$ residues are shown underlined):

caggtgcaac tggttcaatc cggcgccgag gtgaaaaagc ctggcgcctc
cgtgaaagtg tcctgtaagg catctgggta tacgttcaca aattatggta
tgaactgggt gcgacaggca ccagggcagg gactggaatg gatggggtgg
atcaatactt atacaggcga gagtacttat gctgacgatt tcgagggcag
atttgtcttc tccatggaca ccagcgctag taccgcttat ctccagatta
gttctctcaa ggcggaggac acagctgttt atttctgtgc cgcgagagt
ttgtatgact actatagcat ggattactgg ggacaaggta caaccgtgac
agtgagttcc

[0083] The amino acid sequence of the VL Domain of **hLAG-3 mAb 1 VL-1 (SEQ ID NO:96)** is shown below (CDR_L residues are shown underlined):

DIVMTQTPLS LSVTPGQPAS ISC**KSSQSLL HSDGKTYLN**W LLQKPGQSPE RLIY**LVSELD S**GVPDRFSGS GSGTDFTLKI SRVEAEDVGV YYC**WQGTHFP YT**FGGGTKVE IK

[0084] An exemplary polynucleotide that encodes **hLAG-3 mAb 1 VL-1** is **SEQ ID NO:97** (nucleotides encoding the CDR_L residues are shown underlined):

gatatcgtta tgactcagac accactgtca ctgagtgtga ccccaggtca
gcccgctagt atttcctgta aatcatccca gtccctcctg catagcgatg
gaaagaccta tttgaactgg cttctgcaga aaccaggcca aagtccagag
agattgatct acctcgtttc agaactcgac agtggagtgc ccgatcgctt
ctcagggtcc ggctctggga ctgattttac tctcaagatc tcaagagtgg
aggccgagga cgtcggggtt tactactgtt ggcagggtac ccacttccct
tatacatttg gcggaggcac aaaagtggag attaaa

[0085] The amino acid sequence of the VL Domain of **hLAG-3 mAb 1 VL-2 (SEQ ID NO:98)** is shown below (CDR_L residues are shown underlined):

DIVMTQTPLS LSVTPGQPAS ISC**KSSQSLL HSDGKTYLN**W LLQRPGQSPE RLIY**LVSELD S**GVPDRFSGS GSGTDFTLKI SRVEAEDVGV YYC**WQGTHFP YT**FGGGTKVE IK

[0086] An exemplary polynucleotide that encodes **hLAG-3 mAb 1 VL-2** is **SEQ ID NO:99** (nucleotides encoding the CDR_L residues are shown underlined):

gatatcgtta tgactcagac accactgtca ctgagtgtga ccccaggtca
gcccgctagt atttcctgt**a aatcatccca gtccctcctg catagcgatg**
gaaagaccta tttgaactgg cttctgcaga gaccaggcca aagtccagag
agattgatct ac**ctcgtttc agaactcgac agt**ggagtgc ccgatcgctt
ctcagggtcc ggctctggga ctgattttac tctcaagatc tcaagagtgg
aggccgagga cgtcggggtt tactactgt**t ggcagggtac ccacttccct**
tatacatttg gcggaggcac aaaagtggag attaaa

[0087] The amino acid sequence of the VL Domain of **hLAG-3 mAb 1 VL-3 (SEQ ID NO:100)** is shown below (CDR_L residues are shown underlined):

DIVMTQTPLS LSVTPGQPAS ISC**KSSQSLL HSDGKTYLN**W LLQKPGQPPE RLIY**LVSELD S**GVPDRFSGS GSGTDFTLKI SRVEAEDVGV YYC**WQGTHFP YT**FGGGTKVE IK

[0088] An exemplary polynucleotide that encodes **hLAG-3 mAb 1 VL-3** is **SEQ ID NO:101** (nucleotides encoding the CDR_L residues are shown underlined):

gatatcgtta tgactcagac accactgtca ctgagtgtga ccccaggtca
gcccgctagt atttcctgta aatcatccca gtccctcctg catagcgatg
gaaagaccta tttgaactgg cttctgcaga aaccaggcca acgccagag
agattgatct acctcgtttc agaactcgac agtggagtgc ccgatcgctt
ctcagggtcc ggctctggga ctgattttac tctcaagatc tcaagagtgg
aggccgagga cgtcggggtt tactactgtt ggcagggtac ccacttccct
tatacatttg gcggaggcac aaaagtggag attaaa

[0089] The amino acid sequence of the VL Domain of **hLAG-3 mAb 1 VL-4 (SEQ ID NO:102)** is shown below (CDR_L residues are shown underlined):

DIVMTQTPLS LSVTPGQPAS ISCKSSQSLL HSDAKTYLNW LLQKPGQPPE
RLIYLVSELD SGVPDRFSGS GSGTDFTLKI SRVEAEDVGV YYCWQGTHFP
YTFGGGTKVE IK

[0090] An exemplary polynucleotide that encodes **hLAG-3 mAb 1 VL-4** is **SEQ ID NO:103** (nucleotides encoding the CDR_L residues are shown underlined):

gatatcgtta tgactcagac accactgtca ctgagtgtga ccccaggtca
gcccgctagt atttcctgta aatcatccca gtccctcctg catagcgatg
caaagaccta tttgaactgg cttctgcaga aaccaggcca acgccagag
agattgatct acctcgtttc agaactcgac agtggagtgc ccgatcgctt
ctcagggtcc ggctctggga ctgattttac tctcaagatc tcaagagtgg
aggccgagga cgtcggggtt tactactgtt ggcagggtac ccacttccct
tatacatttg gcggaggcac aaaagtggag attaaa

[0091] The CDR_L1 of the VL Domain of hLAG-3 mAb 1 VL-4 comprises an glycine to alanine amino acid substitution and has the amino acid sequence: **KSSQSLLHSDAKTYLN (SEQ ID NO:104)**, the substituted alanine is shown underlined). It is contemplated that a similar substitution may be incorporated into any of the LAG-3 mAb 1 CDR_L1 Domains described above.

B. The Anti-Human LAG-3 Antibody LAG-3 mAb 2

[0092] The amino acid sequence of the VH Domain of LAG-3 mAb 2 **(SEQ ID NO:12)** is shown below (CDR residues are shown underlined):

EVQLQQSGPE LVKPGASVKI SCKTSGYTFT DYNIHWLRQS HGESLEWIGY
IYPYSGDIGY NQKFKNRATL TVDNSSSTAY MDLRSLTSED SAVFYCARWH
RNYFGPWFAY WGQGTPVTVS A

CDR_H1 of LAG-3 mAb 2 **(SEQ ID NO:14)**: **YTFTDYNIH**

VH CDR_H2 of LAG-3 mAb 2 (**SEQ ID NO:15**): **YIYPYSGDIGYNQKFKN**

VH CDR_H3 of LAG-3 mAb 2 (**SEQ ID NO:16**): **WHRNYFGPWFAY**

[0093] An exemplary polynucleotide that encodes the VH Domain of LAG-3 mAb 2 is **SEQ ID NO:13** (nucleotides encoding the CDR_H residues are shown underlined):

gaggtccagc ttcagcagtc aggacctgag ctggtgaaaa ctgggcctc
agtgaagatt tcctgcaaga cttctgga**ta cacatttact gactacaaca**
tacactggtt gaggcagagc catggagaga gccttgagtg gattgga**tat**
atttatcctt acagtggtga tattggatac aaccagaagt tcaagaacag
ggccacattg actgtagaca attcctccag cacagcctac atggatctcc
gcagcctgac atctgaagac tctgcagtct ttttactgtgc aaga**tggcac**
aggaactact ttggcccctg gtttgcttac tggggccaag ggactccggt
cactgtctct gca

[0094] The amino acid sequence of the VL Domain of LAG-3 mAb 2 (**SEQ ID NO:17**) is shown below (CDR_L residues are shown underlined):

DIVLTQSPAS LAVSLGQRAT ISC**KASQSVD YDGESYMN**WY QQKPGQPPKL
LIY**VVSNLES** GIPARFSGSG SGTDFTLNIH PVEEEDAATY YC**QQSSEDPL**
TFGAGTKLEL K

CDR_L1 of LAG-3 mAb 2 (**SEQ ID NO:19**): **KASQSVDYDGESYMN**

CDR_L2 of LAG-3 mAb 2 (**SEQ ID NO:20**): **VVSNLES**

CDR_L3 of LAG-3 mAb 2 (**SEQ ID NO:21**): **QQSSEDPLT**

[0095] An exemplary polynucleotide that encodes the VL Domain of LAG-3 mAb 2 is **SEQ ID NO:18** (nucleotides encoding the CDR_L residues are shown underlined):

gacattgtgc tgacccaatc tccagcttct ttggctgtgt ctctagggca
gagggccacc atctcctgca **aggccagcca aagtgttgat tatgatggtg**
aaagttatat gaactggtac caacagaaac caggacagcc acccaaactc
ctcatttat**g ttgtatccaa tctagaatct** gggatcccag ccaggtttag
tggcagtggg tctgggacag acttcaccct caacatccat cctgtggagg
aggaggatgc tgcaacctat tac**tgtcagc aaagtagtga ggatccgctc**
acgttcggtg ctgggaccaa gctggagctg aaa

C. The Anti-Human LAG-3 Antibody LAG-3 mAb 3

[0096] The amino acid sequence of the VH Domain of LAG-3 mAb 3 (**SEQ ID NO:52**) is shown below (CDR residues are shown underlined):

EVRLQQSGPE LVKPGASVKI SCKASGY**TFT DYNIH**WVRQS HGQSLEWIG**Y**

IYPYNGDTGY NQKFKTKATL TVDNSSNTAY MELRSLASED SAVYYCTR**WS RNYFGPWFAY** WGQGTLVTVS A

CDR$_H$1 of LAG-3 mAb 3 (**SEQ ID NO:54**): **TFTDYNIH**

CDR$_H$2 of LAG-3 mAb 3 (**SEQ ID NO:55**): **YIYPYNGDTGYNQKFKT**

CDR$_H$3 of LAG-3 mAb 3 (**SEQ ID NO:56**): **WSRNYFGPWFAY**

[0097] An exemplary polynucleotide that encodes the VH Domain of LAG-3 mAb 3 is **SEQ ID NO:53** (nucleotides encoding the CDR$_H$ residues are shown underlined):

gaggtccggc ttcagcagtc aggacctgag ctggtgaaac ctggggcctc agtgaagata tcctgcaagg cttctgga**ta cacattcact gactacaaca ttcac**tgggt gaggcagagc catggacaga gccttgagtg gattgga**tat atttatcctt ataatggtga tactggctac aaccagaagt tcaagacc**aa ggccacattg actgtagaca attcctccaa cacagcctac atggaactcc gcagcctggc atctgaagac tctgcagtct attactgtac aaga**tggagc aggaactact ttggcccctg gtttgcttac** tggggccaag ggactctggt cactgtctct gca

[0098] The amino acid sequence of the VL Domain of LAG-3 mAb 3 (**SEQ ID NO:57**) is shown below (CDR$_L$ residues are shown underlined):

DIVLTQSPTS LAVSLGQRAT ISC**KASQSVD YDGDSYMN**WY QQKPGQPPKL LIY**AASNLES** GIPARFSGSG SGTDFTLNIH PVEEEDAATY YC**QQSSEDPL T**FGAGTKLEL K

CDR$_L$1 of LAG-3 mAb 3 (**SEQ ID NO:59**): **KASQSVDYDGDSYMN**

CDR$_L$2 of LAG-3 mAb 3 (**SEQ ID NO:60**): **AASNLES**

CDR$_L$3 of LAG-3 mAb 3 (**SEQ ID NO:61**): **QQSSEDPLT**

[0099] An exemplary polynucleotide that encodes the VL Domain of LAG-3 mAb 3 is **SEQ ID NO:58** (nucleotides encoding the CDR$_H$ residues are shown underlined):

gacattgtgc tgacccaatc tccaacttct ttggctgtgt ctctagggca gagggccacc atctcctgca **aggccagcca aagtgttgat tatgatggtg atagttatat gaac**tggtat caacagaaac caggacagcc acccaaactc ctcatctatg **ctgcatccaa tctagaatct** gggatcccag ccaggtttag tggcagtggg tctgggacag acttcaccct caacatccat cctgtggagg aggaggatgc tgcaacctat tactgt**cagc aaagtagtga ggatccgctc acg**ttcggtg ctgggaccaa gctggagctg aaa

D. The Anti-Human LAG-3 Antibody LAG-3 mAb 4

[00100] The amino acid sequence of the VH Domain of LAG-3 mAb 4 (**SEQ ID NO:62**) is shown below (CDR residues are shown underlined):

EVQLHQSGPE LVKPGASVKI SCKTSG**YTFT DYNIH**WVKQS HGKSLEWIG**Y**
IYPYNGDAGY NQNFKTKATL TVDNSSSTAY MELRSLTSED SAVYYCAR**WN**
MNYFGPWFAY WCQGTLVTVS A

CDR$_H$1 of LAG-3 mAb 4 (**SEQ ID NO:64**): **YTFTDYNIH**

CDR$_H$2 of LAG-3 mAb 4 (**SEQ ID NO:65**): **YIYPYNGDAGYNQNFKT**

CDR$_H$3 of LAG-3 mAb 4 (**SEQ ID NO:66**): **WNMNYFGPWFAY**

[00101] An exemplary polynucleotide that encodes the VH Domain of LAG-4 mAb 4 is **SEQ ID NO:63** (nucleotides encoding the CDR$_H$ residues are shown underlined):

gaggtccagc ttcaccagtc aggacctgag ctggtgaaac ctggggcctc
agtgaagata tcctgcaaga cttctgga**ta cactttcact gactacaaca**
tacactgggt gaagcagagc catggaaaga gccttgagtg gattgga**tat**
atttatcctt acaatggtga tgctggctac aaccagaact tcaagaccaa
ggccacattg actgtagaca attcctccag cacagcctac atggagctcc
gcagcctgac atctgaggac tctgcagtct attactgtgc aaga**tggaac**
atgaactact ttggcccctg gtttgcttac tggggccaag ggactctggt
cactgtctct gcg

[00102] The amino acid sequence of the VL Domain of LAG-3 mAb 4 (**SEQ ID NO:67**) is shown below (CDR$_L$ residues are shown underlined):

DIVLTQSPAS LAVSLGQRAT ISC**KASQSVD YDGVTYIN**WY QQKPGQPPKL
LIF**AASNLES** GIPARFSGSG SGTDFTLNIH PVEEEDAATY YC**QQSNEDPL**
TFGAGTKLEL K

CDR$_L$1 of LAG-3 mAb 4 (**SEQ ID NO:69**): **KASQSVDYDGVTYIN**

CDR$_L$2 of LAG-3 mAb 4 (**SEQ ID NO:70**): **AASNLES**

CDR$_L$3 of LAG-3 mAb 4 (**SEQ ID NO:71**): **QQSNEDPLT**

[00103] An exemplary polynucleotide that encodes the VL Domain of LAG-3 mAb 4 is **SEQ ID NO:68** (nucleotides encoding the CDR$_L$ residues are shown underlined):

gacattgtgc tgacccaatc tccagcttct ttggctgtgt ctctagggca
gagggccacc atctcctgc**a aggccagcca aagtgttgat tatgatggtg**
ttacttatat caactggtac caacagaaac caggacagcc acccaaactc
ctcatctttg **ctgcatccaa tctagaatct** gggatcccag ccaggtttag

tggcagtggg tctgggacag acttcaccct caacatccat cctgtggagg
aggaggatgc tgcaacctat tactgt**cagc aaagtaatga ggatccgctc**
acgttcggtg ctgggaccaa gctggagctg aaa

E. The Anti-Human LAG-3 Antibody LAG-3 mAb 5

[00104] The amino acid sequence of the VH Domain of LAG-3 mAb 5 (**SEQ ID NO:72**) is shown below (CDR_H residues are shown underlined):

EVQLQQSGPE LVKPGASVKI SCKASG**YTFT DYNIH**WVKQS PGKSLEWIGY
IYPYSGDFGY NQKFKSKATL TVDNSSSTAY MDLRSLTSED SAVFYCAR**WH**
RNYFGPWFAY WGQGTLVTVS A

CDR_H1 of LAG-3 mAb 5 (**SEQ ID NO:74**): **YTFTDYNIH**

CDR_H2 of LAG-3 mAb 5 (**SEQ ID NO:75**): **YIYPYSGDFGYNQKFKS**

CDR_H3 of LAG-3 mAb 5 (**SEQ ID NO:76**): **WHRNYFGPWFAY**

[00105] An exemplary polynucleotide that encodes the VH Domain of LAG-3 mAb 5 is **SEQ ID NO:73** (nucleotides encoding the CDR_H residues are shown underlined):

gaggtccagc ttcagcagtc aggacctgag ctggtgaaac ctggggcctc
agtgaagatt tcctgcaaag cttctgga**ta cacatttact gactacaaca**
tacactgggt gaagcagagc cctggaaaga gccttgaatg gattgga**tat**
atttatcctt acagtggtga ttttggatac aaccagaagt tcaagagcaa
ggccacattg actgtagaca attcctccag cacagcctac atggatctcc
gcagcctgac atctgaggac tctgcagtct tttactgtgc aaga**tggcac**
aggaactact ttggcccctg gtttgcttac tggggccaag ggactctggt
cactgtctct gca

[00106] The amino acid sequence of the VL Domain of LAG-3 mAb 5 (**SEQ ID NO:77**) is shown below (CDR_L residues are shown underlined):

DIVLTQSPAS LAVSLGQRAT ISC**KASQSVD YDGESYMN**WY QQKPGQPPKL
LIY**VVSNLES** GIPARFSGSG SGTDFTLNIH PVEEEDAATY YC**QQSSEDPL**
TFGAGTKLEL K

CDR_L1 of LAG-3 mAb 5 (**SEQ ID NO:79**): **KASQSVDYDGESYMN**

CDR_L2 of LAG-3 mAb 5 (**SEQ ID NO:80**): **VVSNLES**

CDR_L3 of LAG-3 mAb 5 (**SEQ ID NO:81**): **QQSSEDPLT**

[00107] An exemplary polynucleotide that encodes the VL Domain of LAG-3 mAb 5 is **SEQ ID NO:78** (nucleotides encoding the CDR_L residues are shown underlined):

gacattgtgc tgacccaatc tccagcttct ttggctgtgt ctctagggca
gagggccacc atctcctgca **agccagcca aagtgttgat tatgatggtg**
aaagttatat gaactggtac caacagaaac caggacagcc acccaaactc
ctcatttat**g ttgtttccaa tctagaatct** gggatccccag ccaggtttag
tggcagtggg tctgggacag acttcaccct caacatccat cctgtggagg
aggaggatgc tgcaacctat tactgt**cagc aaagtagtga ggatccgctc**
acgttcggtg ctgggaccaa gctggagctg aaa

F. The Anti-Human LAG-3 Antibody LAG-3 mAb 6

[00108] The amino acid sequence of the VH Domain of LAG-3 mAb 6 (**SEQ ID NO:82**) is shown below (CDR_H residues are shown underlined):

EVLLQQSGPE LVKPGASVKI PCKASG**YTFT DYNMD**WVKQS HGESLEWIG**D**
INPDNGVTIY NQKFEGKATL TVDKSSSTAY MELRSLTSED TAVYYCAR**EA**
DYFYFDYWGQ GTTLTVSS

CDR_H1 of LAG-3 mAb 6 (**SEQ ID NO:84**): **YTFTDYNMD**

CDR_H2 of LAG-3 mAb 6 (**SEQ ID NO:85**): **DINPDNGVTIYNQKFEG**

CDR_H3 of LAG-3 mAb 6 (**SEQ ID NO:86**): **EADYFYFDY**

[00109] An exemplary polynucleotide that encodes the VH Domain of LAG-3 mAb 6 is **SEQ ID NO:83** (nucleotides encoding the CDR_H residues are shown underlined):

gaggtcctgc tgcaacagtc tggacctgag ctggtgaagc ctggggcttc
agtgaagata ccctgcaagg cttctgga**ta cacattcact gactacaaca**
tggactgggt gaagcagagc catggagaga gccttgagtg gattgga**gat**
attaatcctg acaatggtgt tactatctac aaccagaagt ttgagggcaa
ggccacactg actgtagaca agtcctccag tacagcctac atggagctcc
gcagcctgac atctgaggac actgcagtct attactgtgc aaga**gaggcg**
gattacttct actttgacta ctggggccaa ggcaccactc tcacagtctc
ctca

[00110] The amino acid sequence of the VL Domain of LAG-3 mAb 6 (**SEQ ID NO:87**) is shown below (CDR residues are shown underlined):

DIVMTQSHRF MSTSVGDRVS ITC**KASQDVS SVVA**WYQQKP GQSPKLLIF**S**
ASYRYTGVPD RFTGSGSGTD FTFTISSVQA ADLAVYYCQQ **HYSTPWT**FGG
GTKLEIK

CDR_L1 of LAG-3 mAb 6 (**SEQ ID NO:89**): **KASQDVSSVVA**

CDR_L2 of LAG-3 mAb 6 (**SEQ ID NO:90**): **SASYRYT**

CDR_L3 of LAG-3 mAb 6 (**SEQ ID NO:91**): **HYSTPWT**

[00111] An exemplary polynucleotide that encodes the VL Domain of LAG-3 mAb 6 is **SEQ ID NO:88** (nucleotides encoding the CDRs are shown underlined):

gacattgtga tgacccagtc tcacagattc atgtccacat cagttggaga
cagggtcagc atcacctgc**a aggccagtca ggatgtgagt tctgttgtag**
cctggtatca acagaaacca ggacaatctc ctaaattact gattttt**cg**
gcatcctacc ggtacactgg agtccctgat cgcttcactg gcagtggatc
tgggacggat ttcactttca ccatcagcag tgtgcaggct gcagacctgg
cagtttatta ctgt**cagcaa cattatagta ctccgtggac g**ttcggtgga
ggcaccaagc tggaaatcaa a

[00112] Minor changes to the amino acid sequence of the VH and/or VL Domains provided herein are contemplated. For example, the C-terminal amino acid residue of any of the VH and/or VL Domains described herein may be substituted to facilitate sub-cloning.

V. Anti-Human LAG-3 Antibodies LAG-3 mAb 1, LAG-3 mAb 2, LAG-3 mAb 3, LAG-3 mAb 4, LAG-3 mAb 5, and/or LAG-3 mAb 6 and Their Derivatives Having an Engineered Fc Region

[00113] In traditional immune function, the interaction of antibody-antigen complexes with cells of the immune system results in a wide array of responses, ranging from effector functions such as antibody dependent cytotoxicity, mast cell degranulation, and phagocytosis to immunomodulatory signals such as regulating lymphocyte proliferation and antibody secretion. All of these interactions are initiated through the binding of the Fc Region of antibodies or immune complexes to specialized cell surface receptors on hematopoietic cells. The diversity of cellular responses triggered by antibodies and immune complexes results from the structural heterogeneity of the three Fc receptors: FcγRI (CD64), FcγRII (CD32), and FcγRIII (CD16). FcγRI (CD64), FcγRIIA (CD32A) and FcγRIII (CD16) are activating (*i.e.*, immune system enhancing) receptors; FcγRIIB (CD32B) is an inhibiting (*i.e.*, immune system dampening) receptor. The amino acid sequence of exemplary IgG1 (**SEQ ID NO:22**), IgG2 (**SEQ ID NO:23**), IgG3 (**SEQ ID NO: 27**), and IgG4 (**SEQ ID NO:24**) are presented above.

[00114] Modification of the Fc Region normally leads to an altered phenotype, for example altered serum half-life, altered stability, altered susceptibility to cellular enzymes or altered effector function. It may be desirable to modify the antibody of the invention with respect to effector function, so as to enhance the effectiveness of the antibody in treating cancer, for example. Reduction or elimination of effector function is desirable in certain cases, for example in the case of antibodies whose mechanism of action involves blocking or antagonism, but not killing of the cells bearing a target antigen. Increased effector function is generally desirable when directed to undesirable cells, such as tumor and foreign cells, where the FcγRs are expressed at low levels, for example, tumor-specific B cells with low levels of FcγRIIB (*e.g.*, non-Hodgkins lymphoma, CLL, and Burkitt's lymphoma). In said embodiments, molecules of the invention with conferred or altered effector function activity are useful for the treatment and/or prevention of a disease, disorder or infection where an enhanced efficacy of effector function activity is desired.

[00115] In certain embodiments, the LAG-3-binding molecules of the present invention comprise an Fc Region that possesses one or more modifications (*e.g.*, substitutions, deletions, or insertions) to the sequence of amino acids of a wild-type Fc Region (*e.g.*, **SEQ ID NO:1**), which reduce the affinity and avidity of the Fc Region and, thus, the molecule of the invention, for one or more FcγR receptors. In other embodiments, the molecules of the invention comprise an Fc Region that possesses one or more modifications to the amino acids of the wild-type Fc Region, which increase the affinity and avidity of the Fc Region and, thus, the molecule of the invention, for one or more FcγR receptors. In other embodiments, the molecules comprise a variant Fc Region wherein said variant confers or mediates increased antibody dependent cell mediated cytotoxicity (ADCC) activity and/or an increased binding to FcγRIIA, relative to a molecule comprising no Fc Region or comprising a wild-type Fc Region. In alternate embodiments, the molecules comprise a variant Fc Region wherein said variant confers or mediates decreased ADCC activity (or other effector function) and/or an increased binding to FcγRIIB, relative to a molecule comprising no Fc Region or comprising a wild-type Fc Region. In some embodiments, the invention encompasses LAG-3-binding molecules comprising a variant Fc Region, which variant Fc Region does not show a detectable binding to any FcγR, relative to a comparable molecule

comprising the wild-type Fc Region. In other embodiments, the invention encompasses LAG-3-binding molecules comprising a variant Fc Region, which variant Fc Region only binds a single FcγR, preferably one of FcγRIIA, FcγRIIB, or FcγRIIIA. Any such increased affinity and/or avidity is preferably assessed by measuring *in vitro* the extent of detectable binding to the FcγR or FcγR-related activity in cells that express low levels of the FcγR when binding activity of the parent molecule (without the modified Fc Region) cannot be detected in the cells, or in cells which express non-FcγR receptor target antigens at a density of 30,000 to 20,000 molecules/cell, at a density of 20,000 to 10,000 molecules/cell, at a density of 10,000 to 5,000 molecules/cell, at a density of 5,000 to 1,000 molecules/cell, at a density of 1,000 to 200 molecules/cell or at a density of 200 molecules/cell or less (but at least 10, 50, 100 or 150 molecules/cell).

[00116] The LAG-3-binding molecules of the present invention may comprise altered affinities for an activating and/or inhibitory Fcγ receptor. In one embodiment, the LAG-3-binding molecule comprises a variant Fc Region that has increased affinity for FcγRIIB and decreased affinity for FcγRIIIA and/or FcγRIIA, relative to a comparable molecule with a wild-type Fc Region. In another embodiment, the LAG-3-binding molecule of the present invention comprise a variant Fc Region, which has decreased affinity for FcγRIIB and increased affinity for FcγRIIIA and/or FcγRIIA, relative to a comparable molecule with a wild-type Fc Region. In yet another embodiment, the LAG-3-binding molecules of the present invention comprise a variant Fc Region that has decreased affinity for FcγRIIB and decreased affinity for FcγRIIIA and/or FcγRIIA, relative to a comparable molecule with a wild-type Fc Region. In still another embodiment, the LAG-3-binding molecules of the present invention comprise a variant Fc Region, which has unchanged affinity for FcγRIIB and decreased (or increased) affinity for FcγRIIIA and/or FcγRIIA, relative to a comparable molecule with a wild-type Fc Region.

[00117] In certain embodiments, the LAG-3-binding molecules of the present invention comprise a variant Fc Region having an altered affinity for FcγRIIIA and/or FcγRIIA such that the immunoglobulin has an enhanced effector function. Non-limiting examples of effector cell functions include antibody dependent cell mediated cytotoxicity, antibody dependent phagocytosis, phagocytosis, opsonization,

opsonophagocytosis, cell binding, rosetting, C1q binding, and complement dependent cell mediated cytotoxicity.

[00118] In a preferred embodiment, the alteration in affinity or effector function is at least 2-fold, preferably at least 4-fold, at least 5-fold, at least 6-fold, at least 7-fold, at least 8-fold, at least 9-fold, at least 10-fold, at least 50-fold, or at least 100-fold, relative to a comparable molecule comprising a wild-type Fc Region. In other embodiments of the invention, the variant Fc Region immunospecifically binds one or more FcRs with at least 65%, preferably at least 70%, 75%, 80%, 85%, 90%, 95%, 100%, 125%, 150%, 175%, 200%, 225%, or 250% greater affinity relative to a molecule comprising a wild-type Fc Region. Such measurements can be in vivo or in vitro assays, and in a preferred embodiment are in vitro assays such as ELISA or surface plasmon resonance assays.

[00119] In different embodiments, the LAG-3-binding molecules of the present invention comprise a variant Fc Region wherein said variant agonizes at least one activity of an FcγR receptor, or antagonizes at least one activity of an FcγR receptor. In a preferred embodiment, the molecules comprise a variant that antagonizes one or more activities of FcγRIIB, for example, B-cell receptor-mediated signaling, activation of B-cells, B-cell proliferation, antibody production, intracellular calcium influx of B cells, cell cycle progression, FcγRIIB-mediated inhibition of FcεRI signaling, phosphorylation of FcγRIIB, SHIP recruitment, SHIP phosphorylation and association with Shc, or activity of one or more downstream molecules (*e.g.*, MAP kinase, JNK, p38, or Akt) in the FcγRIIB signal transduction pathway. In another embodiment, the LAG-3-binding molecules of the present invention comprise a variant that agonizes one or more activities of FcεRI, for example, mast cell activation, calcium mobilization, degranulation, cytokine production, or serotonin release.

[00120] In certain embodiments, the molecules comprise an Fc Region comprising regions from two or more IgG isotypes (*e.g.*, IgG1, IgG2, IgG3 and IgG4). The various IgG isotypes exhibit differing physical and functional properties including serum half-life, complement fixation, FcγR binding affinities and effector function activities (*e.g.*, ADCC, CDC, *etc.*) due to differences in the amino acid sequences of their hinge and/or

Fc Regions, for example as described in Flesch and Neppert (1999) J. Clin. Lab. Anal. 14:141-156; Chappel *et al.* (1993) J. Biol. Chem. 33:25124-25131; Chappel *et al.* (1991) Proc. Natl. Acad. Sci. (U.S.A.) 88:9036-9040; or Brüggemann *et al.* (1987) J. Exp. Med 166:1351-1361. This type of variant Fc Region may be used alone, or in combination with an amino acid modification, to affect Fc-mediated effector function and/or binding activity. In combination, the amino acid modification and IgG hinge/Fc Region may display similar functionality (*e.g.*, increased affinity for FcγRIIA) and may act additively or, more preferably, synergistically to modify the effector functionality in the molecule of the invention, relative to a molecule of the invention comprising a wild-type Fc Region. In other embodiments, the amino acid modification and IgG Fc Region may display opposite functionality (*e.g.*, increased and decreased affinity for FcγRIIA, respectively) and may act to selectively temper or reduce a specific functionality in the molecule of the invention, relative to a molecule of the invention not comprising an Fc Region or comprising a wild-type Fc Region of the same isotype.

[00121] In a preferred specific embodiment, the LAG-3-binding molecules of the present invention comprise a variant Fc Region, wherein said variant Fc Region comprises at least one amino acid modification relative to a wild-type Fc Region, such that said molecule has an altered affinity for an FcR, provided that said variant Fc Region does not have a substitution at positions that make a direct contact with FcγR based on crystallographic and structural analysis of Fc-FcR interactions such as those disclosed by Sondermann *et al.* (2000) Nature 406:267-73. Examples of positions within the Fc Region that make a direct contact with FcγR are amino acid residues 234-239 (hinge region), amino acid residues 265-269 (B/C loop), amino acid residues 297-299 (C'/E loop), and amino acid residues 327-332 (F/G loop). In some embodiments, the molecules of the invention comprise variant Fc Regions comprise modification of at least one residue that does not make a direct contact with an FcγR based on structural and crystallographic analysis, *e.g.*, is not within the Fc-FcγR binding site.

[00122] Variant Fc Regions are well known in the art, and any known Fc variant may be used in the present invention to confer or modify the effector function exhibited by a molecule of the invention comprising an Fc Region (or portion thereof) as functionally assayed, *e.g.*, in an NK dependent or macrophage dependent assay. For

example, Fc Region variants identified as altering effector function are disclosed in PCT Publications No. WO 04/063351; WO 06/088494; WO 07/024249; WO 06/113665; WO 07/021841; WO 07/106707; and WO 2008/140603, and any suitable variant disclosed therein may be used in the present molecules.

[00123] In certain embodiments, the LAG-3-binding molecules of the present invention comprise a variant Fc Region, having one or more amino acid modifications in one or more regions, which modification(s) alter (relative to a wild-type Fc Region) the **Ratio of Affinities** of the variant Fc Region to an activating FcγR (such as FcγRIIA or FcγRIIIA) relative to an inhibiting FcγR (such as FcγRIIB):

$$\text{Ratio of Affinities} = \frac{\text{Wild-Type to Variant Change in Affinity to Fc}\gamma\text{R}_{\text{Activating}}}{\text{Wild-Type to Variant Change in Affinity to Fc}\gamma\text{R}_{\text{Inhibiting}}}$$

[00124] Particularly preferred are LAG-3-binding molecules of the present invention that possess a variant Fc Region (relative to the wild-type Fc Region) in which the Fc variant has a Ratio of Affinities greater than 1. Such molecules have particular use in providing a therapeutic or prophylactic treatment of a disease, disorder, or infection, or the amelioration of a symptom thereof, where an enhanced efficacy of effector cell function (*e.g.*, ADCC) mediated by FcγR is desired, *e.g.*, cancer or infectious disease. In contrast, an Fc variant having a Ratio of Affinities less than 1 mediates decreased efficacy of effector cell function. **Table 1** lists exemplary single, double, triple, quadruple and quintuple mutations by whether their Ratio of Affinities is greater than or less than 1.

Table 1
Exemplary Single and Multiple Mutations Listed by Ratio of Affinities

Single	Double	Triple	Quadruple	Quintuple
Ratio of Affinities > 1				
F243L D270E R292G R292P	F243L & R292P F243L & Y300L F243L & P396L D270E & P396L R292P & Y300L R292P & V305I R292P & P396L Y300L & P396L P396L & Q419H	F243L, P247L & N421K F243L, R292P & Y300L F243L, R292P & V305I F243L, R292P & P396L F243L, Y300L & P396L P247L, D270E & N421K R255L, D270E & P396L D270E, G316D & R416G D270E, K392T & P396L D270E, P396L & Q419H V284M, R292L & K370N R292P, Y300L & P396L	L234F, F243L, R292P & Y300L L235I, F243L, R292P & Y300L L235Q, F243L, R292P & Y300L F243L, P247L, D270E & N421K F243L, R255L, D270E & P396L F243L, D270E, G316D & R416G F243L, D270E, K392T & P396L F243L, D270E, P396L & Q419H F243L, R292P, Y300L, & P396L F243L, R292P, V305I & P396L P247L, D270E, Y300L & N421K R255L, D270E, R292G & P396L R255L, D270E, Y300L & P396L D270E, G316D, P396L & R416G	L235V, F243L, R292P, Y300L & P396L L235P, F243L, R292P, Y300L & P396L F243L, R292P, V305I, Y300L & P396L
Ratio of Affinities < 1				
Y300L P396L	F243L & P396L P247L & N421K R255L & P396L R292P & V305I K392T & P396L P396L & Q419H	F243L, R292P & V305I		

[00125] In a specific embodiment, in variant Fc Regions, any amino acid modifications (*e.g.*, substitutions) at any of positions 235, 240, 241, 243, 244, 247, 262, 263, 269, 298, 328, or 330 and preferably one or more of the following residues: A240, I240, L241, L243, H244, N298, I328 or V330. In a different specific embodiment, in variant Fc Regions, any amino acid modifications (*e.g.*, substitutions) at any of positions 268, 269, 270, 272, 276, 278, 283, 285, 286, 289, 292, 293, 301, 303, 305, 307, 309, 331, 333, 334, 335, 337, 338, 340, 360, 373, 376, 416, 419, 430, 434, 435, 437, 438 or 439 and preferably one or more of the following residues: H280, Q280, Y280, G290, S290, T290, Y290, N294, K295, P296, D298, N298, P298, V298, I300 or L300.

[00126] In a preferred embodiment, in variant Fc Regions that bind an FcγR with an altered affinity, any amino acid modifications (*e.g.*, substitutions) at any of positions 255, 256, 258, 267, 268, 269, 270, 272, 276, 278, 280, 283, 285, 286, 289, 290, 292, 293, 294, 295, 296, 298, 300, 301, 303, 305, 307, 309, 312, 320, 322, 326, 329, 330, 332, 331, 333, 334, 335, 337, 338, 339, 340, 359, 360, 373, 376, 416, 419, 430, 434, 435, 437, 438 or 439. Preferably, the variant Fc Region has any of the following residues: A256, N268, Q272, D286, Q286, S286, A290, S290, A298, M301, A312, E320, M320, Q320, R320, E322, A326, D326, E326, N326, S326, K330, T339, A333, A334, E334, I334, L334, M334, Q334, V334, K335, Q335, A359, A360 or A430.

[00127] In a different embodiment, in variant Fc Regions that bind an FcγR (via its Fc Region) with a reduced affinity, any amino acid modifications (*e.g.*, substitutions) at any of positions 252, 254, 265, 268, 269, 270, 278, 289, 292, 293, 294, 295, 296, 298, 300, 301, 303, 322, 324, 327, 329, 333, 335, 338, 340, 373, 376, 382, 388, 389, 414, 416, 419, 434, 435, 437, 438 or 439.

[00128] In a different embodiment, in variant Fc Regions that bind an FcγR (via its Fc Region) with an enhanced affinity, any amino acid modifications (*e.g.*, substitutions) at any of positions 280, 283, 285, 286, 290, 294, 295, 298, 300, 301, 305, 307, 309, 312, 315, 331, 333, 334, 337, 340, 360, 378, 398 or 430. In a different embodiment, in variant Fc Regions that binds FcγRIIA with an enhanced affinity, any of the following residues: A255, A256, A258, A267, A268, N268, A272, Q272, A276, A280, A283,

A285, A286, D286, Q286, S286, A290, S290, M301, E320, M320, Q320, R320, E322, A326, D326, E326, S326, K330, A331, Q335, A337 or A430.

[00129] Preferred variants include one or more modifications at any of positions: 228, 230, 231, 232, 233, 234, 235, 239, 240, 241, 243, 244, 245, 247, 262, 263, 264, 265, 266, 271, 273, 275, 281, 284, 291, 296, 297, 298, 299, 302, 304, 305, 313, 323, 325, 326, 328, 330 or 332.

[00130] Particularly preferred variants include one or more modifications selected from groups A-AI:

A	228E, 228K, 228Y or 228G;
B	230A, 230E, 230Y or 230G;
C	231E, 231K, 231Y, 231P or 231G;
D	232E, 232K, 232Y, 232G;
E	233D;
F	234I or 234F;
G	235D, 235Q, 235P, 235I or 235V;
H	239D, 239E, 239N or 239Q;
I	240A, 240I, 240M or 240T;
J	243R, 243, 243Y, 243L, 243Q, 243W, 243H or 243I;
K	244H;
L	245A;
M	247G, 247V or 247L;
N	262A, 262E, 262I, 262T, 262E or 262F;
O	263A, 263I, 263M or 263T;
P	264F, 264E, 264R, 264I, 264A, 264T or 264W;
Q	265F, 265Y, 265H, 265I, 265L, 265T, 265V, 265N or 265Q;
R	266A, 266I, 266M or 266T;
S	271D, 271E, 271N, 271Q, 271K, 271R, 271S, 271T, 271H, 271A, 271V, 271L, 271I, 271F, 271M, 271Y, 271W or 271G;
T	273I;
U	275L or 275W;
V	281D, 281K, 281Y or 281P;
W	284E, 284N, 284T, 284L, 284Y or284M;
X	291D, 291E, 291Q, 291T, 291H, 291I or 291G;
Y	299A, 299D, 299E, 299F, 299G, 299H, 299I, 299K, 299L, 299M, 299N, 299P, 299Q, 299R, 299S, 299V, 299W or 299Y;
Z	302I;
AA	304D, 304N, 304T, 304H or 304L
AB	305I;
AC	313F;
AD	323I;
AE	325A, 325D, 325E, 325G, 325H, 325I, 325L, 325K, 325R, 325S, 325F, 325M, 325T, 325V, 325Y, 325W or 325P;
AF	328D, 328Q, 328K, 328R, 328S, 328T, 328V, 328I, 328Y, 328W, 328P, 328G, 328A, 328E, 328F, 328H, 328M or 328N;
AG	330L, 330Y, 330I or 330V;
AH	332A, 332D, 332E, 332H, 332N, 332Q, 332T, 332K, 332R, 332S, 332V, 332L, 332F, 332M, 332W, 332P, 332G or 332Y; and
AI	336E, 336K or 336Y

[00131] Still more particularly preferred variants include one or more modifications selected from Groups 1-105:

Group	Variant	Group	Variant
1	A330L / I332E	54	S239D / D265L / N297D / I332E
2	D265F / N297E / I332E	55	S239D / D265T / N297D / I332E
3	D265Y / N297D / I332E	56	S239D / D265V / N297D / I332E
4	D265Y / N297D / T299L / I332E	57	S239D / D265Y / N297D / I332E
5	F241E / F243Q / V262T / V264F	58	S239D / I332D
6	F241E / F243Q / V262T / V264E / I332E	59	S239D / I332E
7	F241E / F243R / V262E / V264R	60	S239D / I332E / A330I
8	F241E / F243R / V262E / V264R / I332E	61	S239D / I332N
9	F241E / F243Y / V262T / V264R	62	S239D / I332Q
10	F241E / F243Y / V262T / V264R / I332E	63	S239D / N297D / I332E
11	F241L / F243L / V262I / V264I	64	S239D / N297D / I332E / A330Y
12	F241L / V262I	65	S239D / N297D / I332E / A330Y / F241S / F243H / V262T / V264T
13	F241R / F243Q / V262T / V264R	66	S239D / N297D / I332E / K326E
14	F241R / F243Q / V262T / V264R / I332E	67	S239D / N297D / I332E / L235D
15	F241W / F243W / V262A / V264A	68	S239D / S298A / I332E
16	F241Y / F243Y / V262T / V264T	69	S239D / V264I / A330L / I332E
17	F241Y / F243Y / V262T / V264T / N297D / I332E	70	S239D / V264I / I332E
18	F243L / V262I / V264W	71	S239D / V264I / S298A / I332E
19	P243L / V264I	72	S239E / D265N
20	L328D / I332E	73	S239E / D265Q
21	L328E / I332E	74	S239E / I332D
22	L328H / I332E	75	S239E / I332E
23	L328I / I332E	76	S239E / I332N
24	L328M / I332E	77	S239E / I332Q
25	L328N / I332E	78	S239E / N297D / I332E
26	L328Q / I332E	79	S239E / V264I / A330Y / I332 E
27	L328T / I332E	80	S239E / V264I / I332 E
28	L328V / I332E	81	S239E / V264I / S298A / A330Y / I332E
29	N297D / A330Y / I332E	82	S239N / A330L / I332E
30	N297D / I332E	83	S239N / A330Y / I332E
31	N297D / I332E / S239D / A330L	84	S239N / I332D
32	N297D / S298A / A330Y / I 332E	85	S239N / I332E
33	N297D / T299L / I332E	86	S239N / I332N
34	N297D / T299F / I332E / N297D / T299H / I332E	87	S239N / I332Q
35	N297D / T299I / I332E	88	S239N1S298A / I332E
36	N297D / T299L / I332E	89	S239Q / I332D
37	N297D / T299V / I332E	90	S239Q / I332E
38	N297E / I332E	91	S239Q / I332N
39	N297S / I332E	92	S239Q / I332Q
40	P230A / E233D / I332E	93	S239Q / V264I / I332E
41	P244H / P245A / P247V	94	S298A / I332E
42	S239D / A330L / I332E	95	V264E / N297D / I332E
43	S239D / A330Y / I332E	96	V264I / A330L / I332E

[00131] Still more particularly preferred variants include one or more modifications selected from Groups 1-105:

Group	Variant	Group	Variant
1	A330L / I332E	54	S239D / D265L / N297D / I332E
2	D265F / N297E / I332E	55	S239D / D265T / N297D / I332E
3	D265Y / N297D / I332E	56	S239D / D265V / N297D / I332E
4	D265Y / N297D / T299L / I332E	57	S239D / D265Y / N297D / I332E
5	F241E / F243Q / V262T / V264P	58	S239D / I332D
6	F241E / F243Q / V262T / V264E / I332E	59	S239D / I332E
7	F241E / F243R / V262E / V264R	60	S239D / I332E / A330I
8	F241E / F243R / V262E / V264R / I332E	61	S239D / I332N
9	F241E / F243Y / V262T / V264R	62	S239D / I332Q
10	F241E / F243Y / V262T / V264R / I332E	63	S239D / N297D / I332E
11	F241L / F243L / V262I / V264I	64	S239D / N297D / I332E / A330Y
12	F241L / V262I	65	S239D / N297D / I332E / A330Y / F241S / F243H / V262T / V264T
13	F241R / F243Q / V262T / V264R	66	S239D / N297D / I332E / K326E
14	F241R / F243Q / V262T / V264R / I332E	67	S239D / N297D / I332E / L235D
15	F241W / F243W / V262A / V264A	68	S239D / S298A / I332E
16	F241Y / F243Y / V262T / V264T	69	S239D / V264I / A330L / I332E
17	F241Y / F243Y / V262T / V264T / N297D / I332E	70	S239D / V264I / I332E
18	F243L / V262I / V264W	71	S239D / V264I / S298A / I332E
19	P243L / V264I	72	S239E / D265N
20	L328D / I332E	73	S239E / D265Q
21	L328E / I332E	74	S239E / I332D
22	L328H / I332E	75	S239E / I332E
23	L328I / I332E	76	S239E / I332N
24	L328M / I332E	77	S239E / I332Q
25	L328N / I332E	78	S239E / N297D / I332E
26	L328Q / I332E	79	S239E / V264I / A330Y / I332 E
27	L328T / I332E	80	S239E / V264I / I332 E
28	L328V / I332E	81	S239E / V264I / S298A / A330Y / I332E
29	N297D / A330Y / I332E	82	S239N / A330L / I332E
30	N297D / I332E	83	S239N / A330Y / I332E
31	N297D / I332E / S239D / A330L	84	S239N / I332D
32	N297D / S298A / A330Y / I 332E	85	S239N / I332E
33	N297D / T299L / I332E	86	S239N / I332N
34	N297D / T299F / I332E / N297D / T299H / I332E	87	S239N / I332Q
35	N297D / T299I / I332E	88	S239N1S298A / I332E
36	N297D / T299L / I332E	89	S239Q / I332D
37	N297D / T299V / I332E	90	S239Q / I332E
38	N297E / I332E	91	S239Q / I332N
39	N297S / I332E	92	S239Q / I332Q
40	P230A / E233D / I332E	93	S239Q / V264I / I332E
41	P244H / P245A / P247V	94	S298A / I332E
42	S239D / A330L / I332E	95	V264E / N297D / I332E
43	S239D / A330Y / I332E	96	V264I / A330L / I332E

44	S239D / A330Y / I332E / K326E	97	V264I / A330Y / I332E
45	S239D / A330Y / I332E / K326T	98	V264L / I332E
46	S239D / A330Y / I332E / L234I	99	V264T / S298A / I332E
47	S239D / A330Y / I332E / L235D	100	Y296D / N297D / I332E
48	S239D / A330Y / I332E / V240I	101	Y296E / N297D / I332 E
49	S239D / A330Y / I332E / V264T	102	Y296H / N297D / I332E
50	S239D / A330Y / I332E / V266I	103	Y296N / N297D / I332E
51	S239D / D265F / N297D / I332E	104	Y296Q / N297I / I332E
52	S239D / D265H / N297D / I332E	105	Y296T / N297D / I332E
53	S239D / D265I / N297D / I332E		

[00132] In one embodiment, a LAG-3 binding molecule of the invention will comprise a variant Fc Region having at least one modification in the Fc Region. In certain embodiments, the variant Fc Region comprises at least one substitution selected from the group consisting of L235V, F243L, R292P, Y300L, V305I, and P396L, wherein said numbering is that of the EU index as in Kabat.

[00133] In a specific embodiment, the variant Fc Region comprises:

(A) at least one substitution selected from the group consisting of F243L, R292P, Y300L, V305I, and P396L;

(B) at least two substitutions selected from the group consisting of:

(1) F243L and P396L;

(2) F243L and R292P; and

(3) R292P and V305I;

(C) at least three substitutions selected from the group consisting of:

(1) F243L, R292P and Y300L;

(2) F243L, R292P and V305I;

(3) F243L, R292P and P396L; and

(4) R292P, V305I and P396L;

(D) at least four substitutions selected from the group consisting of:

(1) F243L, R292P, Y300L and P396L; and

(2) F243L, R292P, V305I and P396L; or

(E) at least the five substitutions selected from the group consisting of:

(1) F243L, R292P, Y300L, V305I and P396L; and

(2) L235V, F243L, R292P, Y300L and P396L.

[00134] In another specific embodiment, the variant Fc Region comprises substitutions of:

(A) F243L, R292P, and Y300L;

(B) L235V, F243L, R292P, Y300L, and P396L; or

(C) F243L, R292P, Y300L, V305I, and P396L.

[00135] In other embodiments, the invention encompasses the use of any Fc variant known in the art, such as those disclosed in Jefferis, B.J. *et al.* (2002) "*Interaction Sites On Human IgG-Fc For FcgammaR: Current Models*," Immunol. Lett. 82:57-65; Presta, L.G. *et al.* (2002) "*Engineering Therapeutic Antibodies For Improved Function*," Biochem. Soc. Trans. 30:487-90; Idusogie, E.E. *et al.* (2001) "*Engineered Antibodies With Increased Activity To Recruit Complement*," J. Immunol. 166:2571-75; Shields, R.L. *et al.* (2001) "*High Resolution Mapping Of The Binding Site On Human IgG1 For Fc Gamma RI, Fc Gamma RII, Fc Gamma RIII, And FcRn And Design Of IgG1 Variants With Improved Binding To The Fc gamma R*," J. Biol. Chem. 276:6591-6604; Idusogie, E.E. *et al.* (2000) "*Mapping Of The C1q Binding Site On Rituxan, A Chimeric Antibody With A Human IgG Fc*," J. Immunol. 164:4178-84; Reddy, M.P. *et al.* (2000) "*Elimination Of Fc Receptor-Dependent Effector Functions Of A Modified IgG4 Monoclonal Antibody To Human CD4*," J. Immunol. 164:1925-1933; Xu, D. *et al.* (2000) "*In Vitro Characterization of Five Humanized OKT3 Effector Function Variant Antibodies*," Cell. Immunol. 200:16-26; Armour, K.L. *et al.* (1999) "*Recombinant human IgG Molecules Lacking Fcgamma Receptor I Binding And Monocyte Triggering Activities*," Eur. J. Immunol. 29:2613-24; Jefferis, R. *et al.* (1996) "*Modulation Of Fc(Gamma)R And Human Complement Activation By IgG3-Core Oligosaccharide Interactions*," Immunol. Lett. 54:101-04; Lund, J. *et al.* (1996) "*Multiple Interactions Of IgG With Its Core Oligosaccharide Can Modulate Recognition By Complement And Human Fc Gamma Receptor I And Influence The Synthesis Of Its Oligosaccharide Chains*," J. Immunol. 157:4963-4969; Hutchins *et al.* (1995) "*Improved Biodistribution, Tumor Targeting, And Reduced Immunogenicity In Mice With A Gamma 4 Variant Of Campath-1H*," Proc. Natl. Acad. Sci. (U.S.A.) 92:11980-84; Jefferis, R. *et al.* (1995) "*Recognition Sites On Human IgG For Fc Gamma Receptors: The Role Of Glycosylation*," Immunol. Lett. 44:111-17; Lund, J. *et al.* (1995) "*Oligosaccharide-Protein Interactions In IgG Can Modulate Recognition By Fc Gamma Receptors*," FASEB J. 9:115-19; Alegre, M.L. *et al.* (1994) "*A Non-Activating "Humanized" Anti-CD3 Monoclonal Antibody Retains Immunosuppressive Properties*

In Vivo," Transplantation 57:1537-1543; Lund *et al.* (1992) "*Multiple Binding Sites On The CH2 Domain Of IgG For Mouse Fc Gamma R11*," Mol. Immunol. 29:53-59; Lund *et al.* (1991) "*Human Fc Gamma RI And Fc Gamma RII Interact With Distinct But Overlapping Sites On Human IgG*," J. Immunol. 147:2657-2662; Duncan, A.R. *et al.* (1988) "*Localization Of The Binding Site For The Human High-Affinity Fc Receptor On IgG*," Nature 332:563-564; US Patent Nos. 5,624,821; 5,885,573; 6,194,551; 7,276,586; and 7,317,091; and PCT Publications WO 00/42072 and PCT WO 99/58572.

[00136] In some embodiments, the molecules of the invention further comprise one or more glycosylation sites, so that one or more carbohydrate moieties are covalently attached to the molecule. Preferably, the molecules of the invention with one or more glycosylation sites and/or one or more modifications in the Fc Region confer or have an enhanced antibody mediated effector function, *e.g.*, enhanced ADCC activity, compared to the unmodified antibody. In some embodiments, the invention further comprises molecules comprising one or more modifications of amino acids that are directly or indirectly known to interact with a carbohydrate moiety of the Fc Region, including but not limited to amino acids at positions 241, 243, 244, 245, 245, 249, 256, 258, 260, 262, 264, 265, 296, 299, and 301. Amino acids that directly or indirectly interact with a carbohydrate moiety of an Fc Region are known in the art, *see, e.g.*, Jefferis *et al.*, 1995 *Immunology Letters*, 44: 111-7, which is incorporated herein by reference in its entirety.

[00137] In another embodiment, the invention encompasses molecules that have been modified by introducing one or more glycosylation sites into one or more sites of the molecules, preferably without altering the functionality of the molecules, *e.g.*, binding activity to target antigen or FcγR. Glycosylation sites may be introduced into the variable and/or constant region of the molecules of the invention. As used herein, "glycosylation sites" include any specific amino acid sequence in an antibody to which an oligosaccharide (*i.e.*, carbohydrates containing two or more simple sugars linked together) will specifically and covalently attach. Oligosaccharide side chains are typically linked to the backbone of an antibody via either N-or O-linkages. N-linked glycosylation refers to the attachment of an oligosaccharide moiety to the side chain of

an asparagine residue. O-linked glycosylation refers to the attachment of an oligosaccharide moiety to a hydroxyamino acid, *e.g.*, serine, threonine. The molecules of the invention may comprise one or more glycosylation sites, including N-linked and O-linked glycosylation sites. Any glycosylation site for N-linked or O-linked glycosylation known in the art may be used in accordance with the instant invention. An exemplary N-linked glycosylation site that is useful in accordance with the methods of the present invention is the amino acid sequence: Asn-X-Thr/Ser, wherein X may be any amino acid and Thr/Ser indicates a threonine or a serine. Such a site or sites may be introduced into a molecule of the invention using methods well known in the art to which this invention pertains (see for example, *IN VITRO* MUTAGENESIS, RECOMBINANT DNA: A SHORT COURSE, J. D. Watson, *et al.* W.H. Freeman and Company, New York, 1983, chapter 8, pp. 106-116, which is incorporated herein by reference in its entirety. An exemplary method for introducing a glycosylation site into a molecule of the invention may comprise: modifying or mutating an amino acid sequence of the molecule so that the desired Asn-X-Thr/Ser sequence is obtained.

[00138] In some embodiments, the invention encompasses methods of modifying the carbohydrate content of a molecule of the invention by adding or deleting a glycosylation site. Methods for modifying the carbohydrate content of antibodies (and molecules comprising antibody domains, *e.g.*, Fc Region) are well known in the art and encompassed within the invention, *see*, *e.g.*, U.S. Patent No. 6,218,149; EP 0 359 096 B1; U.S. Publication No. US 2002/0028486; WO 03/035835; U.S. Publication No. 2003/0115614; U.S. Patent No. 6,218,149; U.S. Patent No. 6,472,511; all of which are incorporated herein by reference in their entirety. In other embodiments, the invention encompasses methods of modifying the carbohydrate content of a molecule of the invention by deleting one or more endogenous carbohydrate moieties of the molecule. In a specific embodiment, the invention encompasses shifting the glycosylation site of the Fc Region of an antibody, by modifying positions adjacent to 297. In a specific embodiment, the invention encompasses modifying position 296 so that position 296 and not position 297 is glycosylated.

[00139] Effector function can also be modified by techniques such as by introducing one or more cysteine residues into the Fc Region, thereby allowing interchain disulfide

bond formation in this region to occur, resulting in the generation of a homodimeric antibody that may have improved internalization capability and/or increased complement-mediated cell killing and ADCC (Caron, P.C. *et al.* (1992) "*Engineered Humanized Dimeric Forms Of IgG Are More Effective Antibodies*," J. Exp. Med. 176:1191-1195; Shopes, B. (1992) "*A Genetically Engineered Human IgG Mutant With Enhanced Cytolytic Activity*," J. Immunol. 148(9):2918-2922. Homodimeric antibodies with enhanced antitumor activity may also be prepared using heterobifunctional cross-linkers as described in Wolff, E.A. *et al.* (1993) "*Monoclonal Antibody Homodimers: Enhanced Antitumor Activity In Nude Mice*," Cancer Research 53:2560-2565. Alternatively, an antibody can be engineered which has dual Fc Regions and may thereby have enhanced complement lysis and ADCC capabilities (Stevenson, G.T. *et al.* (1989) "*A Chimeric Antibody With Dual Fc Regions (bisFabFc) Prepared By Manipulations At The IgG Hinge*," Anti-Cancer Drug Design 3:219-230).

[00140] The serum half-life of proteins comprising Fc Regions may be increased by increasing the binding affinity of the Fc Region for FcRn. The term "half-life" as used herein means a pharmacokinetic property of a molecule that is a measure of the mean survival time of the molecules following their administration. Half-life can be expressed as the time required to eliminate fifty percent (50%) of a known quantity of the molecule from the subjects body (*e.g.*, human patient or other mammal) or a specific compartment thereof, for example, as measured in serum, *i.e.*, circulating half-life, or in other tissues. In general, an increase in half-life results in an increase in mean residence time (MRT) in circulation for the molecule administered.

[00141] In some embodiments, the LAG-3-binding molecules of the present invention comprise a variant Fc Region, wherein said variant Fc Region comprises at least one amino acid modification relative to a wild-type Fc Region, such that said molecule has an increased half-life (relative to a wild-type Fc Region).

[00142] In some embodiments, the LAG-3-binding molecules of the present invention comprise a variant Fc Region, wherein said variant Fc Region comprises a half-live extending amino acid substitution at one or more positions selected from the group consisting of 238, 250, 252, 254, 256, 257, 256, 265, 272, 286, 303, 305, 307,

309, 311, 312, 317, 340, 356, 360, 362, 376, 378, 380, 382, 413, 424, 428, 433, 434, as numbered by the EU index as set forth in Kabat. Numerous specific mutations capable of increasing the half-life of an Fc-containing molecule are known in the art and include, for example 252Y, 254T, 256E, and combinations thereof. For example, see the mutations described in U.S. Patent Nos. 6,277,375, 7,083,784; 7,217,797, 8,088,376; U.S. Publication Nos. 2002/0147311; 2007/0148164; and International Publication Nos. WO 98/23289 and WO 09/058492, which are herein incorporated by reference in their entireties. Fc comprising molecules with enhanced half-life also include those with substitution two or more of Fc Region residues 250, 252, 254, 256, 257, 309, 311, 428, 433, 434, (U.S. Pat. Nos. 7,083,784 and 8,088,376).

[00143] In a specific embodiment, the variant Fc Region comprises substitutions of:

(A) 252Y, 254T and 256E;

(B) 250Q and 428L;

(C) T307Q and N434A;

(D) A378V and N434A;

(E) N434A and Y436I; or

(F) V308P/N434A.

VI. Anti-Human LAG-3 Diabodies

[00144] One embodiment of the present invention relates to bispecific diabodies that are capable of binding to a "**first epitope**" and a "**second epitope**," wherein the first epitope is an epitope of human LAG-3 and the second epitope is an epitope of a molecule involved in regulating an immune checkpoint present on the surface of an immune cell, such as a T lymphocyte. The second epitope is preferably not an epitope of LAG-3. In one embodiment, the second epitope is an epitope of B7-H3, B7-H4, BTLA, CD3, CD8, CD16, CD27, CD32, CD40, CD40L, CD47, CD64, CD70, CD80, CD86, CD94, CD137, CD137L, CD226, CTLA-4, Galectin-9, GITR, GITRL, HHLA2, ICOS, ICOSL, KIR, LAG-3, LIGHT, MHC class I or II, NKG2a, NKG2d, OX40, OX40L, PD1H, PD-1, PD-L1, PD-L2, PVR, SIRPa, TCR, TIGIT, TIM-3 or VISTA. In a specific embodiment, the second epitope is CD137, PD-1, OX40, TIGIT, or TIM-3.

1. Bispecific Diabodies Lacking Fc Regions

[00145] One embodiment of the present invention relates to bispecific monovalent diabodies that comprise, and most preferably are composed of, a first polypeptide chain and a second polypeptide chain, whose sequences permit the polypeptide chains to covalently bind to each other to form a covalently associated complex that is capable of simultaneously binding to LAG-3 and the second epitope (*e.g.* B7-H3, B7-H4, BTLA, CD40, CD80, CD86, CD137, CTLA-4, ICOS, KIR, MHC class I or II, OX40, PD-1, PD-L1, TCR, TIM-3, *etc.*).

[00146] The first polypeptide chain of such an embodiment of bispecific monovalent diabodies comprises, in the N-terminal to C-terminal direction, an N-terminus, the VL Domain of a monoclonal antibody capable of binding to either the first or second epitope (*i.e.*, either VL_{LAG-3} or $VL_{Epitope\ 2}$), a first intervening spacer peptide (Linker 1), a VH Domain of a monoclonal antibody capable of binding to either the second epitope (if such first polypeptide chain contains VL_{LAG-3}) or the first epitope (if such first polypeptide chain contains $VL_{Epitope\ 2}$), a cysteine-containing second intervening spacer peptide (Linker 2), a Heterodimer-Promoting Domain and a C-terminus (**Figure 1**). The notation "**VL1**" and "**VH1**" denote respectively, the Variable Light Chain Domain and Variable Heavy Chain Domain that bind the "first" epitope. Similarly, the notation "**VL2**" and "**VH2**"denote respectively, the Variable Light Chain Domain and Variable Heavy Chain Domain that bind the "second" epitope.

[00147] The second polypeptide chain of this embodiment of bispecific monovalent diabodies comprises, in the N-terminal to C-terminal direction, an N-terminus, a VL Domain of a monoclonal antibody capable of binding to either LAG-3 or the second epitope (*i.e.*, either VL_{LAG-3} or $VL_{Epitope\ 2}$, and being the VL Domain not selected for inclusion in the first polypeptide chain of the diabody), an intervening linker peptide (Linker 1), a VH Domain of a monoclonal antibody capable of binding to either the second epitope (if such second polypeptide chain contains VL_{LAG-3}) or to LAG-3 (if such second polypeptide chain contains $VL_{Epitope\ 2}$), a spacer peptide (Linker 2) optionally containing a cysteine residue, a Heterodimer-Promoting Domain, and a C-terminus (**Figure 1**).

[00148] The VL Domain of the first polypeptide chain interacts with the VH Domain of the second polypeptide chain to form a first functional antigen-binding site that is specific for a first antigen (*i.e.*, either LAG-3 or an antigen that contains the second epitope). Likewise, the VL Domain of the second polypeptide chain interacts with the VH Domain of the first polypeptide chain in order to form a second functional antigen-binding site that is specific for a second antigen (*i.e.*, either an antigen that contains the second epitope or LAG-3). Thus, the selection of the VL and VH Domains of the first and second polypeptide chains is coordinated, such that the two polypeptide chains of the diabody collectively comprise VL and VH Domains capable of binding to both an epitope of LAG-3 and to the second epitope (*i.e.*, they comprise VL_{LAG-3}/VH_{LAG-3} and $VL_{Epitope\ 2}/VH_{Epitope\ 2}$).

[00149] Most preferably, the length of the intervening linker peptide (Linker 1, which separates such VL and VH Domains) is selected to substantially or completely prevent the VL and VH Domains of the polypeptide chain from binding to one another. Thus the VL and VH Domains of the first polypeptide chain are substantially or completely incapable of binding to one another. Likewise, the VL and VH Domains of the second polypeptide chain are substantially or completely incapable of binding to one another. A preferred intervening spacer peptide (Linker 1) has the sequence (**SEQ ID NO:33**): GGGSGGGG.

[00150] The cysteine-containing second intervening spacer peptide (Linker 2) will contain 1, 2, 3 or more cysteines. A preferred cysteine-containing spacer peptide (Linker 2) has the sequence is **SEQ ID NO:34**: GGCGGG. Alternatively, Linker 2 does not comprise a cysteine and a Cysteine-Containing Heterodimer-Promoting Domain, as described below is used. Optionally, both a cysteine-containing Linker 2 and a cysteine-containing Heterodimer-Promoting Domain are used.

[00151] The Heterodimer-Promoting Domains may be GVEPKSC (**SEQ ID NO:35**) or VEPKSC (**SEQ ID NO:36**) or AEPKSC (**SEQ ID NO:115**) on one polypeptide chain and GFNRGEC (**SEQ ID NO:37**) or FNRGEC (**SEQ ID NO:38**) on the other polypeptide chain (US2007/0004909).

[00152] More preferably, however, the Heterodimer-Promoting Domains of such diabodies are formed from one, two, three or four tandemly repeated coil domains of opposing charge that comprise a sequence of at least six, at least seven or at least eight charged amino acid residues (Apostolovic, B. *et al.* (2008) "*pH-Sensitivity of the E3/K3 Heterodimeric Coiled Coil*," Biomacromolecules 9:3173–3180; Arndt, K.M. *et al.* (2001) "*Helix-stabilized Fv (hsFv) Antibody Fragments: Substituting the Constant Domains of a Fab Fragment for a Heterodimeric Coiled-coil Domain*," J. Molec. Biol. 312:221-228; Arndt, K.M. *et al.* (2002) "*Comparison of In Vivo Selection and Rational Design of Heterodimeric Coiled Coils*," Structure 10:1235-1248; Boucher, C. *et al.* (2010) "*Protein Detection By Western Blot Via Coiled–Coil Interactions*," Analytical Biochemistry 399:138-140; Cachia, P.J. *et al.* (2004) "*Synthetic Peptide Vaccine Development: Measurement Of Polyclonal Antibody Affinity And Cross-Reactivity Using A New Peptide Capture And Release System For Surface Plasmon Resonance Spectroscopy*," J. Mol. Recognit. 17:540-557; De Crescenzo, G.D. *et al.* (2003) "*Real-Time Monitoring of the Interactions of Two-Stranded de novo Designed Coiled-Coils: Effect of Chain Length on the Kinetic and Thermodynamic Constants of Binding*," Biochemistry 42:1754-1763; Fernandez-Rodriquez, J. *et al.* (2012) "*Induced Heterodimerization And Purification Of Two Target Proteins By A Synthetic Coiled-Coil Tag*," Protein Science 21:511-519; Ghosh, T.S. *et al.* (2009) "*End-To-End And End-To-Middle Interhelical Interactions: New Classes Of Interacting Helix Pairs In Protein Structures*," Acta Crystallographica D65:1032-1041; Grigoryan, G. *et al.* (2008) "*Structural Specificity In Coiled-Coil Interactions*," Curr. Opin. Struc. Biol. 18:477-483; Litowski, J.R. *et al.* (2002) "*Designing Heterodimeric Two-Stranded α-Helical Coiled-Coils: The Effects Of Hydrophobicity And α-Helical Propensity On Protein Folding, Stability, And Specificity*," J. Biol. Chem. 277:37272-37279; Steinkruger, J.D. *et al.* (2012) "*The d'--d--d' Vertical Triad is Less Discriminating Than the a'--a--a' Vertical Triad in the Antiparallel Coiled-coil Dimer Motif*," J. Amer. Chem. Soc. 134(5):2626–2633; Straussman, R. *et al.* (2007) "*Kinking the Coiled Coil – Negatively Charged Residues at the Coiled-coil Interface*," J. Molec. Biol. 366:1232-1242; Tripet, B. *et al.* (2002) "*Kinetic Analysis of the Interactions between Troponin C and the C-terminal Troponin I Regulatory Region and Validation of a New Peptide Delivery/Capture System used for Surface Plasmon Resonance*," J. Molec. Biol.

323:345–362; Woolfson, D.N. (2005) "*The Design Of Coiled-Coil Structures And Assemblies*," Adv. Prot. Chem. 70:79-112; Zeng, Y. *et al.* (2008) "*A Ligand-Pseudoreceptor System Based On de novo Designed Peptides For The Generation Of Adenoviral Vectors With Altered Tropism*," J. Gene Med. 10:355-367).

[00153] Such repeated coil domains may be exact repeats or may have substitutions. For example, the Heterodimer-Promoting Domain of the first polypeptide chain may comprise a sequence of eight negatively charged amino acid residues and the Heterodimer-Promoting Domain of the second polypeptide chain may comprise a sequence of eight negatively charged amino acid residues. It is immaterial which coil is provided to the first or second polypeptide chains, provided that a coil of opposite charge is used for the other polypeptide chain. The positively charged amino acid may be lysine, arginine, histidine, *etc.* and/or the negatively charged amino acid may be glutamic acid, aspartic acid, *etc.* The positively charged amino acid is preferably lysine and/or the negatively charged amino acid is preferably glutamic acid. It is possible for only a single Heterodimer-Promoting Domain to be employed (since such domain will inhibit homodimerization and thereby promote heterodimerization), however, it is preferred for both the first and second polypeptide chains of the diabodies of the present invention to contain Heterodimer-Promoting Domains.

[00154] In a preferred embodiment, one of the Heterodimer-Promoting Domains will comprise four tandem "E-coil" helical domains (**SEQ ID NO:39**: EVAALEK-EVAALEK-EVAALEK-EVAALEK), whose glutamate residues will form a negative charge at pH 7, while the other of the Heterodimer-Promoting Domains will comprise four tandem "K-coil" domains (**SEQ ID NO:40**: KVAALKE-KVAALKE-KVAALKE-KVAALKE), whose lysine residues will form a positive charge at pH 7. The presence of such charged domains promotes association between the first and second polypeptides, and thus fosters heterodimer formation. Especially preferred is a Heterodimer-Promoting Domain in which one of the four tandem "E-coil" helical domains of **SEQ ID NO:39** has been modified to contain a cysteine residue: EVAACEK-EVAALEK-EVAALEK-EVAALEK (**SEQ ID NO:41**). Likewise, especially preferred is a Heterodimer-Promoting Domain in which one of the four tandem "K-

coil" helical domains of **SEQ ID NO:40** has been modified to contain a cysteine residue: **K**VAA**C****K**E-**K**VAA**K****K**E-**K**VAA**K****K**E-**K**VAA**K****K**E (**SEQ ID NO:42**).

[00155] As disclosed in WO 2012/018687, in order to improve the *in vivo* pharmacokinetic properties of diabodies, a diabody may be modified to contain a polypeptide portion of a serum-binding protein at one or more of the termini of the diabody. Most preferably, such polypeptide portion of a serum-binding protein will be installed at the C-terminus of the diabody. Albumin is the most abundant protein in plasma and has a half-life of 19 days in humans. Albumin possesses several small molecule binding sites that permit it to non-covalently bind to other proteins and thereby extend their serum half-lives. The Albumin-Binding Domain 3 (ABD3) of protein G of Streptococcus strain G148 consists of 46 amino acid residues forming a stable three-helix bundle and has broad albumin-binding specificity (Johansson, M.U. *et al.* (2002) "*Structure, Specificity, And Mode Of Interaction For Bacterial Albumin-Binding Modules*," J. Biol. Chem. 277(10):8114-8120. Thus, a particularly preferred polypeptide portion of a serum-binding protein for improving the *in vivo* pharmacokinetic properties of a diabody is the Albumin-Binding Domain (ABD) from streptococcal protein G, and more preferably, the Albumin-Binding Domain 3 (ABD3) of protein G of Streptococcus strain G148 (**SEQ ID NO:43**): LAEAKVLANR ELDKYGVSDY YKNLIDNAKS AEGVKALIDE ILAALP.

[00156] As disclosed in WO 2012/162068 (herein incorporated by reference), "**deimmunized**" variants of **SEQ ID NO:43** have the ability to attenuate or eliminate MHC class II binding. Based on combinational mutation results, the following combinations of substitutions are considered to be preferred substitutions for forming such a deimmunized ABD: 66D/70S +71A; 66S/70S +71A; 66S/70S +79A; 64A/65A/71A; 64A/65A/71A+66S; 64A/65A/71A+66D; 64A/65A/71A+66E; 64A/65A/79A+66S; 64A/65A/79A+66D; 64A/65A/79A+66E. Variant ABDs having the modifications L64A, I65A and D79A or the modifications N66S, T70S and D79A. Variant deimmunized ABD having the amino acid sequence:

LAEAKVLANR ELDKYGVSDY YKNLI**D**$_{66}$NAK**S**$_{70}$ **A**$_{71}$EGVKALIDE ILAALP (**SEQ ID NO:116**),

or the amino acid sequence:

LAEAKVLANR ELDKYGVSDY YKN**A**$_{64}$**A**$_{65}$NNAKT VEGVKALI**A**$_{79}$E ILAALP **(SEQ ID NO:44)**,

or the amino acid sequence:

LAEAKVLANR ELDKYGVSDY YKNLI**S**$_{66}$NAK**S**$_{70}$ VEGVKALI**A**$_{79}$E ILAALP **(SEQ ID NO:45)**,

are particularly preferred as such deimmunized ABD exhibit substantially wild-type binding while providing attenuated MHC class II binding. Thus, the first polypeptide chain of such a diabody having an ABD contains a third linker (Linker 3) preferably positioned C-terminally to the E-coil (or K-coil) Domain of such polypeptide chain so as to intervene between the E-coil (or K-coil) Domain and the ABD (which is preferably a deimmunized ABD). A preferred sequence for such Linker 3 is **SEQ ID NO:46**: GGGS.

2. Bispecific Diabodies Containing Fc Regions

[00157] One embodiment of the present invention relates to bispecific diabodies comprising an Fc Region capable of simultaneously binding to LAG-3 and a second epitope (*e.g.* B7-H3, B7-H4, BTLA, CD40, CD80, CD86, CD137, CTLA-4, ICOS, KIR, MHC class I or II, OX40, PD-1, PD-L1, TCR, TIM-3, etc.). The addition of an IgG CH2-CH3 Domain to one or both of the diabody polypeptide chains, such that the complexing of the diabody chains results in the formation of an Fc Region, increases the biological half-life and/or alters the valency of the diabody. Incorporating an IgG CH2-CH3 Domain onto both of the diabody polypeptides will permit a two-chain bispecific Fc-Region-containing diabody to form (**Figure 2**).

[00158] Alternatively, incorporating an IgG CH2-CH3 Domain onto only one of the diabody polypeptides will permit a more complex four-chain bispecific Fc Region-containing diabody to form (**Figures 3A-3C**). **Figure 3C** shows a representative four-chain diabody possessing the Constant Light (CL) Domain and the Constant Heavy CH1 Domain, however fragments of such domains as well as other polypeptides may alternatively be employed (see, *e.g.*, **Figures 3A and 3B**, United States Patent Publications No. 2013-0295121; 2010-0174053 and 2009-0060910; European Patent

Publication No. EP 2714079; EP 2601216; EP 2376109; EP 2158221 and PCT Publications No. WO 2012/162068; WO 2012/018687; WO 2010/080538). Thus, for example, in lieu of the CH1 Domain, one may employ a peptide having the amino acid sequence CVEPKSC (**SEQ ID NO:35**) VEPKSC (**SEQ ID NO:36**), or AEPKSC (**SEQ ID NO:115**), derived from the hinge domain of a human IgG, and in lieu of the CL Domain, one may employ the C-terminal 6 amino acids of the human kappa light chain, GFNRGEC (**SEQ ID NO:37**) or FNRGEC (**SEQ ID NO:38**). A representative peptide containing four-chain diabody is shown in **Figure 3A**. Alternatively, or in addition, one may employ a peptide comprising tandem coil domains of opposing charge such as the "E-coil" helical domains (**SEQ ID NO:39**: EVAALEK-EVAALEK-EVAALEK-EVAALEK or **SEQ ID NO:41:** EVAACEK-EVAALEK-EVAALEK-EVAALEK); and the "K-coil" domains (**SEQ ID NO:40**: KVAALKE-KVAALKE-KVAALKE-KVAALKE or **SEQ ID NO:42:** KVAACKE-KVAALKE-KVAALKE-KVAALKE). A representative coil domain containing four-chain diabody is shown in **Figure 3B**.

[00159] Additional or alternative linkers that may be employed in the Fc Region-containing diabody molecules of the present invention include: ASTKG (**SEQ ID NO:47**), DKTHTCPPCP (**SEQ ID NO:48**), EPKSCDKTHTCPPCP (**SEQ ID NO:30**), LEPKSS (**SEQ ID NO:49**), APSSS (**SEQ ID NO:31**), and APSSSPME (**SEQ ID NO:50**), GGC, and GGG. **SEQ ID NO:49** may be used in lieu of GGG or GGC for ease of cloning. Additionally, **SEQ ID NO:49** may be immediately followed by **SEQ ID NO:47** to form an alternate linker (LEPKSSDKTHTCPPCP; **SEQ ID NO:51**).

[00160] As provided in Figure 3A-3C, diabodies of the invention may comprise four different chains. The first and third polypeptide chains of such a diabody contain three domains: (i) a VL1-containing Domain, (ii) a VH2-containing Domain, (iii) Heterodimer-Promoting Domain and (iv) a Domain containing a CH2-CH3 sequence. The second and fourth polypeptide contain: (i) a VL2-containing Domain, (ii) a VH1-containing Domain and (iii) a Heterodimer-Promoting Domain, where the Heterodimer-Promoting Domains promote the dimerization of the first/third chains with the second/fourth chains. The VL and/or VH Domains of the third and fourth polypeptide chains, and VL and/or VH Domains of the first and second polypeptide

chains may be the same or different so as to permit tetravalent binding that is either monospecific, bispecific or tetraspecific. The general structure of the polypeptide chains of a representative four-chain Fc Region-containing diabodies of invention is provided in **Table 2**:

Table 2

Bispecific	2nd Chain	NH_2-VL2-VH1-Heterodimer-Promoting Domain-COOH
	1st Chain	NH_2-VL1-VH2-Heterodimer-Promoting Domain-CH2-CH3-COOH
	1st Chain	NH_2-VL1-VH2-Heterodimer-Promoting Domain-CH2-CH3-COOH
	2nd Chain	NH_2-VL2-VH1-Heterodimer-Promoting Domain-COOH
Tetraspecific	2nd Chain	NH_2-VL2-VH1-Heterodimer-Promoting Domain-COOH
	1st Chain	NH_2-VL1-VH2-Heterodimer-Promoting Domain-CH2-CH3-COOH
	3rd Chain	NH_2-VL3-VH4-Heterodimer-Promoting Domain-CH2-CH3-COOH
	4th Chain	NH_2-VL4-VH3-Heterodimer-Promoting Domain-COOH

[00161] In a specific embodiment, diabodies of the present invention are bispecific, tetravalent, Fc-containing diabodies (**Figure 3**) that are composed of four total polypeptide chains. The bispecific, tetravalent, Fc-containing diabodies of the invention comprise two epitope binding domains immunospecific for LAG-3, and two epitope binding domains specific for a second epitope (*e.g.*, B7-H3, B7-H4, BTLA, CD40, CD80, CD86, CD137, CTLA-4, ICOS, KIR, MHC class I or II, OX40, PD-1, PD-L1, TCR, TIM-3, *etc.*).

[00162] In a further embodiment, the bispecific Fc Region-containing diabodies may comprise three polypeptide chains. The first polypeptide of such a diabody contains three domains: (i) a VL1-containing Domain, (ii) a VH2-containing Domain and (iii) a Domain containing a CH2-CH3 sequence. The second polypeptide of such diabodies contains: (i) a VL2-containing Domain, (ii) a VH1-containing Domain and (iii) a Domain that promotes heterodimerization and covalent bonding with the diabody's first polypeptide chain. The third polypeptide of such diabodies comprises a CH2-CH3 sequence. Thus, the first and second polypeptide chains of such diabodies associate together to form a VL1/VH1 binding site that is capable of binding to the first epitope, as well as a VL2/VH2 binding site that is capable of binding to the second epitope. The first and second polypeptides are bonded to one another through a disulfide bond

involving cysteine residues in their respective Third Domains. Notably, the first and third polypeptide chains complex with one another to form an Fc Region that is stabilized via a disulfide bond. Such diabodies have enhanced potency. **Figures 4A** and **4B** illustrate the structures of such diabodies. Such Fc-Region-containing bispecific diabodies may have either of two orientations (**Table 3**):

Table 3

First Orientation	3rd Chain	NH_2-CH2-CH3-COOH
	1st Chain	NH_2-VL1-VH2-Heterodimer-Promoting Domain-CH2-CH3-COOH
	2nd Chain	NH_2-VL2-VH1-Heterodimer-Promoting Domain-COOH
Second Orientation	3rd Chain	NH_2-CH2-CH3-COOH
	1st Chain	NH_2-CH2-CH3-VL1-VH2-Heterodimer-Promoting Domain-COOH
	2nd Chain	NH_2-VL2-VH1-Heterodimer-Promoting Domain-COOH

[00163] The Fc Region of the Fc Region-containing diabodies of the present invention may be either a complete Fc Region (*e.g.*, a complete IgG Fc Region) or only a fragment of a complete Fc Region. Optionally, the Fc Region of the Fc Region-containing diabodies of the present invention lack the C-terminal amino acid residue. Although the Fc Region of the bispecific Fc diabodies of the present invention may possess the ability to bind to one or more Fc receptors (*e.g.*, FcγR(s)), more preferably such Fc Region will cause altered binding to FcγRIA (CD64), FcγRIIA (CD32A), FcγRIIB (CD32B), FcγRIIIA (CD16a) or FcγRIIIB (CD16b) (relative to the binding exhibited by a wild-type Fc Region) or will substantially eliminate the ability of such Fc Region to bind to inhibitory receptor(s). Thus, the Fc Region of the Fc Region-containing diabodies of the present invention may include some or all of the CH2 Domain and/or some or all of the CH3 Domain of a complete Fc Region, or may comprise a variant CH2 and/or a variant CH3 sequence (that may include, for example, one or more insertions and/or one or more deletions with respect to the CH2 or CH3 domains of a complete Fc Region). Such Fc Regions may comprise non-Fc polypeptide portions, or may comprise portions of non-naturally complete Fc Regions, or may comprise non-naturally occurring orientations of CH2 and/or CH3 Domains (such as,

for example, two CH2 domains or two CH3 domains, or in the N-terminal to C-terminal direction, a CH3 Domain linked to a CH2 Domain, *etc.*).

[00164] Fc Region modifications identified as altering effector function are known in the art, including modifications that increase binding to activating receptors (*e.g.*, FcγRIIA (CD16A) and reduce binding to inhibitory receptors (*e.g.*, FcγRIIB (CD32B) (see, *e.g.*, Stavenhagen, J.B. *et al.* (2007) "*Fc Optimization Of Therapeutic Antibodies Enhances Their Ability To Kill Tumor Cells In Vitro And Controls Tumor Expansion In Vivo Via Low-Affinity Activating Fcgamma Receptors*," Cancer Res. 57(18):8882-8890). Exemplary variants of human IgG1 Fc Regions with reduced binding to CD32B and/or increased binding to CD16A contain F243L, R292P, Y300L, V305I or P296L substitutions. These amino acid substitutions may be present in a human IgG1 Fc Region in any combination. In one embodiment, the human IgG1 Fc Region variant contains a F243L, R292P and Y300L substitution. In another embodiment, the human IgG1 Fc Region variant contains a F243L, R292P, Y300L, V305I and P296L substitution.

[00165] In particular, it is preferred for the CH2-CH3 Domains of the polypeptide chains of the Fc Region-containing diabodies of the present invention to exhibit decreased (or substantially no) binding to FcγRIA (CD64), FcγRIIA (CD32A), FcγRIIB (CD32B), FcγRIIIA (CD16a) or FcγRIIIB (CD16b) (relative to the binding exhibited by the wild-type IgG1 Fc Region (**SEQ ID NO:1**). Fc variants and mutant forms capable of mediating such altered binding are described above. In a specific embodiment, the CH2-CH3 Domains of the polypeptide chains of the Fc Region-containing diabodies of the present invention comprise an IgG Fc Region that exhibits reduced ADCC effector function. In a preferred embodiment the CH2-CH3 Domain of the first and/or third polypeptide chains of such diabodies include any 1, 2, or 3, of the substitutions: L234A, L235A, N297Q, and N297G. In another embodiment, the human IgG1 Fc Region variant contains an N297Q substitution, an N297G substitution, L234A and L235A substitutions or a D265A substitution, as these mutations abolish FcR binding. Alternatively, a CH2-CH3 Domain which inherently exhibits decreased (or substantially no) binding to FcγRIIIA (CD16a) and/or reduced effector function (relative to the binding exhibited by the wild-type IgG1 Fc Region (**SEQ ID NO:1**)) is

utilized. In a specific embodiment, the Fc Region-containing diabodies of the present invention comprise an IgG2 Fc Region (**SEQ ID NO:23**) or an IgG4 Fc Region (**SEQ ID:NO:24**). When an IgG4 Fc Region in utilized, the instant invention also encompasses the introduction of a stabilizing mutation, such as S228P, as numbered by the EU index as set forth in Kabat (Lu *et al.*, (2008) "*The Effect Of A Point Mutation On The Stability Of Igg4 As Monitored By Analytical Ultracentrifugation*," J. Pharmaceutical Sciences 97:960-969) to reduce the incidence of strand exchange. Other stabilizing mutations known in the art may be introduced into an IgG4 Fc Region (Peters, P *et al.*, (2012) "*Engineering an Improved IgG4 Molecule with Reduced Disulfide Bond Heterogeneity and Increased Fab Domain Thermal Stability*," J. Biol. Chem., 287:24525-24533; PCT Patent Publication No: WO 2008/145142). Since the N297G, N297Q, L234A, L235A and D265A substitutions abolish effector function, in circumstances in which effector function is desired, these substitutions would preferably not be employed.

[00166] The CH2 and/or CH3 Domains of such polypeptide chains need not be identical in sequence, and advantageously are modified to foster complexing between the two polypeptide chains. For example, an amino acid substitution (preferably a substitution with an amino acid comprising a bulky side group forming a "knob", *e.g.*, tryptophan) can be introduced into the CH2 or CH3 Domain such that steric interference will prevent interaction with a similarly mutated domain and will obligate the mutated domain to pair with a domain into which a complementary, or accommodating mutation has been engineered, *i.e.*, "the hole" (*e.g.*, a substitution with glycine). Such sets of mutations can be engineered into any pair of polypeptides comprising CH2-CH3 Domains that forms an Fc Region. Methods of protein engineering to favor heterodimerization over homodimerization are well known in the art, in particular with respect to the engineering of immunoglobulin-like molecules, and are encompassed herein (see *e.g.*, Ridgway *et al.* (1996) "*'Knobs-Into-Holes' Engineering Of Antibody CH3 Domains For Heavy Chain Heterodimerization,*" Protein Engr. 9:617-621, Atwell *et al.* (1997) "*Stable Heterodimers From Remodeling The Domain Interface Of A Homodimer Using A Phage Display Library,*" J. Mol. Biol. 270: 26-35, and Xie *et al.* (2005) "*A New Format Of Bispecific Antibody: Highly Efficient Heterodimerization, Expression And Tumor Cell Lysis,*" J. Immunol. Methods 296:95-101; each of which

is hereby incorporated herein by reference in its entirety). Preferably the "knob" is engineered into the CH2-CH3 Domains of the first polypeptide chain and the "hole" is engineered into the CH2-CH3 Domains of the third polypeptide chain of diabodies comprising three polypeptide chains. Thus, the "knob" will help in preventing the first polypeptide chain from homodimerizing via its CH2 and/or CH3 Domains. As the third polypeptide chain preferably contains the "hole" substitution it will heterodimerize with the first polypeptide chain as well as homodimerize with itself. A similar strategy may be utilized for diabodies comprising four chains where the "knob" is engineered into the CH2-CH3 Domains of the second polypeptide chain and the "hole" is engineered into the CH2-CH3 Domains of the fourth polypeptide chain.

[00167] A preferred knob is created by modifying a native IgG Fc Region to contain the modification T366W. A preferred hole is created by modifying a native IgG Fc Region to contain the modification T366S, L368A and Y407V. To aid in purifying the third polypeptide chain homodimer from the final bispecific monovalent Fc diabody comprising the first, second and third polypeptide chains, the protein A binding site of the CH2 and CH3 Domains of the third polypeptide chain is preferably mutated by amino acid substitution at position 435 (H435R). Thus, the third polypeptide chain homodimer will not bind to protein A, whereas the bispecific monovalent Fc diabody will retain its ability to bind protein A via the protein A binding site on the first polypeptide chain.

[00168] A preferred sequence for the CH2 and CH3 Domains of the first polypeptide chain of an Fc Region-containing diabody of the present invention will have the "**knob-bearing**" sequence (**SEQ ID NO:25**):

APE**AA**GGPSV FLFPPKPKDT LM1SRTPEVT CVVVDVSHED PEVKFNWYVD GVEVHNAKTK
PREEQYNSTY RVVSVLTVLH QDWLNGKEYK CKVSNKALPA PIEKTISKAK GQPREPQVYT
LPPSREEMTK NQVSL**W**C**L**VK GFYPSDIAVE WESNGQPENN YKTTPPVLDS DGSFFLYSKL
TVDKSRWQQG NVFSCSVMHE ALHN**H**YTQKS LSLSPGK

[00169] A preferred sequence for the CH2 and CH3 Domains of the second polypeptide chain of an Fc Region-containing diabody of the present invention having two polypeptide chains (or the third polypeptide chain of an Fc Region-containing diabody having three polypeptide chains) will have the "**hole-bearing**" sequence (**SEQ ID NO:26**):

APEAAGGPSV FLFPPKPKDT LMISRTPEVT CVVVDVSHED PEVKFNWYVD GVEVHNAKTK
PREEQYNSTY RVVSVLTVLH QDWLNGKEYK CKVSNKALPA PIEKTISKAK GQPREPQVYT
LPPSREEMTK NQVSLSCAVK GFYPSDIAVE WESNGQPENN YKTTPPVLDS DGSFFLVSKL
TVDKSRWQQG NVFSCSVMHE ALHNRYTQKS LSLSPGK

[00170] As will be noted, the CH2-CH3 Domains of **SEQ ID NO:25** and **SEQ ID NO:26** include a substitution at position 234 with alanine and 235 with alanine, and thus form an Fc Region exhibit decreased (or substantially no) binding to FcγR1A (CD64), FcγRIIA (CD32A), FcγRIIB (CD32B), FcγRIIIA (CD16a) or FcγRIIIB (CD16b) (relative to the binding exhibited by the wild-type Fc Region (**SEQ ID NO:1**).

[00171] It is preferred that the first polypeptide chain will have a "knob-bearing" CH2-CH3 sequence, such as that of **SEQ ID NO:25**. However, as will be recognized, a "hole-bearing" CH2-CH3 Domain (*e.g.*, **SEQ ID NO:26**) could be employed in the first polypeptide chain, in which case, a "knob-bearing" CH2-CH3 Domain (*e.g.*, **SEQ ID NO:25**) would be employed in the second polypeptide chain of an Fc Region-containing diabody of the present invention having two polypeptide chains (or the third polypeptide chain of an Fc Region-containing diabody having three or four polypeptide chains).

VII. Reference Antibodies

A. Reference Anti-Human LAG-3 Antibody

[00172] In order to assess and characterize the novel anti-human LAG-3-binding molecules of the present invention, the following reference antibody was employed: **25F7** (BMS-986016, Medarex/BMS, designated herein as "**LAG-3 mAb A**").

1. 25F7 ("LAG-3 mAb A")

[00173] The amino acid sequence of the VH Domain of **25F7** ("**LAG-3 mAb A**") (**SEQ ID NO:99**) is shown below (CDR_H residues are shown underlined):

QVQLQQWGAG LLKPSETLSL TCAVY**GGSFS** **DYYWN**WIRQP PGKGLEWIG**E**
INHNGNTNSN **PSLKS**RVTLS LDTSKNQFSL KLRSVTAADT AVYYCAF**GYS**
DYEYNWFDPW GQGTLVTVSS

[00174] The amino acid sequence of the VL Domain of **25F7** ("**LAG-3 mAb A**") (**SEQ ID NO:58**) is shown below (CDR_L residues are shown underlined):

EIVLTQSPAT LSLSPGERAT LSC**RASQSIS** **SYLA**WYQQKP GQAPRLLIY**D**
ASNRATGIPA RFSGSGSGTD FTLTISSLEP EDFAVYYC**QQ** **RSNWPLT**FGQ
GTNLEIK

B. Reference Anti-PD-1 Antibodies

[00175] In order to assess and characterize the activity of the novel anti-human LAG-3-binding molecules of the present invention in combination with an anti-PD-1 antibody a reference antibody may be used. Antibodies that are immunospecific for PD-1 are known (see, *e.g.*, United States Patents No. 8,008,449; 8,552,154; PCT Patent Publications WO 2012/135408; WO 2012/145549; and WO 2013/014668) and include: nivolumab (also known as 5C4, BMS-936558, ONO-4538, MDX-1106, and marketed as OPDIVO® by Bristol-Myers Squibb) designated herein as "**PD-1 mAb 1**;" pembrolizumab (formerly known as lambrolizumab, also known as MK-3475, SCH-900475, and marketed as KEYTRUDA® by Merck) designated herein as "**PD-1 mAb 2**"; EH12.2H7 (Dana Farber) designated herein as "**PD-1 mAb 3**"; pidilizumab (also known as CT-011, CureTech,) designated herein as "**PD-1 mAb 4**."

1. Nivolumab ("PD-1 mAb 1")

[00176] The amino acid sequence of the Heavy Chain Variable Domain of **PD-1 mAb 1** has the amino acid sequence (**SEQ ID NO:108**) (CDR_H residues are shown underlined):

QVQLVESGGG VVQPGRSLRL DCKAS**GITFS** **NSGMH**WVRQA PGKGLEWVA**V**
IWYDGSKRYY **ADSVKG**RFTI SRDNSKNTLF LQMNSLRAED TAVYYCAT**ND**
DYWGQGTLVT VSS

[00177] The amino acid sequence of the Light Chain Variable Domain of **PD-1 mAb 1** has the amino acid sequence (**SEQ ID NO:109**) (CDR_L residues are shown underlined):

EIVLTQSPAT LSLSPGERAT LSC**RASQSVS** **SYLA**WYQQKP GQAPRLLIY**D**
ASNRATGIPA RFSGSGSGTD FTLTISSLEP EDFAVYYC**QQ** **SSNWPRT**FGQ
GTKVEIK

2. Pembrolizumab ("PD-1 mAb 2")

[00178] The amino acid sequence of the Heavy Chain Variable Domain of **PD-1 mAb 2** has the amino acid sequence (**SEQ ID NO:110**) (CDR$_H$ residues are shown underlined):

QVQLVQSGVE VKKPGASVKV SCKAS**GYTFT** **NYYMY**WVRQA PGQGLEWMG**G**
INPSNGGTNF **NEKFKN**RVTL TTDSSTTTAY MELKSLQFDD TAVYYCAR**RD**
YRFDMGFDYW GQGTTVTVSS

[00179] The amino acid sequence of the Light Chain Variable Domain of **PD-1 mAb 2** has the amino acid sequence (**SEQ ID NO:111**) (CDR$_L$ residues are shown underlined):

EIVLTQSPAT LSLSPGERAT LSC**RASKGVS** **TSGYSYLH**WY QQKPGQAPRL
LIY**LASYLES** GVPARFSGSG SGTDFTLTIS SLEPEDFAVY YC**QHSRDLPL**
TFGGGTKVEIK

3. EH12.2H7 ("PD-1 mAb 3")

[00180] The amino acid sequence of the Heavy Chain Variable Domain of **PD-1 mAb 3** has the amino acid sequence (**SEQ ID NO:112**) (CDR$_H$ residues are shown underlined):

QVQLQQSGAE LAKPGASVQM SCKAS**GYSFT** **SSWIH**WVKQR PGQGLEWIG**Y**
IYPSTGFTEY **NQKFKD**KATL TADKSSSTAY MQLSSLTSED SAVYYCA**RWR**
DSSGYHAMDY WGQGTSVTVSS

[00181] The amino acid sequence of the Light Chain Variable Domain of **PD-1 mAb 3** has the amino acid sequence (**SEQ ID NO:113**) (CDR$_L$ residues are shown underlined):

DIVLTQSPAS LTVSLGQRAT ISC**RASQSVS** **TSGYSYMH**WY QQKPGQPPKL
LIK**FGSNLES** GIPARFSGSG SGTDFTLNIH PVEEEDTATY YC**QHSWEIPY**
TFGGGTKLEI K

4. Pidilizumab ("PD-1 mAb 4")

[00182] The amino acid sequence of the Heavy Chain Variable Domain of **PD-1 mAb 4** has the amino acid sequence (**SEQ ID NO:114**) (CDR$_H$ residues are shown underlined):

QVQLVQSGSE LKKPGASVKI SCKAS**GYTFT** **NYGMN**WVRQA PGQGLQWMG**W**
INTDSGESTY **AEEFKG**RFVF SLDTSVNTAY LQITSLTAED TGMYFCVR**VG**
YDALDYWGQG TLVTVSS

[00183] The amino acid sequence of the Light Chain Variable Domain of **PD-1 mAb 4** has the amino acid sequence (**SEQ ID NO:9**) (CDR_L residues are shown underlined):

EIVLTQSPSS LSASVGDRVT ITC**SARSSVS** **YMH**WFQQKPG KAPKLWIY**RT** **SNLAS**GVPSR FSGSGSGTSY CLTINSLQPE DFATYYC**QQR** **SSFPLT**FGGG TKLEIK

VIII. Methods of Production

[00184] An anti-LAG-3 polypeptide, and other LAG-3 agonists, antagonists and modulators can be created from the polynucleotides and/or sequences of the LAG-3 mAb 1, LAG-3 mAb 2, LAG-3 mAb 3, LAG-3 mAb 4, LAG-3 mAb 5, or LAG-3 mAb 6 antibodies by methods known in the art, for example, synthetically or recombinantly. One method of producing such peptide agonists, antagonists and modulators involves chemical synthesis of the polypeptide, followed by treatment under oxidizing conditions appropriate to obtain the native conformation, that is, the correct disulfide bond linkages. This can be accomplished using methodologies well known to those skilled in the art (see, *e.g.*, Kelley, R. F. *et al.* (1990) In: GENETIC ENGINEERING PRINCIPLES AND METHODS, Setlow, J.K. Ed., Plenum Press, N.Y., vol. 12, pp 1-19; Stewart, J.M *et al.* (1984) SOLID PHASE PEPTIDE SYNTHESIS, Pierce Chemical Co., Rockford, IL; see also United States Patents Nos. 4,105,603; 3,972,859; 3,842,067; and 3,862,925).

[00185] Polypeptides of the invention may be conveniently prepared using solid phase peptide synthesis (Merrifield, B. (1986) "*Solid Phase Synthesis*," Science 232(4748):341-347; Houghten, R.A. (1985) "*General Method For The Rapid Solid-Phase Synthesis Of Large Numbers Of Peptides: Specificity Of Antigen-Antibody Interaction At The Level Of Individual Amino Acids*," Proc. Natl. Acad. Sci. (U.S.A.) 82(15):5131-5135; Ganesan, A. (2006) "*Solid-Phase Synthesis In The Twenty-First Century*," Mini Rev. Med. Chem. 6(1):3-10).

[00186] In yet another alternative, fully human antibodies having one or more of the CDRs of LAG-3 mAb 1, LAG-3 mAb 2, LAG-3 mAb 3, LAG-3 mAb 4, LAG-3 mAb 5, or LAG-3 mAb 6 or which compete with LAG-3 mAb 1, LAG-3 mAb 2, LAG-3 mAb 3, LAG-3 mAb 4, LAG-3 mAb 5, or LAG-3 mAb 6 for binding to human LAG-3 or a soluble form thereof may be obtained through the use of commercially available

mice that have been engineered to express specific human immunoglobulin proteins. Transgenic animals that are designed to produce a more desirable (*e.g.*, fully human antibodies) or more robust immune response may also be used for generation of humanized or human antibodies. Examples of such technology are XENOMOUSE™ (Abgenix, Inc., Fremont, CA) and HUMAB-MOUSE® and TC MOUSE™ (both from Medarex, Inc., Princeton, NJ).

[00187] In an alternative, antibodies may be made recombinantly and expressed using any method known in the art. Antibodies may be made recombinantly by first isolating the antibodies made from host animals, obtaining the gene sequence, and using the gene sequence to express the antibody recombinantly in host cells (*e.g.*, CHO cells). Another method that may be employed is to express the antibody sequence in plants (*e.g.*, tobacco) or transgenic milk. Suitable methods for expressing antibodies recombinantly in plants or milk have been disclosed (see, for example, Peeters *et al.* (2001) "*Production Of Antibodies And Antibody Fragments In Plants*," Vaccine 19:2756; Lonberg, N. *et al.* (1995) "*Human Antibodies From Transgenic Mice*," Int. Rev. Immunol 13:65-93; and Pollock *et al.*(1999) "*Transgenic Milk As A Method For The Production Of Recombinant Antibodies*," J. Immunol Methods 231:147-157). Suitable methods for making derivatives of antibodies, *e.g.*, humanized, single-chain, *etc.* are known in the art. In another alternative, antibodies may be made recombinantly by phage display technology (see, for example, U.S. Patent Nos. 5,565,332; 5,580,717; 5,733,743; 6,265,150; and Winter, G. *et al.* (1994) "*Making Antibodies By Phage Display Technology*," Annu. Rev. Immunol. 12.433-455).

[00188] The antibodies or protein of interest may be subjected to sequencing by Edman degradation, which is well known to those of skill in the art. The peptide information generated from mass spectrometry or Edman degradation can be used to design probes or primers that are used to clone the protein of interest.

[00189] An alternative method of cloning the protein of interest is by "panning" using purified LAG-3 or portions thereof for cells expressing an antibody or protein of interest that possesses one or more of the CDRs LAG-3 mAb 1, LAG-3 mAb 2, LAG-3 mAb 3, LAG-3 mAb 4, LAG-3 mAb 5, or LAG-3 mAb 6 or that competes with LAG-

3 mAb 1, LAG-3 mAb 2, LAG-3 mAb 3, LAG-3 mAb 4, LAG-3 mAb 5, or LAG-3 mAb 6 for binding to human LAG-3. The "panning" procedure may be conducted by obtaining a cDNA library from tissues or cells that express LAG-3, overexpressing the cDNAs in a second cell type, and screening the transfected cells of the second cell type for a specific binding to LAG-3 in the presence or absence of LAG-3 mAb 1, LAG-3 mAb 2, LAG-3 mAb 3, LAG-3 mAb 4, LAG-3 mAb 5, or LAG-3 mAb 6. Detailed descriptions of the methods used in cloning mammalian genes coding for cell surface proteins by "panning" can be found in the art (see, for example, Aruffo, A. *et al.* (1987) "*Molecular Cloning Of A CD28 cDNA By A High-Efficiency COS Cell Expression System*," Proc. Natl. Acad. Sci. (U.S.A.) 84:8573-8577 and Stephan, J. *et al.* (1999) "*Selective Cloning Of Cell Surface Proteins Involved In Organ Development: Epithelial Glycoprotein Is Involved In Normal Epithelial Differentiation*," Endocrinol. 140:5841-5854).

[00190] Vectors containing polynucleotides of interest can be introduced into the host cell by any of a number of appropriate means, including electroporation, transfection employing calcium chloride, rubidium chloride, calcium phosphate, DEAE- dextran, or other substances; microprojectile bombardment; lipofection; and infection (*e.g.*, where the vector is an infectious agent such as vaccinia virus). The choice of introducing vectors or polynucleotides will often depend on features of the host cell.

[00191] Any host cell capable of overexpressing heterologous DNAs can be used for the purpose of isolating the genes encoding the antibody, polypeptide or protein of interest. Non-limiting examples of suitable mammalian host cells include but are not limited to COS, HeLa, and CHO cells. Preferably, the host cells express the cDNAs at a level of about 5-fold higher, more preferably 10-fold higher, even more preferably 20-fold higher than that of the corresponding endogenous antibody or protein of interest, if present, in the host cells. Screening the host cells for a specific binding to LAG-3 is effected by an immunoassay or FACS. A cell overexpressing the antibody or protein of interest can be identified.

[00192] The invention includes polypeptides comprising an amino acid sequence of the antibodies of this invention. The polypeptides of this invention can be made by procedures known in the art. The polypeptides can be produced by proteolytic or other degradation of the antibodies, by recombinant methods (*i.e.*, single or fusion polypeptides) as described above or by chemical synthesis. Polypeptides of the antibodies, especially shorter polypeptides up to about 50 amino acids, are conveniently made by chemical synthesis. Methods of chemical synthesis are known in the art and are commercially available. For example, an anti-LAG-3 polypeptide could be produced by an automated polypeptide synthesizer employing the solid phase method.

[00193] The invention includes variants of LAG-3 mAb 1, LAG-3 mAb 2, LAG-3 mAb 3, LAG-3 mAb 4, LAG-3 mAb 5, or LAG-3 mAb 6 antibodies and their polypeptide fragments that bind to LAG-3, including functionally equivalent antibodies and fusion polypeptides that do not significantly affect the properties of such molecules as well as variants that have enhanced or decreased activity. Modification of polypeptides is routine practice in the art and need not be described in detail herein. Examples of modified polypeptides include polypeptides with conservative substitutions of amino acid residues, one or more deletions or additions of amino acids which do not significantly deleteriously change the functional activity, or use of chemical analogs. Amino acid residues that can be conservatively substituted for one another include but are not limited to: glycine/alanine; serine/threonine; valine/isoleucine/leucine; asparagine/glutamine; aspartic acid/glutamic acid; lysine/arginine; and phenylalanine/tyrosine. These polypeptides also include glycosylated and non-glycosylated polypeptides, as well as polypeptides with other post-translational modifications, such as, for example, glycosylation with different sugars, acetylation, and phosphorylation. Preferably, the amino acid substitutions would be conservative, *i.e.*, the substituted amino acid would possess similar chemical properties as that of the original amino acid. Such conservative substitutions are known in the art, and examples have been provided above. Amino acid modifications can range from changing or modifying one or more amino acids to complete redesign of a region, such as the Variable Domain. Changes in the Variable Domain can alter binding affinity and/or specificity. Other methods of modification include using coupling techniques known in the art, including, but not limited to, enzymatic means, oxidative

substitution and chelation. Modifications can be used, for example, for attachment of labels for immunoassay, such as the attachment of radioactive moieties for radioimmunoassay. Modified polypeptides are made using established procedures in the art and can be screened using standard assays known in the art.

[00194] The invention encompasses fusion proteins comprising one or more of the polypeptides or LAG-3 mAb 1, LAG-3 mAb 2, LAG-3 mAb 3, LAG-3 mAb 4, LAG-3 mAb 5, or LAG-3 mAb 6 antibodies of this invention. In one embodiment, a fusion polypeptide is provided that comprises a light chain, a heavy chain or both a light and heavy chain. In another embodiment, the fusion polypeptide contains a heterologous immunoglobulin constant region. In another embodiment, the fusion polypeptide contains a Light Chain Variable Domain and a Heavy Chain Variable Domain of an antibody produced from a publicly-deposited hybridoma. For purposes of this invention, an antibody fusion protein contains one or more polypeptide domains that specifically bind to LAG-3 and another amino acid sequence to which it is not attached in the native molecule, for example, a heterologous sequence or a homologous sequence from another region.

IX. Uses of the LAG-3-Binding Molecules of the Present Invention

[00195] The present invention encompasses compositions, including pharmaceutical compositions, comprising the LAG-3-binding molecules of the present invention (*e.g.*, anti-LAG-3 antibodies and anti-LAG-3 bispecific diabodies), polypeptides derived from such molecules, polynucleotides comprising sequences encoding such molecules or polypeptides, and other agents as described herein.

[00196] As discussed above, LAG-3 plays an important role in negatively regulating T-cell proliferation, function and homeostasis. The LAG-3-binding molecules of the present invention have the ability to inhibit LAG-3 function, and thus reverse the LAG-3-mediated immune system inhibition. As such, LAG-3 mAb 1, LAG-3 mAb 2, LAG-3 mAb 3, LAG-3 mAb 4, LAG-3 mAb 5, and LAG-3 mAb 6, their humanized derivatives, and molecules comprising their LAG-3-binding fragments (*e.g.*, bispecific diabodies, *etc.*), may be used to block LAG-3-mediated immune system inhibition, and thereby promote the activation of the immune system.

[00197] Bispecific LAG-3-binding molecules of the present invention that bind to LAG-3 and another molecule involved in regulating an immune check point present on the cell surface (*e.g.*, PD1) augment the immune system by blocking immune system inhibition mediated by LAG-3 and such immune check point molecules. Thus, the LAG-3-binding molecules of the invention are useful for augmenting an immune response (*e.g.*, the T-cell mediated immune response) of a subject. In particular, the LAG-3-binding molecules of the invention and may be used to treat any disease or condition associated with an undesirably suppressed immune system, including cancer and diseases that are associated with the presence of a pathogen (*e.g.*, a bacterial, fungal, viral or protozoan infection).

[00198] The cancers that may be treated by the LAG-3-binding molecules of the present inventioninclude cancers characterized by the presence of a cancer cell selected from the group consisting of a cell of: an adrenal gland tumor, an AIDS-associated cancer, an alveolar soft part sarcoma, an astrocytic tumor, bladder cancer, bone cancer, a brain and spinal cord cancer, a metastatic brain tumor, a breast cancer, a carotid body tumors, a cervical cancer, a chondrosarcoma, a chordoma, a chromophobe renal cell carcinoma, a clear cell carcinoma, a colon cancer, a colorectal cancer, a cutaneous benign fibrous histiocytoma, a desmoplastic small round cell tumor, an ependymoma, a Ewing's tumor, an extraskeletal myxoid chondrosarcoma, a fibrogenesis imperfecta ossium, a fibrous dysplasia of the bone, a gallbladder or bile duct cancer, gastric cancer, a gestational trophoblastic disease, a germ cell tumor, a head and neck cancer, hepatocellular carcinoma, an islet cell tumor, a Kaposi's Sarcoma, a kidney cancer, a leukemia, a lipoma/benign lipomatous tumor, a liposarcoma/malignant lipomatous tumor, a liver cancer, a lymphoma, a lung cancer, a medulloblastoma, a melanoma, a meningioma, a multiple endocrine neoplasia, a multiple myeloma, a myelodysplastic syndrome, a neuroblastoma, a neuroendocrine tumors, an ovarian cancer, a pancreatic cancer, a papillary thyroid carcinoma, a parathyroid tumor, a pediatric cancer, a peripheral nerve sheath tumor, a phaeochromocytoma, a pituitary tumor, a prostate cancer, a posterious uveal melanoma, a rare hematologic disorder, a renal metastatic cancer, a rhabdoid tumor, a rhabdomysarcoma, a sarcoma, a skin cancer, a soft-tissue sarcoma, a squamous cell cancer, a stomach cancer, a synovial sarcoma, a testicular

cancer, a thymic carcinoma, a thymoma, a thyroid metastatic cancer, and a uterine cancer.

[00199] In particular, the LAG-3-binding molecules of the present invention may be used in the treatment of colorectal cancer, hepatocellular carcinoma, glioma, kidney cancer, breast cancer, multiple myeloma, bladder cancer, neuroblastoma; sarcoma, non-Hodgkin's lymphoma, non-small cell lung cancer, ovarian cancer, pancreatic cancer and rectal cancer.

[00200] Pathogen-associated diseases that may be treated by the LAG-3-binding molecules of the present invention include chronic viral, bacterial, fungal and parasitic infections. Chronic infections that may be treated by the LAG-3-binding molecules of the present invention include Epstein Barr virus, Hepatitis A Virus (HAV); Hepatitis B Virus (HBV); Hepatitis C Virus (HCV); herpes viruses (*e.g.* HSV-1, HSV-2, HHV-6, CMV), Human Immunodeficiency Virus (HIV), Vesicular Stomatitis Virus (VSV), *Bacilli, Citrobacter, Cholera, Diphtheria, Enterobacter, Gonococci, Helicobacter pylori, Klebsiella, Legionella, Meningococci,* mycobacteria, *Pseudomonas, Pneumonococci,* rickettsia bacteria, *Salmonella, Serratia, Staphylococci, Streptococci, Tetanus, Aspergillus* (fumigatus, niger, *etc.*), *Blastomyces dermatitidis, Candida* (albicans, krusei, glabrata, tropicalis, *etc.*), *Cryptococcus neoformans,* Genus *Mucorales* (mucor, absidia, rhizopus), *Sporothrix schenkii, Paracoccidioides brasiliensis, Coccidioides immitis, Histoplasma capsulatum, Leptospirosis, Borrelia burgdorferi,* helminth parasite (hookworm, tapeworms, flukes, flatworms (*e.g. Schistosomia*), *Giardia lambia, trichinella, Dientamoeba Fragilis, Trypanosoma brucei, Trypanosoma cruzi,* and *Leishmania donovani.*

[00201] The LAG-3-binding molecules of the invention can be combined with an immunogenic agent such as a tumor vaccine. Such vaccines may comprise purified tumor antigens (including recombinant proteins, peptides, and carbohydrate molecules), autologous or allogeneic tumor cells. A number of tumor vaccine strategies have been described (see for example, Palena, C., *et al.*, (2006) "*Cancer vaccines: preclinical studies and novel strategies*," Adv. Cancer Res. 95, 115-145; Mellman, I., *et al.* (2011) "*Cancer immunotherapy comes of age*," Nature 480, 480–489; Zhang, X.

M. *et al.* (2008) "*The anti-tumor immune response induced by a combination of MAGE-3/MAGE-n-derived peptides*," Oncol. Rep. 20, 245–252; Disis, M. L. *et al.* (2002) "*Generation of T-cell immunity to the HER-2/neu protein after active immunization with HER-2/neu peptide-based vaccines*," J. Clin. Oncol. 20, 2624–2632; Vermeij, R. *et al.* (2012) "*Potentiation of a p53-SLP vaccine by cyclophosphamide in ovarian cancer: a single-arm phase II study*," Int. J. Cancer 131, E670–E680). The LAG-3-binding molecules of the invention can be combined with chemotherapeutic regimes. In these instances, it may be possible to reduce the dose of chemotherapeutic reagent administered (Mokyr *et al.* (1998) Cancer Research 58: 5301-5304).

[00202] The LAG-3-binding molecules of the invention can be combined with other immunostimulatory molecules such as antibodies which activate host immune responsiveness to provide for increased levels of T-cell activation. In particular, anti-PD-1 antibodies, anti-PD-L1 antibodies and/or an anti-CTLA-4 antibodies have been demonstrated to active the immune system (see, *e.g.*, del Rio, M-L. *et al.* (2005) "*Antibody-mediated signaling through PD-1 costimulates T cells and enhances CD28-dependent proliferation*," Eur. J. Immunol 35:3545-3560; Barber, D. L. *et al.* (2006) "*Restoring function in exhausted CD8 T cells during chronic viral infection*," Nature 439, 682–687; Iwai, Y. *et al.* (2002) "*Involvement of PD-L1 on tumor cells in the escape from host immune system and tumor immunotherapy by PD-L1 blockade*," Proc. Natl Acad. Sci. USA 99, 12293–12297; Leach, D. R., *et al.*, (1996) "*Enhancement of antitumor immunity by CTLA-4 blockade*," Science 271, 1734-1736). Additional immunostimulatory molecules that may be combined with the LAG-3-binding molecules of the invention include antibodies to molecules on the surface of dendritic cells that activate dendritic cell (DC) function and antigen presentation, anti-CD40 antibodies able to substitute for T-cell helper activity, and activating antibodies to T-cell costimulatory molecules such as PD-1L, CTLA-4, OX-40 4-1BB, and ICOS (see, for example, Ito *et al.* (2000) "*Effective priming of cytotoxic T lymphocyte precursors by subcutaneous administration of peptide antigens in liposomes accompanied by anti-CD40 and anti-CTLA-4 antibodies*,"Immunobiology 201:527-40; U.S. Pat. No. 5,811,097; Weinberg *et al.* (2000) "*Engagement of the OX-40 Receptor In Vivo Enhances Antitumor Immunity*," Immunol 164:2160-2169; Melero *et al.* (1997) "*Monoclonal antibodies against the 4-1BB T-cell activation molecule eradicate*

established tumors," Nature Medicine 3: 682-685; Hutloff *et al.* (1999) "*ICOS is an inducible T-cell co-stimulator structurally and functionally related to CD28*," Nature 397: 263-266; and Moran, A.E. *et al.* (2013) "*The TNFRs OX40, 4-1BB, and CD40 as targets for cancer immunotherapy*," Curr Opin Immunol. 2013 Apr; 25(2): 10.1016/j.coi.2013.01.004), and/or stimulatory Chimeric Antigen Receptors (**CARs**) comprising an antigen binding domain directed against a disease antigen fused to one or more intracellular signaling domains from various costimulatory protein receptors (*e.g.*, CD28, 41BB, ICOS, OX40, *etc.*) which serve to stimulate T-cells upon antigen binding (see, for example, Tettamanti, S. *et al.* (2013) "*Targeting Of Acute Myeloid Leukaemia By Cytokine-Induced Killer Cells Redirected With A Novel CD123-Specific Chimeric* Antigen *Receptor*," Br. J. Haematol. 161:389-401; Gill, S. *et al.* (2014) "*Efficacy Against Human Acute Myeloid Leukemia And Myeloablation Of Normal Hematopoiesis In A Mouse Model Using Chimeric Antigen Receptor-Modified T Cells*," Blood 123(15): 2343-2354; Mardiros, A. *et al.* (2013) "*T Cells Expressing CD123-Specific Chimeric Antigen Receptors Exhibit Specific Cytolytic Effector Functions And Antitumor Effects Against Human Acute Myeloid Leukemia*," Blood 122:3138-3148; Pizzitola, I. *et al.* (2014) "*Chimeric Antigen Receptors Against CD33/CD123 Antigens Efficiently Target Primary Acute Myeloid Leukemia Cells in vivo*," Leukemia doi:10.1038/leu.2014.62).

[00203] LAG-3-binding molecules of the invention can be combined with inhibitory Chimeric Antigen Receptors (**iCARs**) to divert off target immunotherapy responses. iCARs an antigen binding domain directed against a disease antigen fused to one or more intracellular signaling domains from various inhibitory protein receptors (*e.g.*, CTLA-4, PD-1, *etc.*) which serve to constrain T-cell responses upon antigen binding (see, for example, Fedorov V.D. (2013) "*PD-1- and CTLA-4–Based Inhibitory Chimeric Antigen Receptors (iCARs) Divert Off-Target Immunotherapy Responses*," Sci Tranl Med. 5:215ra172 doi:10.1126/scitranslmed.3006597.

[00204] In particular, the anti-LAG-3 antibodies of the invention are used in combination with an anti-CD137 antibody, an anti-OX40 antibody, an anti-PD-1 antibody, an anti-PD-L1 antibody, an anti-TIGIT antibody, an anti TIM-3 antibody and/or a cancer vaccine.

[00205] In addition to their utility in therapy, the LAG-3-binding molecules of the present invention may be detectably labeled and used in the detection of LAG-3 in samples or in the imaging of LAG-3 on cells.

X. Pharmaceutical Compositions

[00206] The compositions of the invention include bulk drug compositions useful in the manufacture of pharmaceutical compositions (*e.g.*, impure or non-sterile compositions) and pharmaceutical compositions (*i.e.*, compositions that are suitable for administration to a subject or patient) that can be used in the preparation of unit dosage forms. Such compositions comprise a prophylactically or therapeutically effective amount of the LAG-3-binding molecules of the present invention, or a combination of such agents and a pharmaceutically acceptable carrier. Preferably, compositions of the invention comprise a prophylactically or therapeutically effective amount of the LAG-3-binding molecules of the present invention and a pharmaceutically acceptable carrier. The invention particularly encompasses such pharmaceutical compositions in which the LAG-3-binding molecule is: a LAG-3 mAb 1, LAG-3 mAb 2, LAG-3 mAb 3, LAG-3 mAb 4, LAG-3 mAb 5, or LAG-3 mAb 6 antibody; a humanized LAG-3 mAb 1; LAG-3 mAb 2, LAG-3 mAb 3, LAG-3 mAb 4 LAG-3 mAb 5, or LAG-3 mAb 6 antibody; a LAG-3-binding fragment of any such antibody; or in which the LAG-3-binding molecule is a bispecific LAG-3 diabody (*e.g.*, a LAG-3 x PD-1 bispecific diabody). Especially encompassed are such molecules that comprise the 3 CDR_{LS} and the 3 CDR_{HS} of LAG-3 mAb 1; or that comprise the 3 CDR_{LS} and the 3 CDR_{HS} of LAG-3 mAb 2; or that comprise the 3 CDR_{LS} and the 3 CDR_{HS} of LAG-3 mAb 3, or that comprise the 3 CDR_{LS} and the 3 CDR_{HS} of LAG-3 mAb 4, or that comprise the 3 CDR_{LS} and the 3 CDR_{HS} of LAG-3 mAb 5, or that comprise the 3 CDR_{LS} and the 3 CDR_{HS} of LAG-3 mAb 6.

[00207] The invention also encompasses such pharmaceutical compositions that additionally include a second therapeutic antibody (*e.g.*, tumor-specific monoclonal antibody) that is specific for a particular cancer antigen, and a pharmaceutically acceptable carrier.

[00208] In a specific embodiment, the term "pharmaceutically acceptable" means approved by a regulatory agency of the Federal or a state government or listed in the U.S. Pharmacopeia or other generally recognized pharmacopeia for use in animals, and more particularly in humans. The term "carrier" refers to a diluent, adjuvant (*e.g.*, Freund's adjuvant (complete and incomplete), excipient, or vehicle with which the therapeutic is administered. Such pharmaceutical carriers can be sterile liquids, such as water and oils, including those of petroleum, animal, vegetable or synthetic origin, such as peanut oil, soybean oil, mineral oil, sesame oil and the like. Water is a preferred carrier when the pharmaceutical composition is administered intravenously. Saline solutions and aqueous dextrose and glycerol solutions can also be employed as liquid carriers, particularly for injectable solutions. Suitable pharmaceutical excipients include starch, glucose, lactose, sucrose, gelatin, malt, rice, flour, chalk, silica gel, sodium stearate, glycerol monostearate, talc, sodium chloride, dried skim milk, glycerol, propylene, glycol, water, ethanol and the like. The composition, if desired, can also contain minor amounts of wetting or emulsifying agents, or pH buffering agents. These compositions can take the form of solutions, suspensions, emulsion, tablets, pills, capsules, powders, sustained-release formulations and the like.

[00209] Generally, the ingredients of compositions of the invention are supplied either separately or mixed together in unit dosage form, for example, as a dry lyophilized powder or water free concentrate in a hermetically sealed container such as an ampoule or sachette indicating the quantity of active agent. Where the composition is to be administered by infusion, it can be dispensed with an infusion bottle containing sterile pharmaceutical grade water or saline. Where the composition is administered by injection, an ampoule of sterile water for injection or saline can be provided so that the ingredients may be mixed prior to administration.

[00210] The compositions of the invention can be formulated as neutral or salt forms. Pharmaceutically acceptable salts include, but are not limited to those formed with anions such as those derived from hydrochloric, phosphoric, acetic, oxalic, tartaric acids, *etc.*, and those formed with cations such as those derived from sodium, potassium, ammonium, calcium, ferric hydroxides, isopropylamine, triethylamine, 2-ethylamino ethanol, histidine, procaine, *etc.*

[00211] The invention also provides a pharmaceutical pack or kit comprising one or more containers filled with a LAG-3-binding molecule of the present invention (and more preferably, a LAG-3 mAb 1, LAG-3 mAb 2, LAG-3 mAb 3, LAG-3 mAb 4, LAG-3 mAb 5, or LAG-3 mAb 6 antibody; a humanized LAG-3 mAb 1, LAG-3 mAb 2, LAG-3 mAb 3, LAG-3 mAb 4, LAG-3 mAb 5, or LAG-3 mAb 6 antibody; a LAG-3-binding fragment of any such antibody; or in which the LAG-3-binding molecule is a bispecific LAG-3 diabody (*e.g.*, a LAG-3 x PD-1 bispecific diabody)). Especially encompassed are such molecules that comprise the 3 CDR_{L}s and the 3 CDR_{H}s of LAG-3 mAb 1; or that comprise the 3 CDR_{L}s and the 3 CDR_{H}s of LAG-3 mAb 2; or that comprise the 3 CDR_{L}s and the 3 CDR_{H}s of LAG-3 mAb 3; or that comprise the 3 CDR_{L}s and the 3 CDR_{H}s of LAG-3 mAb 4; or that comprise the 3 CDR_{L}s and the 3 CDR_{H}s of LAG-3 mAb 5; or that comprise the 3 CDR_{L}s and the 3 CDR_{H}s of LAG-3 mAb 6, alone or with such pharmaceutically acceptable carrier. Additionally, one or more other prophylactic or therapeutic agents useful for the treatment of a disease can also be included in the pharmaceutical pack or kit. The invention also provides a pharmaceutical pack or kit comprising one or more containers filled with one or more of the ingredients of the pharmaceutical compositions of the invention. Optionally associated with such container(s) can be a notice in the form prescribed by a governmental agency regulating the manufacture, use or sale of pharmaceuticals or biological products, which notice reflects approval by the agency of manufacture, use or sale for human administration.

[00212] The present invention provides kits that can be used in the above methods. A kit can comprise any of the LAG-3-binding molecules of the present invention. The kit can further comprise one or more other prophylactic and/or therapeutic agents useful for the treatment of cancer, in one or more containers; and/or the kit can further comprise one or more cytotoxic antibodies that bind one or more cancer antigens associated with cancer. In certain embodiments, the other prophylactic or therapeutic agent is a chemotherapeutic. In other embodiments, the prophylactic or therapeutic agent is a biological or hormonal therapeutic.

XI. Methods of Administration

[00213] The compositions of the present invention may be provided for the treatment, prophylaxis, and amelioration of one or more symptoms associated with a disease, disorder or infection by administering to a subject an effective amount of a fusion protein or a conjugated molecule of the invention, or a pharmaceutical composition comprising a fusion protein or a conjugated molecule of the invention. In a preferred aspect, such compositions are substantially purified (*i.e.*, substantially free from substances that limit its effect or produce undesired side effects). In a specific embodiment, the subject is an animal, preferably a mammal such as non-primate (*e.g.*, bovine, equine, feline, canine, rodent, *etc.*) or a primate (*e.g.*, monkey such as, a cynomolgus monkey, human, *etc.*). In a preferred embodiment, the subject is a human.

[00214] Various delivery systems are known and can be used to administer the compositions of the invention, *e.g.*, encapsulation in liposomes, microparticles, microcapsules, recombinant cells capable of expressing the antibody or fusion protein, receptor-mediated endocytosis (See, *e.g.*, Wu *et al.* (1987) *"Receptor-Mediated In Vitro Gene Transformation By A Soluble DNA Carrier System,"* J. Biol. Chem. 262:4429-4432), construction of a nucleic acid as part of a retroviral or other vector, *etc.*

[00215] Methods of administering a molecule of the invention include, but are not limited to, parenteral administration (*e.g.*, intradermal, intramuscular, intraperitoneal, intravenous and subcutaneous), epidural, and mucosal (*e.g.*, intranasal and oral routes). In a specific embodiment, the LAG-3-binding molecules of the present invention are administered intramuscularly, intravenously, or subcutaneously. The compositions may be administered by any convenient route, for example, by infusion or bolus injection, by absorption through epithelial or mucocutaneous linings (*e.g.*, oral mucosa, rectal and intestinal mucosa, *etc.*) and may be administered together with other biologically active agents. Administration can be systemic or local. In addition, pulmonary administration can also be employed, *e.g.*, by use of an inhaler or nebulizer, and formulation with an aerosolizing agent. See, *e.g.*, U.S. Patent Nos. 6,019,968; 5,985, 320; 5,985,309; 5,934,272; 5,874,064; 5,855,913; 5,290,540; and 4,880,078; and

PCT Publication Nos. WO 92/19244; WO 97/32572; WO 97/44013; WO 98/31346; and WO 99/66903, each of which is incorporated herein by reference in its entirety.

[00216] The invention also provides that the LAG-3-binding molecules of the present invention are packaged in a hermetically sealed container such as an ampoule or sachette indicating the quantity of the molecule. In one embodiment, such molecules are supplied as a dry sterilized lyophilized powder or water free concentrate in a hermetically sealed container and can be reconstituted, *e.g.*, with water or saline to the appropriate concentration for administration to a subject. Preferably, the LAG-3-binding molecules of the present invention are supplied as a dry sterile lyophilized powder in a hermetically sealed container. .

[00217] The lyophilized LAG-3-binding molecules of the present invention should be stored at between 2°C and 8°C in their original container and the molecules should be administered within 12 hours, preferably within 6 hours, within 5 hours, within 3 hours, or within 1 hour after being reconstituted. In an alternative embodiment, such molecules are supplied in liquid form in a hermetically sealed container indicating the quantity and concentration of the molecule, fusion protein, or conjugated molecule. Preferably, such LAG-3-binding molecules when provided in liquid form are supplied in a hermetically sealed container.

[00218] The amount of the composition of the invention which will be effective in the treatment, prevention or amelioration of one or more symptoms associated with a disorder can be determined by standard clinical techniques. The precise dose to be employed in the formulation will also depend on the route of administration, and the seriousness of the condition, and should be decided according to the judgment of the practitioner and each patient's circumstances. Effective doses may be extrapolated from dose-response curves derived from in vitro or animal model test systems.

[00219] For the LAG-3-binding antibodies encompassed by the invention, the dosage administered to a patient is preferably determined based upon the body weight (kg) of the recipient subject. The dosage of a diabody administered is typically from at least about 0.01 μg/kg body weight, at least about 0.05 μg/kg body weight, at least about 0.1 μg/kg body weight, at least about 0.2 μg/kg body weight, at least about 0.5 μg/kg body

weight, at least about 1 μg/kg body weight, at least about 2 μg/kg body weight, at least about 3 μg/kg body weight, at least about 5 μg/kg body weight, at least about 10 μg/kg body weight, at least about 20 μg/kg body weight, at least about 30 μg/kg body weight, at least about 50 μg/kg body weight, 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 1.5 mg/kg body weight, at least about 3 mg/kg body weight, at least about 5 mg/kg body weight, or at least about 10 mg/kg body weight. The calculated dose will be administered based on the patient's body weight at baseline. Significant (> 10%) change in body weight from baseline or established plateau weight should prompt recalculation of dose.

[00220] For the LAG-3-binding bispecific diabodies encompassed by the invention, the dosage administered to a patient is preferably determined based upon the body weight (kg) of the recipient subject. The dosage of a diabody administered is typically from at least about 0.3 ng/kg per day to about 0.9 ng/kg per day, from at least about 1 ng/kg per day to about 3 ng/kg per day, from at least about 3 ng/kg per day to about 9 ng/kg per day, from at least about 10 ng/kg per day to about 30 ng/kg per day, from at least about 30 ng/kg per day to about 90 ng/kg per day, from at least about 100 ng/kg per day to about 300 ng/kg per day, from at least about 200 ng/kg per day to about 600 ng/kg per day, from at least about 300 ng/kg per day to about 900 ng/kg per day, from at least about 400 ng/kg per day to about 800 ng/kg per day, from at least about 500 ng/kg per day to about 1000 ng/kg per day, from at least about 600 ng/kg per day to about 1000 ng/kg per day, from at least about 700 ng/kg per day to about 1000 ng/kg per day, from at least about 800 ng/kg per day to about 1000 ng/kg per day, from at least about 900 ng/kg per day to about 1000 ng/kg per day, or at least about 1,000 ng/kg per day. The calculated dose will be administered based on the patient's body weight at baseline. Significant (≥ 10%) change in body weight from baseline or established plateau weight should prompt recalculation of dose.

[00221] In another embodiment, the patient is administered a treatment regimen comprising one or more doses of such prophylactically or therapeutically effective amount of a LAG-3-binding molecule of the present invention, wherein the treatment regimen is administered over 2 days, 3 days, 4 days, 5 days, 6 days or 7 days. In certain embodiments, the treatment regimen comprises intermittently administering doses of

the prophylactically or therapeutically effective amount of the LAG-3-binding molecules of the present invention (for example, administering a dose on day 1, day 2, day 3 and day 4 of a given week and not administering doses of the prophylactically or therapeutically effective amount of the LAG-3-binding molecule (and particularly, a LAG-3 mAb 1, LAG-3 mAb 2, LAG-3 mAb 3, LAG-3 mAb 4, LAG-3 mAb 5, LAG-3 mAb 6 antibody; a humanized LAG-3 mAb 1, LAG-3 mAb 2, LAG-3 mAb 3, LAG-3 mAb 4, LAG-3 mAb 5, or LAG-3 mAb 6 antibody; a LAG-3-binding fragment of any such antibody; or in which the LAG-3-binding molecule is a bispecific LAG-3 diabody (*e.g.*, a LAG-3 x PD-1 bispecific Fc diabody). Especially encompassed is the administration (on day 5, day 6 and day 7 of the same week) of molecules that comprise the the 3 CDR_{LS} and the 3 CDR_{HS} of LAG-3 mAb 1; or that comprise the 3 CDR_{LS} and the 3 CDR_{HS} of LAG-3 mAb 2; or that comprise the 3 CDR_{LS} and the 3 CDR_{HS} of LAG-3 mAb 3; or that comprise the 3 CDR_{LS} and the 3 CDR_{HS} of LAG-3 mAb 4; or that comprise the 3 CDR_{LS} and the 3 CDR_{HS} of LAG-3 mAb 5; or that comprise the 3 CDR_{LS} and the 3 CDR_{HS} of LAG-3 mAb 6. Typically, there are 1, 2, 3, 4, 5 or more courses of treatment. Each course may be the same regimen or a different regimen.

[00222] In another embodiment, the administered dose escalates over the first quarter, first half or first two-thirds or three-quarters of the regimen(s) (*e.g.*, over the first, second, or third regimens of a 4 course treatment) until the daily prophylactically or therapeutically effective amount of the LAG-3-binding molecule is achieved. **Table 4** provides 5 examples of different dosing regimens described above for a typical course of treatment with a diabody.

Table 4

Regimen	Day	Diabody Dosage (ng diabody per kg subject weight per day)				
1	1, 2, 3, 4	100	100	100	100	100
	5, 6, 7	none	none	none	none	none
2	1, 2, 3, 4	300	500	700	900	1,000
	5, 6, 7	none	none	none	none	none
3	1, 2, 3, 4	300	500	700	900	1,000
	5, 6, 7	none	none	none	none	none
4	1, 2, 3, 4	300	500	700	900	1,000
	5, 6, 7	none	none	none	none	none

[00223] The dosage and frequency of administration of a LAG-3-binding molecule of the present invention may be reduced or altered by enhancing uptake and tissue penetration of the molecule by modifications such as, for example, lipidation.

[00224] The dosage of a LAG-3-binding molecule of the invention administered to a patient may be calculated for use as a single agent therapy. Alternatively, the molecule may be used in combination with other therapeutic compositions and the dosage administered to a patient are lower than when said molecules are used as a single agent therapy.

[00225] The pharmaceutical compositions of the invention may be administered locally to the area in need of treatment; this may be achieved by, for example, and not by way of limitation, local infusion, by injection, or by means of an implant, said implant being of a porous, non-porous, or gelatinous material, including membranes, such as sialastic membranes, or fibers. Preferably, when administering a molecule of the invention, care must be taken to use materials to which the molecule does not absorb.

[00226] The compositions of the invention can be delivered in a vesicle, in particular a liposome (*See* Langer (1990) *"New Methods Of Drug Delivery,"* Science 249:1527-1533); Treat *et al.*, in LIPOSOMES IN THE THERAPY OF INFECTIOUS DISEASE AND CANCER, Lopez-Berestein and Fidler (eds.), Liss, New York, pp. 353- 365 (1989); Lopez-Berestein, ibid., pp. 3 17-327).

[00227] The compositions of the invention can be delivered in a controlled-release or sustained-release system. Any technique known to one of skill in the art can be used to produce sustained-release formulations comprising one or more of the LAG-3-binding molecule(s) of the invention. *See, e.g.*, U.S. Patent No. 4,526,938; PCT publication WO 91/05548; PCT publication WO 96/20698; Ning *et al.* (1996) *"Intratumoral Radioimmunotheraphy Of A Human Colon Cancer Xenograft Using A Sustained-Release Gel,"* Radiotherapy & Oncology 39:179-189, Song *et al.* (1995) *"Antibody Mediated Lung Targeting Of Long-Circulating Emulsions,"* PDA Journal of Pharmaceutical Science & Technology 50:372-397; Cleek *et al.* (1997) *"Biodegradable Polymeric Carriers For A bFGF Antibody For Cardiovascular*

Application," Pro. Int'l. Symp. Control. Rel. Bioact. Mater. 24:853-854; and Lam *et al.* (1997) *"Microencapsulation Of Recombinant Humanized Monoclonal Antibody For Local Delivery,"* Proc. Int'l. Symp. Control Rel. Bioact. Mater. 24:759-760, each of which is incorporated herein by reference in its entirety. In one embodiment, a pump may be used in a controlled-release system (*See* Langer, *supra*; Sefton, (1987) *"Implantable Pumps,"* CRC Crit. Rev. Biomed. Eng. 14:201-240; Buchwald *et al.* (1980) *"Long-Term, Continuous Intravenous Heparin Administration By An Implantable Infusion Pump In Ambulatory Patients With Recurrent Venous Thrombosis,"* Surgery 88:507-516; and Saudek *et al.* (1989) *"A Preliminary Trial Of The Programmable Implantable Medication System For Insulin Delivery,"* N. Engl. J. Med. 321:574-579). In another embodiment, polymeric materials can be used to achieve controlled-release of the molecules (*see e.g.*, MEDICAL APPLICATIONS OF CONTROLLED RELEASE, Langer and Wise (eds.), CRC Pres., Boca Raton, Florida (1974); CONTROLLED DRUG BIOAVAILABILITY, DRUG PRODUCT DESIGN AND PERFORMANCE, Smolen and Ball (eds.), Wiley, New York (1984); Levy *et al.* (1985) *"Inhibition Of Calcification Of Bioprosthetic Heart Valves By Local Controlled-Release Diphosphonate,"* Science 228:190-192; During *et al.* (1989) *"Controlled Release Of Dopamine From A Polymeric Brain Implant: In Vivo Characterization,"* Ann. Neurol. 25:351-356; Howard *et al.* (1989) *"Intracerebral Drug Delivery In Rats With Lesion-Induced Memory Deficits,"* J. Neurosurg. 7(1):105-112); U.S. Patent No. 5,679,377; U.S. Patent No. 5,916,597; U.S. Patent No. 5,912,015; U.S. Patent No. 5,989,463; U.S. Patent No. 5,128,326; PCT Publication No. WO 99/15154; and PCT Publication No. WO 99/20253). Examples of polymers used in sustained-release formulations include, but are not limited to, poly(2-hydroxy ethyl methacrylate), poly(methyl methacrylate), poly(acrylic acid), poly(ethylene-co-vinyl acetate), poly(methacrylic acid), polyglycolides (PLG), polyanhydrides, poly(N-vinyl pyrrolidone), poly(vinyl alcohol), polyacrylamide, poly(ethylene glycol), polylactides (PLA), poly(lactide-co-glycolides) (PLGA), and polyorthoesters. A controlled-release system can be placed in proximity of the therapeutic target (*e.g.*, the lungs), thus requiring only a fraction of the systemic dose (see, *e.g.*, Goodson, in MEDICAL APPLICATIONS OF CONTROLLED RELEASE, *supra*, vol. 2, pp. 115-138 (1984)). Polymeric compositions useful as controlled-release implants can be used according to

Dunn *et al.* (See U.S. 5,945,155). This particular method is based upon the therapeutic effect of the in situ controlled-release of the bioactive material from the polymer system. The implantation can generally occur anywhere within the body of the patient in need of therapeutic treatment. A non-polymeric sustained delivery system can be used, whereby a non-polymeric implant in the body of the subject is used as a drug delivery system. Upon implantation in the body, the organic solvent of the implant will dissipate, disperse, or leach from the composition into surrounding tissue fluid, and the non-polymeric material will gradually coagulate or precipitate to form a solid, microporous matrix (See U.S. 5,888,533).

[00228] Controlled-release systems are discussed in the review by Langer (1990, *"New Methods Of Drug Delivery,"* Science 249:1527-1533). Any technique known to one of skill in the art can be used to produce sustained-release formulations comprising one or more therapeutic agents of the invention. See, *e.g.*, U.S. Patent No. 4,526,938; International Publication Nos. WO 91/05548 and WO 96/20698; Ning *et al.* (1996) *"Intratumoral Radioimmunotheraphy Of A Human Colon Cancer Xenograft Using A Sustained-Release Gel,"* Radiotherapy & Oncology 39:179-189, Song *et al.* (1995) *"Antibody Mediated Lung Targeting Of Long-Circulating Emulsions,"* PDA Journal of Pharmaceutical Science & Technology 50:372-397; Cleek *et al.* (1997) *"Biodegradable Polymeric Carriers For A bFGF Antibody For Cardiovascular Application,"* Pro. Int'l. Symp. Control. Rel. Bioact. Mater. 24:853-854; and Lam *et al.* (1997) *"Microencapsulation Of Recombinant Humanized Monoclonal Antibody For Local Delivery,"* Proc. Int'l. Symp. Control Rel. Bioact. Mater. 24:759-760, each of which is incorporated herein by reference in its entirety.

[00229] Where the composition of the invention is a nucleic acid encoding a LAG-3-binding molecule of the present invention, the nucleic acid can be administered *in vivo* to promote expression of its encoded LAG-3-binding molecule by constructing it as part of an appropriate nucleic acid expression vector and administering it so that it becomes intracellular, *e.g.*, by use of a retroviral vector (*See* U.S. Patent No. 4,980,286), or by direct injection, or by use of microparticle bombardment (*e.g.*, a gene gun; Biolistic, Dupont), or coating with lipids or cell surface receptors or transfecting agents, or by administering it in linkage to a homeobox-like peptide which is known to enter

the nucleus (See *e.g.*, Joliot *et al.* (1991) *"Antennapedia Homeobox Peptide Regulates Neural Morphogenesis,"* Proc. Natl. Acad. Sci. (U.S.A.) 88:1864-1868), *etc.* Alternatively, a nucleic acid can be introduced intracellularly and incorporated within host cell DNA for expression by homologous recombination.

[00230] Treatment of a subject with a therapeutically or prophylactically effective amount of a LAG-3-binding molecule of the present invention can include a single treatment or, preferably, can include a series of treatments. In a preferred example, a subject is treated with such a diabody one time per week for between about 1 to 10 weeks, preferably between 2 to 8 weeks, more preferably between about 3 to 7 weeks, and even more preferably for about 4, 5, or 6 weeks. The pharmaceutical compositions of the invention can be administered once a day, twice a day, or three times a day. Alternatively, the pharmaceutical compositions can be administered once a week, twice a week, once every two weeks, once a month, once every six weeks, once every two months, twice a year or once per year. It will also be appreciated that the effective dosage of the molecules used for treatment may increase or decrease over the course of a particular treatment.

Examples:

[00231] Having now generally described the invention, the same will be more readily understood through reference to the following examples, which are provided by way of illustration and are not intended to be limiting of the present invention unless specified. It will be apparent to those skilled in the art that many modifications, both to materials and methods, can be practiced without departing from the scope of the present disclosure.

A. Example 1: Characterization of Anti-Human LAG-3 Monoclonal Antibodies LAG-3 mAb 1, LAG-3 mAb 2, LAG-3 mAb 3, LAG-3 mAb 4, LAG-3 mAb 5, and LAG-3 mAb 6

[00232] Six murine monoclonal antibodies were isolated as being capable of immunospecifically binding to both human and cynomolgus monkey LAG-3, and accorded the designations "**LAG-3 mAb 1**," "**LAG-3 mAb 2**," "**LAG-3 mAb 3**," "**LAG-3 mAb 4**," "**LAG-3 mAb 5**," and "**LAG-3 mAb 6.**"The CDRs of these

antibodies were found to differ and are provided above. LAG-3 mAb 1 was humanized yielded two humanized VH Domains, designated herein as "**hLAG-3 mAb 1 VH-1,**" and "**hLAG-3 mAb 1 VH-2**," and four humanized VL Domains designated herein as "**hLAG-3 mAb 1 VL-1**," "**hLAG-3 mAb 1 VL-2**," "**hLAG-3 mAb 1 VL-3**," and "**hLAG-3 mAb 1 VL-4**. The humanized heavy and light chains may be used in any combination and particular combinations of humanized chains are referred to by reference to the specific VH/VL Domains, for example a humanized antibody comprising hLAG-3 mAb 1 VH-1 and hLAG-3 mAb 1 VL-2 is specifically referred to as "**hLAG-3 mAb 1(1,2)**." The humanized mAbs were engineered with either a human IgG1 Fc Region (comprising the L234A/L235A (AA) substitutions) or a human IgG4 Fc Region (comprising the S228P substitution)

[00233] The binding kinetics of the antibodies LAG-3 mAb 1, LAG-3 mAb 2, LAG-3 mAb 3, LAG-3 mAb 4, LAG-3 mAb 5, LAG-3 mAb 6, several humanized and a reference antibody LAG-3 mAb A was investigated using Biacore analysis. The anti-LAG-3 antibodies were captured and were incubated with His-tagged soluble human LAG-3 (shLAG-3-His) and the kinetics of binding was determined via Biacore analysis. The calculated ka, kd and KD are presented in **Table 5**.

Table 5

Anti-LAG-3 Antibody	k_a ($x10^5$)	k_d ($x10^{-4}$)	KD (nM)
LAG-3 mAb A	8.7	5.4	0.6
LAG-3 mAb 1[a]	20	0.26	0.013
LAG-3 mAb 1[b]	31	0.27	0.01
LAG-3 mAb 2[a]	11	21	1.9
LAG-3 mAb 3[a]	7.7	34	4.4
LAG-3 mAb 4[a]	12	9.3	0.8
LAG-3 mAb 5[a]	14	13	0.9
LAG-3 mAb 6[a]	42	0.84	0.02
hLAG-3 mAb 1 (1.2)[d, e]	23	0.13	0.01
hLAG-3 mAb 1 (2.2)[d, e]	8.2	2.6	0.3
hLAG-3 mAb 1 (1.1)[d, e]	17	0.74	0.04
hLAG-3 mAb 1 (1.4)[c,e]	16	0.59	0.04
hLAG-3 mAb 1 (1.4)[c,f]	17	0.86	0.05

a = captured on immobilized Fab2 goat-anti-mouse Fc
b = captured on immobilized Protein G
c = captured on immobilized Fab2 goat anti-human Fc
d = captured on immobilized Protein A
e = human IgG1 (AA)
f – IgG4 (S228P)

[00234] The results demonstrate that LAG-3 mAb 1 and LAG-3 mAb 6 exhibit better binding kinetics relative to reference antibody LAG-3 mAb A.

[00235] The epitope specificity of LAG-3 mAb 1, LAG-3 mAb 6 and the reference antibody LAG-3 mAb A was examined using Biacore analysis. In order to determine whether the antibodies bound to different LAG-3 epitopes, shLAG-3-His was captured by mouse anti-PentaHis antibody immobilized on the CM5 sensor chip according to the procedure recommended by the manufacturer. Briefly, the carboxyl groups on the sensor chip surface were activated with an injection of a solution containing 0.2 M N-ethyl-N-(3dietylamino-propyl) carbodimide and 0.05 M N-hydroxy-succinimide. Anti-PentaHis antibody was injected over the activated CM5 surface in 10 mM sodium-acetate, pH 5.0, at a flow rate 5 μL/min, followed by 1 M ethanolamine for deactivation of remaining amine-reactive groups.. Binding experiments were performed in HBS-EP buffer, which contains 10 mM HEPES, pH 7.4, 150 mM NaCl, 3 mM EDTA, and 0.005% P20 surfactant. Each antibody (LAG-3 mAb 1, LAG-3 mAb 6 and LAG-2 mAb A) was preinjected over captured hLAG3-His for 180 seconds at a flow rate of 5 μL/min at concentration of 1μM followed by running buffer and injection of competing antibody at the same conditions. Binding response of competing antibody was compared to its binding response to hLAG3-His preinjected with buffer to identify antibodies competing for the same epitope. Regeneration of the immobilized anti-PentaHis surface was performed by pulse injection of 10 mM glycine, pH 1.5.Reference curves were obtained by injection of analytes over the treated surface with no immobilized protein. Binding curves were generated by BIAevaluation software v4.1 from real-time sensogram data.

[00236] The results of this experiment are shown in **Figure 5.** The results of this experiment indicate that the biotinylated antibody LAG3 mAb A was capable of binding to shLAG-3-His even in the presence of excess amounts of the non-biotinylated antibodies LAG-3 mAb 1 and LAG-3 mAb 6. While, LAG-3 mAb 1 blocked the binding of LAG-3 mAb 6. Thus, the results show that LAG-3 mAb 1 and LAG-3 mAb 6 likely bind to the same, or over lapping epitopes of LAG-3. In contrast, both LAG-3 mAb 1 and LAG-3 mAb 6 bind to an epitope that is distinct from that of LAG-3 mAb A.

[00237] In order to further characterize the anti-LAG3 antibodies, their ability to block binding between LAG-3 and MHC class II was assessed in two different assays. In one assay the ability of the antibodies to block the binding of a soluble human LAG3-Fc fusion protein to MHC class II immobilized on a surface was examined. For this assay LAG-3 mAb 1, LAG-3 mAb 2, LAG-3 mAb 3, LAG-3 mAb 4, and LAG-3 mAb 5 each (at 0-67 nM) were mixed with a soluble human LAG-3-Fc fusion protein, (at 5 μg/mL) and were separately incubated with immobilized MHC class II (1 μg/mL). The amount of LAG-3 binding to the immobilized MHC class II was assessed using a goat anti-human Fc gamma-HRP secondary antibody. The results of this experiment are shown in **Figure 6**.

[00238] In another assay the ability of the anti-LAG-3 antibodies of the present invention to block the binding of a soluble human LAG3-Fc fusion protein to native MHC class II on a cell surface was examined. For this assay LAG-3 mAb 1, LAG-3 mAb 2, LAG-3 mAb 3, LAG-3 mAb 4, LAG-3 mAb 5, and the reference antibody LAG-3 mAb A each (at 0.1-26.7 ng/ml) were mixed with a biotinylated-soluble human LAG-3-Fc fusion protein, (at 0.5 μg/ml) and were separately incubated with MHC II-positive Daudi cells. The amount of LAG-3 binding to the surface of the Daudi cells was determined using a PE-conjugated Streptavidin secondary antibody by FACS analysis. In additional separate experiments LAG-3 mAb 1, LAG-3 mAb 6 and LAG-3 mAb A; or LAG-3 mAb 1 and the humanized antibodies, hLAG-3 mAb 1 (1.6), hLAG-3 mAb 1 (1.2), hLAG-3 mAb 1 (2.2), and LAG-3 mAb (1.1) were assayed as described above. The results of these experiments are shown in **Figure 7, Panels A-7**, respectively.

[00239] The results of these inhibition assays (**Figure 6** and **Figure 7**) show that all the anti-LAG-3 antibodies tested blocked the binding of a shLAG-Fc fusion protein to bind MHC class II.

B. Example 2: Flow-Cytometry Methodology

[00240] Experiments to determine expression levels of checkpoint inhibitors: PD-1 and LAG-3 on cells in the experiments described below used the following appropriately fluorescent labeled commercial antibodies (phycoerythrin-cyanine7 (PE-

Cy7)-conjugated anti-CD4 [clone SK3] or fluorescein isothiocyanate (FITC)-conjugated anti-CD4 [clone RPA-T4], phycoerythrin (PE)-conjugated anti-LAG-3 [clone 3DS223H], phycoerythrin (PE)-conjugated anti-PD-1 [clone EH12.2H7] or allophycocyanin (APC)-conjugated (eBiosciences, or BioLegend)) and the appropriate isotype controls. All antibodies were used at the manufacturer's recommended concentrations. Cell staining was performed in FACS buffer (10% FCS in PBS) on ice for 30 minutes in the dark for the addition of primary antibodies. After two washes, cells were either stained with the appropriate secondary reagent on ice for 30 minutes in the dark or immediately analyzed on a flow cytometer. To exclude dead cells, all samples were co-stained with a viability dye: 7-Aminoactinomycin D (7-AAD) (BD Biosciences, or BioLegend,) or 4',6-Diamidino-2-Phenylindole, Dihydrochloride (DAPI) (Life Technologies) All samples were analyzed on either a FACS Calibur or Fortessa Flow Cytometer (BD Biosciences) and analyzed using FlowJo Software (TreeStar, Ashland, OR).

C. Example 3: LAG-3 Expression Binding To Stimulated T-Cells

[00241] LAG-3 expression and the ability of the isolated LAG-3 antibodies to specifically bind LAG-3 on the surface of CD3/CD28-stimulated T-cells was examined. T-cells were obtained from peripheral blood mononuclear cell (PBMCs), briefly, PBMCs were purified using the Ficoll-Paque Plus (GE Healthcare) density gradient centrifugation method according to manufacturer's instructions from whole blood obtained under informed consent from healthy donors (Biological Specialty Corporation) and T cells were then purified using the Dynabeads® Untouched Human T Cells Kit (Life Technologies) according to manufacturer's instructions. For stimulation, isolated T cells were cultured for 10-14 days in the presence of recombinant human IL-2 30 U/ml] (Peprotech) and Dynabeads® Human T cell Activator beads (Life Technologies) according to manufacturer's instructions. LAG-3 expression on freshly isolated unstimulated CD4+ T cells, and stimulated CD4+ T cells (taken from culture at day 11 or 14), was examined by flow cytometry as described above, using FITC-conjugated anti-CD4 and PE-conjugated anti-LAG-3.

[00242] The results of these studies are shown in **Figure 8**. No LAG-3 expression was observed on unstimulated CD4+ T-cells (**Figure 8, Panel A**). However, CD3/CD8 stimulated CD4+ T-cells from two different donors (D:58468 and D:43530) exhibited a dramatic increase in LAG-3 expression (**Figure 8, Panels B** and **C**).

[00243] The ability of LAG-3 mAb 1, LAG-3 mAb 2, LAG-3 mAb 3, LAG-3 mAb 4, and LAG-3 mAb 5, to specifically bind to stimulated CD4+ T cells was investigated. Stimulated T cells (prepared as described above from donor D:58468) taken from culture at day 14, and fresh unstimulated cells (prepared as described above from donor D:43530) were subjected to flow cytometry using the isolated anti-LAG-3 antibodies and the following appropriately fluorescent labeled secondary reagent (PC-conjugated anti-mouse-IgG (H+L) (Jackson ImmunoResearch Labs)) and FITC-conjugated anti-CD4. As shown in **Figure 9** and **Figure 10** each of the anti-LAG-3 antibodies examined bound only to stimulated, but not to unstimulated CD4+ T-cells.

[00244] The results of these studies demonstrate that LAG-3 is upregulated on stimulated T-cells and that the anti-LAG-3 antibodies of the present invention specifically bind only stimulated T-cells.

D. Example 4: Functional Activity of anti-LAG Antibodies

[00245] *Staphylococcus aureus* enterotoxin type B (SEB) is a microbial superantigen capable of activating a large proportion of T-cells (5-30%). SEB binds to MHC II outside the peptide binding grove and thus is MHC II dependent, but unrestricted and TCR mediated. SEB-stimulation of T-cells results in oligoclonal T-cell proliferation and cytokine production (although donor variability may be observed). The expression of anti-LAG-3 and anti-PD-1 antibodies alone and in combination on SEB-stimulated PMBCs was examined.

[00246] PBMCs purified as described above were cultured in RPMI-media + 10% heat inactivated FBS + 1% Penicillin/Streptomycin in T-25 bulk flasks for 2-3 days alone or with SEB (Sigma-Aldrich) at 0.1 ng/ml (primary stimulation). At the end of the first round of SEB-stimulation, PBMCs were washed twice with PBS and immediately plated in 96-well tissue culture plates at a concentration of 1-5 x 10^5

cells/well in media alone, media with SEB at 0.1 ng/ml (secondary stimulation) and no antibody, or media with SEB and a control IgG antibody, and cultured for an additional 2-3 days. At 48 hours post-primary bulk SEB-stimulation were examined for PD-1 and LAG-3 expression by flow cytometry using PE-conjugated anti-LAG-3 and FITC-conjugated anti-CD3; or APC-conjugated anti-PD-1 and FITC-conjugated anti-CD3. At day 5, post-secondary culture in 96-well plate with SEB-stimulation, wells treated with no antibody or with control antibody were examined using flow cytometry for PD-1 and LAG-3 expression using PE-conjugated anti-LAG-3 and APC-conjugated anti-PD-1.

[00247] Flow cytometry results from two representative donors (D:34765 and D:53724) are shown in **Figure 11** (D:34765) and **Figure 12** (D:53724). These results demonstrate that LAG-3 and PD-1 are upregulated by 48 hours post-SEB-stimulation with a further enhancement seen at day 5 post culture with SEB-stimulation. In these studies Donor 1 had more LAG-3/PD-1 double positive cells after SEB-stimulation. The addition of a control antibody post-SEB-stimulation did not alter LAG-3 or PD-1 expression (**Figures 11** and **12**, compare **Panels C** and **D**).

[00248] Upregulation of the immune check point proteins LAG-3 and PD-1 following SEB-stimulation of PBMCs limits cytokine release upon restimulation. The ability of LAG-3 mAb 1, LAG-3 mAb 3, LAG-3 mAb 4, LAG-3 mAb 6, a PD-1 monoclonal antibody designated "**PD-1 mAb 5**", and the reference antibodies PD-1 mAb 1 (comprising the 235A/235A Fc variant (AA)), PD-1 mAb 2, LAG-3 mAb A, and the commercial anti-LAG3 antibody 17B4 (#LS-C18692, LS Bio, designated "**LAG-3 mAb B**") to enhance cytokine release through checkpoint inhibition was examined.

[00249] PBMCs were stimulated with SEB as described above except during the secondary stimulations cells were plated with no antibody, with an isotype control antibody, or with anti-LAG-3 and anti-PD-1 antibodies alone or in combination. At the end of the second stimulation, supernatants were harvested to measure cytokine secretion using human DuoSet ELISA Kits for IFNγ, TNFα, IL-10, and IL-4 (R&D Systems) according to the manufacture's instructions. **Figure 13** shows the IFNγ

(**Figure 13A**) and TNFα (**Figure 13B**), secretion profiles from SEB-stimulated PBMCs from a representative donor (D:38166), treated with no antibody or one of the following antibodies: isotype control, PD-1 mAb 1, PD-1 mAb 2, LAG-3 mAb A, LAG-3 mAb B, LAG-3 mAb 1, LAG-3 mAb 3, LAG-3 mAb 4, or LAG-3 mAb 6. For this study the antibodies were utilized at 0.009, 0.039, 0.156, 0.625, 2.5, 10, and 40 μg/ml. **Figure 14** shows the IFNγ secretion profiles from SEB-stimulated PBMCs from another representative donor (D:58108), treated with: no antibody; isotype control antibody; PD-1 mAb 2 and/or LAG-3 mAb A; PD-1 mAb 5 and/or LAG-3 mAb 1; or PD-1 mAb 5 and/or LAG-3 mAb 3. For this study the antibodies were used at 10 μg/ml.

[00250] The results of these studies demonstrate that anti-PD-1 antibodies dramatically enhanced IFNγ (**Figure 13A** and **Figure 14**), and TNFα (**Figure 13B**) production from SEB-stimulated PBMCs upon restimulation. Surprisingly, LAG-3 mAb 1 was also seen to increase cytokine production across multiple donors to levels comparable to the anti-PD-1 antibodies while the reference anti-LAG-3 mAbs, LAG-3 mAb A and LAG-3 mAb B and several of the isolated antibodies (LAG-3 mAb 3, LAG-4, and LAG-6) provided only a slight enhancement of cytokine release. In addition, the combination of anti-LAG-3 antibodies with anti-PD-1 resulted in a further enhancement of cytokine release (**Figure 14**, **Panels A** and **B**) from SEB-stimulated PBMCs upon restimulation. LAG-3 mAb 1 provided the largest enhancement in cytokine release when combined with an anti-PD-1 antibody.

[00251] All publications and patents mentioned in this specification are herein incorporated by reference to the same extent as if each individual publication or patent application was specifically and individually indicated to be incorporated by reference in its entirety. While the invention has been described in connection with specific embodiments thereof, it will be understood that it is capable of further modifications and this application is intended to cover any variations, uses, or adaptations of the invention following, in general, the principles of the invention and including such departures from the present disclosure as come within known or customary practice within the art to which the invention pertains and as may be applied to the essential features hereinbefore set forth.

What Is Claimed Is:

Claim 1. An anti-human LAG-3-binding molecule that comprises a Variable Heavy Chain Domain and a Variable Light Chain Domain, wherein:

said Variable Heavy Chain Domain comprises a $CDR_{H}1$ Domain, a $CDR_{H}2$ Domain and a $CDR_{H}3$ Domain, and said Variable Light Chain Domain comprises a $CDR_{L}1$ Domain, a $CDR_{L}2$ Domain, and a $CDR_{L}3$ Domain, wherein:

(A) (1) the $CDR_{H}1$ Domain, $CDR_{H}2$ Domain, and $CDR_{H}3$ Domain are the Heavy Chain CDRs of LAG-3 mAb 1, and respectively have the amino acid sequences: **SEQ ID NO:4**, **SEQ ID NO:5**, and **SEQ ID NO:6**; and

(2) the $CDR_{L}1$ Domain, $CDR_{L}2$ Domain, and $CDR_{L}3$ Domain are the Light Chain CDRs of LAG-3 mAb 1, and respectively have the amino acid sequences: **SEQ ID NO:9**, **SEQ ID NO:10**, and **SEQ ID NO:11**;

or

(B) (1) the $CDR_{H}1$ Domain, $CDR_{H}2$ Domain, and $CDR_{H}3$ Domain are the Heavy Chain CDRs of LAG-3 mAb 2, and respectively have the amino acid sequences: **SEQ ID NO:14**, **SEQ ID NO:15**, and **SEQ ID NO:16**; and

(2) the $CDR_{L}1$ Domain, $CDR_{L}2$ Domain, and $CDR_{L}3$ Domain are the Light Chain CDRs of LAG-3 mAb 2, and, respectively have the amino acid sequences: **SEQ ID NO:19**, **SEQ ID NO:20**, and **SEQ ID NO:21**;

or

(C) (1) the $CDR_{H}1$ Domain, $CDR_{H}2$ Domain, and $CDR_{H}3$ Domain are the Heavy Chain CDRs of LAG-3 mAb 3, and respectively have the amino acid sequences: **SEQ ID NO:54**, **SEQ ID NO:55**, and **SEQ ID NO:56**; and

(2) the $CDR_{L}1$ Domain, $CDR_{L}2$ Domain, and $CDR_{L}3$ Domain are the Light Chain CDRs of LAG-3 mAb 3,

and, respectively have the amino acid sequences: **SEQ ID NO:59**, **SEQ ID NO:60**, and **SEQ ID NO:61**;

or

(D) (1) the $CDR_{H}1$ Domain, $CDR_{H}2$ Domain, and $CDR_{H}3$ Domain are the Heavy Chain CDRs of LAG-3 mAb 4, and respectively have the amino acid sequences: **SEQ ID NO:64**, **SEQ ID NO:65**, and **SEQ ID NO:66**; and

(2) the $CDR_{L}1$ Domain, $CDR_{L}2$ Domain, and $CDR_{L}3$ Domain are the Light Chain CDRs of LAG-3 mAb 4, and, respectively have the amino acid sequences: **SEQ ID NO:69**, **SEQ ID NO:70**, and **SEQ ID NO:71**;

or

(E) (1) the $CDR_{H}1$ Domain, $CDR_{H}2$ Domain, and $CDR_{H}3$ Domain are the Heavy Chain CDRs of LAG-3 mAb 5, and respectively have the amino acid sequences: **SEQ ID NO:74**, **SEQ ID NO:75**, and **SEQ ID NO:76**; and

(2) the $CDR_{L}1$ Domain, $CDR_{L}2$ Domain, and $CDR_{L}3$ Domain are the Light Chain CDRs of LAG-3 mAb 5, and, respectively have the amino acid sequences: **SEQ ID NO:79**, **SEQ ID NO:80**, and **SEQ ID NO:81**;

or

(F) (1) the $CDR_{H}1$ Domain, $CDR_{H}2$ Domain, and $CDR_{H}3$ Domain are the Heavy Chain CDRs of LAG-3 mAb 6 VH1, and respectively have the amino acid sequences: **SEQ ID NO:84**, **SEQ ID NO:85**, and **SEQ ID NO:86**; and

(2) the $CDR_{L}1$ Domain, $CDR_{L}2$ Domain, and $CDR_{L}3$ Domain are the Light Chain CDRs of LAG-3 mAb 6, and, respectively have the amino acid sequences: **SEQ ID NO:89**, **SEQ ID NO:90**, and **SEQ ID NO:91**;

or

(G) (1) the CDR_H1 Domain, CDR_H2 Domain, and CDR_H3 Domain are the Heavy Chain CDRs of LAG-3 mAb 1, and respectively have the amino acid sequences: **SEQ ID NO:4**, **SEQ ID NO:5**, and **SEQ ID NO:6**; and

(2) the CDR_L1 Domain, CDR_L2 Domain, and CDR_L3 Domain are the Light Chain CDRs of hLAG-3 mAb 1, and respectively have the amino acid sequences: **SEQ ID NO:104**, **SEQ ID NO:10**, and **SEQ ID NO:11**.

Claim 2. The anti-human LAG-3-binding molecule of any one of claims 1, wherein said molecule is an antibody.

Claim 3. The anti-human LAG-3-binding molecule of claim 2, wherein said molecule is a chimeric antibody or a humanized antibody.

Claim 4. The anti-human LAG-3-binding molecule of claim 1 or 3, wherein said molecule comprises a Heavy Chain Variable Domain having the amino acid sequence of **SEQ ID NO:92** or **SEQ ID NO:94**.

Claim 5. The anti-human LAG-3-binding molecule of of any one of claims 1,3 or 4, wherein said molecule comprises a Light Chain Variable Domain having the amino acid sequence of **SEQ ID NO:96, SEQ ID NO:98, SEQ ID NO:100** or **SEQ ID NO:102**.

Claim 6. The anti-human LAG-3-binding molecule of any one of claims 1-5, wherein said molecule is a bispecific binding molecule, capable of simultaneously binding to human LAG-3 and to a second epitope.

Claim 7. The anti-human LAG-3-binding molecule of claim 6, wherein said second epitope is an epitope of a molecule involved in regulating an immune check point present on the surface of an immune cell.

Claim 8. The anti-human LAG-3-binding molecule of claim 6, wherein said second epitope is an epitope of B7-H3, B7-H4, BTLA, CD40, CD40L, CD47, CD70, CD80, CD86, CD94, CD137, CD137L, CD226, CTLA-

4, Galectin-9, GITR, GITRL, HHLA2, ICOS, ICOSL, KIR, LAG-3, LIGHT, MHC class I or II, NKG2a, NKG2d, OX40, OX40L, PD1H, PD-1, PD-L1, PD-L2, PVR, SIRPa, TCR, TIGIT, TIM-3 or VISTA.

Claim 9. The anti-human LAG-3-binding molecule of any one of claims 6-8, wherein said molecule is a diabody, said diabody being a covalently bonded complex that comprises two, or three or four different polypeptide chains.

Claim 10. The anti-human LAG-3-binding molecule of claim 9, wherein said molecule comprises an Fc Region.

Claim 11. The anti-human LAG-3-binding molecule of claim 9, wherein said molecule comprises an Albumin-Binding Domain (ABD).

Claim 12. The anti-human LAG-3-binding molecule of claim 11, wherein said ABD is a deimmunized ABD.

Claim 13. The anti-human LAG-3-binding molecule of any one of claims 2-5 or 10, wherein said Fc Region is a variant Fc Region that comprises one or more amino acid modifications that reduces the affinity of the variant Fc Region for an FcγR or stabilize said Fc Region.

Claim 14. The anti-human LAG-3-binding molecule of claim 13, wherein said modifications comprise the substitution of L234A; L235A; or L234A and L235A, wherein said numbering is that of the EU index as in Kabat.

Claim 15. The anti-human LAG-3-binding molecule of any one of claims 1-14, wherein said molecule is used to stimulate a T-cell mediate immune response of a subject in need thereof.

Claim 16. The anti-human LAG-3-binding molecule of any one of claims 1-14, wherein said molecule is used in the treatment of a disease or condition associated with a suppressed immune system.

Claim 17. The anti-human LAG-3-binding molecule of claim 16, wherein the disease or condition is cancer or an infection.

Claim 18. The anti-human LAG-3-binding molecule of claim 17, wherein said cancer is characterized by the presence of a cancer cell selected from the group consisting of a cell of: an adrenal gland tumor, an AIDS-associated cancer, an alveolar soft part sarcoma, an astrocytic tumor, bladder cancer, bone cancer, a brain and spinal cord cancer, a metastatic brain tumor, a breast cancer, a carotid body tumors, a cervical cancer, a chondrosarcoma, a chordoma, a chromophobe renal cell carcinoma, a clear cell carcinoma, a colon cancer, a colorectal cancer, a cutaneous benign fibrous histiocytoma, a desmoplastic small round cell tumor, an ependymoma, a Ewing's tumor, an extraskeletal myxoid chondrosarcoma, a fibrogenesis imperfecta ossium, a fibrous dysplasia of the bone, a gallbladder or bile duct cancer, gastric cancer, a gestational trophoblastic disease, a germ cell tumor, a head and neck cancer, hepatocellular carcinoma, an islet cell tumor, a Kaposi's Sarcoma, a kidney cancer, a leukemia, a lipoma/benign lipomatous tumor, a liposarcoma/malignant lipomatous tumor, a liver cancer, a lymphoma, a lung cancer, a medulloblastoma, a melanoma, a meningioma, a multiple endocrine neoplasia, a multiple myeloma, a myelodysplastic syndrome, a neuroblastoma, a neuroendocrine tumors, an ovarian cancer, a pancreatic cancer, a papillary thyroid carcinoma, a parathyroid tumor, a pediatric cancer, a peripheral nerve sheath tumor, a phaeochromocytoma, a pituitary tumor, a prostate cancer, a posterious uveal melanoma, a rare hematologic disorder, a renal metastatic cancer, a rhabdoid tumor, a rhabdomysarcoma, a sarcoma, a skin cancer, a soft-tissue sarcoma, a squamous cell cancer, a stomach cancer, a synovial sarcoma, a testicular cancer, a thymic carcinoma, a thymoma, a thyroid metastatic cancer, and a uterine cancer.

Claim 19. The anti-human LAG-3-binding molecule of claim 43, wherein said cancer is colorectal cancer, hepatocellular carcinoma, glioma, kidney

cancer, breast cancer, multiple mycloma, bladder cancer, neuroblastoma; sarcoma, non-Hodgkin's lymphoma, non-small cell lung cancer, ovarian cancer, pancreatic cancer, rectal cancer, acute myeloid leukemia (AML), chronic myelogenous leukemia (CML), acute B lymphoblastic leukemia (B-ALL), chronic lymphocytic leukemia (CLL), hairy cell leukemia (HCL), blastic plasmacytoid dendritic cell neoplasm (BPDCN), non-Hodgkin's lymphomas (NHL), including mantel cell leukemia (MCL), and small lymphocytic lymphoma (SLL), Hodgkin's lymphoma, systemic mastocytosis, or Burkitt's lymphoma.

Claim 20. The anti-human LAG-3-binding molecule of any one of claims 1-14, wherein said molecule is detectably labeled and is used in the detection of LAG-3.

Abstract of the Invention:

The present invention is directed to the anti-LAG-3 antibodies, LAG-3 mAb 1, LAG-3 mAb 2, LAG-3 mAb 4, LAG-3 mAb 5, and LAG-3 mAb 6, and to humanized and chimeric versions of such antibodies. The invention additionally pertains to LAG-3-binding molecules that comprise LAG-3 binding fragments of such anti-LAG-3 antibodies, immunocongugates, and to bispecific molecules, including diabodies, BiTEs, bispecific antibodies, *etc.*, that comprise (i) such LAG-3-binding fragments, and (ii) a domain capable of binding an epitope of a molecule involved in regulating an immune check point present on the surface of an immune cells. The present invention also pertains to methods of detecting LAG-3, as well as methods of using molecules that bind LAG-3 for stimulating immune responses.

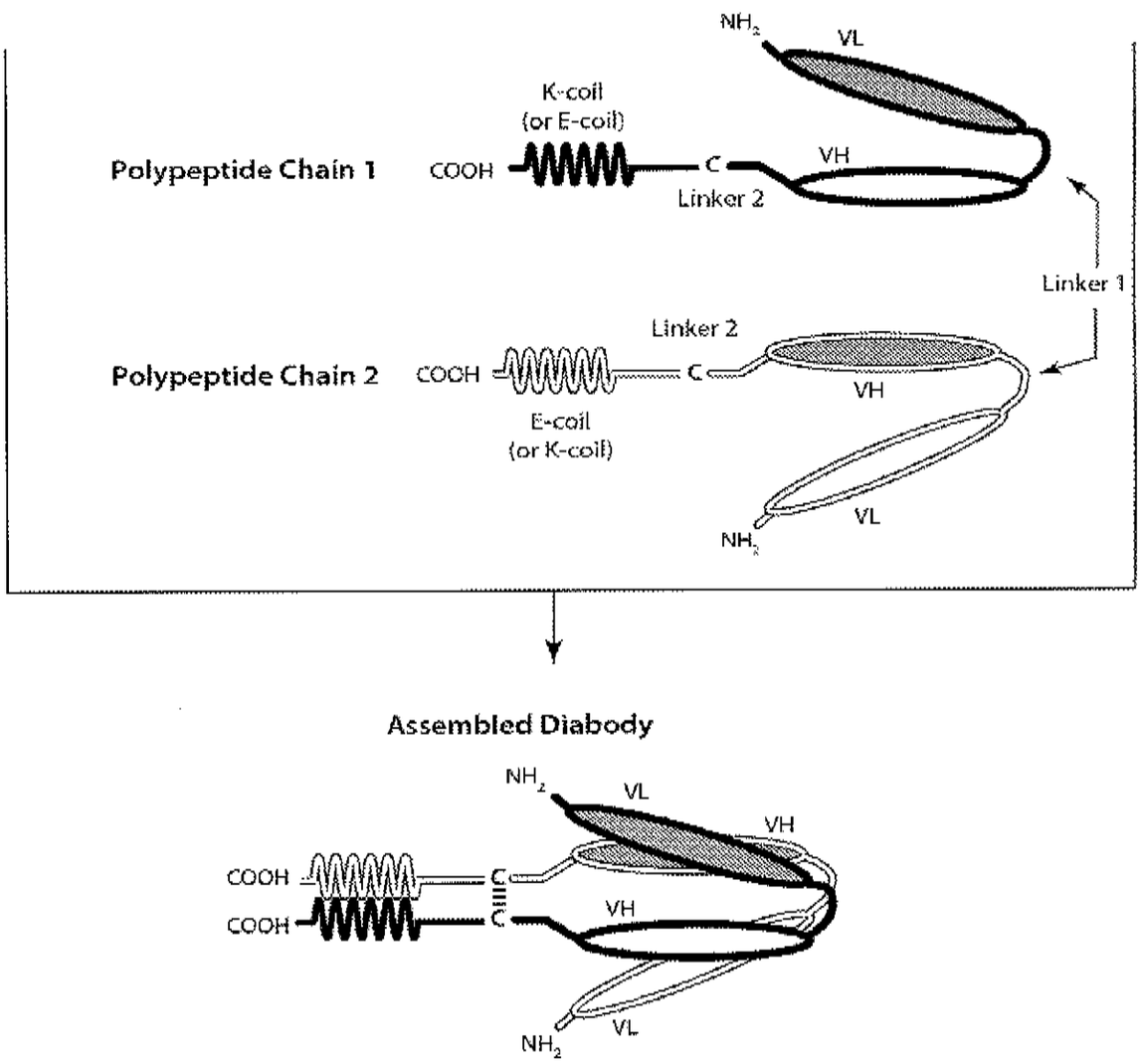


Figure 1

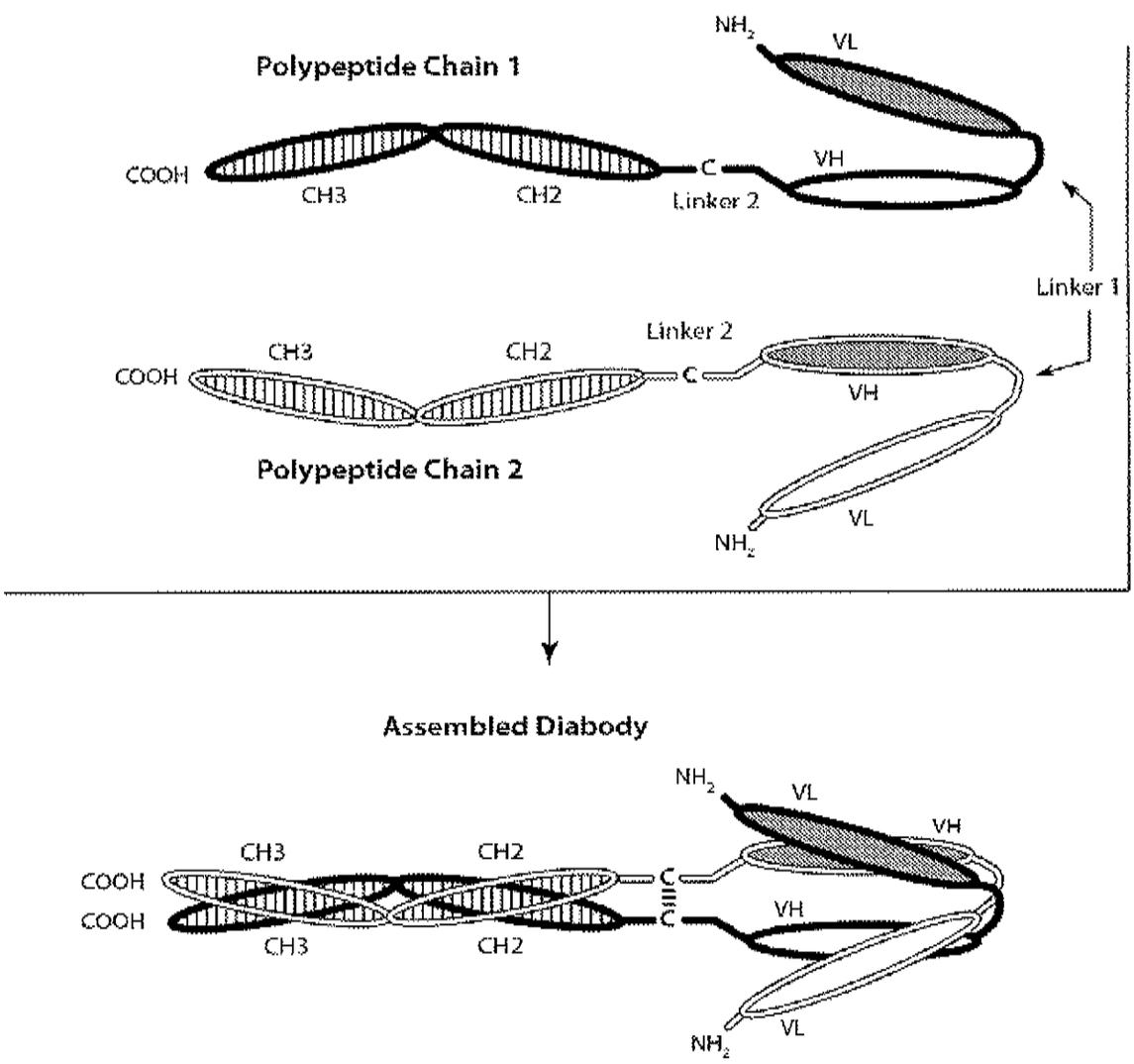


Figure 2

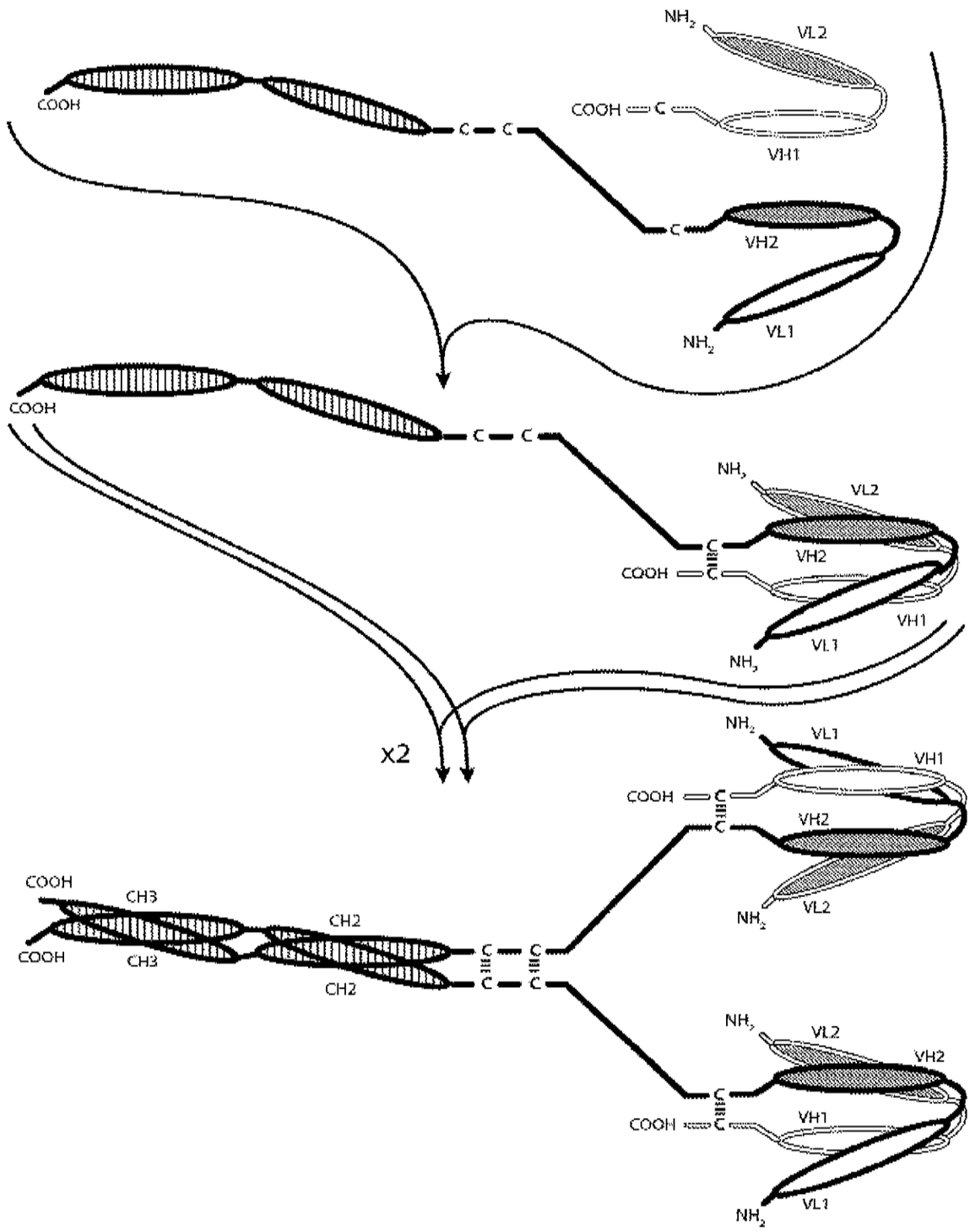


Figure 3A

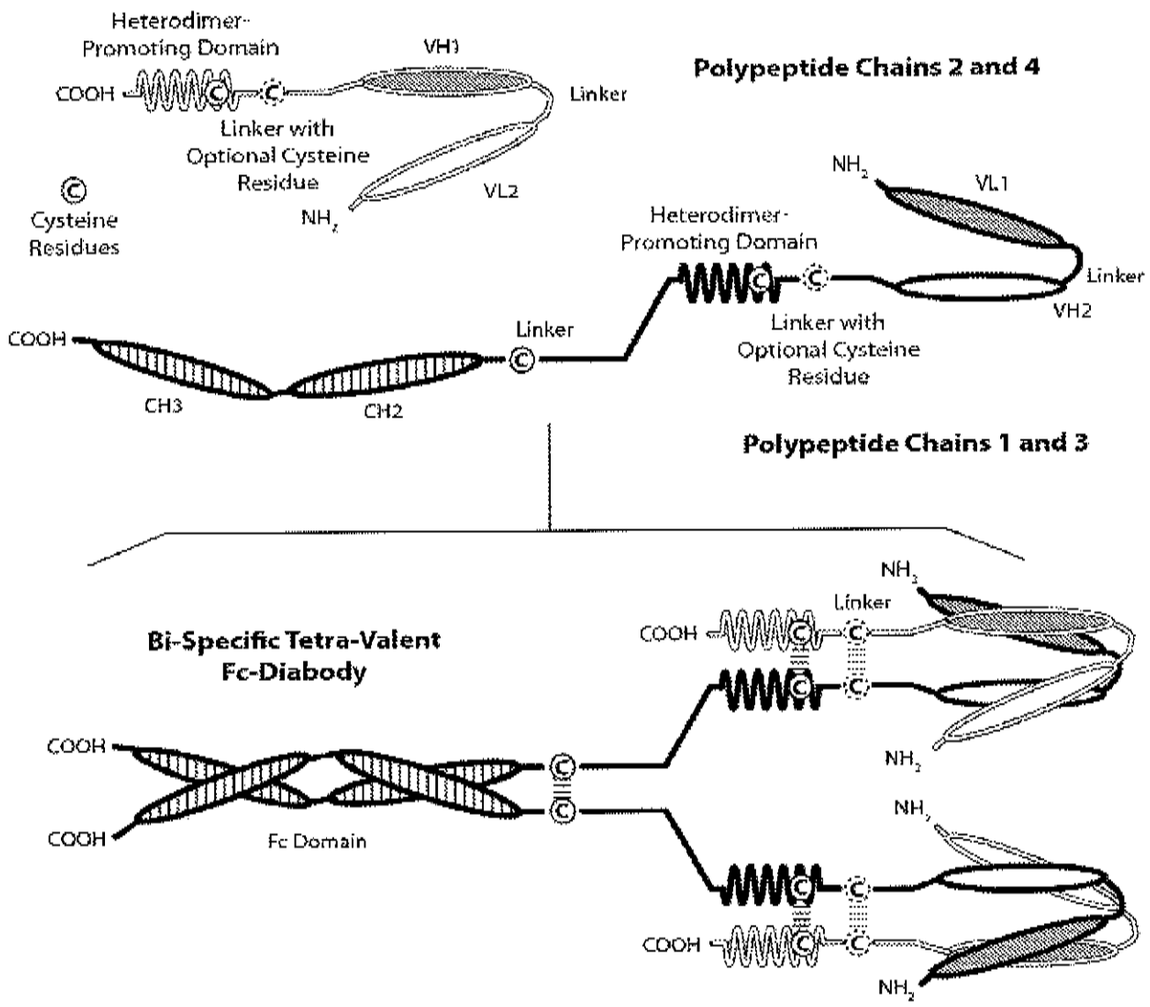


Figure 3B

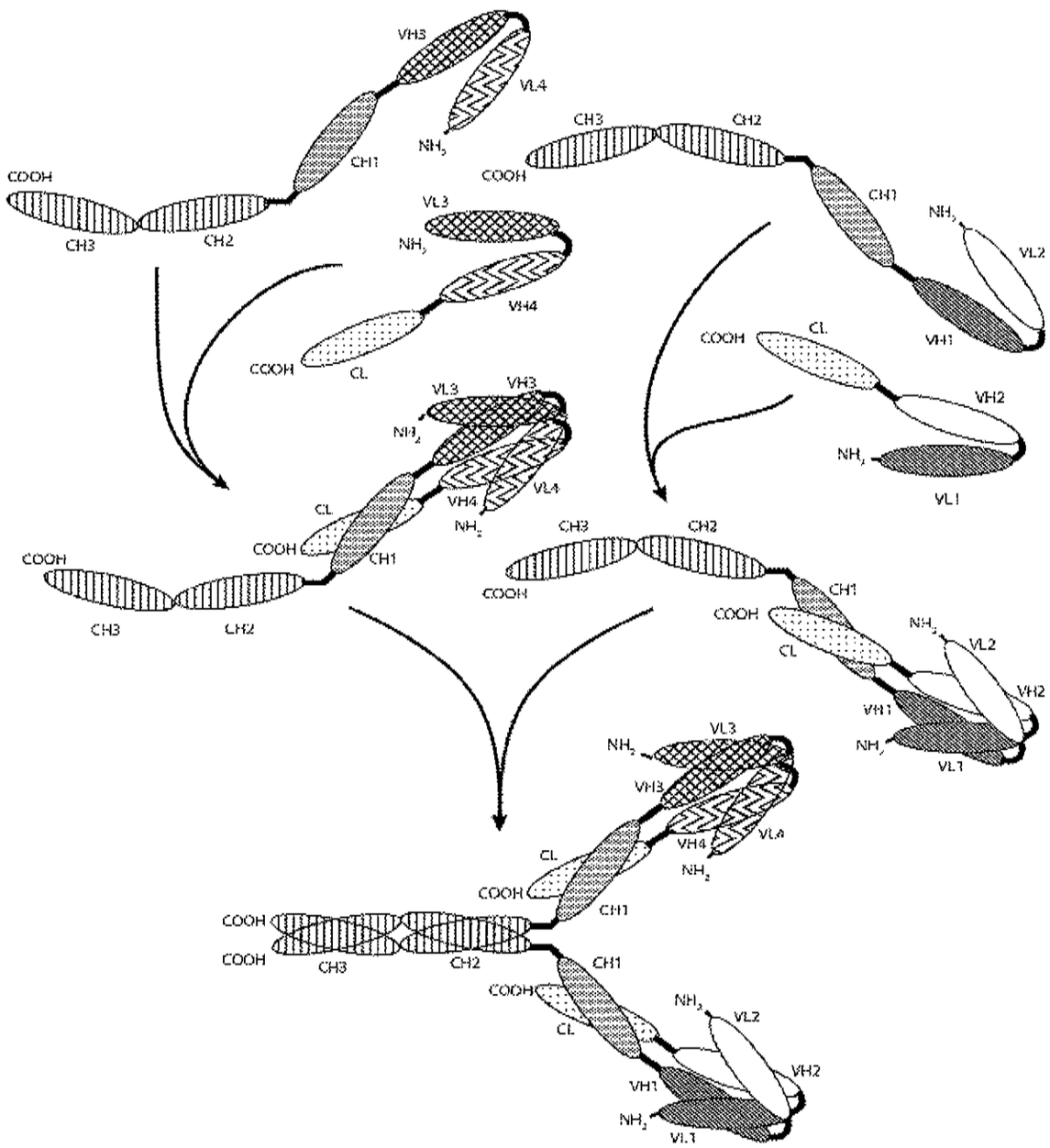


Figure 3C

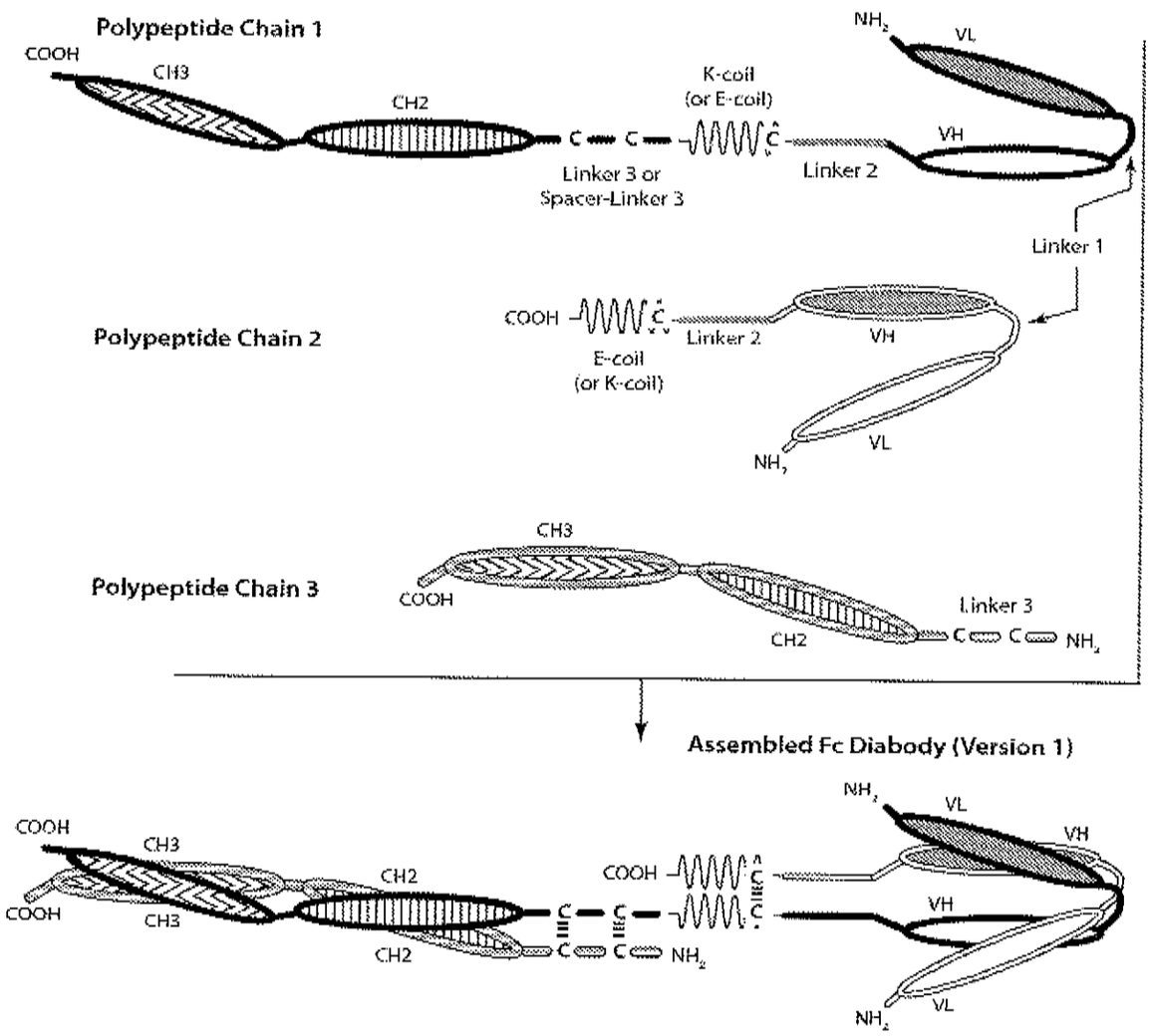


Figure 4A

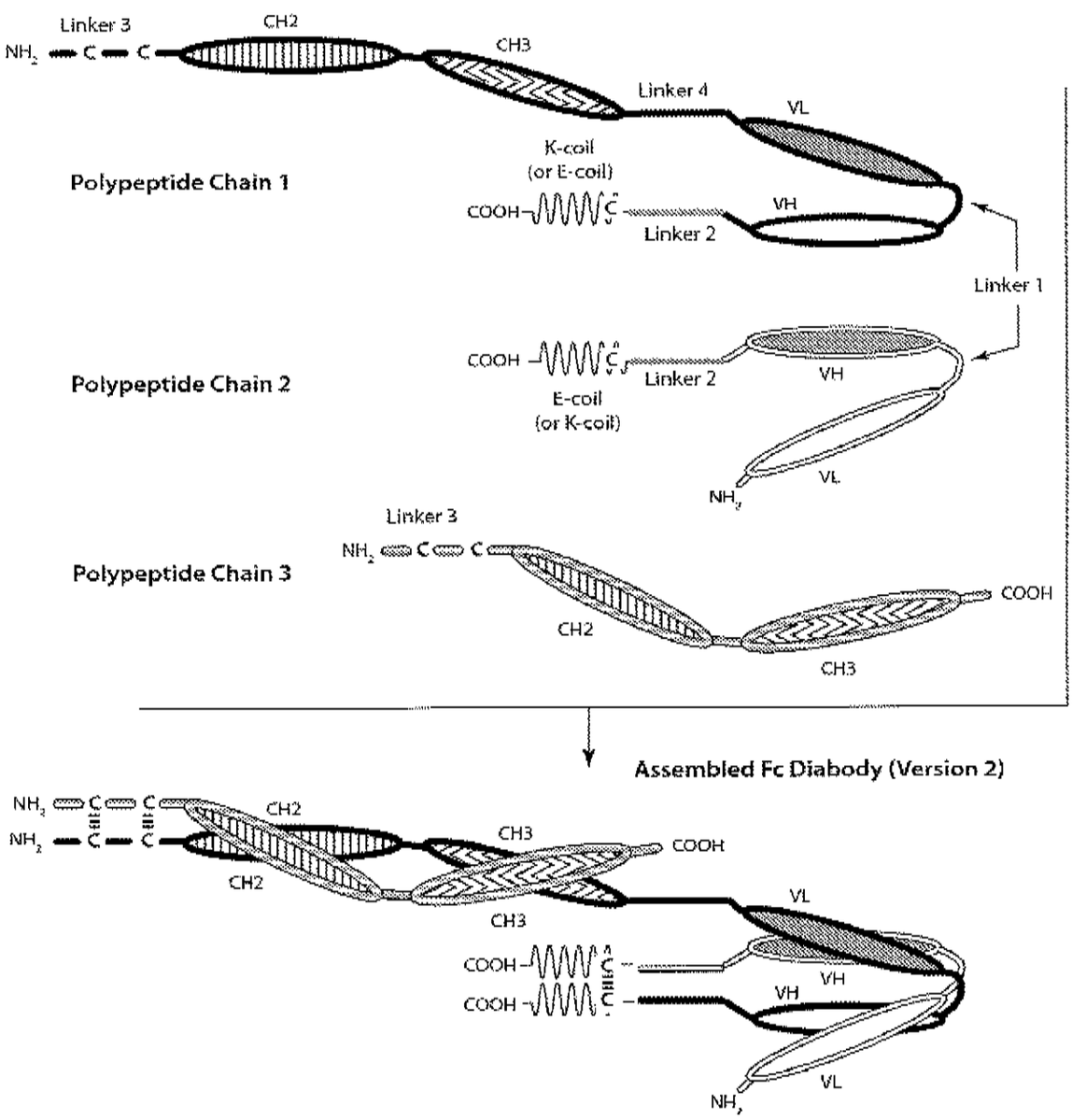


Figure 4B

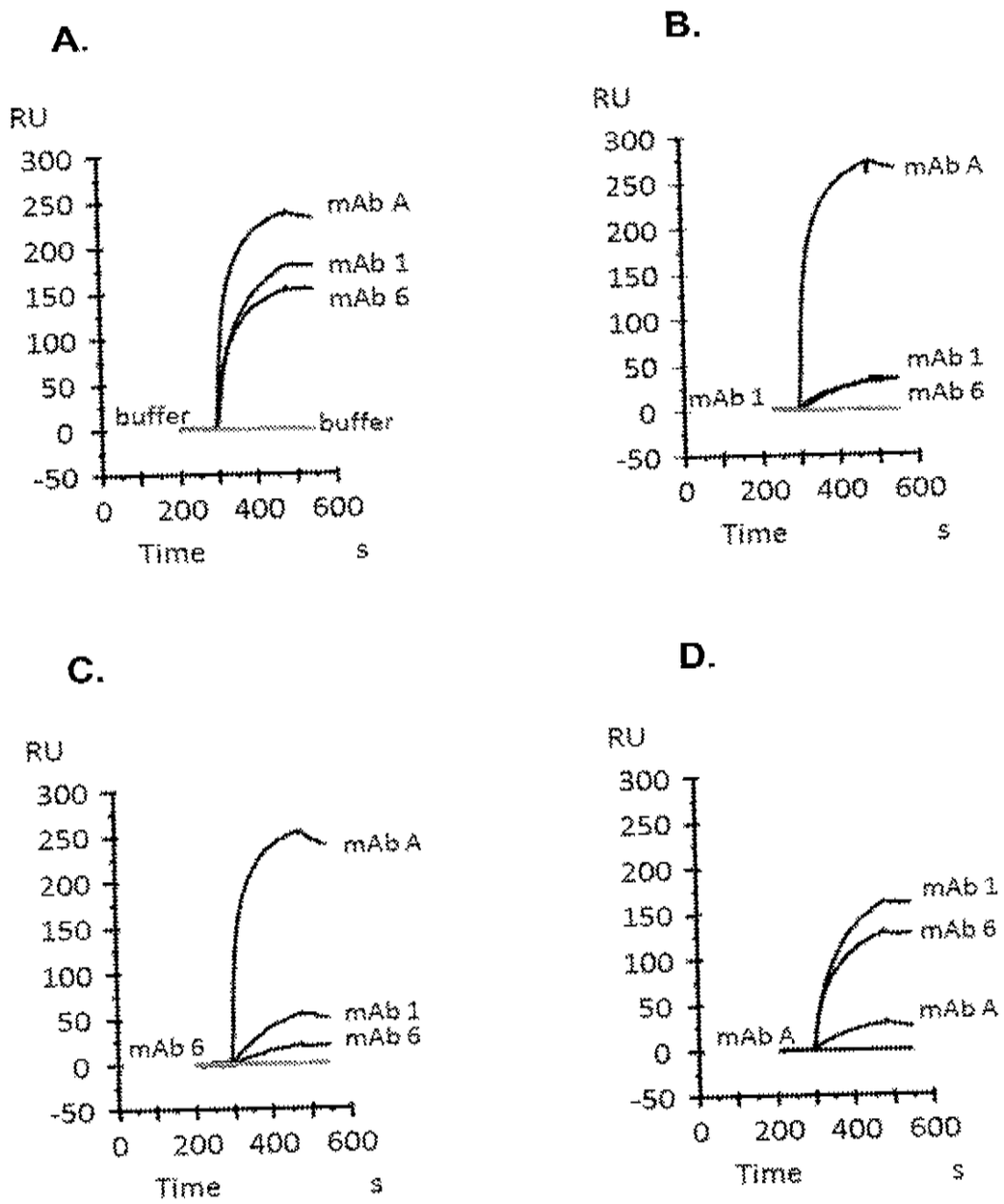


Figure 5

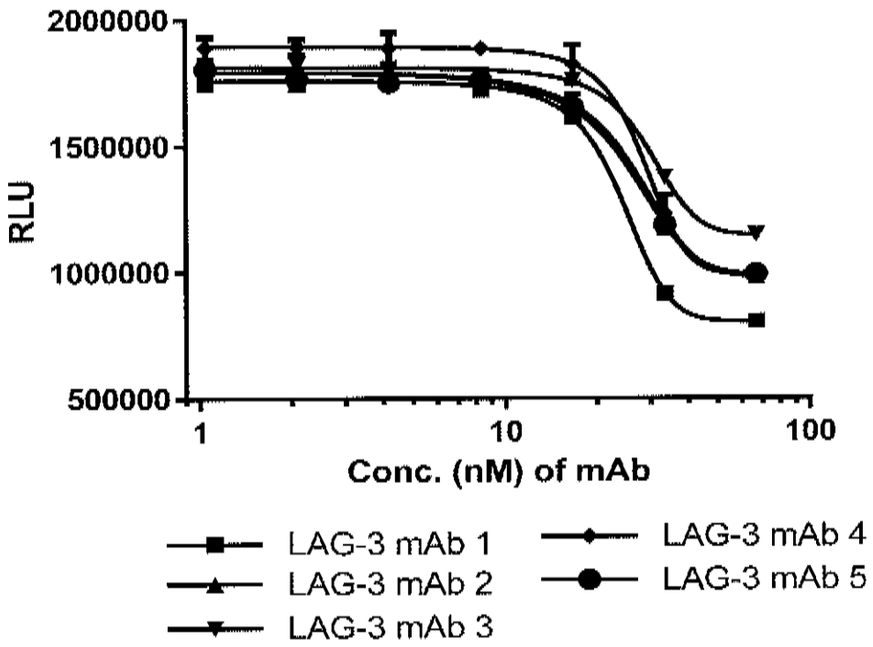


Figure 6

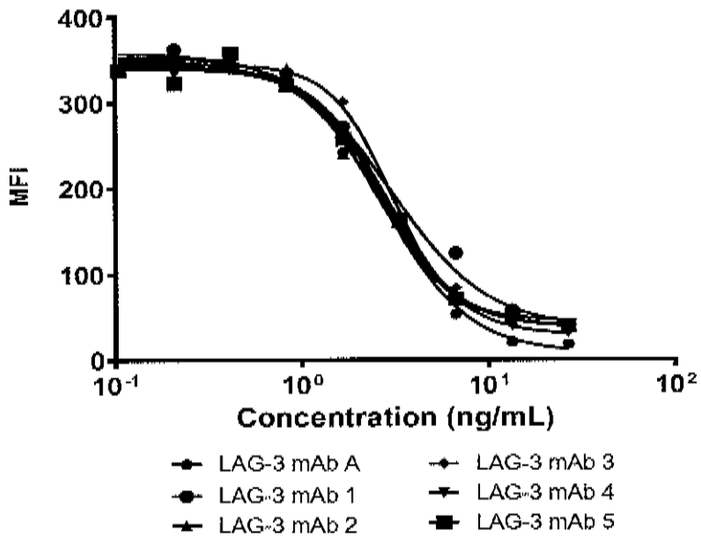


Figure 7A

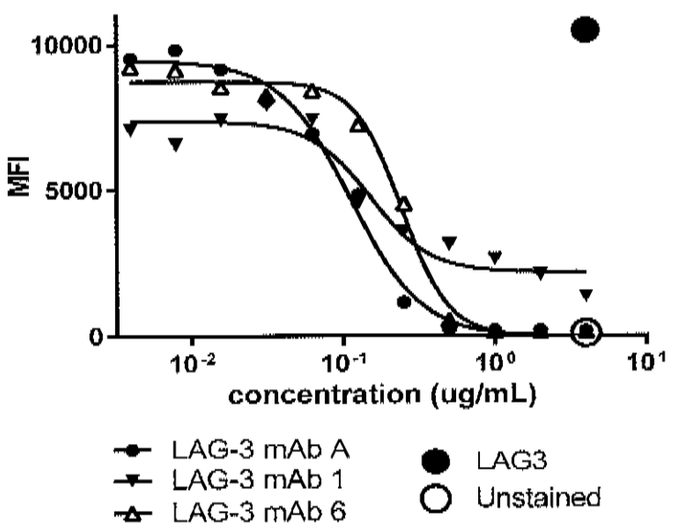


Figure 7B

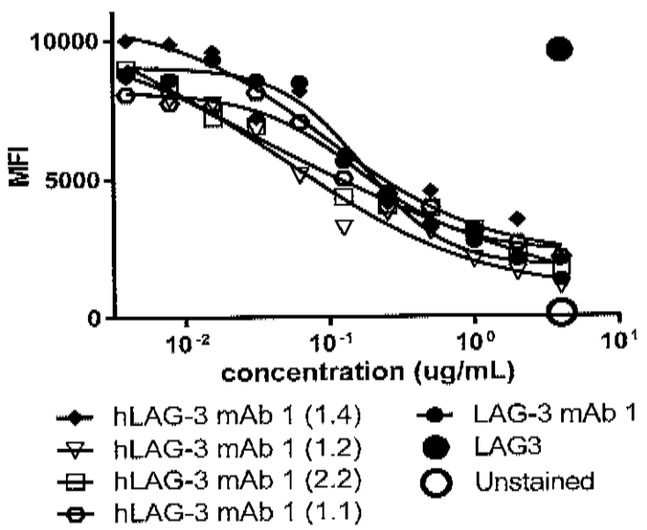
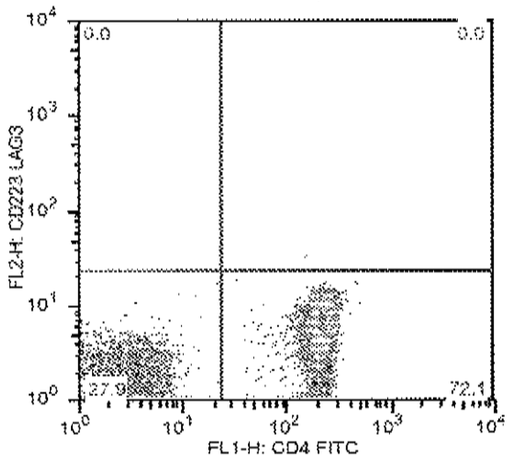
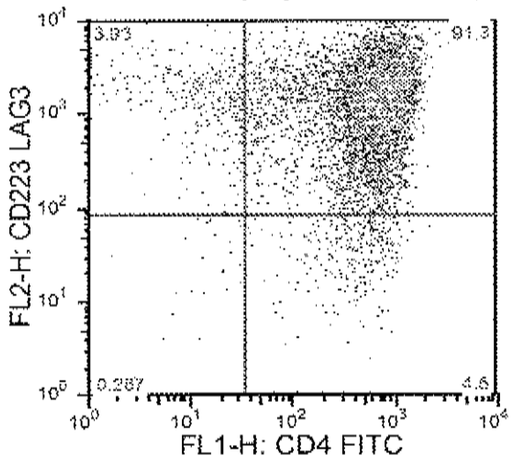


Figure 7C

A. Unstimulated (D:58468)



B. Stimulated (day 11, D:58468)



C. Stimulated (day 14, D:43530)

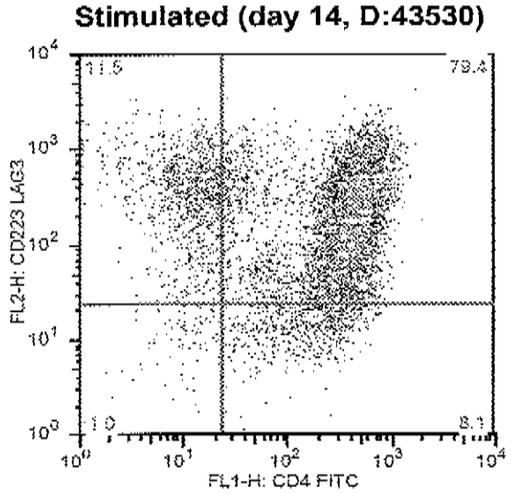


Figure 8

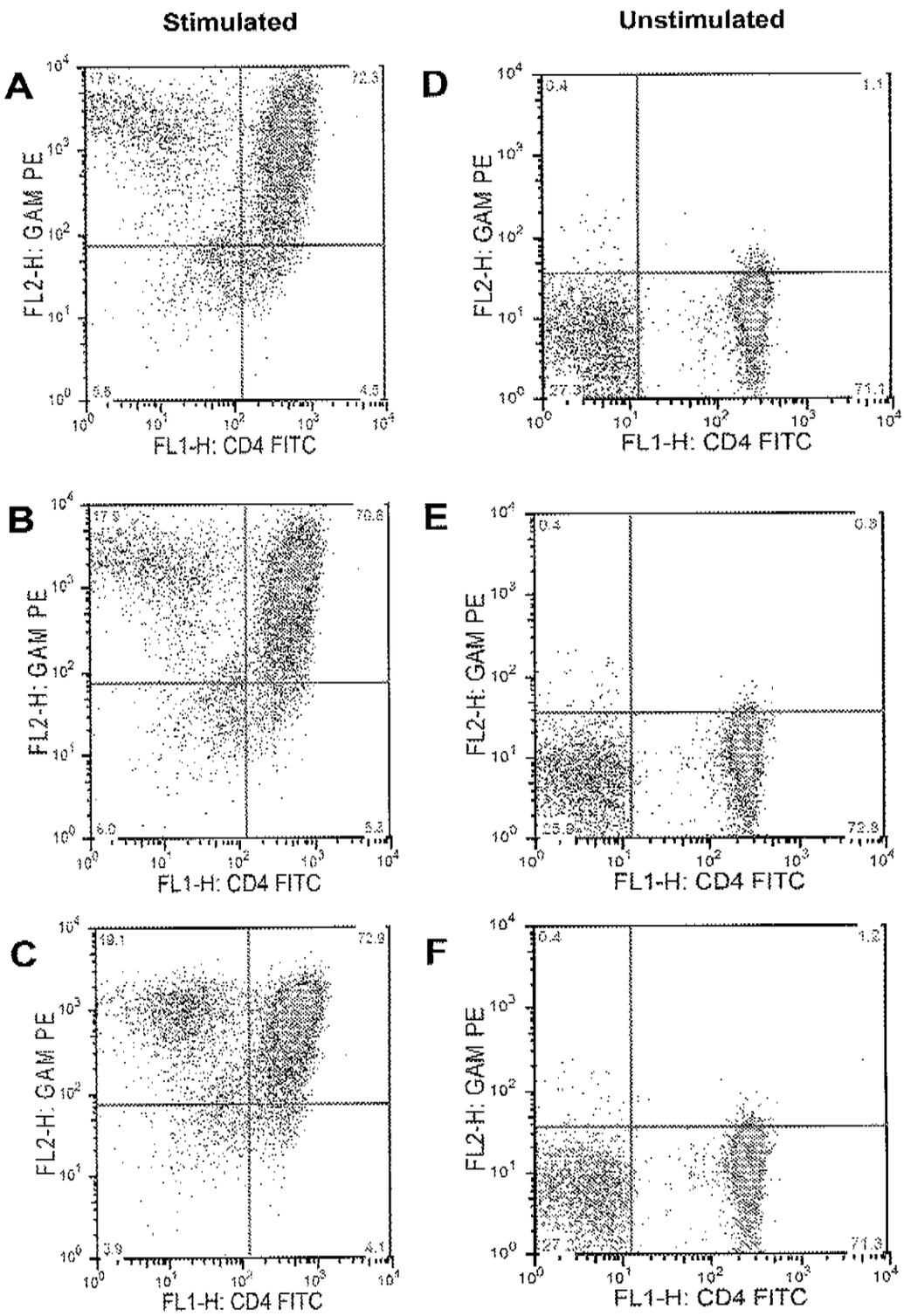


Figure 9

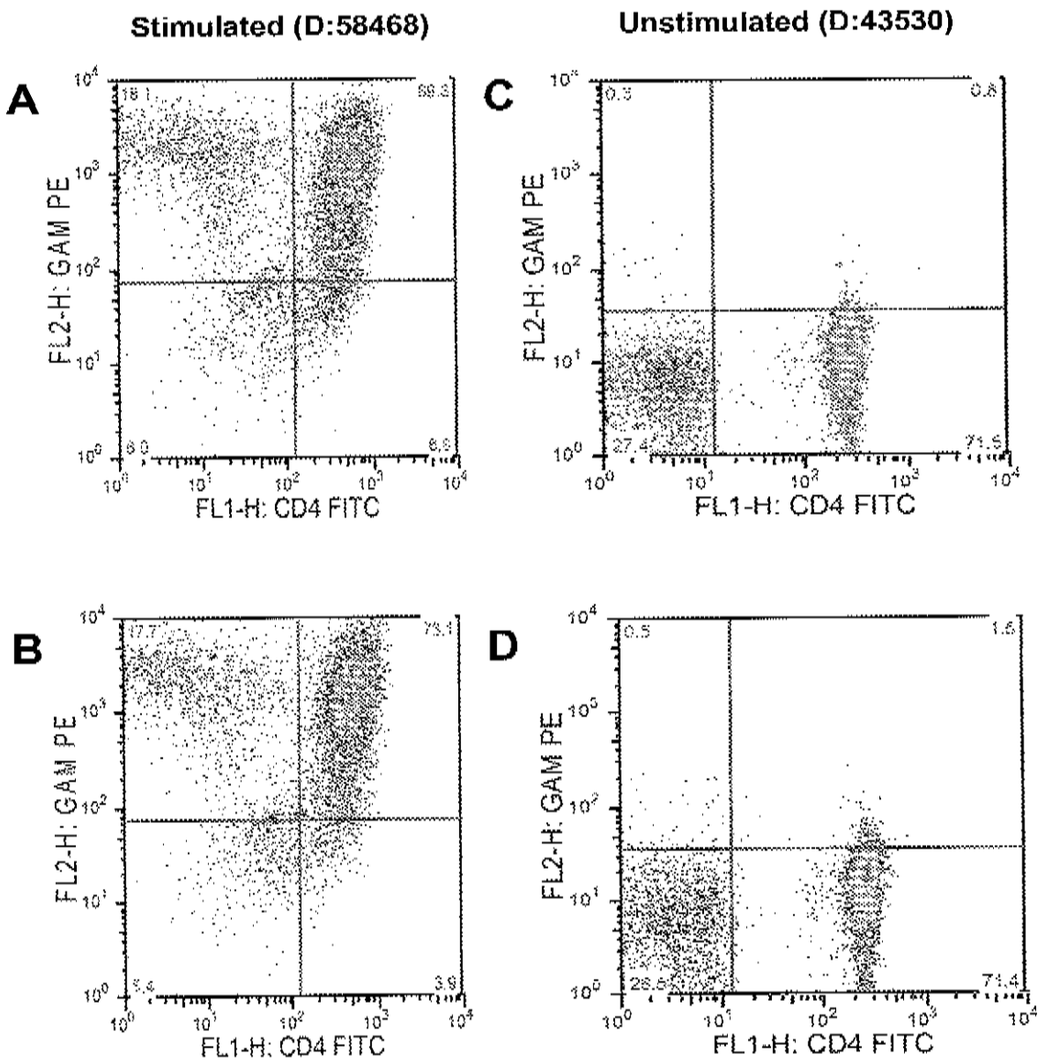


Figure 10

D:34765

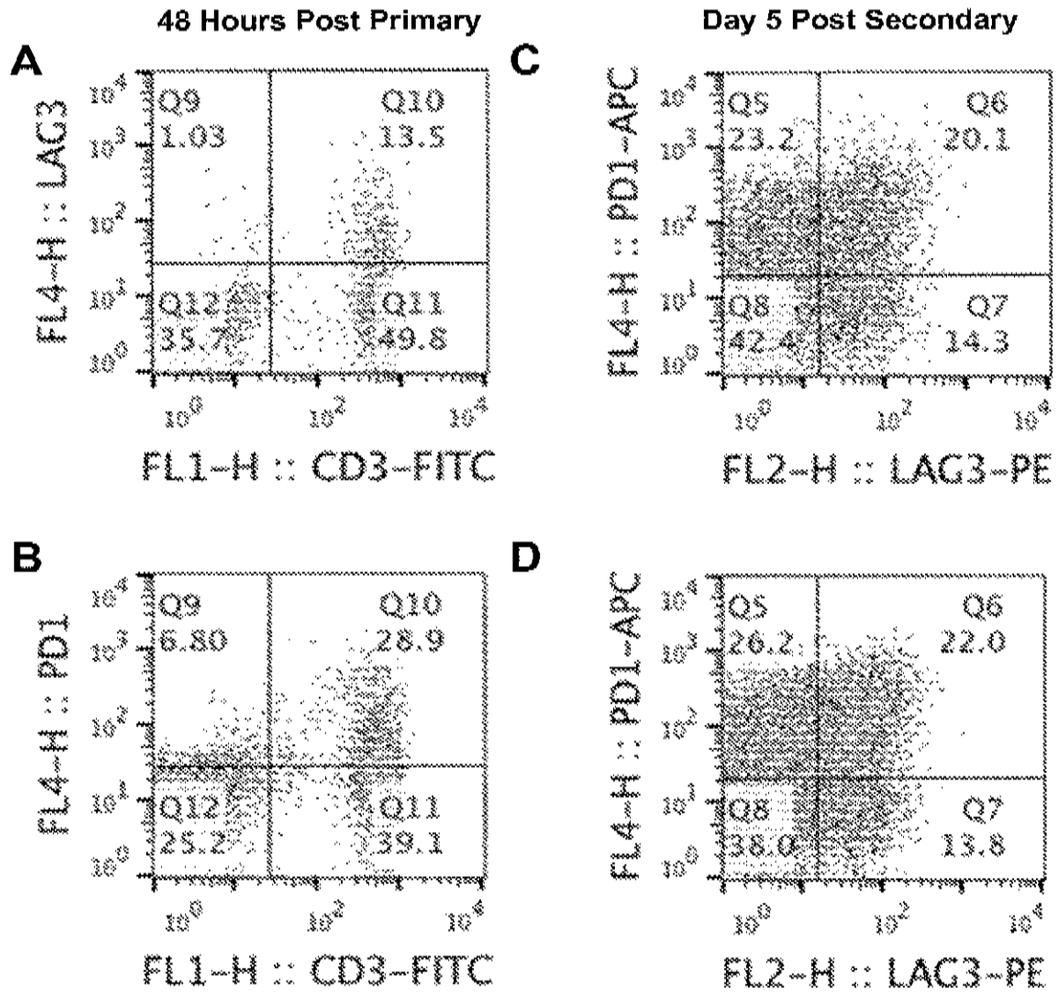


Figure 11

D:53724

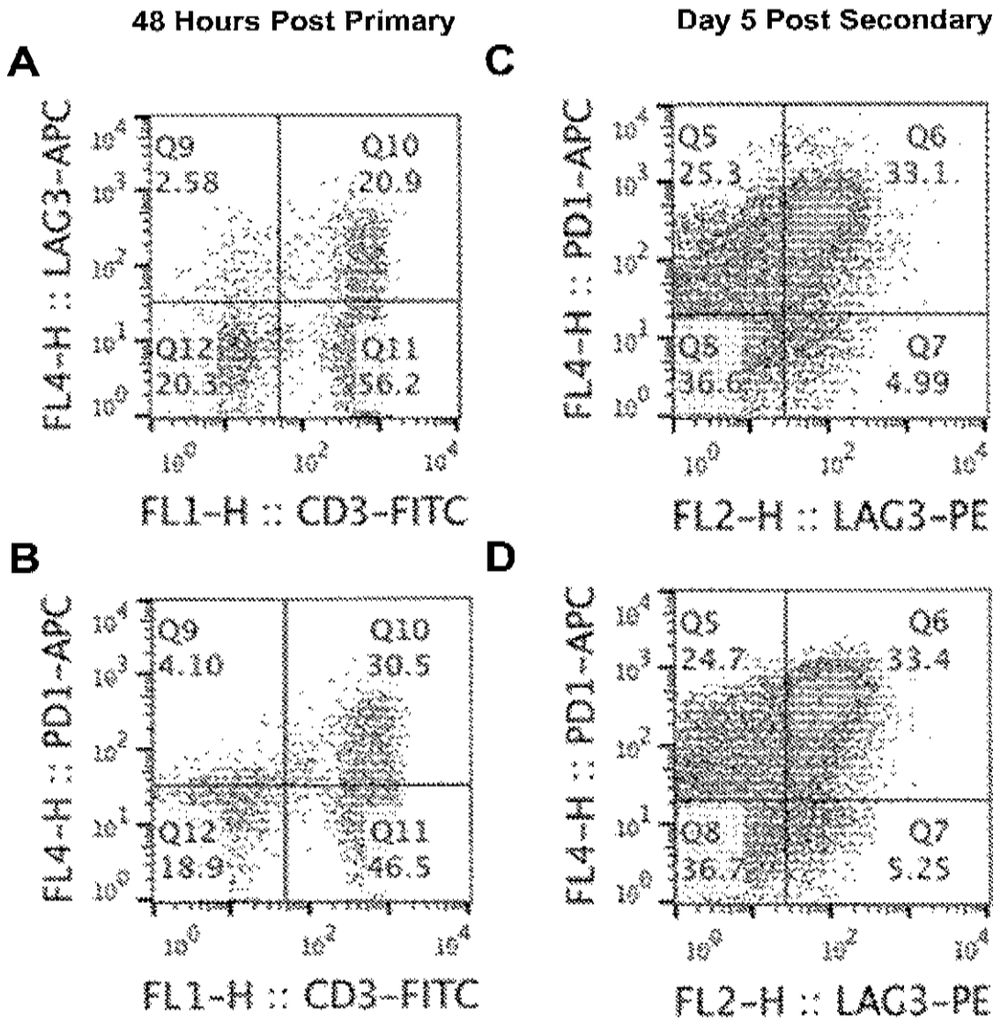


Figure 12

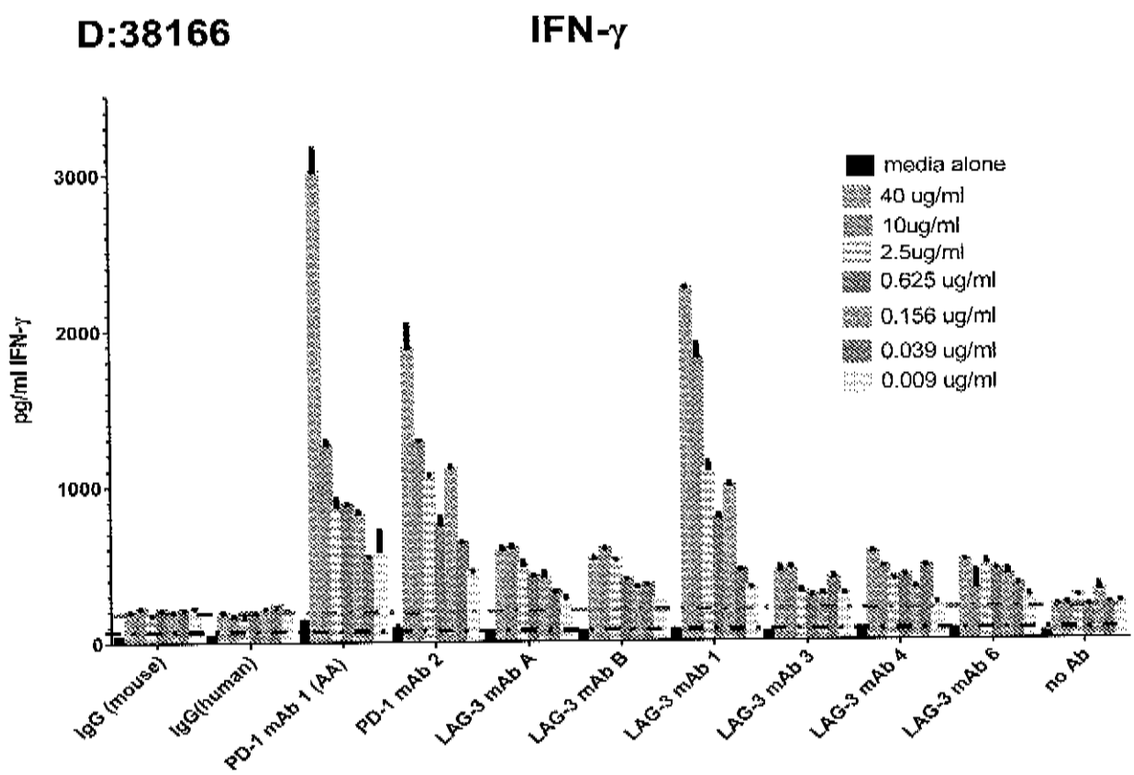


Figure 13A

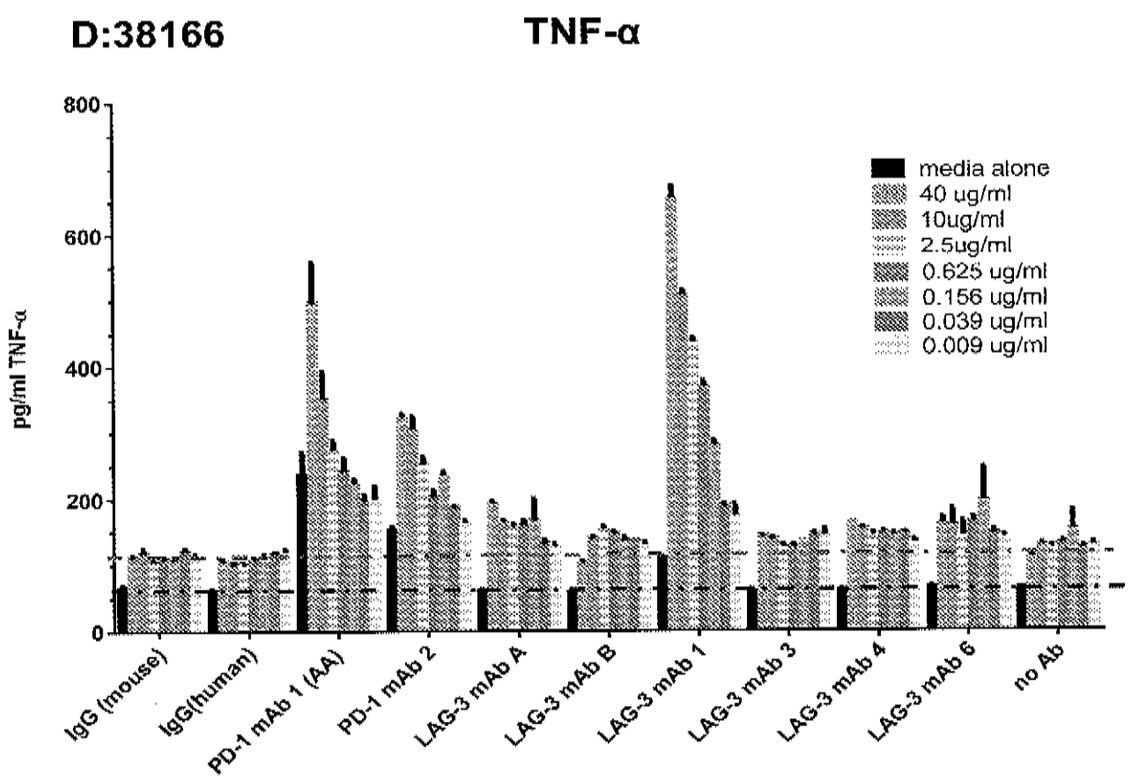


Figure 13B

D:58108

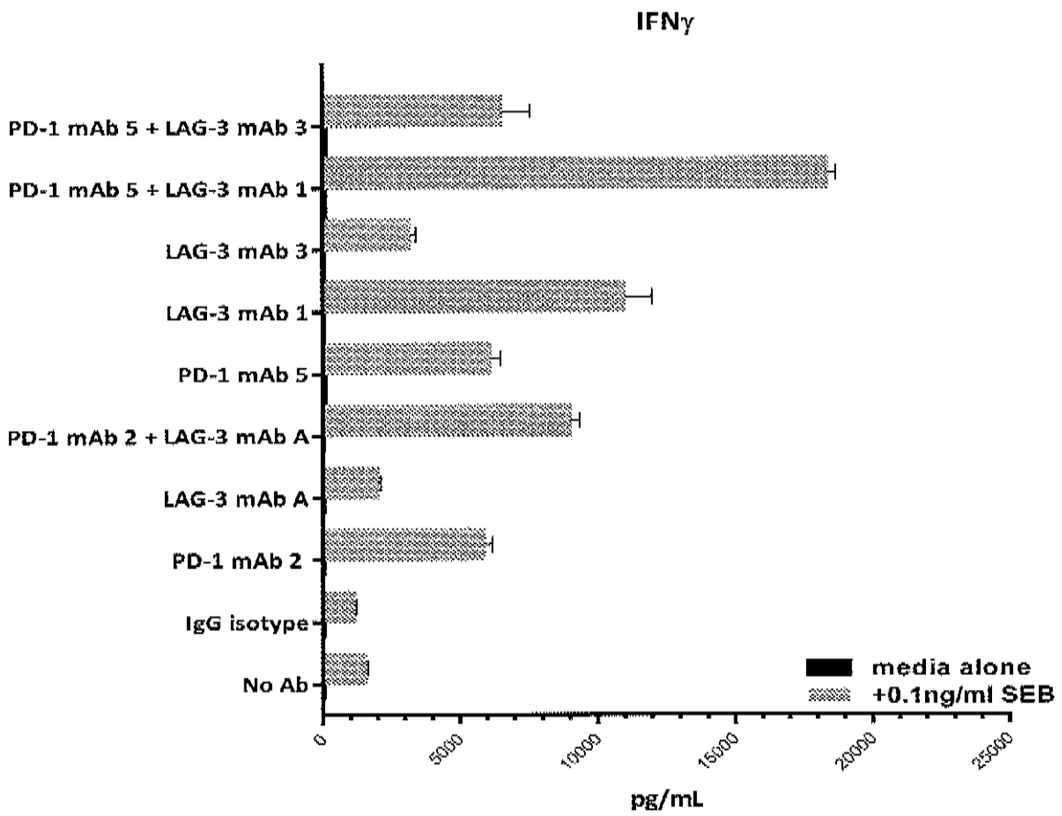


Figure 14

PTO/AIA/14 (12-13)
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pplication Data Sheet 37 CFR 1.76	Attorney Docket Number	1301.0121P
	Application Number	
itle of Invention	LAG-3-Binding Molecules and Methods of Use Thereof	

ne application data sheet is part of the provisional or nonprovisional application for which it is being submitted. The following form contains the bliographic data arranged in a format specified by the United States Patent and Trademark Office as outlined in 37 CFR 1.76.
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ecrecy Order 37 CFR 5.2

☐ Portions or all of the application associated with this Application Data Sheet may fall under a Secrecy Order pursuant to 37 CFR 5.2 (Paper filers only. Applications that fall under Secrecy Order may not be filed electronically.)

ventor Information:

Remove

nventor 1

egal Name

Prefix	Given Name	Middle Name	Family Name	Suffix
	Leslie	S.	Johnson	

Residence Information (Select One) ◉ US Residency ○ Non US Residency ○ Active US Military Service

City	Damestown	State/Province	MD	Country of Residence i	US

lailing Address of Inventor:

Address 1	14411 Poplar Hill Road		
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Inventor 2

Legal Name

Prefix	Given Name	Middle Name	Family Name	Suffix
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Inventor 3

Legal Name

Prefix	Given Name	Middle Name	Family Name	Suffix
	Doug		Smith	

Residence Information (Select One) ◉ US Residency ○ Non US Residency ○ Active US Military Service

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Application Data Sheet 37 CFR 1.76	Attorney Docket Number	1301.0121P
	Application Number	
Title of Invention	LAG-3-Binding Molecules and Methods of Use Thereof	

City	San Mateo	State/Province	CA	Country of Residence i	US

Mailing Address of Inventor:

Address 1	429 W. Hillsdale Blvd.		
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Postal Code	94403	Country i	US

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Inventor 4

Legal Name

Prefix	Given Name	Middle Name	Family Name	Suffix
	Ross		La Motte-Mohs	

Residence Information (Select One) (•) US Residency ( ) Non US Residency ( ) Active US Military Service

City	Gaithersburg	State/Province	MD	Country of Residence i	US

Mailing Address of Inventor:

Address 1	111 Teachers Way		
Address 2	#4102		
City	Gaithersburg	State/Province	MD
Postal Code	20877	Country i	US

Remove

Inventor 5

Legal Name

Prefix	Given Name	Middle Name	Family Name	Suffix
	Kalpana		Shah	

Residence Information (Select One) (•) US Residency ( ) Non US Residency ( ) Active US Military Service

City	Boyds	State/Province	MD	Country of Residence i	US

Mailing Address of Inventor:

Address 1	13013 Ethel Rose Way		
Address 2			
City	Boyds	State/Province	MD
Postal Code	20841	Country i	US

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Correspondence Information:

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☐ **An Address is being provided for the correspondence Information of this application.**

Application Data Sheet 37 CFR 1.76	Attorney Docket Number	1301.0121P
	Application Number	
Title of Invention	LAG-3-Binding Molecules and Methods of Use Thereof	

Customer Number	66522		
Email Address	epatent@ipatlaw.com	Add Email	Remove Email

Application Information:

Title of the Invention	LAG-3-Binding Molecules and Methods of Use Thereof		
Attorney Docket Number	1301.0121P	Small Entity Status Claimed	☒
Application Type	Provisional		
Subject Matter	Utility		
Total Number of Drawing Sheets (if any)	19	Suggested Figure for Publication (if any)	

Filing By Reference :

Only complete this section when filing an application by reference under 35 U.S.C. 111(c) and 37 CFR 1.57(a). Do not complete this section if application papers including a specification and any drawings are being filed. Any domestic benefit or foreign priority information must be provided in the appropriate section(s) below (i.e., "Domestic Benefit/National Stage Information" and "Foreign Priority Information").

For the purposes of a filing date under 37 CFR 1.53(b), the description and any drawings of the present application are replaced by this reference to the previously filed application, subject to conditions and requirements of 37 CFR 1.57(a).

Application number of the previously filed application	Filing date (YYYY-MM-DD)	Intellectual Property Authority or Country i

Publication Information:

☐ Request Early Publication (Fee required at time of Request 37 CFR 1.219)

☐ **Request Not to Publish.** I hereby request that the attached application not be published under 35 U.S.C. 122(b) and certify that the invention disclosed in the attached application **has not and will not** be the subject of an application filed in another country, or under a multilateral international agreement, that requires publication at eighteen months after filing.

Representative Information:

Representative information should be provided for all practitioners having a power of attorney in the application. Providing this information in the Application Data Sheet does not constitute a power of attorney in the application (see 37 CFR 1.32).
Either enter Customer Number or complete the Representative Name section below. If both sections are completed the customer Number will be used for the Representative Information during processing.

Please Select One:	◉ Customer Number	○ US Patent Practitioner	○ Limited Recognition (37 CFR 11.9)
Customer Number	66522		

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Application Data Sheet 37 CFR 1.76	Attorney Docket Number	1301.0121P
	Application Number	
Title of Invention	LAG-3-Binding Molecules and Methods of Use Thereof	

Domestic Benefit/National Stage Information:

This section allows for the applicant to either claim benefit under 35 U.S.C. 119(e), 120, 121, or 365(c) or indicate National Stage entry from a PCT application. Providing this information in the application data sheet constitutes the specific reference required by 35 U.S.C. 119(e) or 120, and 37 CFR 1.78.
When referring to the current application, please leave the application number blank.

Prior Application Status			Remove
Application Number	Continuity Type	Prior Application Number	Filing Date (YYYY-MM-DD)

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Foreign Priority Information:

This section allows for the applicant to claim priority to a foreign application. Providing this information in the application data sheet constitutes the claim for priority as required by 35 U.S.C. 119(b) and 37 CFR 1.55(d). When priority is claimed to a foreign application that is eligible for retrieval under the priority document exchange program (PDX) the information will be used by the Office to automatically attempt retrieval pursuant to 37 CFR 1.55(h)(1) and (2). Under the PDX program, applicant bears the ultimate responsibility for ensuring that a copy of the foreign application is received by the Office from the participating foreign intellectual property office, or a certified copy of the foreign priority application is filed, within the time period specified in 37 CFR 1.55(g)(1).

			Remove
Application Number	Country	Filing Date (YYYY-MM-DD)	Access Code (if applicable)

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Statement under 37 CFR 1.55 or 1.78 for AIA (First Inventor to File) Transition Applications

☐ This application (1) claims priority to or the benefit of an application filed before March 16, 2013 and (2) also contains, or contained at any time, a claim to a claimed invention that has an effective filing date on or after March 16, 2013.
NOTE: By providing this statement under 37 CFR 1.55 or 1.78, this application, with a filing date on or after March 16, 2013, will be examined under the first inventor to file provisions of the AIA.

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Application Data Sheet 37 CFR 1.76	Attorney Docket Number	1301.0121P
	Application Number	
Title of Invention	LAG-3-Binding Molecules and Methods of Use Thereof	

Authorization to Permit Access:

☐ Authorization to Permit Access to the Instant Application by the Participating Offices

If checked, the undersigned hereby grants the USPTO authority to provide the European Patent Office (EPO), the Japan Patent Office (JPO), the Korean Intellectual Property Office (KIPO), the World Intellectual Property Office (WIPO), and any other intellectual property offices in which a foreign application claiming priority to the instant patent application is filed access to the instant patent application. See 37 CFR 1.14(c) and (h). This box should not be checked if the applicant does not wish the EPO, JPO, KIPO, WIPO, or other intellectual property office in which a foreign application claiming priority to the instant patent application is filed to have access to the instant patent application.

In accordance with 37 CFR 1.14(h)(3), access will be provided to a copy of the instant patent application with respect to: 1) the instant patent application-as-filed; 2) any foreign application to which the instant patent application claims priority under 35 U.S.C. 119(a)-(d) if a copy of the foreign application that satisfies the certified copy requirement of 37 CFR 1.55 has been filed in the instant patent application; and 3) any U.S. application-as-filed from which benefit is sought in the instant patent application.

In accordance with 37 CFR 1.14(c), access may be provided to information concerning the date o f filing this Authorization.

Applicant Information:

Providing assignment information in this section does not substitute for compliance with any requirement of part 3 of Title 37 of CFR to have an assignment recorded by the Office.

Applicant 1 Remove

If the applicant is the inventor (or the remaining joint inventor or inventors under 37 CFR 1.45), this section should not be completed. The information to be provided in this section is the name and address of the legal representative who is the applicant under 37 CFR 1.43; or the name and address of the assignee, person to whom the inventor is under an obligation to assign the invention, or person who otherwise shows sufficient proprietary interest in the matter who is the applicant under 37 CFR 1.46. If the applicant is an applicant under 37 CFR 1.46 (assignee, person to whom the inventor is obligated to assign, or person who otherwise shows sufficient proprietary interest) together with one or more joint inventors, then the joint inventor or inventors who are also the applicant should be identified in this section.

Clear

◉ Assignee	○ Legal Representative under 35 U.S.C. 117	○ Joint Inventor
○ Person to whom the inventor is obligated to assign.	○ Person who shows sufficient proprietary interest	

If applicant is the legal representative, indicate the authority to file the patent application, the inventor is:

Name of the Deceased or Legally Incapacitated Inventor :	
If the Applicant is an Organization check here. ☒	
Organization Name	MacroGenics, Inc.

Application Data Sheet 37 CFR 1.76	Attorney Docket Number	1301.0121P
	Application Number	

Title of Invention	LAG-3-Binding Molecules and Methods of Use Thereof

Mailing Address Information For Applicant:			
Address 1	9640 Medical Center Drive		
Address 2			
City	Rockville	**State/Province**	MD
Country i	US	Postal Code	20850
Phone Number		Fax Number	
Email Address			

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Assignee Information including Non-Applicant Assignee Information:

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Remove

If the Assignee or Non-Applicant Assignee is an Organization check here. ☒

Organization Name	MacroGenics, Inc.

Mailing Address Information For Assignee including Non-Applicant Assignee:			
Address 1	9640 Medical Center Drive		
Address 2			
City	Rockville	**State/Province**	MD
Country i	US	Postal Code	20850
Phone Number		Fax Number	
Email Address			

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Signature:

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Signature	/Lisa Kreppel/	Date (YYYY-MM-DD)	2015-06-08

Application Data Sheet 37 CFR 1.76	Attorney Docket Number	1301.0121P
	Application Number	
Title of Invention	LAG-3-Binding Molecules and Methods of Use Thereof	

First Name	Lisa	Last Name	Kreppel	Registration Number	56970

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7. A record from this system of records may be disclosed, as a routine use, to the Administrator, General Services, or his/her designee, during an inspection of records conducted by GSA as part of that agency's responsibility to recommend improvements in records management practices and programs, under authority of 44 U.S.C. 2904 and 2906. Such disclosure shall be made in accordance with the GSA regulations governing inspection of records for this purpose, and any other relevant (i.e., GSA or Commerce) directive. Such disclosure shall not be used to make determinations about individuals.

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