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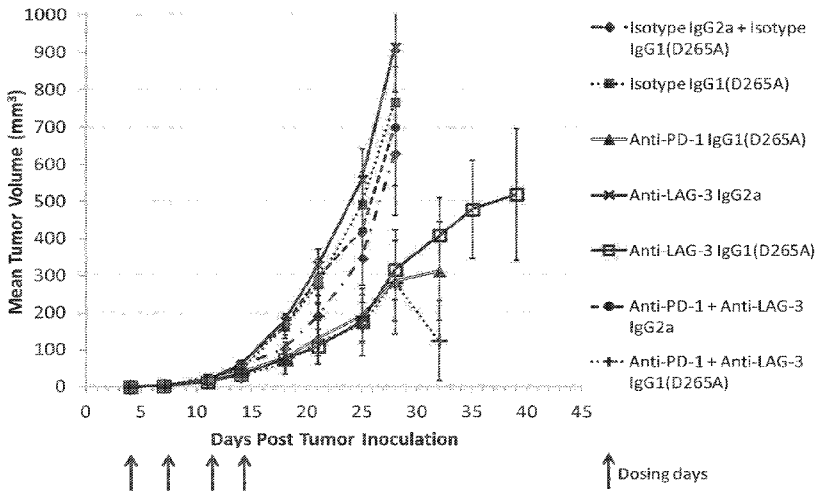
(54) Title: ANTIBODIES DIRECTED AGAINST LYMPHOCYTE ACTIVATION GENE 3 (LAG-3)

Fig. 1A



(57) Abstract: The invention relates to an isolated immunoglobulin heavy chain polypeptide and an isolated immunoglobulin light chain polypeptide that bind to a protein encoded by the Lymphocyte Activation Gene-3 (LAG-3). The invention provides a LAG-3-binding agent that comprises the aforementioned immunoglobulin heavy chain polypeptide and immunoglobulin light chain polypeptide. The invention also provides related vectors, compositions, and methods of using the LAG-3-binding agent to treat a disorder or disease that is responsive to LAG-3 inhibition, such as cancer or an infectious disease.

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ANTIBODIES DIRECTED AGAINST LYMPHOCYTE ACTIVATION GENE 3 (LAG-3)

INCORPORATION-BY-REFERENCE OF MATERIAL SUBMITTED ELECTRONICALLY

[0001] Incorporated by reference in its entirety herein is a computer-readable nucleotide/amino acid sequence listing submitted concurrently herewith and identified as follows: One 182,600 Byte ASCII (Text) file named "723163_ST25.TXT," created on February 2, 2016.

BACKGROUND OF THE INVENTION

[0002] Lymphocyte Activation Gene-3 (LAG-3), which is also known as CD223, is a member of the immunoglobulin supergene family and is structurally and genetically related to CD4. LAG-3 is expressed on T-cells, B cells, natural killer (NK) cells and plasmacytoid dendritic cells (pDCs). Like CD4, LAG-3 has been demonstrated to interact with MHC Class II molecules (Baixeras et al., *J. Exp. Med.*, *176*: 327-337 (1992)), but binds at a distinct site (Huard et al., *Proc. Natl. Acad. Sci. USA*, *94*(11): 5744-5749 (1997)). In particular, for example, a LAG-3 immunoglobulin fusion protein (sLAG-3Ig) directly and specifically binds via LAG-3 to MHC class II on the cell surface (Huard et al., *Eur. J. Immunol.*, *26*: 1180-1186 (1996)).

[0003] LAG-3 is upregulated following T-cell activation, and modulates T-cell function as well as T-cell homeostasis (Sierro et al., *Expert Opin. Ther. Targets*, *15*(1):91-101 (2011)). The LAG-3/MHC class II interaction may play a role in down-regulating antigen-dependent stimulation of CD4+ T lymphocytes, as demonstrated in *in vitro* studies of antigen-specific T-cell responses in which the addition of anti-LAG-3 antibodies led to increased T-cell proliferation, higher expression of activation antigens such as CD25, and higher concentrations of cytokines such as interferon-gamma and interleukin-4 (Huard et al., *Eur. J. Immunol.*, *24*: 3216-3221 (1994)). CD4+CD25+ regulatory T-cells (Treg) also have been shown to express LAG-3 upon activation and antibodies to LAG-3 inhibit suppression by induced Treg cells, both *in vitro* and *in vivo*, suggesting that LAG-3 contributes to the suppressor activity of Treg cells (Huang et al. *Immunity*, *21*: 503-513 (2004)). Furthermore, LAG-3 has been shown to negatively regulate T-cell homeostasis by regulatory T-cells in both T-cell-dependent and independent mechanisms (Workman, C. J. and Vignali, D. A., *J. Immunol.*, *174*: 688-695 (2005)).

[0004] Subsets of conventional T-cells that are anergic or display impaired functions express LAG-3, and LAG-3+ T-cells are enriched at tumor sites and during chronic viral infections. However, while LAG-3 knockout mice have been shown to mount normal virus-specific CD4+ and CD8+ T-cell responses, suggesting a non-essential role for LAG-3, blockade of the PD-1/PD-L1 pathway combined with LAG-3 blockade improved viral control as compared with PD-L1 blockade alone (Blackburn et al., *Nat. Immunol.*, *10*: 29-37 (2009); and Richter et al., *Int. Immunol.*, *22*: 13-2 (2010)).

[0005] In a self-tolerance/tumor mouse model where transgenic CD8+ T-cells were rendered unresponsive/anergic *in vivo*, LAG-3 blockade or deficiency in CD8+ T-cells enhanced T-cell proliferation, T-cell recruitment and effector functions at the tumor site (Grosso et al., *J. Clin. Invest.*, *117*: 3383-92 (2007)).

[0006] Inhibition of LAG-3 activity, such as through use of monoclonal antibodies, is currently under investigation as a therapeutic approach to treat viral infections and melanoma based on preclinical studies. For example, addition of soluble huLAG-3 fused to an Fc region enhanced the proliferation of antigen-specific T-cells to viral and tumor antigens, such as influenza matrix protein or melanoma antigen recognized by T-cells (MART-1), in PBMCs of healthy or cancer patients (Casati et al., *J. Immunol*, *180*: 3782-3788 (2008)).

[0007] There is a need for additional antagonists of LAG-3 (e.g., an antibody) that binds LAG-3 with high affinity and effectively neutralizes LAG-3 activity. The invention provides such LAG-3-binding agents.

BRIEF SUMMARY OF THE INVENTION

[0008] The invention provides an isolated immunoglobulin heavy chain polypeptide which comprises the amino acid sequence Glu Val Gln Leu Val Gln Ser Gly Ala Glu Val Lys Lys Pro Gly Ala Thr Val Lys Ile Ser Cys Lys Ala Ser Gly Phe Xaa1 Ile Xaa2 Asp Asp Tyr Ile His Trp Val Xaa3 Gln Ala Pro Gly Lys Gly Leu Glu Trp Xaa4 Gly Trp Ile Asp Xaa5 Xaa6 Asn Xaa7 Asp Ser Xaa8 Tyr Xaa9 Ser Lys Phe Xaa10 Gly Arg Val Thr Ile Thr Val Asp Thr Ser Thr Xaa11 Thr Ala Tyr Met Xaa12 Leu Ser Ser Leu Arg Ser Glu Asp Thr Ala Val Tyr Tyr Cys Thr Tyr Ala Phe Gly Gly Tyr Trp Gly Gln Gly Thr Thr Val Thr Val Ser Ser (SEQ ID NO: 181), wherein (a) Xaa1 is asparagine (Asn) or serine (Ser), (b) Xaa2 is lysine (Lys), tyrosine (Tyr), or asparagine

(Asn), (c) Xaa3 is lysine (Lys) or glutamine (Gln), (d) Xaa4 is isoleucine (Ile) or methionine (Met), (e) Xaa5 is alanine (Ala) or proline (Pro), (f) Xaa6 is glutamic acid (Glu) or methionine (Met), (g) Xaa6 is glycine (Gly), asparagine (Asn), or aspartic acid (Asp), (h) Xaa8 is glutamic acid (Glu) or glutamine (Q), (i) Xaa9 is alanine (Ala) or serine (Ser), (j) Xaa10 is glutamine (Gln) or arginine (Arg), (k) Xaa11 is aspartic acid (Asp) or asparagine (Asn), and (l) Xaa12 is glutamine (Gln) or lysine (Lys)..

[0009] The invention provides an isolated immunoglobulin heavy chain polypeptide which comprises the amino acid sequence Gln Val Gln Leu Gln Gln Trp Gly Ala Xaa1 Leu Leu Lys Pro Ser Glu Thr Leu Ser Leu Xaa2 Cys Xaa3 Val Tyr Gly Gly Xaa4 Phe Xaa5 Gly Tyr Tyr Trp Xaa6 Trp Ile Arg Gln Pro Pro Xaa7 Lys Gly Leu Glu Trp Ile Gly Glu Ile Asn His Ser Gly Xaa8 Thr Asn Tyr Asn Pro Ser Leu Lys Ser Arg Val Thr Ile Ser Val Asp Thr Ser Lys Asn Gln Xaa9 Ser Leu Lys Leu Xaa10 Xaa11 Val Thr Ala Ala Asp Thr Ala Val Tyr Tyr Cys Xaa12 Arg Glu Gly Xaa13 Tyr Gly Asp Tyr Asp Tyr Trp Gly Gln Gly Thr Leu Val Thr Val Ser Ser (SEQ ID NO: 35), wherein (a) Xaa1 is arginine (Arg) or glycine (Gly), (b) Xaa2 is threonine (Thr) or isoleucine (Ile), (c) Xaa3 is threonine (Thr) or alanine (Ala), (d) Xaa4 is serine (Ser) or phenylalanine (Phe), (e) Xaa5 is serine (Ser) or phenylalanine (Phe), (f) Xaa6 is serine (Ser) or isoleucine (Ile), (g) Xaa7 is glycine (Gly) or arginine (Arg), (h) Xaa8 is serine (Ser) or asparagine (Asn), (i) Xaa9 is phenylalanine (Phe) or leucine (Leu), (j) Xaa10 is asparagine (Asn) or serine (Ser), (k) Xaa11 is serine (Ser) or phenylalanine (Phe), (l) Xaa12 is alanine (Ala) or valine (Val), and (m) Xaa13 is aspartic acid (Asp) or asparagine (Asn).

[0010] The invention further provides an isolated immunoglobulin heavy chain polypeptide comprising SEQ ID NO: 190 or 191.

[0011] The invention provides an isolated immunoglobulin light chain polypeptide which comprises the amino acid sequence Asp Xaa1 Val Met Thr Gln Thr Pro Leu Ser Leu Ser Val Thr Pro Gly Gln Pro Ala Ser Ile Ser Cys Arg Xaa2 Ser Gln Ser Leu Val His Ser Asp Xaa3 Xaa4 Thr Tyr Leu His Trp Tyr Leu Gln Lys Pro Gly Gln Ser Pro Gln Leu Leu Ile Tyr Xaa Xaa Ser Asn Arg Phe Ser Gly Val Pro Asp Arg Phe Ser Gly Ser Gly Ser Gly Thr Asp Phe Thr Leu Lys Ile Ser Arg Val Glu Ala Glu Asp Val Gly Val Tyr Phe Cys Xaa Gln Ser Thr Xaa Val Pro Tyr Ala Phe Gly Gly Gly Thr Lys Val Glu Ile Lys Arg Thr (SEQ ID NO: 57), wherein (a) Xaa1 is valine (Val) or isoleucine (Ile), (b) Xaa2 is cysteine (Cys) or serine (Ser), (c) Xaa3 is glycine (Gly) or

serine (Ser), (d) Xaa4 is asparagine (Asn) or aspartic acid (Asp), (e)Xaa5 is lysine (Lys), glycine (Gly), asparagine (Asn), serine (Ser), or leucine (Leu), (f) Xaa6 is valine (Val) or isoleucine (Ile), (g) Xaa7 is serine (Ser), alanine (Ala), or glycine (Gly), and (h) Xaa8 is histidine (His) or tyrosine (Tyr).

[0012] The invention provides an isolated immunoglobulin light chain polypeptide which comprises the amino acid sequence Asp Ile Gln Met Thr Gln Ser Pro Ser Ser Leu Ser Ala Ser Val Gly Asp Arg Val Thr Ile Thr Cys Gln Ala Ser Gln Asp Ile Ser Asn Tyr Leu Asn Trp Tyr Gln Gln Lys Pro Gly Lys Ala Pro Lys Leu Leu Ile Xaa1 Xaa2 Xaa3 Xaa4 Xaa5 Leu Glu Thr Gly Val Pro Ser Arg Phe Ser Gly Ser Gly Ser Gly Thr Asp Phe Thr Phe Thr Ile Ser Ser Leu Gln Pro Glu Asp Ile Ala Val Tyr Tyr Cys Gln Gln Ser Tyr Ser Xaa6 Leu Ile Thr Phe Gly Gln Gly Thr Arg Leu Glu Ile Lys Arg Thr Val Ala Ala Pro Ser Val (SEQ ID NO: 89), wherein (a) the subsequence Xaa1 Xaa2 Xaa3 Xaa4 Xaa5 is deleted or is Tyr-Asp-Ala-Ser-Asn, and (b) Xaa6 is threonine (Thr) or isoleucine (Ile).

[0013] The invention also provides isolated immunoglobulin light chain polypeptide comprising SEQ ID NO: 196 or 197.

[0014] In addition, the invention provides isolated or purified nucleic acid sequences encoding the foregoing immunoglobulin polypeptides, vectors comprising such nucleic acid sequences, LAG-3-binding agents comprising the foregoing immunoglobulin polypeptides, nucleic acid sequences encoding such LAG-3-binding agents, vectors comprising such nucleic acid sequences, isolated cells comprising such vectors, compositions comprising such LAG-3-binding agents or such vectors with a pharmaceutically acceptable carrier, and methods of treating cancer or infectious diseases in mammals by administering effective amounts of such compositions to mammals.

BRIEF DESCRIPTION OF THE DRAWINGS

[0015] Figure 1A is a graph of mean tumor volume over time in mice implanted with Colon26 colon adenocarcinoma cells and injected with the indicated antibodies. Each data plot in the figure refers to the indicated treatment group.

[0016] Figure 1B is a graph of tumor volume over time of individual animals in three treatment groups of mice implanted with Colon26 colon adenocarcinoma cells and injected with

the indicated antibodies. Each data plot in the graphs refers to an individual animal in the treatment group.

[0017] Figure 2A depicts IL-2 secretion by CD4+ T-cells in a mixed lymphocyte reaction (MLR) assay at varying concentrations of Anti PD-1 or Anti-LAG-3 antibodies.

[0018] Figure 2B depicts LAG-3 and PD-1 expression on CD4+ T-cells prior to (naïve) or subsequent to (24, 48, and 72 hour) exposure to dendritic cells.

DETAILED DESCRIPTION OF THE INVENTION

[0019] The invention provides an isolated immunoglobulin heavy chain polypeptide and/or an isolated immunoglobulin light chain polypeptide, or a fragment (e.g., antigen-binding fragment) thereof. The term "immunoglobulin" or "antibody," as used herein, refers to a protein that is found in blood or other bodily fluids of vertebrates, which is used by the immune system to identify and neutralize foreign objects, such as bacteria and viruses. The polypeptide is "isolated" in that it is removed from its natural environment. In a preferred embodiment, an immunoglobulin or antibody is a protein that comprises at least one complementarity determining region (CDR). The CDRs form the "hypervariable region" of an antibody, which is responsible for antigen binding (discussed further below). A whole immunoglobulin typically consists of four polypeptides: two identical copies of a heavy (H) chain polypeptide and two identical copies of a light (L) chain polypeptide. Each of the heavy chains contains one N-terminal variable (V_H) region and three C-terminal constant (C_H1, C_H2, and C_H3) regions, and each light chain contains one N-terminal variable (V_L) region and one C-terminal constant (C_L) region. The light chains of antibodies can be assigned to one of two distinct types, either kappa (κ) or lambda (λ), based upon the amino acid sequences of their constant domains. In a typical immunoglobulin, each light chain is linked to a heavy chain by disulphide bonds, and the two heavy chains are linked to each other by disulphide bonds. The light chain variable region is aligned with the variable region of the heavy chain, and the light chain constant region is aligned with the first constant region of the heavy chain. The remaining constant regions of the heavy chains are aligned with each other.

[0020] The variable regions of each pair of light and heavy chains form the antigen binding site of an antibody. The V_H and V_L regions have the same general structure, with each region comprising four framework (FW or FR) regions. The term "framework region," as used herein, refers to the relatively conserved amino acid sequences within the variable region which are located between the hypervariable or complementary determining regions (CDRs). There are four framework regions in each variable domain, which are designated FR1, FR2, FR3, and FR4. The framework regions form the β sheets that provide the structural framework of the variable region (see, e.g., C.A. Janeway et al. (eds.), *Immunobiology, 5th Ed.*, Garland Publishing, New York, NY (2001)).

[0021] The framework regions are connected by three complementarity determining regions (CDRs). As discussed above, the three CDRs, known as CDR1, CDR2, and CDR3, form the "hypervariable region" of an antibody, which is responsible for antigen binding. The CDRs form loops connecting, and in some cases comprising part of, the beta-sheet structure formed by the framework regions. While the constant regions of the light and heavy chains are not directly involved in binding of the antibody to an antigen, the constant regions can influence the orientation of the variable regions. The constant regions also exhibit various effector functions, such as participation in antibody-dependent complement-mediated lysis or antibody-dependent cellular toxicity via interactions with effector molecules and cells.

[0022] The isolated immunoglobulin heavy chain polypeptide and the isolated immunoglobulin light chain polypeptide of the invention desirably bind to the protein encoded by the Lymphocyte Activation Gene-3 (LAG-3) (also referred to herein as "LAG-3 protein"). As discussed above, LAG-3 is a 498 amino acid protein that negatively regulates T-cell function and homeostasis (Triebel et al., *J. Exp. Med.*, *171*(5): 1393-1405 (1990); and Triebel F., *Trends Immunol.*, *24*(12): 619-22 (2003)). LAG-3 is a member of the immunoglobulin supergene family and is structurally and genetically related to CD4. The intra-cytoplasmic region of LAG-3 has been shown to interact with a protein denoted LAP, which is thought to be a signal transduction molecule involved in the downregulation of the CD3/TCR activation pathway (Iouzalen et al., *Eur. J. Immunol.*, *31*: 2885-2891 (2001)). Furthermore, CD4+CD25+ regulatory T-cells (Treg) have been shown to express LAG-3 upon activation and antibodies to LAG-3 inhibit suppression by induced Treg cells, both *in vitro* and *in vivo*, suggesting that LAG-3 contributes to the

suppressor activity of Treg cells (Huang et al., *Immunity*, *21*: 503-513 (2004)). However, a recent study suggests that LAG-3 expression on CD4+ T-cells renders them more susceptible to suppression by Tregs, rather than making Tregs more suppressive (see Durham et al., *PLoS ONE*, *9*(11): e109080 (2014)). In certain circumstances, LAG-3 also has been shown to have immunostimulatory effects (see, e.g., Prigent et al., *Eur. J. Immunol.*, *29*: 3867-3876 (1999)); El Mir and Triebel, *J. Immunol.*, *164*: 5583-5589 (2000)); and Casati et al., *Cancer Res.*, *66*: 4450-4460 (2006)). The inventive isolated immunoglobulin heavy chain polypeptide and the inventive isolated immunoglobulin light chain polypeptide can form an agent that binds to LAG-3 and another antigen, resulting in a "dual reactive" binding agent (e.g., a dual reactive antibody). For example, the agent can bind to LAG-3 and to another negative regulator of the immune system such as, for example, programmed death 1 (PD-1) and/or T-cell immunoglobulin domain and mucin domain 3 protein (TIM-3).

[0023] Antibodies which bind to LAG-3, and components thereof, are known in the art (see, e.g., U.S. Patent Application Publication Nos. 2010/0233183, 2011/0150892, and 2014/0093511). Anti-LAG-3 antibodies also are commercially available from sources such as, for example, Abcam (Cambridge, MA), and R&D Systems, Inc. (Minneapolis, MN).

[0024] The invention provides an isolated immunoglobulin heavy chain polypeptide which comprises the amino acid sequence Glu Val Gln Leu Val Gln Ser Gly Ala Glu Val Lys Lys Pro Gly Ala Thr Val Lys Ile Ser Cys Lys Ala Ser Gly Phe Xaa1 Ile Xaa2 Asp Asp Tyr Ile His Trp Val Xaa3 Gln Ala Pro Gly Lys Gly Leu Glu Trp Xaa4 Gly Trp Ile Asp Xaa5 Xaa6 Asn Xaa7 Asp Ser Xaa8 Tyr Xaa9 Ser Lys Phe Xaa10 Gly Arg Val Thr Ile Thr Val Asp Thr Ser Thr Xaa11 Thr Ala Tyr Met Xaa12 Leu Ser Ser Leu Arg Ser Glu Asp Thr Ala Val Tyr Tyr Cys Thr Tyr Ala Phe Gly Gly Tyr Trp Gly Gln Gly Thr Thr Val Thr Val Ser Ser (SEQ ID NO: 181), wherein (a) Xaa1 is asparagine (Asn) or serine (Ser), (b) Xaa2 is lysine (Lys), tyrosine (Tyr), or asparagine (Asn), (c) Xaa3 is lysine (Lys) or glutamine (Gln), (d) Xaa4 is isoleucine (Ile) or methionine (Met), (e) Xaa5 is alanine (Ala) or proline (Pro), (f) Xaa6 is glutamic acid (Glu) or methionine (Met), (g) Xaa6 is glycine (Gly), asparagine (Asn), or aspartic acid (Asp), (h) Xaa8 is glutamic acid (Glu) or glutamine (Q), (i) Xaa9 is alanine (Ala) or serine (Ser), (j) Xaa10 is glutamine (Gln) or arginine (Arg), (k) Xaa11 is aspartic acid (Asp) or asparagine (Asn), and (l) Xaa12 is glutamine (Gln) or lysine (Lys).

[0025] In another aspect, the immunoglobulin heavy chain polypeptide comprises, consists of, or consists essentially of the amino acid sequence Glu Val Gln Leu Val Gln Ser Gly Ala Glu Val Lys Lys Pro Gly Ala Thr Val Lys Ile Ser Cys Lys Ala Ser Gly Phe Xaa1 Ile Xaa2 Asp Asp Tyr Ile His Trp Val Xaa3 Gln Ala Pro Gly Lys Gly Leu Glu Trp Xaa4 Gly Trp Ile Asp Xaa5 Glu Asn Xaa6 Asp Ser Glu Tyr Xaa7 Ser Lys Phe Xaa8 Gly Arg Val Thr Ile Thr Val Asp Thr Ser Thr Xaa9 Thr Ala Tyr Met Glu Leu Ser Ser Leu Arg Ser Glu Asp Thr Ala Val Tyr Tyr Cys Thr Tyr Ala Phe Gly Gly Tyr Trp Gly Gln Gly Thr Thr Val Thr Val Ser Ser (SEQ ID NO: 1), wherein (a) Xaa1 is asparagine (Asn) or serine (Ser), (b) Xaa2 is lysine (Lys), tyrosine (Tyr), or asparagine (Asn), (c) Xaa3 is lysine (Lys) or glutamine (Gln), (d) Xaa4 is isoleucine (Ile) or methionine (Met), (e) Xaa5 is alanine (Ala) or proline (Pro), (f) Xaa6 is glycine (Gly), asparagine (Asn), or aspartic acid (Asp), (g) Xaa7 is alanine (Ala) or serine (Ser), (h) Xaa8 is glutamine (Gln) or arginine (Arg), and (i) Xaa9 is aspartic acid (Asp) or asparagine (Asn).

[0026] In one embodiment, the isolated immunoglobulin heavy chain polypeptide comprises, consists of, or consists essentially of an amino acid sequence of any one of SEQ ID NO: 2, SEQ ID NO: 3, SEQ ID NO: 4, SEQ ID NO: 5, SEQ ID NO: 6, SEQ ID NO: 7, SEQ ID NO: 8, SEQ ID NO: 9, SEQ ID NO: 10, SEQ ID NO: 11, SEQ ID NO: 12, SEQ ID NO: 13, SEQ ID NO: 14, SEQ ID NO: 15, SEQ ID NO: 16, SEQ ID NO: 17, SEQ ID NO: 18, SEQ ID NO: 19, SEQ ID NO: 20, SEQ ID NO: 21, SEQ ID NO: 22, SEQ ID NO: 23, SEQ ID NO: 24, SEQ ID NO: 25, SEQ ID NO: 26, SEQ ID NO: 27, SEQ ID NO: 28, SEQ ID NO: 29, SEQ ID NO: 30, SEQ ID NO: 31, SEQ ID NO: 32, SEQ ID NO: 33, SEQ ID NO: 34, SEQ ID NO: 182, SEQ ID NO: 183, SEQ ID NO: 184, SEQ ID NO: 185, or SEQ ID NO: 186.

[0027] The invention also provides an immunoglobulin heavy chain polypeptide that comprises, consists of, or consists essentially of the amino acid sequence Gln Val Gln Leu Gln Gln Trp Gly Ala Xaa1 Leu Leu Lys Pro Ser Glu Thr Leu Ser Leu Xaa2 Cys Xaa3 Val Tyr Gly Gly Xaa4 Phe Xaa5 Gly Tyr Tyr Trp Xaa6 Trp Ile Arg Gln Pro Pro Xaa7 Lys Gly Leu Glu Trp Ile Gly Glu Ile Asn His Ser Gly Xaa8 Thr Asn Tyr Asn Pro Ser Leu Lys Ser Arg Val Thr Ile Ser Val Asp Thr Ser Lys Asn Gln Xaa9 Ser Leu Lys Leu Xaa10 Xaa11 Val Thr Ala Ala Asp Thr Ala Val Tyr Tyr Cys Xaa12 Arg Glu Gly Xaa13 Tyr Gly Asp Tyr Asp Tyr Trp Gly Gln Gly Thr Leu Val Thr Val Ser Ser (SEQ ID NO: 35), wherein (a) Xaa1 is arginine (Arg) or glycine (Gly), (b) Xaa2 is threonine (Thr) or isoleucine (Ile), (c) Xaa3 is threonine (Thr) or alanine (Ala), (d) Xaa4

is serine (Ser) or phenylalanine (Phe), (e) Xaa5 is serine (Ser) or phenylalanine (Phe), (f) Xaa6 is serine (Ser) or isoleucine (Ile), (g) Xaa7 is glycine (Gly) or arginine (Arg), (h) Xaa8 is serine (Ser) or asparagine (Asn), (i) Xaa9 is phenylalanine (Phe) or leucine (Leu), (j) Xaa10 is asparagine (Asn) or serine (Ser), (k) Xaa11 is serine (Ser) or phenylalanine (Phe), (l) Xaa12 is alanine (Ala) or valine (Val), and (m) Xaa13 is aspartic acid (Asp) or asparagine (Asn).

[0028] In one embodiment, the isolated immunoglobulin heavy chain polypeptide comprises, consists of, or consists essentially of an amino acid sequence of any one of SEQ ID NO: 36, SEQ ID NO: 37, SEQ ID NO: 38, SEQ ID NO: 39, SEQ ID NO: 40, SEQ ID NO: 41, SEQ ID NO: 42, SEQ ID NO: 43, SEQ ID NO: 44, SEQ ID NO: 45, SEQ ID NO: 46, SEQ ID NO: 47, SEQ ID NO: 48, SEQ ID NO: 49, SEQ ID NO: 50, SEQ ID NO: 51, SEQ ID NO: 52, SEQ ID NO: 53, SEQ ID NO: 54, SEQ ID NO: 55, or SEQ ID NO: 56.

[0029] In another embodiment, there is provided an isolated immunoglobulin heavy chain polypeptide which comprises SEQ ID NO: 190 or 191. Examples of such a polypeptide include those comprising any one of SEQ ID NOs: 192-195.

[0030] When the inventive immunoglobulin heavy chain polypeptide consists essentially of an amino acid sequence of any one of SEQ ID NO: 1-SEQ ID NO: 56, SEQ ID NOS: 182-186, or SEQ ID NOS: 190-195, additional components can be included in the polypeptide that do not materially affect the polypeptide (e.g., protein moieties such as biotin that facilitate purification or isolation). When the inventive immunoglobulin heavy chain polypeptide consists of an amino acid sequence of any one of SEQ ID NO: 1-SEQ ID NO: 56, the polypeptide does not comprise any additional components (i.e., components that are not endogenous to the inventive immunoglobulin heavy chain polypeptide).

[0031] The invention provides an isolated immunoglobulin heavy chain polypeptide which comprises an amino acid sequence that is at least 90% identical (e.g., at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98%, at least 99%, or 100% identical) to any one of SEQ ID NO: 1-56. Nucleic acid or amino acid sequence "identity," as described herein, can be determined by comparing a nucleic acid or amino acid sequence of interest to a reference nucleic acid or amino acid sequence. The percent identity is the number of nucleotides or amino acid residues that are the same (i.e., that are identical) as between the sequence of interest and the reference sequence divided by the length of the longest

sequence (i.e., the length of either the sequence of interest or the reference sequence, whichever is longer). A number of mathematical algorithms for obtaining the optimal alignment and calculating identity between two or more sequences are known and incorporated into a number of available software programs. Examples of such programs include CLUSTAL-W, T-Coffee, and ALIGN (for alignment of nucleic acid and amino acid sequences), BLAST programs (e.g., BLAST 2.1, BL2SEQ, and later versions thereof) and FASTA programs (e.g., FASTA3x, FASTM, and SSEARCH) (for sequence alignment and sequence similarity searches). Sequence alignment algorithms also are disclosed in, for example, Altschul et al., *J. Molecular Biol.*, *215*(3): 403-410 (1990), Beigert et al., *Proc. Natl. Acad. Sci. USA*, *106*(10): 3770-3775 (2009), Durbin et al., eds., *Biological Sequence Analysis: Probalistic Models of Proteins and Nucleic Acids*, Cambridge University Press, Cambridge, UK (2009), Soding, *Bioinformatics*, *21*(7): 951-960 (2005), Altschul et al., *Nucleic Acids Res.*, *25*(17): 3389-3402 (1997), and Gusfield, *Algorithms on Strings, Trees and Sequences*, Cambridge University Press, Cambridge UK (1997)).

[0032] In another embodiment, the invention provides an immunoglobulin light chain polypeptide that comprises, consists of, or consists essentially of, an isolated immunoglobulin light chain polypeptide which comprises the amino acid sequence Asp Xaa1 Val Met Thr Gln Thr Pro Leu Ser Leu Ser Val Thr Pro Gly Gln Pro Ala Ser Ile Ser Cys Arg Xaa2 Ser Gln Ser Leu Val His Ser Asp Xaa3 Xaa4 Thr Tyr Leu His Trp Tyr Leu Gln Lys Pro Gly Gln Ser Pro Gln Leu Leu Ile Tyr Xaa Xaa Ser Asn Arg Phe Ser Gly Val Pro Asp Arg Phe Ser Gly Ser Gly Ser Gly Thr Asp Phe Thr Leu Lys Ile Ser Arg Val Glu Ala Glu Asp Val Gly Val Tyr Phe Cys Xaa Gln Ser Thr Xaa Val Pro Tyr Ala Phe Gly Gly Gly Thr Lys Val Glu Ile Lys Arg Thr (SEQ ID NO: 57), wherein (a) Xaa1 is valine (Val) or isoleucine (Ile), (b) Xaa2 is cysteine (Cys) or serine (Ser), (c) Xaa3 is glycine (Gly) or serine (Ser), (d) Xaa4 is asparagine (Asn) or aspartic acid (Asp), (e)Xaa5 is lysine (Lys), glycine (Gly), asparagine (Asn), serine (Ser), or leucine (Leu), (f) Xaa6 is valine (Val) or isoleucine (Ile), (g) Xaa7 is serine (Ser), alanine (Ala), or glycine (Gly), and (h) Xaa8 is histidine (His) or tyrosine (Tyr).

[0033] In one embodiment, the isolated immunoglobulin light chain polypeptide comprises, consists of , or consists essentially of an amino acid sequence of any one of SEQ ID NO: 58, SEQ ID NO: 59, SEQ ID NO: 60, SEQ ID NO: 61, SEQ ID NO: 62, SEQ ID NO: 63, SEQ ID

NO: 64, SEQ ID NO: 65, SEQ ID NO: 66, SEQ ID NO: 67, SEQ ID NO: 68, SEQ ID NO: 69, SEQ ID NO: 70, SEQ ID NO: 71, SEQ ID NO: 72, SEQ ID NO: 73, SEQ ID NO: 74, SEQ ID NO: 75, SEQ ID NO: 76, SEQ ID NO: 77, SEQ ID NO: 78, SEQ ID NO: 79, SEQ ID NO: 80, SEQ ID NO: 81, SEQ ID NO: 82, SEQ ID NO: 83, SEQ ID NO: 84, SEQ ID NO: 85, SEQ ID NO: 86, SEQ ID NO: 87, SEQ ID NO: 88, SEQ ID NO: 187, SEQ ID NO: 188, or SEQ ID NO: 189.

[0034] The invention provides an isolated immunoglobulin light chain polypeptide which comprises, consists essentially of, or consists of the amino acid sequence Asp Ile Gln Met Thr Gln Ser Pro Ser Ser Leu Ser Ala Ser Val Gly Asp Arg Val Thr Ile Thr Cys Gln Ala Ser Gln Asp Ile Ser Asn Tyr Leu Asn Trp Tyr Gln Gln Lys Pro Gly Lys Ala Pro Lys Leu Leu Ile Xaa1 Xaa2 Xaa3 Xaa4 Xaa5 Leu Glu Thr Gly Val Pro Ser Arg Phe Ser Gly Ser Gly Ser Gly Thr Asp Phe Thr Phe Thr Ile Ser Ser Leu Gln Pro Glu Asp Ile Ala Val Tyr Tyr Cys Gln Gln Ser Tyr Ser Xaa6 Leu Ile Thr Phe Gly Gln Gly Thr Arg Leu Glu Ile Lys Arg Thr Val Ala Ala Pro Ser Val (SEQ ID NO: 89), wherein (a) the subsequence Xaa1 Xaa2 Xaa3 Xaa4 Xaa5 is deleted or is Tyr-Asp-Ala-Ser-Asn, and (b) Xaa6 is threonine (Thr) or isoleucine (Ile).

[0035] The inventive immunoglobulin light chain polypeptide can include or lack the subsequence Xaa1 Xaa2 Xaa3 Xaa4 Xaa5 at positions 49-53 of SEQ ID NO: 89 when Xaa6 is threonine (Thr) or isoleucine (Ile). When the inventive immunoglobulin light chain polypeptide comprises the subsequence Xaa1 Xaa2 Xaa3 Xaa4 Xaa5, each of Xaa1, Xaa2, Xaa3, Xaa4, and Xaa5 can be any suitable amino acid residue. Preferably, Xaa1 is tyrosine (Tyr), Xaa2 is aspartic acid (Asp), Xaa3 is alanine (Ala), Xaa4 is serine (Ser), and Xaa5 is asparagine (Asn). A preferred amino acid sequence of an immunoglobulin light chain polypeptide which includes the subsequence Xaa1 Xaa2 Xaa3 Xaa4 Xaa5 comprises SEQ ID NO: 90. When the immunoglobulin light chain polypeptide lacks the subsequence Xaa1 Xaa2 Xaa3 Xaa4 Xaa5, the immunoglobulin light chain polypeptide preferably comprises the amino acid sequence SEQ ID NO: 91 or SEQ ID NO: 92.

[0036] In another embodiment, provided is an isolated immunoglobulin light chain polypeptide which comprises SEQ ID NO: 196 or 197. Examples of such a polypeptide include those comprising any one of SEQ ID NOs: 198-200.

[0037] When the inventive immunoglobulin light chain polypeptide consists essentially of an amino acid sequence of any one of SEQ ID NO: 57-SEQ ID NO: 92, SEQ ID NOs: 187-189, or SEQ ID NOs: 196-200, additional components can be included in the polypeptide that do not materially affect the polypeptide (e.g., protein moieties such as biotin that facilitate purification or isolation). When the inventive immunoglobulin light chain polypeptide consists of an amino acid sequence of any one of SEQ ID NO: 57-SEQ ID NO: 92, the polypeptide does not comprise any additional components (i.e., components that are not endogenous to the inventive immunoglobulin light chain polypeptide).

[0038] The invention provides an isolated immunoglobulin light chain polypeptide which comprises an amino acid sequence that is at least 90% identical (e.g., at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98%, at least 99%, or 100% identical) to any one of SEQ ID NO: 57-SEQ ID NO: 92. Nucleic acid or amino acid sequence "identity" can be determined using the methods described herein.

[0039] One or more amino acids of the aforementioned immunoglobulin heavy chain polypeptides and/or light chain polypeptides can be replaced or substituted with a different amino acid. An amino acid "replacement" or "substitution" refers to the replacement of one amino acid at a given position or residue by another amino acid at the same position or residue within a polypeptide sequence.

[0040] Amino acids are broadly grouped as "aromatic" or "aliphatic." An aromatic amino acid includes an aromatic ring. Examples of "aromatic" amino acids include histidine (H or His), phenylalanine (F or Phe), tyrosine (Y or Tyr), and tryptophan (W or Trp). Non-aromatic amino acids are broadly grouped as "aliphatic." Examples of "aliphatic" amino acids include glycine (G or Gly), alanine (A or Ala), valine (V or Val), leucine (L or Leu), isoleucine (I or Ile), methionine (M or Met), serine (S or Ser), threonine (T or Thr), cysteine (C or Cys), proline (P or Pro), glutamic acid (E or Glu), aspartic acid (A or Asp), asparagine (N or Asn), glutamine (Q or Gln), lysine (K or Lys), and arginine (R or Arg).

[0041] Aliphatic amino acids may be sub-divided into four sub-groups. The "large aliphatic non-polar sub-group" consists of valine, leucine, and isoleucine. The "aliphatic slightly-polar sub-group" consists of methionine, serine, threonine, and cysteine. The "aliphatic polar/charged sub-group" consists of glutamic acid, aspartic acid, asparagine, glutamine, lysine, and arginine.

The "small-residue sub-group" consists of glycine and alanine. The group of charged/polar amino acids may be sub-divided into three sub-groups: the "positively-charged sub-group" consisting of lysine and arginine, the "negatively-charged sub-group" consisting of glutamic acid and aspartic acid, and the "polar sub-group" consisting of asparagine and glutamine.

[0042] Aromatic amino acids may be sub-divided into two sub-groups: the "nitrogen ring sub-group" consisting of histidine and tryptophan and the "phenyl sub-group" consisting of phenylalanine and tyrosine.

[0043] The amino acid replacement or substitution can be conservative, semi-conservative, or non-conservative. The phrase "conservative amino acid substitution" or "conservative mutation" refers to the replacement of one amino acid by another amino acid with a common property. A functional way to define common properties between individual amino acids is to analyze the normalized frequencies of amino acid changes between corresponding proteins of homologous organisms (Schulz and Schirmer, *Principles of Protein Structure*, Springer-Verlag, New York (1979)). According to such analyses, groups of amino acids may be defined where amino acids within a group exchange preferentially with each other, and therefore resemble each other most in their impact on the overall protein structure (Schulz and Schirmer, *supra*).

[0044] Examples of conservative amino acid substitutions include substitutions of amino acids within the sub-groups described above, for example, lysine for arginine and vice versa such that a positive charge may be maintained, glutamic acid for aspartic acid and vice versa such that a negative charge may be maintained, serine for threonine such that a free -OH can be maintained, and glutamine for asparagine such that a free -NH_2 can be maintained.

[0045] "Semi-conservative mutations" include amino acid substitutions of amino acids within the same groups listed above, but not within the same sub-group. For example, the substitution of aspartic acid for asparagine, or asparagine for lysine, involves amino acids within the same group, but different sub-groups. "Non-conservative mutations" involve amino acid substitutions between different groups, for example, lysine for tryptophan, or phenylalanine for serine, etc.

[0046] In addition, one or more amino acids can be inserted into the aforementioned immunoglobulin heavy chain polypeptides and/or light chain polypeptides. Any number of any suitable amino acids can be inserted into the amino acid sequence of the immunoglobulin heavy

chain polypeptide and/or light chain polypeptide. In this respect, at least one amino acid (e.g., 2 or more, 5 or more, or 10 or more amino acids), but not more than 20 amino acids (e.g., 18 or less, 15 or less, or 12 or less amino acids), can be inserted into the amino acid sequence of the immunoglobulin heavy chain polypeptide and/or light chain polypeptide. Preferably, 1-10 amino acids (e.g., 1, 2, 3, 4, 5, 6, 7, 8, 9, or 10 amino acids) are inserted into the amino acid sequence of the immunoglobulin heavy chain polypeptide and/or light chain polypeptide. In this respect, the amino acid(s) can be inserted into any one of the aforementioned immunoglobulin heavy chain polypeptides and/or light chain polypeptides in any suitable location. Preferably, the amino acid(s) are inserted into a CDR (e.g., CDR1, CDR2, or CDR3) of the immunoglobulin heavy chain polypeptide and/or light chain polypeptide.

[0047] The inventive isolated immunoglobulin heavy chain polypeptide and light chain polypeptides are not limited to polypeptides comprising the specific amino acid sequences described herein. Indeed, the immunoglobulin heavy chain polypeptide or light chain polypeptide can be any heavy chain polypeptide or light chain polypeptide that competes with the inventive immunoglobulin heavy chain polypeptide or light chain polypeptide for binding to LAG-3. In this respect, for example, the immunoglobulin heavy chain polypeptide or light chain polypeptide can be any heavy chain polypeptide or light chain polypeptide that binds to the same epitope of LAG-3 recognized by the heavy and light chain polypeptides described herein. Antibody competition can be assayed using routine peptide competition assays which utilize ELISA, Western blot, or immunohistochemistry methods (see, e.g., U.S. Patents 4,828,981 and 8,568,992; and Braitbard et al., *Proteome Sci.*, *4*: 12 (2006)).

[0048] The invention provides an isolated LAG-3-binding agent comprising, consisting essentially of, or consisting of one or more of the inventive isolated amino acid sequences described herein. By "LAG-3-binding agent" is meant a molecule, preferably a proteinaceous molecule, which binds specifically to the LAG-3 protein. Preferably, the LAG-3-binding agent is an antibody or a fragment (e.g., immunogenic fragment) thereof. The LAG-3-binding agent of the invention comprises, consists essentially of, or consists of the inventive isolated immunoglobulin heavy chain polypeptide and/or the inventive isolated immunoglobulin light chain polypeptide. In one embodiment, the LAG-3-binding agent comprises, consists essentially of, or consists of the inventive immunoglobulin heavy chain polypeptide or the inventive

immunoglobulin light chain polypeptide. In another embodiment, the LAG-3-binding agent comprises, consists essentially of, or consists of the inventive immunoglobulin heavy chain polypeptide and the inventive immunoglobulin light chain polypeptide.

[0049] Any amino acid residue of the inventive immunoglobulin heavy chain polypeptide and/or the inventive immunoglobulin light chain polypeptide can be replaced, in any combination, with a different amino acid residue, or can be deleted or inserted, so long as the biological activity of the LAG-3-binding agent is enhanced or improved as a result of the amino acid replacements, insertions, and/or deletions. The "biological activity" of an LAG-3-binding agent refers to, for example, binding affinity for a particular LAG-3 epitope, neutralization or inhibition of LAG-3 binding to its receptor(s), neutralization or inhibition of LAG-3 activity *in vivo* (e.g., IC_{50}), pharmacokinetics, and cross-reactivity (e.g., with non-human homologs or orthologs of the LAG-3 protein, or with other proteins or tissues). Other biological properties or characteristics of an antigen-binding agent recognized in the art include, for example, avidity, selectivity, solubility, folding, immunotoxicity, expression, and formulation. The aforementioned properties or characteristics can be observed, measured, and/or assessed using standard techniques including, but not limited to, ELISA, competitive ELISA, surface plasmon resonance analysis (BIACORE™), or KINEXA™, *in vitro* or *in vivo* neutralization assays, receptor-ligand binding assays, cytokine or growth factor production and/or secretion assays, and signal transduction and immunohistochemistry assays.

[0050] The terms "inhibit" or "neutralize," as used herein with respect to the activity of a LAG-3-binding agent, refer to the ability to substantially antagonize, prohibit, prevent, restrain, slow, disrupt, alter, eliminate, stop, or reverse the progression or severity of, for example, the biological activity of LAG-3, or a disease or condition associated with LAG-3. The isolated LAG-3-binding agent of the invention preferably inhibits or neutralizes the activity of LAG-3 by at least about 20%, about 30%, about 40%, about 50%, about 60%, about 70%, about 80%, about 90%, about 95%, about 100%, or a range defined by any two of the foregoing values.

[0051] The isolated LAG-3-binding agent of the invention can be a whole antibody, as described herein, or an antibody fragment. The terms "fragment of an antibody," "antibody fragment," and "functional fragment of an antibody" are used interchangeably herein to mean one or more fragments of an antibody that retain the ability to specifically bind to an antigen

(see, generally, Holliger et al., *Nat. Biotech.*, *23*(9): 1126-1129 (2005)). The isolated LAG-3-binding agent can contain any LAG-3-binding antibody fragment. The antibody fragment desirably comprises, for example, one or more CDRs, the variable region (or portions thereof), the constant region (or portions thereof), or combinations thereof. Examples of antibody fragments include, but are not limited to, (i) a Fab fragment, which is a monovalent fragment consisting of the V_L, V_H, C_L, and CH_1 domains, (ii) a $F(ab')_2$ fragment, which is a bivalent fragment comprising two Fab fragments linked by a disulfide bridge at the hinge region, (iii) a Fv fragment consisting of the V_L and V_H domains of a single arm of an antibody, (iv) a Fab' fragment, which results from breaking the disulfide bridge of an $F(ab')_2$ fragment using mild reducing conditions, (v) a disulfide-stabilized Fv fragment (dsFv), and (vi) a domain antibody (dAb), which is an antibody single variable region domain (VH or VL) polypeptide that specifically binds antigen.

[0052] In embodiments where the isolated LAG-3-binding agent comprises a fragment of the immunoglobulin heavy chain or light chain polypeptide, the fragment can be of any size so long as the fragment binds to, and preferably inhibits the activity of, LAG-3. In this respect, a fragment of the immunoglobulin heavy chain polypeptide desirably comprises between about 5 and 18 (e.g., about 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, or a range defined by any two of the foregoing values) amino acids. Similarly, a fragment of the immunoglobulin light chain polypeptide desirably comprises between about 5 and 18 (e.g., about 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, or a range defined by any two of the foregoing values) amino acids.

[0053] When the LAG-3-binding agent is an antibody or antibody fragment, the antibody or antibody fragment desirably comprises a heavy chain constant region (F_c) of any suitable class. Preferably, the antibody or antibody fragment comprises a heavy chain constant region that is based upon wild-type IgG1, IgG2, or IgG4 antibodies, or variants thereof. In some embodiments, the LAG-3 binding agent comprises an Fc region engineered to reduce or eliminate effector functions of the antibody. Engineered Fc regions with reduced or abrogated effector function are known in the art and commercially available, as are techniques for engineering Fc regions to reduce or eliminate effector function, any of which can be used in conjunction with the invention.

[0054] The LAG-3-binding agent also can be a single chain antibody fragment. Examples of single chain antibody fragments include, but are not limited to, (i) a single chain Fv (scFv), which is a monovalent molecule consisting of the two domains of the Fv fragment (i.e., V_L and V_H) joined by a synthetic linker which enables the two domains to be synthesized as a single polypeptide chain (see, e.g., Bird et al., *Science, 242*: 423-426 (1988); Huston et al., *Proc. Natl. Acad. Sci. USA, 85*: 5879-5883 (1988); and Osbourn et al., *Nat. Biotechnol., 16*: 778 (1998)) and (ii) a diabody, which is a dimer of polypeptide chains, wherein each polypeptide chain comprises a V_H connected to a V_L by a peptide linker that is too short to allow pairing between the V_H and V_L on the same polypeptide chain, thereby driving the pairing between the complementary domains on different V_H -V_L polypeptide chains to generate a dimeric molecule having two functional antigen binding sites. Antibody fragments are known in the art and are described in more detail in, e.g., U.S. Patent Application Publication 2009/0093024 A1.

[0055] The isolated LAG-3-binding agent also can be an intrabody or fragment thereof. An intrabody is an antibody which is expressed and which functions intracellularly. Intrabodies typically lack disulfide bonds and are capable of modulating the expression or activity of target genes through their specific binding activity. Intrabodies include single domain fragments such as isolated V_H and V_L domains and scFvs. An intrabody can include sub-cellular trafficking signals attached to the N or C terminus of the intrabody to allow expression at high concentrations in the sub-cellular compartments where a target protein is located. Upon interaction with a target gene, an intrabody modulates target protein function and/or achieves phenotypic/functional knockout by mechanisms such as accelerating target protein degradation and sequestering the target protein in a non-physiological sub-cellular compartment. Other mechanisms of intrabody-mediated gene inactivation can depend on the epitope to which the intrabody is directed, such as binding to the catalytic site on a target protein or to epitopes that are involved in protein-protein, protein-DNA, or protein-RNA interactions.

[0056] The isolated LAG-3-binding agent also can be an antibody conjugate. In this respect, the isolated LAG-3-binding agent can be a conjugate of (1) an antibody, an alternative scaffold, or fragments thereof, and (2) a protein or non-protein moiety comprising the LAG-3-binding agent. For example, the LAG-3-binding agent can be all or part of an antibody conjugated to a peptide, a fluorescent molecule, or a chemotherapeutic agent.

[0057] The isolated LAG-3-binding agent can be, or can be obtained from, a human antibody, a non-human antibody, or a chimeric antibody. By "chimeric" is meant an antibody or fragment thereof comprising both human and non-human regions. Preferably, the isolated LAG-3-binding agent is a humanized antibody. A "humanized" antibody is a monoclonal antibody comprising a human antibody scaffold and at least one CDR obtained or derived from a non-human antibody. Non-human antibodies include antibodies isolated from any non-human animal, such as, for example, a rodent (e.g., a mouse or rat). A humanized antibody can comprise, one, two, or three CDRs obtained or derived from a non-human antibody. In one embodiment of the invention, CDRH3 of the inventive LAG-3-binding agent is obtained or derived from a mouse monoclonal antibody, while the remaining variable regions and constant region of the inventive LAG-3-binding agent are obtained or derived from a human monoclonal antibody.

[0058] A human antibody, a non-human antibody, a chimeric antibody, or a humanized antibody can be obtained by any means, including via *in vitro* sources (e.g., a hybridoma or a cell line producing an antibody recombinantly) and *in vivo* sources (e.g., rodents). Methods for generating antibodies are known in the art and are described in, for example, Köhler and Milstein, *Eur. J. Immunol.*, *5*: 511-519 (1976); Harlow and Lane (eds.), *Antibodies: A Laboratory Manual*, CSH Press (1988); and Janeway et al. (eds.), *Immunobiology, 5th Ed.*, Garland Publishing, New York, NY (2001)). In certain embodiments, a human antibody or a chimeric antibody can be generated using a transgenic animal (e.g., a mouse) wherein one or more endogenous immunoglobulin genes are replaced with one or more human immunoglobulin genes. Examples of transgenic mice wherein endogenous antibody genes are effectively replaced with human antibody genes include, but are not limited to, the Medarex HUMAB-MOUSE™, the Kirin TC MOUSE™, and the Kyowa Kirin KM-MOUSE™ (see, e.g., Lonberg, *Nat. Biotechnol.*, *23(9)*: 1117-25 (2005), and Lonberg, *Handb. Exp. Pharmacol.*, *181*: 69-97 (2008)). A humanized antibody can be generated using any suitable method known in the art (see, e.g., An, Z. (ed.), *Therapeutic Monoclonal Antibodies: From Bench to Clinic*, John Wiley & Sons, Inc., Hoboken, New Jersey (2009)), including, e.g., grafting of non-human CDRs onto a human antibody scaffold (see, e.g., Kashmiri et al., *Methods*, *36*(1): 25-34 (2005); and Hou et al., *J. Biochem.*, *144*(1): 115-120 (2008)). In one embodiment, a humanized antibody can be

produced using the methods described in, e.g., U.S. Patent Application Publication 2011/0287485 A1.

[0059] In one embodiment, a CDR (e.g., CDR1, CDR2, or CDR3) or a variable region of the immunoglobulin heavy chain polypeptide and/or the immunoglobulin light chain polypeptide described herein can be transplanted (i.e., grafted) into another molecule, such as an antibody or non-antibody polypeptide, using either protein chemistry or recombinant DNA technology. In this regard, the invention provides an isolated LAG-3-binding agent comprising at least one CDR of an immunoglobulin heavy chain and/or light chain polypeptide as described herein. The isolated LAG-3-binding agent can comprise one, two, or three CDRs of an immunoglobulin heavy chain and/or light chain variable region as described herein.

[0060] In a preferred embodiment, the LAG-3-binding agent binds an epitope of LAG-3 which blocks the binding of LAG-3 to MHC Class II molecules and inhibits LAG-3-mediated signaling. For example, the LAG-3 binding agent can bind to one or more of the four Ig-like extracellular domains (D1-D4) of the LAG-3 protein (see, e.g, Triebel et al., *J. Exp. Med.*, *171*(5): 1393-1405 (1990); and Bruniquel et al., *Immunogenetics*, *47*: 96-98 (1997)). Preferably, the LAG-3 binding agent binds to domain 1 (D1) and/or domain (D2) of the LAG-3 protein. The invention also provides an isolated or purified epitope of LAG-3 which blocks the binding of LAG-3 to MHC Class II molecules in an indirect or allosteric manner.

[0061] The invention also provides one or more isolated or purified nucleic acid sequences that encode the inventive immunoglobulin heavy chain polypeptide, the inventive immunoglobulin light chain polypeptide, and the inventive LAG-3-binding agent.

[0062] The term "nucleic acid sequence" is intended to encompass a polymer of DNA or RNA, i.e., a polynucleotide, which can be single-stranded or double-stranded and which can contain non-natural or altered nucleotides. The terms "nucleic acid" and "polynucleotide" as used herein refer to a polymeric form of nucleotides of any length, either ribonucleotides (RNA) or deoxyribonucleotides (DNA). These terms refer to the primary structure of the molecule, and thus include double- and single-stranded DNA, and double- and single-stranded RNA. The terms include, as equivalents, analogs of either RNA or DNA made from nucleotide analogs and modified polynucleotides such as, though not limited to, methylated and/or capped polynucleotides. Nucleic acids are typically linked via phosphate bonds to form nucleic acid

sequences or polynucleotides, though many other linkages are known in the art (e.g., phosphorothioates, boranophosphates, and the like).

[0063] The invention further provides a vector comprising one or more nucleic acid sequences encoding the inventive immunoglobulin heavy chain polypeptide, the inventive immunoglobulin light chain polypeptide, and/or the inventive LAG-3-binding agent. The vector can be, for example, a plasmid, episome, cosmid, viral vector (e.g., retroviral or adenoviral), or phage. Suitable vectors and methods of vector preparation are well known in the art (see, e.g., Sambrook et al., *Molecular Cloning, a Laboratory Manual, 3rd edition*, Cold Spring Harbor Press, Cold Spring Harbor, N.Y. (2001), and Ausubel et al., *Current Protocols in Molecular Biology*, Greene Publishing Associates and John Wiley & Sons, New York, N.Y. (1994)).

[0064] In addition to the nucleic acid sequence encoding the inventive immunoglobulin heavy polypeptide, the inventive immunoglobulin light chain polypeptide, and/or the inventive LAG-3-binding agent, the vector preferably comprises expression control sequences, such as promoters, enhancers, polyadenylation signals, transcription terminators, signal peptides (e.g., the osteonectin signal peptide), internal ribosome entry sites (IRES), and the like, that provide for the expression of the coding sequence in a host cell. Exemplary expression control sequences are known in the art and described in, for example, Goeddel, *Gene Expression Technology: Methods in Enzymology*, Vol. 185, Academic Press, San Diego, Calif. (1990).

[0065] A large number of promoters, including constitutive, inducible, and repressible promoters, from a variety of different sources are well known in the art. Representative sources of promoters include for example, virus, mammal, insect, plant, yeast, and bacteria, and suitable promoters from these sources are readily available, or can be made synthetically, based on sequences publicly available, for example, from depositories such as the ATCC as well as other commercial or individual sources. Promoters can be unidirectional (i.e., initiate transcription in one direction) or bi-directional (i.e., initiate transcription in either a 3' or 5' direction). Non-limiting examples of promoters include, for example, the T7 bacterial expression system, pBAD (araA) bacterial expression system, the cytomegalovirus (CMV) promoter, the SV40 promoter, the RSV promoter. Inducible promoters include, for example, the Tet system (U.S. Patents 5,464,758 and 5,814,618), the Ecdysone inducible system (No et al., *Proc. Natl. Acad. Sci., 93*: 3346-3351 (1996)), the T-REX™ system (Invitrogen, Carlsbad, CA), LACSWITCH™ system

(Stratagene, San Diego, CA), and the Cre-ERT tamoxifen inducible recombinase system (Indra et al., *Nuc. Acid. Res.*, *27*: 4324-4327 (1999); *Nuc. Acid. Res.*, *28*: e99 (2000); U.S. Patent 7,112,715; and Kramer & Fussenegger, *Methods Mol. Biol.*, *308*: 123-144 (2005)).

[0066] The term "enhancer" as used herein, refers to a DNA sequence that increases transcription of, for example, a nucleic acid sequence to which it is operably linked. Enhancers can be located many kilobases away from the coding region of the nucleic acid sequence and can mediate the binding of regulatory factors, patterns of DNA methylation, or changes in DNA structure. A large number of enhancers from a variety of different sources are well known in the art and are available as or within cloned polynucleotides (from, e.g., depositories such as the ATCC as well as other commercial or individual sources). A number of polynucleotides comprising promoters (such as the commonly-used CMV promoter) also comprise enhancer sequences. Enhancers can be located upstream, within, or downstream of coding sequences.

[0067] The vector also can comprise a "selectable marker gene." The term "selectable marker gene," as used herein, refers to a nucleic acid sequence that allow cells expressing the nucleic acid sequence to be specifically selected for or against, in the presence of a corresponding selective agent. Suitable selectable marker genes are known in the art and described in, e.g., International Patent Application Publications WO 1992/008796 and WO 1994/028143; Wigler et al., *Proc. Natl. Acad. Sci. USA*, *77*: 3567-3570 (1980); O'Hare et al., *Proc. Natl. Acad. Sci. USA*, *78*: 1527-1531 (1981); Mulligan & Berg, *Proc. Natl. Acad. Sci. USA*, *78*: 2072-2076 (1981); Colberre-Garapin et al., *J. Mol. Biol.*, *150*: 1-14 (1981); Santerre et al., *Gene*, *30*: 147-156 (1984); Kent et al., *Science*, *237*: 901-903 (1987); Wigler et al., *Cell*, *11*: 223-232 (1977); Szybalska & Szybalski, *Proc. Natl. Acad. Sci. USA*, *48*: 2026-2034 (1962); Lowy et al., *Cell*, *22*: 817-823 (1980); and U.S. Patents 5,122,464 and 5,770,359.

[0068] In some embodiments, the vector is an "episomal expression vector" or "episome," which is able to replicate in a host cell, and persists as an extrachromosomal segment of DNA within the host cell in the presence of appropriate selective pressure (see, e.g., Conese et al., *Gene Therapy*, *11*: 1735-1742 (2004)). Representative commercially available episomal expression vectors include, but are not limited to, episomal plasmids that utilize Epstein Barr Nuclear Antigen 1 (EBNA1) and the Epstein Barr Virus (EBV) origin of replication (oriP). The vectors pREP4, pCEP4, pREP7, and pcDNA3.1 from Invitrogen (Carlsbad, CA) and pBK-CMV

from Stratagene (La Jolla, CA) represent non-limiting examples of an episomal vector that uses T-antigen and the SV40 origin of replication in lieu of EBNA1 and oriP.

[0069] Other suitable vectors include integrating expression vectors, which may randomly integrate into the host cell's DNA, or may include a recombination site to enable the specific recombination between the expression vector and the host cell's chromosome. Such integrating expression vectors may utilize the endogenous expression control sequences of the host cell's chromosomes to effect expression of the desired protein. Examples of vectors that integrate in a site specific manner include, for example, components of the flp-in system from Invitrogen (Carlsbad, CA) (e.g., pcDNA™5/FRT), or the cre-lox system, such as can be found in the pExchange-6 Core Vectors from Stratagene (La Jolla, CA). Examples of vectors that randomly integrate into host cell chromosomes include, for example, pcDNA3.1 (when introduced in the absence of T-antigen) from Life Technologies (Carlsbad, CA), UCOE from Millipore (Billerica, MA), and pCI or pFN10A (ACT) FLEXI™ from Promega (Madison, WI).

[0070] Viral vectors also can be used. Representative commercially available viral expression vectors include, but are not limited to, the adenovirus-based Per.C6 system available from Crucell, Inc. (Leiden, The Netherlands), the lentiviral-based pLP1 from Invitrogen (Carlsbad, CA), and the retroviral vectors pFB-ERV plus pCFB-EGSH from Stratagene (La Jolla, CA).

[0071] Nucleic acid sequences encoding the inventive amino acid sequences can be provided to a cell on the same vector (i.e., in *cis*). A unidirectional promoter can be used to control expression of each nucleic acid sequence. In another embodiment, a combination of bidirectional and unidirectional promoters can be used to control expression of multiple nucleic acid sequences. Nucleic acid sequences encoding the inventive amino acid sequences alternatively can be provided to the population of cells on separate vectors (i.e., in *trans*). Each of the nucleic acid sequences in each of the separate vectors can comprise the same or different expression control sequences. The separate vectors can be provided to cells simultaneously or sequentially.

[0072] The vector(s) comprising the nucleic acid(s) encoding the inventive amino acid sequences can be introduced into a host cell that is capable of expressing the polypeptides encoded thereby, including any suitable prokaryotic or eukaryotic cell. As such, the invention

provides an isolated cell comprising the inventive vector. Preferred host cells are those that can be easily and reliably grown, have reasonably fast growth rates, have well characterized expression systems, and can be transformed or transfected easily and efficiently.

[0073] Examples of suitable prokaryotic cells include, but are not limited to, cells from the genera *Bacillus* (such as *Bacillus subtilis* and *Bacillus brevis*), *Escherichia* (such as *E. coli*), *Pseudomonas*, *Streptomyces*, *Salmonella*, and *Erwinia*. Particularly useful prokaryotic cells include the various strains of *Escherichia coli* (e.g., K12, HB101 (ATCC No. 33694), DH5α, DH10, MC1061 (ATCC No. 53338), and CC102).

[0074] Preferably, the vector is introduced into a eukaryotic cell. Suitable eukaryotic cells are known in the art and include, for example, yeast cells, insect cells, and mammalian cells. Examples of suitable yeast cells include those from the genera *Kluyveromyces*, *Pichia*, *Rhinosporidium*, *Saccharomyces*, and *Schizosaccharomyces*. Preferred yeast cells include, for example, *Saccharomyces cerivisae* and *Pichia pastoris*.

[0075] Suitable insect cells are described in, for example, Kitts et al., *Biotechniques*, *14*: 810-817 (1993); Lucklow, *Curr. Opin. Biotechnol.*, *4*: 564-572 (1993); and Lucklow et al., *J. Virol.*, *67*: 4566-4579 (1993). Preferred insect cells include Sf-9 and HI5 (Invitrogen, Carlsbad, CA).

[0076] Preferably, mammalian cells are utilized in the invention. A number of suitable mammalian host cells are known in the art, and many are available from the American Type Culture Collection (ATCC, Manassas, VA). Examples of suitable mammalian cells include, but are not limited to, Chinese hamster ovary cells (CHO) (ATCC No. CCL61), CHO DHFR-cells (Urlaub et al., *Proc. Natl. Acad. Sci. USA*, *97*: 4216-4220 (1980)), human embryonic kidney (HEK) 293 or 293T cells (ATCC No. CRL1573), and 3T3 cells (ATCC No. CCL92). Other suitable mammalian cell lines are the monkey COS-1 (ATCC No. CRL1650) and COS-7 cell lines (ATCC No. CRL1651), as well as the CV-1 cell line (ATCC No. CCL70). Further exemplary mammalian host cells include primate cell lines and rodent cell lines, including transformed cell lines. Normal diploid cells, cell strains derived from in vitro culture of primary tissue, as well as primary explants, are also suitable. Other suitable mammalian cell lines include, but are not limited to, mouse neuroblastoma N2A cells, HeLa, mouse L-929 cells, and BHK or HaK hamster cell lines, all of which are available from the ATCC. Methods for

selecting suitable mammalian host cells and methods for transformation, culture, amplification, screening, and purification of cells are known in the art.

[0077] In one embodiment, the mammalian cell is a human cell. For example, the mammalian cell can be a human lymphoid or lymphoid derived cell line, such as a cell line of pre-B lymphocyte origin. Examples of human lymphoid cells lines include, without limitation, RAMOS (CRL-1596), Daudi (CCL-213), EB-3 (CCL-85), DT40 (CRL-2111), 18-81 (Jack et al., *Proc. Natl. Acad. Sci. USA, 85*: 1581-1585 (1988)), Raji cells (CCL-86), PER.C6 cells (Crucell Holland B.V., Leiden, The Netherlands), and derivatives thereof.

[0078] A nucleic acid sequence encoding the inventive amino acid sequence may be introduced into a cell by "transfection," "transformation," or "transduction." "Transfection," "transformation," or "transduction," as used herein, refer to the introduction of one or more exogenous polynucleotides into a host cell by using physical or chemical methods. Many transfection techniques are known in the art and include, for example, calcium phosphate DNA co-precipitation (see, e.g., Murray E.J. (ed.), *Methods in Molecular Biology, Vol. 7, Gene Transfer and Expression Protocols*, Humana Press (1991)); DEAE-dextran; electroporation; cationic liposome-mediated transfection; tungsten particle-facilitated microparticle bombardment (Johnston, *Nature*, *346*: 776-777 (1990)); and strontium phosphate DNA co-precipitation (Brash et al., *Mol. Cell Biol.*, *7*: 2031-2034 (1987)). Phage or viral vectors can be introduced into host cells, after growth of infectious particles in suitable packaging cells, many of which are commercially available.

[0079] The invention provides a composition comprising an effective amount of the inventive immunoglobulin heavy chain polypeptide, the inventive immunoglobulin light chain polypeptide, the inventive LAG-3-binding agent, the inventive nucleic acid sequence encoding any of the foregoing, or the inventive vector comprising the inventive nucleic acid sequence. Preferably, the composition is a pharmaceutically acceptable (e.g., physiologically acceptable) composition, which comprises a carrier, preferably a pharmaceutically acceptable (e.g., physiologically acceptable) carrier, and the inventive amino acid sequences, antigen-binding agent, or vector. Any suitable carrier can be used within the context of the invention, and such carriers are well known in the art. The choice of carrier will be determined, in part, by the particular site to which the composition may be administered and the particular method used to

administer the composition. The composition optionally can be sterile. The composition can be frozen or lyophilized for storage and reconstituted in a suitable sterile carrier prior to use. The compositions can be generated in accordance with conventional techniques described in, e.g., Remington: *The Science and Practice of Pharmacy, 21st Edition*, Lippincott Williams & Wilkins, Philadelphia, PA (2001).

[0080] The invention further provides a method of treating a disorder in a mammal that is responsive to LAG-3 inhibition or neutralization. The method comprises administering the aforementioned composition to a mammal having a disorder that is responsive to LAG-3 inhibition or neutralization, whereupon the disorder is treated in the mammal. A disorder that is "responsive to LAG-3 inhibition" or "responsive to LAG-3 neutralization" refers to any disease or disorder in which a decrease in LAG-3 levels or activity has a therapeutic benefit in mammals, preferably humans, or the improper expression (e.g., overexpression) or increased activity of LAG-3 causes or contributes to the pathological effects of the disease or disorder. Disorders that are responsive to LAG-3 inhibition include, for example, cancer and infectious diseases. The inventive method can be used to treat any type of cancer known in the art, such as, for example, melanoma, renal cell carcinoma, lung cancer, bladder cancer, breast cancer, cervical cancer, colon cancer, gall bladder cancer, laryngeal cancer, liver cancer, thyroid cancer, stomach cancer, salivary gland cancer, prostate cancer, pancreatic cancer, or Merkel cell carcinoma (see, e.g., Bhatia et al., *Curr. Oncol. Rep., 13*(6): 488-497 (2011)). The inventive method can be used to treat any type of infectious disease (i.e., a disease or disorder caused by a bacterium, a virus, a fungus, or a parasite). Examples of infectious diseases that can be treated by the inventive method include, but are not limited to, diseases caused by a human immunodeficiency virus (HIV), a respiratory syncytial virus (RSV), an influenza virus, a dengue virus, a hepatitis B virus (HBV, or a hepatitis C virus (HCV)). Administration of a composition comprising the inventive immunoglobulin heavy chain polypeptide, the inventive immunoglobulin light chain polypeptide, the inventive LAG-3-binding agent, the inventive nucleic acid sequence encoding any of the foregoing, or the inventive vector comprising the inventive nucleic acid sequence induces an immune response against a cancer or infectious disease in a mammal. An "immune response" can entail, for example, antibody production and/or the activation of immune effector cells (e.g., T-cells).

[0081] As used herein, the terms "treatment," "treating," and the like refer to obtaining a desired pharmacologic and/or physiologic effect. Preferably, the effect is therapeutic, i.e., the effect partially or completely cures a disease and/or adverse symptom attributable to the disease. To this end, the inventive method comprises administering a "therapeutically effective amount" of the LAG-3-binding agent. A "therapeutically effective amount" refers to an amount effective, at dosages and for periods of time necessary, to achieve a desired therapeutic result. The therapeutically effective amount may vary according to factors such as the disease state, age, sex, and weight of the individual, and the ability of the LAG-3-binding agent to elicit a desired response in the individual. For example, a therapeutically effective amount of a LAG-3-binding agent of the invention is an amount which decreases LAG-3 bioactivity in a human.

[0082] Alternatively, the pharmacologic and/or physiologic effect may be prophylactic, i.e., the effect completely or partially prevents a disease or symptom thereof. In this respect, the inventive method comprises administering a "prophylactically effective amount" of the LAG-3-binding agent. A "prophylactically effective amount" refers to an amount effective, at dosages and for periods of time necessary, to achieve a desired prophylactic result (e.g., prevention of disease onset).

[0083] A typical dose can be, for example, in the range of 1 pg/kg to 20 mg/kg of animal or human body weight; however, doses below or above this exemplary range are within the scope of the invention. The daily parenteral dose can be about 0.00001 μg/kg to about 20 mg/kg of total body weight (e.g., about 0.001 μg /kg, about 0.1 μg /kg , about 1 μg /kg, about 5 μg /kg, about 10 μg/kg, about 100 μg /kg, about 500 μg/kg, about 1 mg/kg, about 5 mg/kg, about 10 mg/kg, or a range defined by any two of the foregoing values), preferably from about 0.1 μg/kg to about 10 mg/kg of total body weight (e.g., about 0.5 μg/kg, about 1 μg/kg, about 50 μg/kg, about 150 μg/kg, about 300 μg/kg, about 750 μg/kg, about 1.5 mg/kg, about 5 mg/kg, or a range defined by any two of the foregoing values), more preferably from about 1 μg/kg to 5 mg/kg of total body weight (e.g., about 3 μg/kg, about 15 μg/kg, about 75 μg/kg, about 300 μg/kg, about 900 μg/kg, about 2 mg/kg, about 4 mg/kg, or a range defined by any two of the foregoing values), and even more preferably from about 0.5 to 15 mg/kg body weight per day (e.g., about 1 mg/kg, about 2.5 mg/kg, about 3 mg/kg, about 6 mg/kg, about 9 mg/kg, about 11 mg/kg, about 13 mg/kg, or a range defined by any two of the foregoing values). Therapeutic or prophylactic

efficacy can be monitored by periodic assessment of treated patients. For repeated administrations over several days or longer, depending on the condition, the treatment can be repeated until a desired suppression of disease symptoms occurs. However, other dosage regimens may be useful and are within the scope of the invention. The desired dosage can be delivered by a single bolus administration of the composition, by multiple bolus administrations of the composition, or by continuous infusion administration of the composition.

[0084] The composition comprising an effective amount of the inventive immunoglobulin heavy chain polypeptide, the inventive immunoglobulin light chain polypeptide, the inventive LAG-3-binding agent, the inventive nucleic acid sequence encoding any of the foregoing, or the inventive vector comprising the inventive nucleic acid sequence can be administered to a mammal using standard administration techniques, including oral, intravenous, intraperitoneal, subcutaneous, pulmonary, transdermal, intramuscular, intranasal, buccal, sublingual, or suppository administration. The composition preferably is suitable for parenteral administration. The term "parenteral," as used herein, includes intravenous, intramuscular, subcutaneous, rectal, vaginal, and intraperitoneal administration. More preferably, the composition is administered to a mammal using peripheral systemic delivery by intravenous, intraperitoneal, or subcutaneous injection.

[0085] Once administered to a mammal (e.g., a cross-reactive human), the biological activity of the inventive LAG-3-binding agent can be measured by any suitable method known in the art. For example, the biological activity can be assessed by determining the stability of a particular LAG-3-binding agent. In one embodiment of the invention, the LAG-3-binding agent (e.g., an antibody) has an *in vivo* half life between about 30 minutes and 45 days (e.g., about 30 minutes, about 45 minutes, about 1 hour, about 2 hours, about 4 hours, about 6 hours, about 10 hours, about 12 hours, about 1 day, about 5 days, about 10 days, about 15 days, about 25 days, about 35 days, about 40 days, about 45 days, or a range defined by any two of the foregoing values). In another embodiment, the LAG-3-binding agent has an *in vivo* half life between about 2 hours and 20 days (e.g., about 5 hours, about 10 hours, about 15 hours, about 20 hours, about 2 days, about 3 days, about 7 days, about 12 days, about 14 days, about 17 days, about 19 days, or a range defined by any two of the foregoing values). In another embodiment, the LAG-3-binding agent has an *in vivo* half life between about 10 days and about 40 days (e.g., about 10 days, about 13

days, about 16 days, about 18 days, about 20 days, about 23 days, about 26 days, about 29 days, about 30 days, about 33 days, about 37 days, about 38 days, about 39 days, about 40 days, or a range defined by any two of the foregoing values).

[0086] The biological activity of a particular LAG-3-binding agent also can be assessed by determining its binding affinity to LAG-3 or an epitope thereof. The term "affinity" refers to the equilibrium constant for the reversible binding of two agents and is expressed as the dissociation constant (K_D). Affinity of a binding agent to a ligand, such as affinity of an antibody for an epitope, can be, for example, from about 1 picomolar (pM) to about 100 micromolar (μM) (e.g., from about 1 picomolar (pM) to about 1 nanomolar (nM), from about 1 nM to about 1 micromolar (μM), or from about 1 μM to about 100 μM). In one embodiment, the LAG-3-binding agent can bind to an LAG-3protein with a K_D less than or equal to 1 nanomolar (e.g., 0.9 nM, 0.8 nM, 0.7 nM, 0.6 nM, 0.5 nM, 0.4 nM, 0.3 nM, 0.2 nM, 0.1 nM, 0.05 nM, 0.025 nM, 0.01 nM, 0.001 nM, or a range defined by any two of the foregoing values). In another embodiment, the LAG-3-binding agent can bind to LAG-3 with a K_D less than or equal to 200 pM (e.g., 190 pM, 175 pM, 150 pM, 125 pM, 110 pM, 100 pM, 90 pM, 80 pM, 75 pM, 60 pM, 50 pM, 40 pM, 30 pM, 25 pM, 20 pM, 15 pM, 10 pM, 5 pM, 1 pM, or a range defined by any two of the foregoing values). Immunoglobulin affinity for an antigen or epitope of interest can be measured using any art-recognized assay. Such methods include, for example, fluorescence activated cell sorting (FACS), separable beads (e.g., magnetic beads), surface plasmon resonance (SPR), solution phase competition (KINEXA™), antigen panning, and/or ELISA (see, e.g., Janeway et al. (eds.), *Immunobiology*, 5th ed., Garland Publishing, New York, NY, 2001).

[0087] The LAG-3-binding agent of the invention may be administered alone or in combination with other drugs (e.g., as an adjuvant). For example, the LAG-3-binding agent can be administered in combination with other agents for the treatment or prevention of the diseases disclosed herein. In this respect, the LAG-3-binding agent can be used in combination with at least one other anticancer agent including, for example, any chemotherapeutic agent known in the art, ionization radiation, small molecule anticancer agents, cancer vaccines, biological therapies (e.g., other monoclonal antibodies, cancer-killing viruses, gene therapy, and adoptive T-cell transfer), and/or surgery. When the inventive method treats an infectious disease, the LAG-3-binding agent can be administered in combination with at least one anti-bacterial agent

or at least one anti-viral agent. In this respect, the anti-bacterial agent can be any suitable antibiotic known in the art. The anti-viral agent can be any vaccine of any suitable type that specifically targets a particular virus (e.g., live-attenuated vaccines, subunit vaccines, recombinant vector vaccines, and small molecule anti-viral therapies (e.g., viral replication inhibitors and nucleoside analogs).

[0088] In another embodiment, the inventive LAG-3 binding agent can be administered in combination with other agents that inhibit immune checkpoint pathways. For example, the inventive LAG-3 binding agent can be administered in combination with agents that inhibit or antagonize the programmed death 1 (PD-1), T-cell immunoglobulin domain and mucin domain 3 protein (TIM-3), and cytotoxic T-lymphocyte-associated protein 4 (CTLA-4) pathways. Combination treatments that simultaneously target two or more of these immune checkpoint pathways have demonstrated improved and potentially synergistic antitumor activity (see, e.g., Sakuishi et al., *J. Exp. Med.*, *207*: 2187-2194 (2010); Ngiow et al., *Cancer Res.*, *71*: 3540-3551 (2011); and Woo et al., *Cancer Res.*, *72*: 917-927 (2012)). In one embodiment, the inventive LAG-3 binding agent is administered in combination with an antibody that binds to TIM-3 and/or an antibody that binds to PD-1. In this respect, the inventive method of treating a cancer or an infectious disease in a mammal can further comprise administering to the mammal a composition comprising (i) an antibody that binds to a TIM-3 protein and (ii) a pharmaceutically acceptable carrier or a composition comprising (i) an antibody that binds to a PD-1 protein and (ii) a pharmaceutically acceptable carrier.

[0089] In addition to therapeutic uses, the LAG-3-binding agent described herein can be used in diagnostic or research applications. In this respect, the LAG-3-binding agent can be used in a method to diagnose a disorder or disease in which the improper expression (e.g., overexpression) or increased activity of LAG-3 causes or contributes to the pathological effects of the disease or disorder. In a similar manner, the LAG-3-binding agent can be used in an assay to monitor LAG-3 protein levels in a subject being tested for a disease or disorder that is responsive to LAG-3 inhibition. Research applications include, for example, methods that utilize the LAG-3-binding agent and a label to detect an LAG-3 protein in a sample, e.g., in a human body fluid or in a cell or tissue extract. The LAG-3-binding agent can be used with or without modification, such as covalent or non-covalent labeling with a detectable moiety. For example, the detectable

moiety can be a radioisotope (e.g., ^{3}H, ^{14}C, ^{32}P, ^{35}S, or ^{125}I), a fluorescent or chemiluminescent compound (e.g., fluorescein isothiocyanate, rhodamine, or luciferin), an enzyme (e.g., alkaline phosphatase, beta-galactosidase, or horseradish peroxidase), or prosthetic groups. Any method known in the art for separately conjugating an antigen-binding agent (e.g., an antibody) to a detectable moiety may be employed in the context of the invention (see, e.g., Hunter et al., *Nature, 194*: 495-496 (1962); David et al., *Biochemistry, 13*: 1014-1021 (1974); Pain et al., *J. Immunol. Meth., 40*: 219-230 (1981); and Nygren, *J. Histochem. and Cytochem., 30*: 407-412 (1982)).

[0090] LAG-3 protein levels can be measured using the inventive LAG-3-binding agent by any suitable method known in the art. Such methods include, for example, radioimmunoassay (RIA), and FACS. Normal or standard expression values of LAG-3 can be established using any suitable technique, e.g., by combining a sample comprising, or suspected of comprising, LAG-3 with a LAG-3-specific antibody under conditions suitable to form an antigen-antibody complex. The antibody is directly or indirectly labeled with a detectable substance to facilitate detection of the bound or unbound antibody. Suitable detectable substances include various enzymes, prosthetic groups, fluorescent materials, luminescent materials, and radioactive materials (see, e.g., Zola, *Monoclonal Antibodies: A Manual of Techniques*, CRC Press, Inc. (1987)). The amount of LAG-3 polypeptide expressed in a sample is then compared with a standard value.

[0091] The LAG-3-binding agent can be provided in a kit, i.e., a packaged combination of reagents in predetermined amounts with instructions for performing a diagnostic assay. If the LAG-3-binding agent is labeled with an enzyme, the kit desirably includes substrates and cofactors required by the enzyme (e.g., a substrate precursor which provides a detectable chromophore or fluorophore). In addition, other additives may be included in the kit, such as stabilizers, buffers (e.g., a blocking buffer or lysis buffer), and the like. The relative amounts of the various reagents can be varied to provide for concentrations in solution of the reagents which substantially optimize the sensitivity of the assay. The reagents may be provided as dry powders (typically lyophilized), including excipients which on dissolution will provide a reagent solution having the appropriate concentration.

[0092] The following examples further illustrate the invention but, of course, should not be construed as in any way limiting its scope.

EXAMPLE 1

[0093] This example demonstrates a method of generating monoclonal antibodies directed against human LAG-3.

[0094] The gene encoding the extracellular domain (ECD) of human LAG-3 was fused to either mouse IgG2a (human LAG-3 mIgG2a Fc) or a disabled form of wasabi fluorescent protein (dWFP human LAG-3) to produce antigen for use in mouse immunization and hybridoma screening. Specifically, female Swiss Webster (SWR) mice were purchased from Harlan Laboratories, Inc. (Indianapolis, IN) and divided into two groups. After six days of acclimatization, one group of animals was immunized with four to six doses of purified human LAG-3 mIgG2a Fc at 50 μg/mouse at intervals of three to four weeks using complete Freund's adjuvant (CFA) or incomplete Freunds adjuvant (IFA). The second group of animals was injected with four to six doses at intervals of three to four weeks alternating between human LAG-3 mIgG2a Fc or dWFP human LAG-3 ECD. CFA or IFA was also used as adjuvant in the second group. Animals were bled for measurement of the serum titer to human LAG-3 as assessed by binding to cell surface human LAG-3. CHO-S cells were transfected with a full length human LAG-3 extracellular domain fused to the H-2Kk transmembrane domain (CHO-S huLAG-3 ECD cells). Sera were diluted from 1:1,000 – 1:1,000,000 and incubated with the CHO-S huLAG-3 ECD cells for 30 minutes at 4 °C. Cells were centrifuged, washed once with PBS/1% BSA, and incubated with PE-conjugated (Southern Biotech, Birmingham, Alabama) or ALEXAFLUOR™ 647- (Jackson Immunoresearch, West Grove, PA) labeled goat anti-mouse IgG (H+L) for 30 minutes at 4°C. Cells were washed twice in PBS/1%BSA, resuspended in PBS/1%BSA, and analyzed on a BD FACSARRAY™ Bioanalyzer (BD Biosciences, Franklin Lakes, NJ). Based on titer readings, one animal from each group was boosted 3 days prior to spleen collection. Single cell suspensions were prepared from spleen tissue and used for generation of hybridomas by cell fusion using standard techniques. Two different myeloma cell lines were used for fusion, F0 (described in de St. Groth and Scheidegger, J. *Immunol. Methods, 35*: 1-21 (1980)) and P3X63Ag8.653 (described in Kearney et al., *J. Immunol., 123*: 1548-1550 (1979)).

[0095] Hybridoma supernatants were screened for binding to CHO-S huLAG-3 ECD cells and compared to binding to untransfected CHO-S cells as described above. Based upon binding CHO-S huLAG-3 ECD cells, hybridomas were transferred to 48-well plates and expanded.

[0096] Supernatants were then tested for the ability to block binding of human LAG-3 mIgG2a Fc labeled with DyL650 (human LAG-3 mIgG2a Fc DyL650) to Daudi cells, which is a B-cell line that endogenously expresses high levels of MHCII (the LAG-3 receptor). Briefly, human LAG-3 mIgG2a Fc DyL650 was pre-incubated with control IgG or anti-human LAG-3 candidate monoclonal antibodies prior to addition to Daudi cells. Blocking was measured by reduction in fluorescence to Daudi cells using a BD FACSARRAY™ Bioanalyzer. These hybridomas were then subcloned and expanded to plate for generation of exhaust supernatant. Antibodies were subsequently purified and retested to confirm both binding to CHO-S huLAG-3 ECD cells and blocking ability in the Daudi assay.

[0097] The results of this example confirm the production of anti-LAG-3 monoclonal antibodies using hybridoma cell technology.

EXAMPLE 2

[0098] This example describes the design and generation of CDR-grafted and chimeric anti-LAG-3 monoclonal antibodies.

[0099] Antibodies from the hybridomas described in Example 1 were isotyped, subjected to RT-PCR for cloning the antibody heavy chain variable region (V_H) and light chain variable region (V_L), and sequenced. Specifically, RNA was isolated from cell pellets of hybridoma clones (1 x10^6 cells/pellet) using the RNEASY™ kit (Qiagen, Venlo, Netherlands), and cDNA was prepared using oligo-dT-primed SUPERSCRIPT™ III First-Strand Synthesis System (Life Technologies, Carlsbad, CA). PCR amplification of the V_L utilized a pool of degenerate mouse V_L forward primers (see Kontermann and Dubel, eds., *Antibody Engineering*, Springer-Verlag, Berlin (2001)) and a mouse κ constant region reverse primer. PCR amplification of the V_H utilized a pool of degenerate mouse V_H forward primers (Kontermann and Dubel, *supra*) and a mouse γ1 or γ2a constant region reverse primer (based on isotyping of purified antibody from each clone) with the protocol recommended in the SUPERSCRIPT™ III First-Stand Synthesis

System (Life Technologies, Carlsbad, CA). PCR products were purified and cloned into pcDNA3.3-TOPO (Life Technologies, Carlsbad, CA).

[00100] Individual colonies from each cell pellet were selected and sequenced using standard Sanger sequencing methodology (Genewiz, Inc., South Plainfield, NJ). Variable region sequences were examined and aligned with the closest human heavy chain or light chain V-region germline sequence. Three antibodies were selected for CDR-grafting, which were denoted (1) 5.B11, (2) 5.D7, and (3) 1.E10.

[00101] CDR-grafted antibody sequences were designed by cloning CDR residues from each of the above-described mouse antibodies into the closest human germline homolog. CDR-grafted antibody variable regions were synthesized and expressed with human IgG1/κ constant regions for analysis. In addition, mouse:human chimeric antibodies were constructed using the variable regions of the above-described mouse antibodies linked to human IgG1/κ constant regions. Chimeric and CDR-grafted antibodies were characterized for binding to CHO-S huLAG-3 ECD cells and for activity in the human LAG-3 ECD/Daudi blocking assay as described above.

[00102] The functional antagonist activity of chimeric and CDR-grafted antibodies also was tested in a human $CD4^+$ T-cell:dendritic cell mixed lymphocyte reaction (MLR) assay in which activation of $CD4^+$ T-cells in the presence of anti-LAG-3 antibodies is assessed by measuring IL-2 secretion. Because LAG-3 is a negative regulator of T-cell function, antagonism of LAG-3 was expected to result in increased T-cell activation as measured by increased IL-2 production. The 5.B11, 5.D7, and 1.E10 CDR-grafted antibodies demonstrated antagonistic activity in the MLR assay as measured by an increase in IL-2 activity.

[00103] The results of this example demonstrate a method of generating chimeric and CDR-grafted monoclonal antibodies that specifically bind to and inhibit LAG-3.

EXAMPLE 3

[0100] This example demonstrates affinity maturation of humanized monoclonal antibodies directed against human LAG-3.

[0101] CDR-grafted antibodies derived from two of the original murine monoclonal antibodies described in Example 2, 5.D7 and 1.E10, were subjected to affinity maturation via in

silico somatic hypermutation (iSHM). This method incorporates mutations as predicted by computational analysis comparing *in vivo* matured antibody sequences, as downloaded from NCBI, and comparing them to germline human IGHV, IGKV, and IGLV sequences and their allelic forms (as described in Bowers et al., *J. Biol. Chem.*, *288*(11):7688-7696 (2013)). The LAG-3 binding properties of resultant antibodies were assayed using surface plasmon resonance (SPR) as well as ability to bind to CHO-S huLAG-3 ECD cells as described previously. Solution-based affinity analyses were also performed on using a KINEXA™ 3000 assay (Sapidyne Instruments, Boise, Idaho), and results were analyzed using KINEXA™ Pro Software 3.2.6. Experimental parameters were selected to reach a maximum signal with antibody alone between 0.8 and 1.2 V, while limiting nonspecific binding signal with buffer alone to less than 10% of the maximum signal. Azlactone beads (50 mg) were coated with antigen by diluting in a solution of human or cynoWFP-LAG-3 (50 µg/mL in 1 mL) in 50 mM Na_2CO_3. The solution was rotated at room temperature for 2 hours, and beads were pelleted in a picofuge and washed twice with blocking solution (10 mg/mL BSA, 1 M Tris-HCl, pH 8.0). Beads were resuspended in blocking solution (1 mL), rotated at room temperature for 1 hour, and diluted in 25 volumes PBS/0.02% NaN_3. For affinity measurement, the secondary antibody was ALEXFLUOR™ 647 dye-anti-human IgG (500 ng/mL). Sample antibody concentrations were held constant (50 pM or 75 pM), while human or cynomolgus WFP-LAG-3 antigen was titrated using a three-fold dilutions series from 1 µM to 17 pM. All samples were diluted in PBS, 0.2% NaN_3, 1 mg/mL BSA and allowed to equilibrate at room temperature for 30 hours. Additionally, samples containing only antibody and only buffer were tested in order to determine maximum signal and nonspecific binding signal, respectively.

[0102] Thermal stability of the selected antibodies was assessed using a Thermofluor assay as described in McConnell et al., *Protein Eng. Des. Sel.*, *26*: 151 (2013). This assay assesses stability through the ability of a hydrophobic fluorescent dye to bind to hydrophobic patches on the protein surface which are exposed as the protein unfolds. The temperature at which 50% of the protein unfolds (Tm) is determined to measure thermal stability. This assay demonstrated that 5.D7 monoclonal antibody variants had acceptable melting temperatures (T_ms) (i.e., greater than 70°C) that were suitable for drug development.

[0103] De-risking of potential issues related to *in vivo* pharmokinetics of the tested antibodies was undertaken through assessment of non-specific binding to target negative cells (see, e.g., Hotzel et al., *mAbs*, *4*: 753-760 (2012)). Antibodies were tested for binding to HEK 293f cells using a flow cytometry-based assay. The results indicated that non-specific binding was low for 5.D7 and could be further eliminated through second step purification.

[0104] The results of this example confirm a method of affinity maturing humanized monoclonal antibodies directed against LAG-3.

EXAMPLE 4

[0105] This example demonstrates a method of identifying antibodies directed against human LAG-3 from an evolvable library.

[0106] An IgG evolvable library, based on germline sequence V-gene segments joined to human donor-derived recombined (D)J regions, was constructed as described in Bowers et al. *Proc. Natl. Acad. Sci. USA*, *108*(51): 20455-20460 (2011). IgG heavy chain (HC) and light chain (LC) were cloned into separate episomal vectors (Horlick et al., *Gene*, *243*(1-2): 187-194 (2000)), with each vector encoding a distinct antibiotic selectable marker. The HC vector was formatted such that antibody was presented both on the cell surface as well as secreted into the tissue culture medium (Horlick et al., *J. Biol. Chem.*, *288*(27): 19861-19869 (2013)). The diverse sets of HCs and LCs were co-transfected into HEK293 cells and expanded to approximately 10^9 cells. The cell library was then subjected to two rounds each of negative selection against streptavidin (SA)-coupled magnetic beads alone (catalog # 11047, Life Technologies, Carlsbad, CA) and irrelevant biotinylated antigen coated with SA-coupled magnetic beads. One round of positive selection was then performed using either magnetic beads coated directly with human LAG-3 mIgG2a Fc or with SA-coupled magnetic beads coated with biotinylated LAG-3 ECD mIgG1 Fc. The positively selected cells were diluted and plated in 96-well format at an approximate density of 1-10 cells/well. Resulting colonies were expanded into daughter plates and a portion of each population was tested for binding to LAG-3 ECD mIgG1 Fc DyL650 by FACSARRAY™ analysis. Antibodies secreted into the supernatant also were tested by BIACORE™ for ability to bind to LAG-3 ECD mIgG1 Fc.

[0107] Cells that showed specific staining to human LAG-3 mIgG2a Fc DyL650 by FACSARRAY™ analysis and/or binding by BIACORE™ were expanded for sorting and submitted for sequencing to recover the specific HC/LC combinations capable of binding to human LAG-3. The open reading frames (ORFs) encoding the HCs and LCs of the antibodies found in the cell populations were rescued by PCR. Generally, multiple HC/LC sequences were found by sequencing. In some cases the desired HC/LC combinations were identified by enriching cells expressing monoclonal antibodies of interest by first FACS sorting with human LAG-3 mIgG2a Fc DyL650. Populations of cells exhibiting high antibody expression and positive for binding to human LAG-3 mIgG2a Fc DyL650 were isolated and subjected to subsequent sequence analysis. Overall, 12 different HC/LC pairs were identified as potential specific anti-LAG-3 antibody hits suitable for further characterization. These strategies were labeled A1/A14, A2, A3/A17, A4/A19, A5/A16, A6, A8/A20, A9, A10/A15, A11, A12, and A13.

[0108] Antibodies also were characterized for their ability to bind to cynomolgus monkey LAG-3 protein (cyno LAG-3). As these germline antibodies identified from the library were too weak to bind to antigen expressed on the cell surface, soluble antigen similar to the human antigen was labeled with DyL650 (cyno LAG-3 mIgG2a Fc DyL650) and then incubated with HEK293 cells displaying antibody strategies on the cell surface. Eight antibody strategies identified from the evolvable library were tested and demonstrated an ability to bind to cyno LAG-3 ECD mIgG1 Fc.

[0109] The results of this example confirm that monoclonal antibodies directed against human and non-human LAG-3 can be identified using an evolvable library.

EXAMPLE 5

[0110] This example demonstrates affinity maturation of antibodies directed against human LAG-3 identified using an evolvable library.

[0111] Stable cell lines co-expressing the HC and LC of each antibody identified from the evolvable library described in Example 4 were transfected with activation induced cytidine deaminase (AID) to initiate *in vitro* SHM. AID was also transfected directly into the original mixed population of cells expanded from the library screen. In all cases, cell populations were

stained for both IgG expression and binding to antigen, collected by flow cytometry as a bulk population, and then expanded for sequence analysis by next generation sequencing (NGS). This process was repeated iteratively to accumulate SHM-derived mutations in the variable regions of both the heavy and light chains, and their derivatives, for each strategy. Improvements in affinity were monitored by (1) SPR, (2) ability to bind to CHO-S huLAG-3 ECD cells, and (3) activity in the MLR assay. As the affinity of each antibody improved, the stringency of selection was increased until affinity goals were achieved through the identification and recombination of novel mutations.

[0112] Thermal stability of the selected antibodies was assessed using a Thermofluor assay as described above. This assay demonstrated that select monoclonal antibodies from the A17 strategy had acceptable T_ms that were suitable for drug development. Antibodies also were tested for binding to HEK 293f cells using a flow cytometry-based assay. The results indicated that non-specific binding was low for select A17 candidates.

[0113] Selected antibodies were tested for the ability to block binding of human LAG-3 mIgG2a Fc labeled with DyL650 (human LAG-3 mIgG2a Fc DyL650) to Daudi cells, as described above. A dose range of neutralizing antibodies was preincubated with the soluble LAG-3 and analyzed by flow cytometry. Certain affinity-matured anti-LAG-3 antibodies completely inhibited the interaction of soluble LAG-3 with MHCII.

[0114] The results of this example confirm a method of affinity maturing monoclonal antibodies directed against LAG-3 identified using an evolvable library.

EXAMPLE 6

[0115] This example demonstrates that an inventive anti-LAG-3 monoclonal antibody can inhibit LAG-3 signaling and enhance T-cell activation *in vitro* alone and in combination with an anti-PD-1 antibody or an anti-TIM-3 antibody.

[0116] To establish parameters for anti-LAG-3 and anti-PD-1 combination studies, the anti-PD-1 antibody APE02058 was titrated in a dose-response in the human CD4+ T-cell MLR assay described above. Based on the results from titrating the anti-PD-1 antibody in multiple MLR assays, 133pM (approximate EC50) and 13pM (approximate EC10) were selected for testing in combination for antagonist studies with the anti-LAG-3 monoclonal antibody. In combination

with 133pM or 13.3pM of anti PD-1, the EC50 of the anti-LAG-3 monoclonal antibody decreased from 690pM (anti-LAG-3 only) to 40pM (+133pM anti-PD-1) or 200pM (+13.3pM anti-PD-1), which was a 17-fold and 3-fold increase in potency, respectively.

[0117] To establish parameters for anti-LAG-3 and anti-TIM-3 combination studies, the anti-LAG-3 antibody APE05505 was titrated in a dose response in the human CD4+ T-cell MLR assay described above. Based on the results from titrating the anti-LAG-3 antibody in multiple MLR assays, 2nM (approximate EC50) and 0.2nM (approximate EC10) were selected for testing in combination for antagonist studies with the anti-TIM-3 monoclonal antibody. In combination with 2nM or 0.2nM of anti LAG-3, the EC50 of the anti-LAG-3 mAb decreased from 11nM (anti-LAG-3 only) to 6nM (+ 0.2nM anti-TIM-3) or 3nM (+2nM anti-TIM-3), which was a 1.8-fold and 3.6-fold increase in potency, respectively.

[0118] The results of this example demonstrate that the inventive LAG-3 binding agent can inhibit LAG-3 biological activity alone and in combination with antagonists of other negative regulators of the immune system.

EXAMPLE 7

[0119] This example demonstrates that an inventive anti-LAG-3 monoclonal antibody can inhibit LAG-3 signaling and enhance T-cell activation *in vivo* in combination with an anti-PD-1 antibody.

[0120] The activity of an anti-mouse LAG-3 surrogate monoclonal antibody (mAb C9B7W, BioXcell, West Lebanon, New Hampshire) was tested alone or in combination with an anti-mouse PD-1 surrogate monoclonal antibody (mAb RMP1-14, BioXcell, West Lebanon, New Hampshire) in the MC38 syngeneic tumor model. Groups of ten animals were injected subcutaneously with 1 x 10^6 MC38 cells. Ten days after inoculation, animals were randomized for tumor size. Mice were treated with 5mg/kg of anti-PD-1 monoclonal antibody and/or 10mg/kg or anti-LAG-3 monoclonal antibody on days 1, 4, 8, and 11, totaling four doses of each antibody or combination of antibodies. Tumors were measured twice weekly to assess response to treatment. The anti-PD-1 + anti-LAG-3 combination was more efficacious in reducing tumor growth than each single agent alone. Complete response was observed in all ten animals of the group treated with the combination, as compared to seven animals in the PD-1-only group and no

animals in the anti-LAG-3-only group. Nine animals showing a complete response from the combination group were then rechallenged by subcutaneous innoculation with 4 x 10^6 MC38 cells. None of the animals in the rechallenged group developed measurable tumor, while all control naive mice injected with the same amount of cells grew palpable tumor.

[0121] The activities of the surrogate monoclonal antibodies described above also were tested alone or in combination in the Colon26 syngeneic tumor model. Groups of 12 animals were injected subcutaneously with 5 x 10^5 Colon26 cells. Mice were treated with 10 mg/kg of anti-PD-1 antibody and/or 10 mg/kg of anti-LAG-3 antibody on days 4, 7, 11, and 14, totaling four doses of each antibody or combination of antibodies. Tumors were measured twice weekly to assess response to treatment. The anti-PD-1 + anti-LAG-3 combination was more efficacious for tumor growth than each single agent alone. Complete response was observed in 10 out of 12 animals in the combination group, as compared to three animals in the PD-1-only group and one animal in the anti-LAG-3-only group. Nine animals showing complete response from the combination group were then rechallenged with 5x10^5 Colon26 cells. None of the animals in the rechallenged group developed measurable tumor, while all the control naive mice injected with the same amount of cells grew palpable tumor.

[0122] The results of this example demonstrate that the inventive LAG-3 binding agent, in combination with antagonists of other negative regulators of the immune system, can inhibit LAG-3 biological activity *in vivo*.

EXAMPLE 8

[0123] This example demonstrates the effect of antibody isotype on anti-tumor activity of an anti-LAG-3 antibody alone or in combination with an anti-PD-1 antibody in a syngeneic mouse tumor model.

[0124] Surrogate antibodies recognizing mouse LAG-3 of IgG1 (D265A) and IgG2a isotypes were created after sequencing and cloning the variable regions of an anti-mouse LAG-3 neutralizing antibody (mAb C9B7W, BioXcell, West Lebanon, NH) from a rat hybridoma cell line and cloning into a mouse IgG1 or mouse IgG2a expression vector. These antibodies were then tested for efficacy both alone and in combination with a mouse IgG1 (D265A) surrogate antibody recognizing mouse PD-1, similarly created from a purchased rat antibody from BioXcel

(mAb RMP1-14, West Lebanon, NH). Specifically, Colon26 colon adenocarcinoma cells (5 x10^5 s.c.) were implanted into Balb/c mice and grown for 3 days. Mice were randomized into seven groups of 12 animals/group and dosed with each antibody or antibody combination on days 4, 7, 11, and 14 as set forth in Table 1. Mice injected with matched isotype antibodies served as a control. Tumor volumes were measured twice weekly until the end of the study.

[0125] Table 1

Group	Treatment	Dose
1	Isotype IgG2a + Isotype IgG1(D265A)	10 mg/kg, 1 mg/kg
2	Isotype IgG1 (D265A)	10 mg/kg
3	Anti-mPD-1 IgG1(D265A)	1 mg/kg
4	Anti-mLAG-3 IgG2a	10 mg/kg
5	Anti-mLAG-3 IgG1(D265A)	10 mg/kg
6	Anti-mPD-1 IgG1(D265A) + Anti-mLAG-3 IgG2a	1 mg/kg, 10 mg/kg
7	Anti-mPD-1 IgG1(D265A)+ Anti-mLAG-3 IgG1(D265A)	1 mg/kg, 10 mg/kg

[0126] Results for this experiment are shown in Figures 1A and 1B, which show that a single-agent anti-mouse LAG-3 antibody with minimal effector function (i.e., IgG1 (D265A)) has anti-tumor efficacy as compared with an anti-mouse LAG-3 antibody with effector function (i.e., IgG2a), which has no apparent effect on tumor growth.

[0127] In addition, Figure 1A shows an anti-mouse LAG-3 antibody with minimal effector function (i.e., IgG1(D265A)) in combination with a regimen of an anti-mouse PD-1 IgG1(D265A) antibody exhibited increased anti-tumor activity compared with the anti-mouse PD-1 IgG1(D265A) antibody alone. However, an anti-mouse LAG-3 antibody with in-tact effector function (IgG2a) in combination with an anti-mouse PD-1 antibody was less efficacious than anti-mouse PD-1 IgG1 (D265A) alone, suggesting that the effector function of the antibody possibly interfered with anti-mouse PD-1 mediated efficacy.

[0128] Figure 1B provides graphs of tumor volume over time for individual animals from treatment group 3 (anti-mouse PD-1 IgG1(D265A) antibody treated animals), group 7 (combination of anti-mouse PD-1 IgG1(D265A) antibody with anti-mouse LAG-3 IgG1(D265A) antibody), and group 6 (combination of anti-mouse PD-1 IgG1(D265A) antibody with anti-mouse LAG-3 IgG2 antibody). In group 7 (anti-mouse PD-1 IgG1(D265A) antibody with anti-

mouse LAG-3 IgG1(D265A)), 8/12 animals had no visible tumor growth by the end of the study. By contrast, only 3/12 animals in group 6 (anti-mouse PD-1 IgG1(D265A) antibody with anti-mouse LAG-3 IgG2 antibody) had no visible tumor by the end of the study. In group 3 (anti-mouse PD-1 IgG1 (D265A) alone), 6/12 animals were tumor free by the end of study, suggesting possible interference by the effector function of the anti-mouse LAG-3 IgG2 antibody when dosed in combination with the anti-mouse PD-1 IgG1 (D265A) antibody.

[0129] The results of this example demonstrate that anti-mouse LAG-3 and anti-mouse PD-1 antibodies without effector function, alone and in combination, can inhibit tumor growth in a mouse syngeneic tumor model. Efficacy was not observed using an anti-mouse LAG-3 antibody with effector function and furthermore may interfere with anti-PD-1 mediated efficacy.

EXAMPLE 9

[0130] This example demonstrates that an inventive anti-LAG-3 monoclonal antibody inhibitory activity can be differentiated from that of an anti-PD-1 monoclonal antibody in a mixed lymphocyte reaction based upon time of harvest and correlates with PD-1 and LAG-3 expression.

[0131] A functional LAG-3 antagonist antibody was tested in a human CD4+ T-cell mixed lymphocyte reaction (MLR) assay in which activation of CD4+ T-cells in the presence of anti-LAG-3 antibodies is assessed by measuring IL-2 secretion. The anti-LAG-3 antibody was tested side by side with an antagonistic anti-PD-1 antibody, wherein the antibodies were added and/or harvested at different timepoints. Specifically, isolated peripheral blood monocytes from a human donor were differentiated into dendritic cells (DCs) and then mixed with CD4+ T-cells isolated from a second donor. Inhibitory antibodies were added either at the start of the co-culture or 24 hours after the start of the co-culture. IL-2 levels were measured at 24 and 48 hours after antibody addition.

[0132] Antagonism of LAG-3 and PD-1 was expected to result in increased T-cell activation as measured by increased IL-2 production. When added at the start of the assay, the anti-PD-1 antibody increased IL-2 secretion at both 24 and 48 hours post antibody addition, while the anti-LAG-3 antibody increased IL-2 secretion when measured at 48 hours in the MLR assay, but not at 24 hours. When inhibitory anti-LAG-3 or anti-PD-1 antibodies were added at 24 hours after

starting the co-culture and harvested at 72 hours, both antibodies were active and the EC50 appeared to be equivalent (Figure 2A). This correlates with expression as increased PD-1 expression is observed at 24 – 72 hours, while LAG-3 appears to be expressed later in the assay at 48 and 72 hours (Figure 2B).

[0133] The results of this example demonstrate that the effects of LAG-3 inhibition correlates with target expression, and that LAG-3 expression occurs temporally later than PD-1.

[0134] All references, including publications, patent applications, and patents, cited herein are hereby incorporated by reference to the same extent as if each reference were individually and specifically indicated to be incorporated by reference and were set forth in its entirety herein.

[0135] The use of the terms "a" and "an" and "the" and "at least one" and similar referents in the context of describing the invention (especially in the context of the following claims) are to be construed to cover both the singular and the plural, unless otherwise indicated herein or clearly contradicted by context. The use of the term "at least one" followed by a list of one or more items (for example, "at least one of A and B") is to be construed to mean one item selected from the listed items (A or B) or any combination of two or more of the listed items (A and B), unless otherwise indicated herein or clearly contradicted by context. The terms "comprising," "having," "including," and "containing" are to be construed as open-ended terms (i.e., meaning "including, but not limited to,") unless otherwise noted. Recitation of ranges of values herein are merely intended to serve as a shorthand method of referring individually to each separate value falling within the range, unless otherwise indicated herein, and each separate value is incorporated into the specification as if it were individually recited herein. All methods described herein can be performed in any suitable order unless otherwise indicated herein or otherwise clearly contradicted by context. The use of any and all examples, or exemplary language (e.g., "such as") provided herein, is intended merely to better illuminate the invention and does not pose a limitation on the scope of the invention unless otherwise claimed. No language in the specification should be construed as indicating any non-claimed element as essential to the practice of the invention.

[0136] Preferred embodiments of this invention are described herein, including the best mode known to the inventors for carrying out the invention. Variations of those preferred embodiments may become apparent to those of ordinary skill in the art upon reading the

foregoing description. The inventors expect skilled artisans to employ such variations as appropriate, and the inventors intend for the invention to be practiced otherwise than as specifically described herein. Accordingly, this invention includes all modifications and equivalents of the subject matter recited in the claims appended hereto as permitted by applicable law. Moreover, any combination of the above-described elements in all possible variations thereof is encompassed by the invention unless otherwise indicated herein or otherwise clearly contradicted by context.

CLAIM(S):

1. An isolated immunoglobulin heavy chain polypeptide which comprises the amino acid sequence Glu Val Gln Leu Val Gln Ser Gly Ala Glu Val Lys Lys Pro Gly Ala Thr Val Lys Ile Ser Cys Lys Ala Ser Gly Phe Xaa1 Ile Xaa2 Asp Asp Tyr Ile His Trp Val Xaa3 Gln Ala Pro Gly Lys Gly Leu Glu Trp Xaa4 Gly Trp Ile Asp Xaa5 Xaa6 Asn Xaa7 Asp Ser Xaa8 Tyr Xaa9 Ser Lys Phe Xaa10 Gly Arg Val Thr Ile Thr Val Asp Thr Ser Thr Xaa11 Thr Ala Tyr Met Xaa12 Leu Ser Ser Leu Arg Ser Glu Asp Thr Ala Val Tyr Tyr Cys Thr Tyr Ala Phe Gly Gly Tyr Trp Gly Gln Gly Thr Thr Val Thr Val Ser Ser (SEQ ID NO: 181), wherein

(a) Xaa1 is asparagine (Asn) or serine (Ser),

(b) Xaa2 is lysine (Lys), tyrosine (Tyr), or asparagine (Asn),

(c) Xaa3 is lysine (Lys) or glutamine (Gln),

(d) Xaa4 is isoleucine (Ile) or methionine (Met),

(e) Xaa5 is alanine (Ala) or proline (Pro),

(f) Xaa6 is glutamic acid (Glu) or methionine (Met),

(g) Xaa7 is glycine (Gly), asparagine (Asn), or aspartic acid (Asp),

(h) Xaa8 is glutamic acid (Glu) or glutamine (Gln)

(i) Xaa9 is alanine (Ala) or serine (Ser),

(j) Xaa10 is glutamine (Gln) or arginine (Arg),

(k) Xaa11 is aspartic acid (Asp) or asparagine (Asn), and

(l) Xaa12 is glutamic acid (Glu) or lysine (Lys).

2. The isolated immunoglobulin heavy chain polypeptide of claim 1, which comprises the amino acid sequence Glu Val Gln Leu Val Gln Ser Gly Ala Glu Val Lys Lys Pro Gly Ala Thr Val Lys Ile Ser Cys Lys Ala Ser Gly Phe Xaa1 Ile Xaa2 Asp Asp Tyr Ile His Trp Val Xaa3 Gln Ala Pro Gly Lys Gly Leu Glu Trp Xaa4 Gly Trp Ile Asp Xaa5 Glu Asn Xaa6 Asp Ser Glu Tyr Xaa7 Ser Lys Phe Xaa8 Gly Arg Val Thr Ile Thr Val Asp Thr Ser Thr Xaa9 Thr Ala Tyr Met Glu Leu Ser Ser Leu Arg Ser Glu Asp Thr Ala Val Tyr Tyr Cys Thr Tyr Ala Phe Gly Gly Tyr Trp Gly Gln Gly Thr Thr Val Thr Val Ser Ser (SEQ ID NO: 1), wherein

(a) Xaa1 is asparagine (Asn) or serine (Ser),

(b) Xaa2 is lysine (Lys), tyrosine (Tyr), or asparagine (Asn),

(c) Xaa3 is lysine (Lys) or glutamine (Gln),

(d) Xaa4 is isoleucine (Ile) or methionine (Met),

(e) Xaa5 is alanine (Ala) or proline (Pro),

(f) Xaa6 is glycine (Gly), asparagine (Asn), or aspartic acid (Asp),

(g) Xaa7 is alanine (Ala) or serine (Ser),

(h) Xaa8 is glutamine (Gln) or arginine (Arg), and

(i) Xaa9 is aspartic acid (Asp) or asparagine (Asn).

3. The isolated immunoglobulin heavy chain polypeptide of claim 1 or 2, which comprises the amino acid sequence of any one of SEQ ID NO: 2 – SEQ ID NO: 34 or SEQ ID NO: 182-186.

4. An isolated immunoglobulin heavy chain polypeptide which comprises the amino acid sequence Gln Val Gln Leu Gln Gln Trp Gly Ala Xaa1 Leu Leu Lys Pro Ser Glu Thr Leu Ser Leu Xaa2 Cys Xaa3 Val Tyr Gly Gly Xaa4 Phe Xaa5 Gly Tyr Tyr Trp Xaa6 Trp Ile Arg Gln Pro Pro Xaa7 Lys Gly Leu Glu Trp Ile Gly Glu Ile Asn His Ser Gly Xaa8 Thr Asn Tyr Asn Pro Ser Leu Lys Ser Arg Val Thr Ile Ser Val Asp Thr Ser Lys Asn Gln Xaa9 Ser Leu Lys Leu Xaa10 Xaa11 Val Thr Ala Ala Asp Thr Ala Val Tyr Tyr Cys Xaa12 Arg Glu Gly Xaa13 Tyr Gly Asp Tyr Asp Tyr Trp Gly Gln Gly Thr Leu Val Thr Val Ser Ser (SEQ ID NO: 35), wherein

(a) Xaa1 is arginine (Arg) or glycine (Gly),

(b) Xaa2 is threonine (Thr) or isoleucine (Ile),

(c) Xaa3 is threonine (Thr) or alanine (Ala),

(d) Xaa4 is serine (Ser) or phenylalanine (Phe),

(e) Xaa5 is serine (Ser) or phenylalanine (Phe),

(f) Xaa6 is serine (Ser) or isoleucine (Ile),

(g) Xaa7 is glycine (Gly) or arginine (Arg),

(h) Xaa8 is serine (Ser) or asparagine (Asn),

(i) Xaa9 is phenylalanine (Phe) or leucine (Leu),

(j) Xaa10 is asparagine (Asn) or serine (Ser),

(k) Xaa11 is serine (Ser) or phenylalanine (Phe),

(l) Xaa12 is alanine (Ala) or valine (Val), and

(m) Xaa13 is aspartic acid (Asp) or asparagine (Asn).

5. The isolated immunoglobulin heavy chain polypeptide of claim 4, which comprises the amino acid sequence of any one of SEQ ID NO: 36 – SEQ ID NO: 56.

6. An isolated immunoglobulin light chain polypeptide which comprises the amino acid sequence Asp Xaa1 Val Met Thr Gln Thr Pro Leu Ser Leu Ser Val Thr Pro Gly Gln Pro Ala Ser Ile Ser Cys Arg Xaa2 Ser Gln Ser Leu Val His Ser Asp Xaa3 Xaa4 Thr Tyr Leu His Trp Tyr Leu Gln Lys Pro Gly Gln Ser Pro Gln Leu Leu Ile Tyr Xaa Xaa Ser Asn Arg Phe Ser Gly Val Pro Asp Arg Phe Ser Gly Ser Gly Ser Gly Thr Asp Phe Thr Leu Lys Ile Ser Arg Val Glu Ala Glu Asp Val Gly Val Tyr Phe Cys Xaa Gln Ser Thr Xaa Val Pro Tyr Ala Phe Gly Gly Gly Thr Lys Val Glu Ile Lys Arg Thr (SEQ ID NO: 57), wherein

(a) Xaa1 is valine (Val) or isoleucine (Ile),

(b) Xaa2 is cysteine (Cys) or serine (Ser),

(c) Xaa3 is glycine (Gly) or serine (Ser),

(d) Xaa4 is asparagine (Asn) or aspartic acid (Asp),

(e) Xaa5 is lysine (Lys), glycine (Gly), asparagine (Asn), serine (Ser), or leucine (Leu),

(f) Xaa6 is valine (Val) or isoleucine (Ile),

(g) Xaa7 is serine (Ser), alanine (Ala), or glycine (Gly), and

(h) Xaa8 is histidine (His) or tyrosine (Tyr).

7. The isolated immunoglobulin light chain polypeptide of claim 6, which comprises the amino acid sequence of any one of SEQ ID NO: 58 – SEQ ID NO: 88 or 187-189.

8. An isolated immunoglobulin light chain polypeptide which comprises the amino acid sequence Asp Ile Gln Met Thr Gln Ser Pro Ser Ser Leu Ser Ala Ser Val Gly Asp Arg Val Thr Ile Thr Cys Gln Ala Ser Gln Asp Ile Ser Asn Tyr Leu Asn Trp Tyr Gln Gln Lys Pro Gly Lys Ala Pro Lys Leu Leu Ile Xaa1 Xaa2 Xaa3 Xaa4 Xaa5 Leu Glu Thr Gly Val Pro Ser Arg Phe Ser Gly Ser Gly Ser Gly Thr Asp Phe Thr Phe Thr Ile Ser Ser Leu Gln Pro Glu Asp Ile Ala Val Tyr Tyr Cys Gln Gln Ser Tyr Ser Xaa6 Leu Ile Thr Phe Gly Gln Gly Thr Arg Leu Glu Ile Lys Arg Thr Val Ala Ala Pro Ser Val (SEQ ID NO: 89), wherein

(a) the subsequence Xaa1 Xaa2 Xaa3 Xaa4 Xaa5 is deleted or is Tyr-Asp-Ala-Ser-Asn, and

(b) Xaa6 is threonine (Thr) or isoleucine (Ile).

9. The isolated immunoglobulin light chain polypeptide of claim 8, wherein the subsequence Xaa1 Xaa2 Xaa3 Xaa4 Xaa5 is Tyr-Asp-Ala-Ser-Asn.

10. The isolated immunoglobulin light chain polypeptide of claim 9, which comprises the amino acid sequence of SEQ ID NO: 90.

11. The isolated immunoglobulin light chain polypeptide of claim 8, wherein the subsequence Xaa1 Xaa2 Xaa3 Xaa4 Xaa5 is deleted.

12. The isolated immunoglobulin light chain polypeptide of claim 11, which comprises the amino acid sequence of SEQ ID NO: 91 or SEQ ID NO: 92.

13. An isolated immunoglobulin heavy chain polypeptide which comprises SEQ ID NO: 190 or 191.

14. The isolated immunoglobulin heavy chain polypeptide of claim 13 comprising any one of SEQ ID NOs: 192-195.

15. An isolated immunoglobulin light chain polypeptide which comprises SEQ ID NO: 196 or 197.

16. The isolated immunoglobulin light chain polypeptide of claim 15 comprising any one of SEQ ID NOs: 198-200.

17. An isolated nucleic acid sequence encoding the immunoglobulin heavy chain polypeptide of any one of claims 1-5, 13, or 14.

18. An isolated nucleic acid sequence encoding the immunoglobulin light chain polypeptide of any one of claims 6-12, 15, or 16.

19. A vector comprising the isolated nucleic acid sequence of claim 17 or claim 18.

20. A Lymphocyte Activation Gene-3 (LAG-3)-binding agent comprising the immunoglobulin heavy chain polypeptide of any one of claims 1-5, 13, or 14 and/or the immunoglobulin light chain polypeptide of any one of claims 6-12, 15, or 16.

21. The LAG-3 binding agent of claim 20, which comprises the immunoglobulin heavy chain polypeptide of any one of claims 1-5, 13, or 14 and the immunoglobulin light chain polypeptide of any one of claims 6-12, 15, or 16.

22. The LAG-3 binding agent of claim 20, which comprises the immunoglobulin heavy chain polypeptide of any one of claims 1-5, 13, or 14 or the immunoglobulin light chain polypeptide of any one of claims 6-12, 15, or 16.

23. The LAG-3 binding agent of claim 20, which comprises (a) an immunoglobulin heavy chain polypeptide of any of claims 1-3, and an immunoglobulin light chain polypeptide of claim 6 or 7; (b) an immunoglobulin heavy chain polypeptide of claim 4 or 5, and an immunoglobulin light chain polypeptide of any of claims 8-12; or (c) an immunoglobulin heavy chain polypeptide of claim 13 or 14, and an immunoglobulin light chain polypeptide of claim 15 or 16.

24. The LAG-3-binding agent of any one of claims 20-23, which is an antibody, an antibody conjugate, or an antigen-binding fragment thereof.

25. The LAG-3-binding agent of claim 24, which is a $F(ab')_2$ fragment, a Fab' fragment, a Fab fragment, a Fv fragment, a scFv fragment, a dsFv fragment, a dAb fragment, or a single chain binding polypeptide.

26. The LAG-3 binding agent of any one of claims 20-25, which binds to the extracellular domain 1 (D1) and/or the extracellular domain 2 (D2) of the LAG-3 protein.

27. The LAG-3 binding agent of any one of claims 20-26, wherein the LAG-3 binding agent comprises an Fc region with reduced or abrogated effector function.

28. An isolated nucleic acid sequence encoding the LAG-3-binding agent of any one of claims 20-27.

29. A vector comprising the isolated nucleic acid sequence of claim 28.

30. An isolated cell comprising the vector of claim 29.

31. A composition comprising (a) the LAG-3-binding agent of any one of claims 20-27 or the vector of claim 29 and (b) a pharmaceutically acceptable carrier.

32. A method of treating a disorder in a mammal that is responsive to LAG-3 inhibition, which method comprises administering an effective amount of the composition of claim 31 to a mammal having a disorder that is responsive to LAG-3 inhibition, whereupon the disorder is treated in the mammal.

33. The method of claim 32, wherein the disorder is cancer.

34. The method of claim 33, wherein the cancer is melanoma, renal cell carcinoma, lung cancer, bladder cancer, breast cancer, cervical cancer, colon cancer, gall bladder cancer, laryngeal cancer, liver cancer, thyroid cancer, stomach cancer, salivary gland cancer, prostate cancer, pancreatic cancer, or Merkel cell carcinoma.

35. The method of claim 32, wherein the disorder is an infectious disease.

36. The method of claim 35, wherein the infectious disease is caused by a virus or a bacterium.

37. The method of claim 36, wherein the virus is human immunodeficiency virus (HIV), respiratory syncytial virus (RSV), influenza virus, dengue virus, or hepatitis B virus (HBV).

38. The method of any one of claims 32-37, wherein the half-life of the LAG-3-binding agent in the mammal is between 30 minutes and 45 days.

39. The method of any one of claims 32-38, wherein the LAG-3-binding agent binds to LAG-3 with a K_D between about 1 picomolar (pM) and about 100 micromolar (μM).

40. The method of any one of claims 32-39, further comprising administering a PD-1 binding agent and/or a TIM-3 binding agent to the mammal.

41. The method of claim 40, wherein the PD-1 binding agent and/or TIM-3 binding agent is an antibody, an antibody conjugate, or an antigen-binding fragment thereof.

Fig. 1A

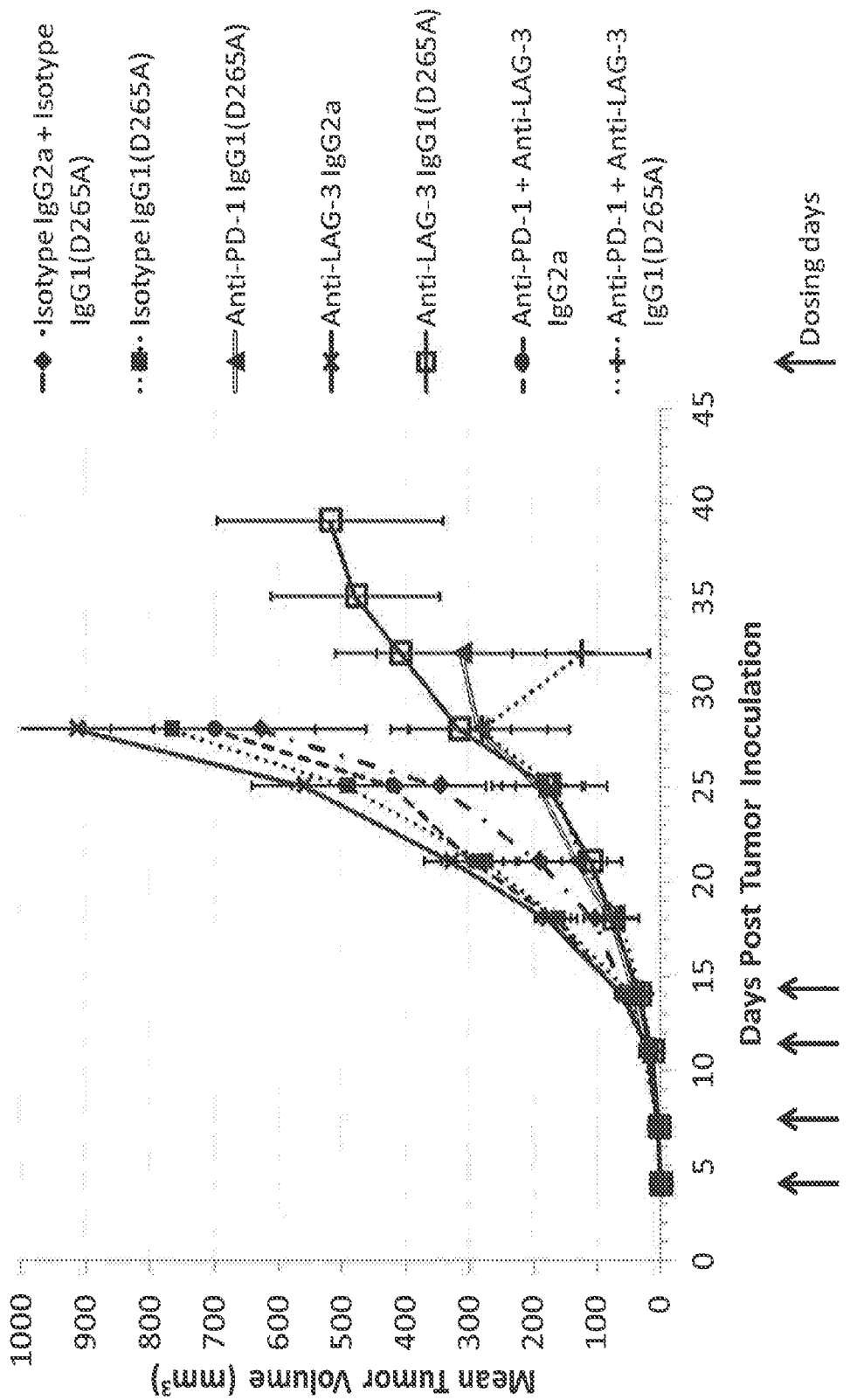

Isotype IgG2a + Isotype IgG1(D265A)
Isotype IgG1(D265A)
Anti-PD-1 IgG1(D265A)
Anti-LAG-3 IgG2a
Anti-LAG-3 IgG1(D265A)
Anti-PD-1 + Anti-LAG-3 IgG2a
Anti-PD-1 + Anti-LAG-3 IgG1(D265A)
Dosing days
Mean Tumor Volume (mm³)
1000
900
800
700
600
500
400
300
200
100
0
0
5
10
15
20
25
30
35
40
45
Days Post Tumor Inoculation

Fig. 1B

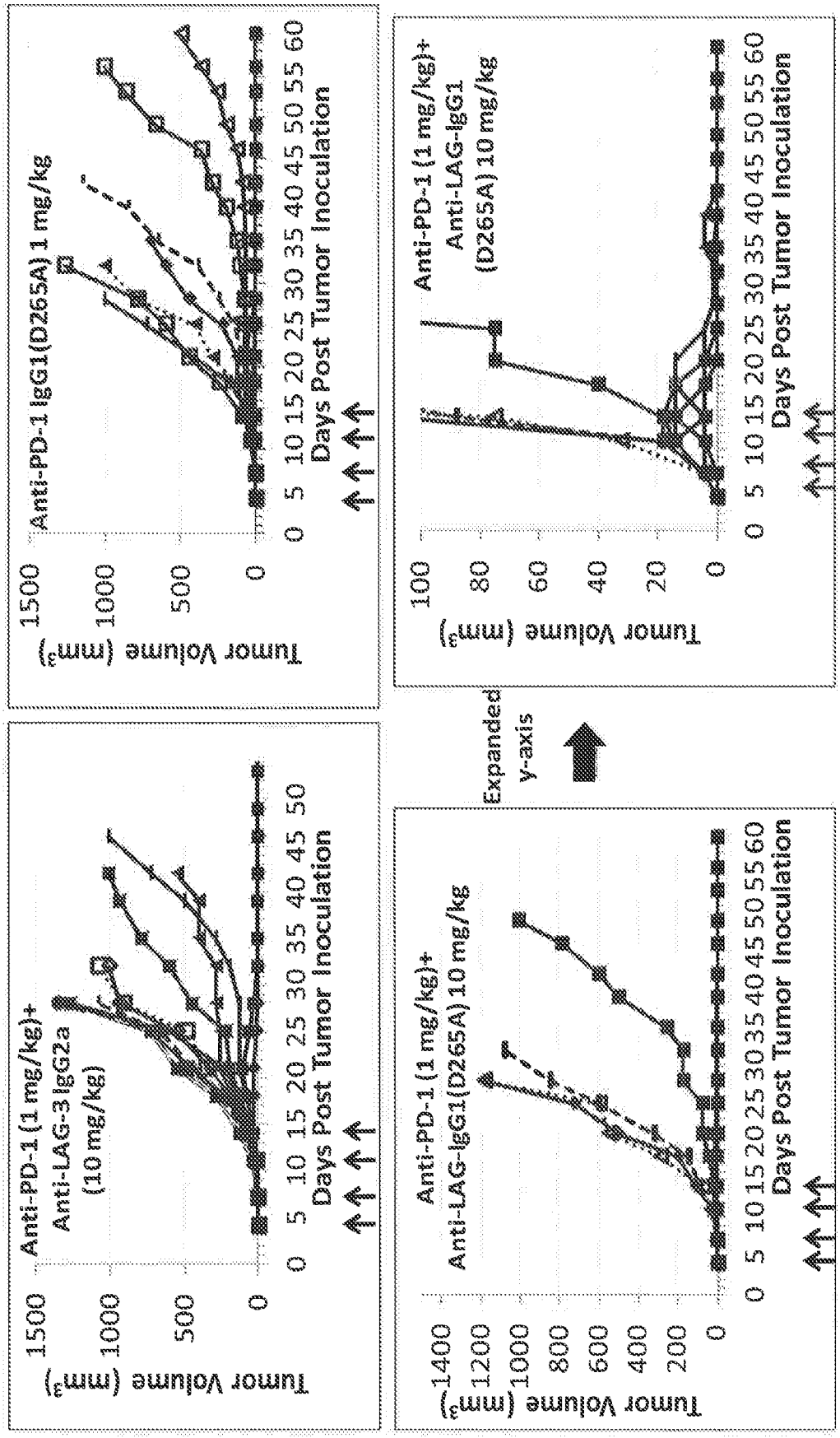
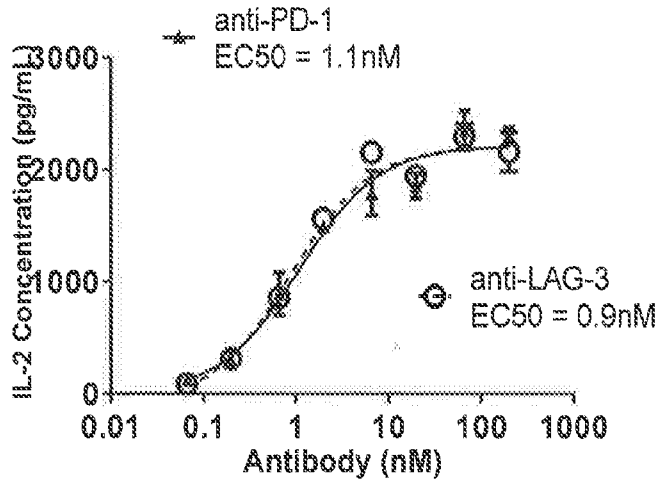
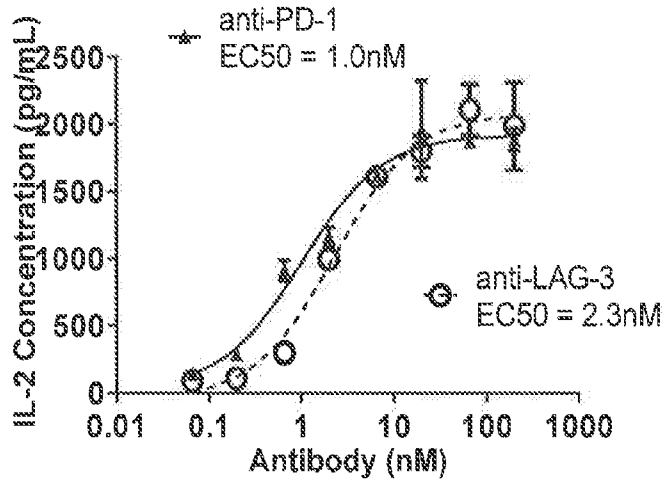

Anti-PD-1 IgG1(D265A) 1 mg/kg
Tumor Volume (mm³)
1500
1000
500
0
0 5 10 15 20 25 30 35 40 45 50 55 60
Days Post Tumor Inoculation
Anti-PD-1 (1 mg/kg)+
Anti-LAG-3 IgG2a
(10 mg/kg)
0 5 10 15 20 25 30 35 40 45 50
Anti-PD-1 (1 mg/kg)+
Anti-LAG-IgG1(D265A) 10 mg/kg
1400
1200
1000
800
600
400
200
Expanded
y-axis
Anti-PD-1 (1 mg/kg)+
Anti-LAG-IgG1
(D265A) 10 mg/kg
100
80
60
40
20

Fig. 2A

Addition @ t=24 hour
Harvest @ 72 hour



Addition @ t=0
Harvest @ 48 hour



Addition @ t=0
Harvest @ 24 hour

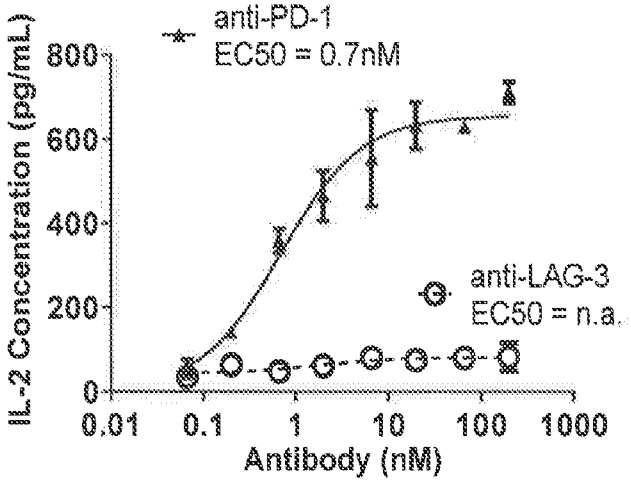
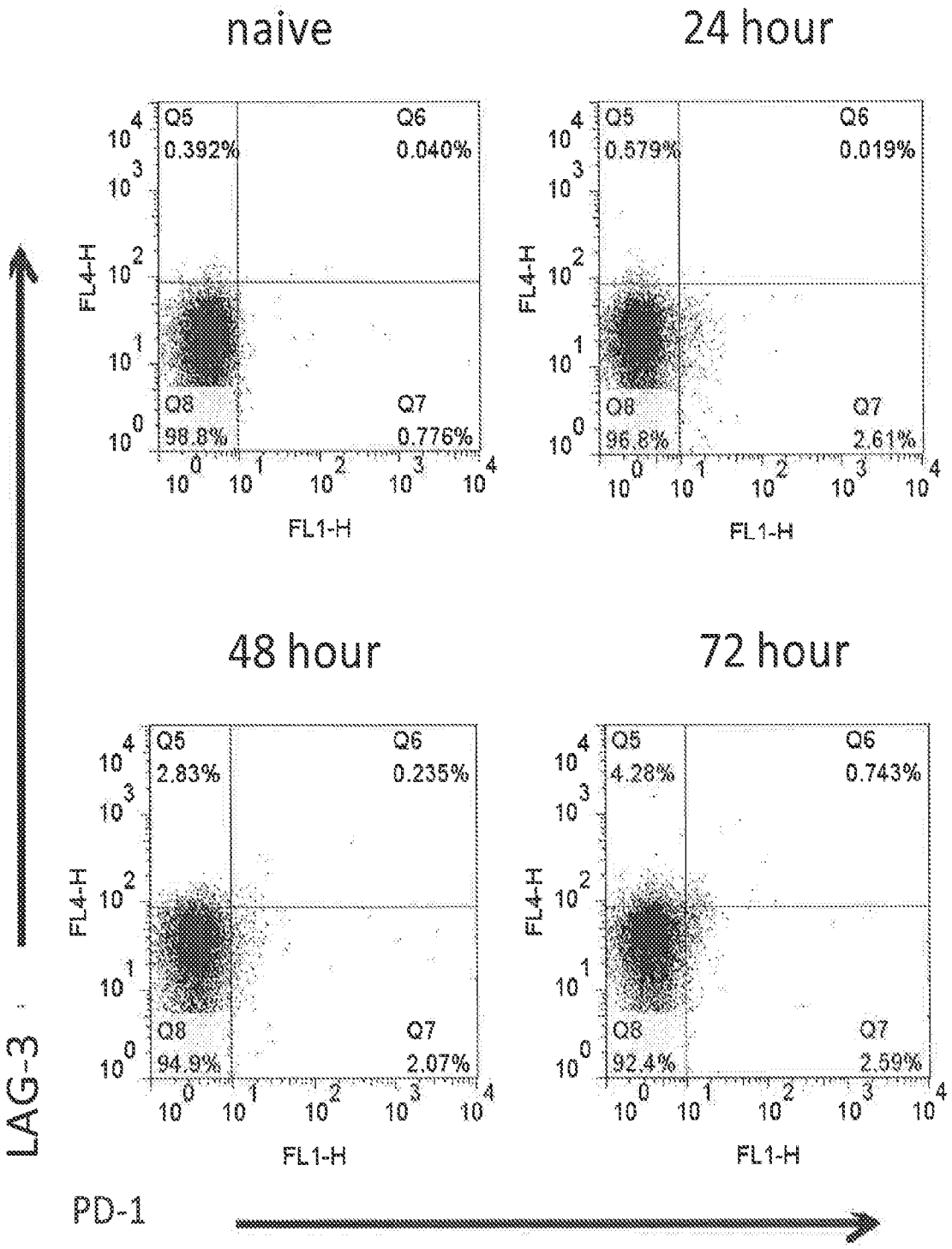


Fig. 2B


naive
Q5
0.392%
Q6
0.040%
Q8
98.8%
Q7
0.776%
24 hour
Q5
0.579%
Q6
0.019%
Q8
96.8%
Q7
2.61%
48 hour
Q5
2.83%
Q6
0.235%
Q8
94.9%
Q7
2.07%
72 hour
Q5
4.28%
Q6
0.743%
Q8
92.4%
Q7
2.59%
FL4-H
FL1-H
LAG-3
PD-1

(19)中华人民共和国国家知识产权局

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(54)发明名称

针对淋巴细胞活化基因3(LAG-3)的抗体

(57)摘要

本发明涉及分离的免疫球蛋白重链多肽和分离的免疫球蛋白轻链多肽,所述分离的免疫球蛋白重链多肽和分离的免疫球蛋白轻链多肽结合由淋巴细胞活化基因-3(LAG-3)编码的蛋白。本发明提供了LAG-3结合剂,所述LAG-3结合剂包括上述免疫球蛋白重链多肽和免疫球蛋白轻链多肽。本发明还提供了相关载体、组合物和使用所述LAG-3结合剂治疗对LAG-3的抑制有应答的紊乱或疾病(例如癌症或感染性疾病)的方法。

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1.一种分离的免疫球蛋白重链多肽,其包括以下氨基酸序列:Glu Val Gln Leu Val Gln Ser Gly Ala Glu Val Lys Lys Pro Gly Ala Thr Val Lys Ile Ser Cys Lys Ala Ser Gly Phe Xaa1 Ile Xaa2 Asp Asp Tyr Ile His Trp Val Xaa3 Gln Ala Pro Gly Lys Gly Leu Glu Trp Xaa4 Gly Trp Ile Asp Xaa5 Xaa6 Asn Xaa7 Asp Ser Xaa8Tyr Xaa9 Ser Lys Phe Xaa10 Gly Arg ValThr Ile Thr Val Asp Thr Ser Thr Xaa11 Thr Ala Tyr Met Xaa12 Leu Ser Ser Leu Arg Ser Glu Asp Thr Ala Val Tyr Tyr Cys Thr Tyr Ala Phe Gly Gly Tyr Trp Gly Gln Gly Thr Thr Val Thr Val Ser Ser(SEQ ID NO:181),其中

(a)Xaa1是天冬酰胺(Asn)或丝氨酸(Ser),

(b)Xaa2是赖氨酸(Lys)、酪氨酸(Tyr)、或天冬酰胺(Asn),

(c)Xaa3是赖氨酸(Lys)或谷氨酰胺(Gln),

(d)Xaa4是异亮氨酸(Ile)或甲硫氨酸(Met),

(e)Xaa5是丙氨酸(Ala)或脯氨酸(Pro),

(f)Xaa6是谷氨酸(Glu)或甲硫氨酸(Met),

(g)Xaa7是甘氨酸(Gly)、天冬酰胺(Asn)、或天冬氨酸(Asp)

(h)Xaa8是谷氨酸(Glu)或谷氨酰胺(Gln)

(i)Xaa9是丙氨酸(Ala)或丝氨酸(Ser),

(j)Xaa10是谷氨酰胺(Gln)或精氨酸(Arg),

(k)Xaa11是天冬氨酸(Asp)或天冬酰胺(Asn),以及

(l)Xaa12是谷氨酸(Glu)或赖氨酸(Lys)。

2.根据权利要求1所述的分离的免疫球蛋白重链多肽,其包括以下氨基酸序列:Glu Val Gln Leu Val Gln Ser Gly Ala Glu Val Lys Lys Pro Gly Ala Thr Val Lys Ile Ser Cys Lys Ala Ser Gly Phe Xaa1 Ile Xaa2 Asp Asp Tyr Ile His Trp Val Xaa3 Gln Ala Pro Gly Lys Gly Leu Glu Trp Xaa4 Gly Trp Ile Asp Xaa5 Glu Asn Xaa6 Asp Ser Glu Tyr Xaa7 Ser Lys Phe Xaa8 Gly Arg Val Thr Ile Thr Val Asp Thr Ser Thr Xaa9 Thr Ala Tyr Met Glu Leu Ser Ser Leu Arg Ser Glu Asp Thr Ala Val Tyr Tyr Cys Thr Tyr Ala Phe Gly Gly Tyr Trp Gly Gln Gly Thr Thr Val Thr Val Ser Ser(SEQ ID NO:1),其中

(a)Xaa1是天冬酰胺(Asn)或丝氨酸(Ser),

(b)Xaa2是赖氨酸(Lys)、酪氨酸(Tyr)、或天冬酰胺(Asn),

(c)Xaa3是赖氨酸(Lys)或谷氨酰胺(Gln),

(d)Xaa4是异亮氨酸(Ile)或甲硫氨酸(Met),

(e)Xaa5是丙氨酸(Ala)或脯氨酸(Pro),

(f)Xaa6是甘氨酸(Gly)、天冬酰胺(Asn)、或天冬氨酸(Asp)

(g)Xaa7是丙氨酸(Ala)或丝氨酸(Ser),

(h)Xaa8是谷氨酰胺(Gln)或精氨酸(Arg),以及

(i)Xaa9是天冬氨酸(Asp)或天冬酰胺(Asn)。

3.根据权利要求1或权利要求2所述的分离的免疫球蛋白重链多肽,其包括SEQ ID NO:2-SEQ ID NO:34或者SEQ ID NO:182-186中的任一项所示的氨基酸序列。

4.一种分离的免疫球蛋白重链多肽,其包括以下氨基酸序列:Gln Val Gln Leu Gln Gln Trp Gly Ala Xaa1 Leu Leu Lys Pro Ser Glu Thr Leu Ser Leu Xaa2 Cys Xaa3 Val Tyr Gly Gly Xaa4 Phe Xaa5 Gly Tyr Tyr Trp Xaa6 Trp Ile Arg Gln Pro Pro Xaa7 Lys Gly Leu Glu Trp Ile Gly Glu Ile Asn His Ser Gly Xaa8 Thr Asn Tyr Asn Pro Ser Leu Lys Ser Arg ValThr Ile Ser Val Asp Thr Ser Lys Asn Gln Xaa9 Ser Leu Lys Leu Xaa10 Xaa11 Val Thr Ala Ala Asp Thr Ala Val Tyr Tyr Cys Xaa12 Arg Glu Gly Xaa13 Tyr Gly Asp Tyr Asp Tyr Trp Gly Gln Gly Thr Leu Val Thr Val Ser Ser(SEQ ID NO:35),其中

(a)Xaa1是精氨酸(Arg)或甘氨酸(Gly),

(b)Xaa2是苏氨酸(Thr)或异亮氨酸(Ile),

(c)Xaa3是苏氨酸(Thr)或丙氨酸(Ala),

(d)Xaa4是丝氨酸(Ser)或苯丙氨酸(Phe),

(e)Xaa5是丝氨酸(Ser)或苯丙氨酸(Phe),

(f)Xaa6是丝氨酸(Ser)或异亮氨酸(Ile),

(g)Xaa7是甘氨酸(Gly)或精氨酸(Arg),

(h)Xaa8是丝氨酸(Ser)或天冬酰胺(Asn),

(i)Xaa9是苯丙氨酸(Phe)或亮氨酸(Leu),

(j)Xaa10是天冬酰胺(Asn)或丝氨酸(Ser),

(k)Xaa11是丝氨酸(Ser)或苯丙氨酸(Phe),

(l)Xaa12是丙氨酸(Ala)或缬氨酸(Val),以及

(m)Xaa13是天冬氨酸(Asp)或天冬酰胺(Asn)。

5.根据权利要求4所述的分离的免疫球蛋白重链多肽,其包括SEQ ID NO:36-SEQ ID NO:56中的任一项所示的氨基酸序列。

6.一种分离的免疫球蛋白轻链多肽,其包括以下氨基酸序列:Asp Xaa1 Val Met Thr Gln Thr Pro Leu Ser Leu Ser Val Thr Pro Gly Gln Pro Ala Ser Ile Ser Cys Arg Xaa2 Ser Gln Ser Leu Val His Ser Asp Xaa3 Xaa4 Thr Tyr Leu His Trp Tyr Leu Gln Lys Pro Gly Gln Ser Pro Gln Leu Leu Ile Tyr Xaa Xaa Ser Asn Arg Phe Ser Gly Val Pro Asp Arg Phe Ser Gly Ser Gly Ser Gly Thr Asp Phe Thr Leu Lys Ile Ser Arg Val Glu Ala Glu Asp Val Gly Val Tyr Phe Cys Xaa Gln Ser Thr Xaa Val Pro Tyr Ala Phe Gly Gly Gly Thr Lys Val Glu Ile Lys Arg Thr(SEQ ID NO:57),其中

(a)Xaa1是缬氨酸(Val)或异亮氨酸(Ile),

(b)Xaa2是半胱氨酸(Cys)或丝氨酸(Ser),

(c)Xaa3是甘氨酸(Gly)或丝氨酸(Ser),

(d)Xaa4是天冬酰胺(Asn)或天冬氨酸(Asp),

(e)Xaa5是赖氨酸(Lys)、甘氨酸(Gly)、天冬酰胺(Asn)、丝氨酸(Ser)、或亮氨酸(Leu)

(f)Xaa6是缬氨酸(Val)或异亮氨酸(Ile),

(g)Xaa7是丝氨酸(Ser)、丙氨酸(Ala)、或甘氨酸(Gly),以及

(h)Xaa8是组氨酸(His)或酪氨酸(Tyr)。

7.根据权利要求6所述的分离的免疫球蛋白轻链多肽，其包括SEQ ID NO:58-SEQ ID NO:88或187-189中的任一项所示的氨基酸序列。

8.一种分离的免疫球蛋白轻链多肽，其包括以下氨基酸序列:Asp Ile Gln Met Thr Gln Ser Pro Ser Ser Leu Ser Ala Ser Val Gly Asp Arg Val Thr Ile Thr Cys Gln Ala Ser Gln Asp Ile Ser Asn Tyr Leu Asn Trp Tyr Gln Gln Lys Pro Gly Lys Ala Pro Lys Leu Leu Ile Xaa1 Xaa2 Xaa3 Xaa4 Xaa5 Leu Glu Thr Gly Val Pro Ser Arg Phe Ser Gly Ser Gly Ser Gly Thr Asp Phe Thr Phe Thr Ile Ser Ser Leu Gln Pro Glu Asp Ile Ala Val Tyr Tyr Cys Gln Gln Ser Tyr Ser Xaa6 Leu Ile Thr Phe Gly Gln Gly Thr Arg Leu Glu Ile Lys Arg Thr Val Ala Ala Pro Ser Val(SEQ ID NO:89)，其中

(a)所述子序列Xaa1Xaa2Xaa3Xaa4Xaa5缺失或者是Tyr-Asp-Ala-Ser-Asn，以及

(b)Xaa6是苏氨酸(Thr)或异亮氨酸(Ile)。

9.根据权利要求8所述的分离的免疫球蛋白轻链多肽，其中所述子序列Xaa1 Xaa2 Xaa3 Xaa4 Xaa5是Tyr-Asp-Ala-Ser-Asn。

10.根据权利要求9所述的分离的免疫球蛋白轻链多肽，其包括SEQ ID NO:90所示的氨基酸序列。

11.根据权利要求8所述的分离的免疫球蛋白轻链多肽，其中所述序列Xaa1 Xaa2 Xaa3 Xaa4 Xaa5缺失。

12.根据权利要求11所述的分离的免疫球蛋白轻链多肽，其包括SEQ ID NO:91或SEQ ID NO:92所示的氨基酸序列。

13.一种分离的免疫球蛋白重链多肽，其包括SEQ ID NO:190或191。

14.根据权利要求13所述的分离的免疫球蛋白重链多肽，其包括SEQ ID NO:192-195中的任一项。

15.一种分离的免疫球蛋白轻链多肽，其包括SEQ ID NO:196或197。

16.根据权利要求15所述的分离的免疫球蛋白轻链多肽，其包括SEQ ID NO:198-200中的任一项。

17.一种分离的核酸序列，其编码权利要求1-5、13或14中的任一项所述的免疫球蛋白重链多肽。

18.一种分离的核酸序列，其编码权利要求6-12、15或16中的任一项所述的免疫球蛋白轻链多肽。

19.一种载体，其包括权利要求17或权利要求18所述的分离的核酸序列。

20.一种淋巴细胞活化基因-3(LAG-3)结合剂，其包括权利要求1-5、13或14中的任一项所述的免疫球蛋白重链多肽和/或权利要求6-12、15或16中的任一项所述的免疫球蛋白轻链多肽。

21.根据权利要求20所述的LAG-3结合剂，其包括权利要求1-5、13或14中的任一项所述的免疫球蛋白重链多肽和权利要求6-12、15或16中的任一项所述的免疫球蛋白轻链多肽。

22.根据权利要求20所述的LAG-3结合剂，其包括权利要求1-5、13或14中的任一项所述的免疫球蛋白重链多肽或权利要求6-12、15或16中的任一项所述的免疫球蛋白轻链多肽。

23.根据权利要求20所述的LAG-3结合剂，其包括(a)权利要求1-3中的任一项所述的免

疫球蛋白重链多肽和权利要求6或7中所述的免疫球蛋白轻链多肽；(b)权利要求4或5中所述的免疫球蛋白重链多肽和权利要求8-12中的任一项所述的免疫球蛋白轻链多肽；或者(c)权利要求13或14中所述的免疫球蛋白重链多肽和权利要求15或16中所述的免疫球蛋白轻链多肽。

24.根据权利要求20-23中的任一项所述的LAG-3结合剂，其是抗体、抗体缀合物、或者其抗原结合片段。

25.根据权利要求24所述的LAG-3结合剂，其是$F(ab')_2$片段、Fab'片段、Fab片段、Fv片段、scFv片段、dsFv片段、dAb片段、或者单链结合多肽。

26.根据权利要求20-25中的任一项所述的LAG-3结合剂，其结合所述LAG-3蛋白的胞外结构域1(D1)和/或胞外结构域2(D2)。

27.根据权利要求20-26中的任一项所述的LAG-3结合剂，其中所述LAG-3结合剂包括Fc区，所述Fc区具有降低或消除的效应子功能。

28.一种分离的核酸序列，其编码权利要求20-27中的任一项所述的LAG-3结合剂。

29.一种载体，其包括权利要求28所述的分离的核酸序列。

30.一种分离的细胞，其包括权利要求29所述的载体。

31.一种组合物，其包括(a)权利要求20-27中的任一项所述的LAG-3结合剂或权利要求29中所述的载体，和(b)药学上可接受的运载体。

32.一种在哺乳动物中治疗对LAG-3的抑制有应答的疾病的方法，所述方法包括向具有对LAG-3的抑制有应答的疾病的哺乳动物施用有效量的根据权利要求31所述的组合物，从而在所述哺乳动物中治疗所述疾病。

33.根据权利要求32所述的方法，其中所述疾病是癌症。

34.根据权利要求33所述的方法。其中所述癌症是黑色素瘤、肾细胞癌、肺癌、膀胱癌、乳腺癌、子宫颈癌、结肠癌、胆囊癌、喉癌、肝癌、甲状腺癌、胃癌、唾液腺癌、前列腺癌、胰腺癌或默克尔细胞癌。

35.根据权利要求32所述的方法，其中所述疾病是感染性疾病。

36.根据权利要求35所述的方法，其中所述感染性疾病由病毒或细菌引起。

37.根据权利要求36所述的方法，其中所述病毒是人免疫缺陷病毒(HIV)、呼吸道合胞病毒(RSV)、流感病毒、登革热病毒、或者乙型肝炎病毒(HBV)。

38.根据权利要求32-37中任一项所述的方法，其中所述LAG-3结合剂在所述哺乳动物中的半衰期在30分钟到45天之间。

39.根据权利要求32-38中任一项所述的方法，其中所述LAG-3结合剂以约1皮摩尔(pM)和约100微摩尔(μM)之间的K_D结合LAG-3。

40.根据权利要求32-39中任一项所述的方法，其还包括向所述哺乳动物施用PD-1结合剂和/或TIM-3结合剂。

41.根据权利要求40所述的方法，其中所述PD-1结合剂和/或TIM-3结合剂是抗体、抗体缀合物、或其抗原结合片段。

针对淋巴细胞活化基因3(LAG-3)的抗体

[0001] 通过电子形式提交的材料的引用并入

[0002] 与本文同时提交的计算机可读的核苷酸/氨基酸序列表通过引用整体并入本文，并被确认如下：在2016年2月2日创建的命名为“723163_ST25.TXT”的182,600字节的一个ASCII(文本)文件。

背景技术

[0003] 淋巴细胞活化基因-3(LAG-3)(也称为CD223)是免疫球蛋白超基因家族的成员，并且在结构和遗传学上与CD4相关。LAG-3在T细胞、B细胞、自然杀伤(NK)细胞和浆细胞样树突状细胞(pDC)上表达。与CD4类似，LAG-3已被证明与MHC II类分子相互作用(Baixeras等，J.Exp.Med.，176:327-337(1992))，但在不同的位点结合(Huard等，Proc.Natl.Acad.Sci.USA,94(11):5744-5749(1997))。例如，特别地，LAG-3免疫球蛋白融合蛋白(sLAG-3Ig)通过LAG-3直接地且特异性地结合细胞表面上的MHC II类分子。

[0004] 在T细胞活化后，LAG-3上调并调节T细胞功能以及T细胞稳态(Sierro等,Expert Opin.Ther.Targets,15(1):91-101(2011))。抗原特异性T细胞应答的体外研究证明，LAG-3/MHCII类分子的相互作用可能在下调CD4+T淋巴细胞的抗原依赖性刺激中起作用，在该研究中，加入抗LAG-3的抗体导致了升高的T细胞增殖，活化抗原(如，CD25)的更高表达，以及更高浓度的细胞因子(如，干扰素γ和白细胞介素4)((Huard等,Eur.J.Immunol.,24:3216-3221(1994))。CD4+CD25+调节性T细胞(Treg)也被证明在体外和体内条件下，在被激活时表达LAG-3且抗LAG-3的抗体抑制由经诱导的Treg细胞导致的压制作用，表明LAG-3有助于Treg细胞的抑制子活性(Huang等Immunity,21:503-513(2004))。此外，LAG-3已被证明通过调节性T细胞以T细胞依赖和不依赖型的机制负调节T细胞稳态(Workman,C.J.和Vignali,D.A.,J.Immunol.,174:688-695(2005))。

[0005] 常规T细胞中的无能的或者显示出功能受损的亚群表达LAG-3，并且LAG-3+T细胞在肿瘤部位和在慢性病毒感染期间富集。然而，虽然LAG-3敲除小鼠已被证明产生正常的病毒特异性的CD4+和CD8+T细胞应答，从而表明LAG-3的作用是非必需的，但是与单独阻断PD-L1相比，PD-1/PD-L1通路的阻断结合LAG-3阻断改善了对病毒的控制(Blackburn等，Nat.Immunol.,10:29-37(2009)；和Richter等,Int.Immunol.,22:13-2(2010))。

[0006] 在使得转基因CD8+T细胞在体内无应答/无能的自体耐受性/肿瘤小鼠模型中，CD8+T细胞中LAG-3的阻断或缺陷增强了肿瘤部位的T细胞增殖、T细胞募集和效应子功能(Grosso等,J.Clin.Invest.,117:3383-92(2007))。

[0007] 在临床前研究的基础上，目前正在研究抑制LAG-3的活性(例如，通过使用单克隆抗体)来治疗病毒感染和黑色素瘤的治疗方法。例如，加入与Fc区融合的可溶性huLAG-3增强了健康个体或癌症患者的PBMC中抗原特异性T细胞针对病毒和肿瘤抗原(例如，流感病毒基质蛋白或者T细胞识别的黑色素瘤抗原(MART-1))的增殖(Casati等,J.Immunol,180:3782-3788(2008))。

[0008] 需要另外的以高亲和力结合LAG-3并有效中和LAG-3活性的LAG-3拮抗剂(例如，抗

体)。本发明提供了这样的LAG-3结合剂。

[0009] 发明简述

[0010] 本发明提供了一种分离的免疫球蛋白重链多肽,其包括以下氨基酸序列Glu Val Gln Leu Val Gln Ser Gly Ala Glu Val Lys Lys Pro Gly Ala Thr Val Lys Ile Ser Cys Lys Ala Ser Gly Phe Xaa1 Ile Xaa2 Asp Asp Tyr Ile His Trp Val Xaa3 Gln Ala Pro Gly Lys Gly Leu Glu Trp Xaa4 Gly Trp Ile Asp Xaa5 Xaa6 Asn Xaa7 Asp Ser Xaa8 Tyr Xaa9 Ser Lys Phe Xaa10 Gly Arg Val Thr Ile Thr Val Asp Thr Ser Thr Xaa11 Thr Ala Tyr Met Xaa12 Leu Ser Ser Leu Arg Ser Glu Asp Thr Ala Val Tyr Tyr Cys Thr Tyr Ala Phe Gly Gly Tyr Trp Gly Gln Gly Thr Thr Val Thr Val Ser Ser(SEQ ID NO:181),其中(a)Xaa1是天冬酰胺(Asn)或丝氨酸(Ser),(b)Xaa2是赖氨酸(Lys)、酪氨酸(Tyr)或天冬酰胺(Asn),(c)Xaa3是赖氨酸(Lys)或谷氨酰胺(Gln),(d)Xaa4是异亮氨酸(Ile)或甲硫氨酸(Met),(e)Xaa5是丙氨酸(Ala)或脯氨酸(Pro),(f)Xaa6是谷氨酸(Glu)或甲硫氨酸(Met),(g)Xaa6是甘氨酸(Gly)、天冬酰胺(Asn)或天冬氨酸(Asp),(h)Xaa8是谷氨酸(Glu)或谷氨酰胺(Q),(i)Xaa9是丙氨酸(Ala)或丝氨酸(Ser),(j)Xaa10是谷氨酰胺(Gln)或精氨酸(Arg),(k)Xaa11是天冬氨酸(Asp)或天冬酰胺(Asn),以及(l)Xaa12是谷氨酰胺(Gln)或赖氨酸(Lys)。

[0011] 本发明提供了一种分离的免疫球蛋白重链多肽,其包括以下氨基酸序列:Gln Val Gln Leu Gln Gln Trp Gly Ala Xaa1 Leu Leu Lys Pro Ser Glu Thr Leu Ser Leu Xaa2 Cys Xaa3 Val Tyr Gly Gly Xaa4 Phe Xaa5 Gly Tyr Tyr Trp Xaa6 Trp Ile Arg Gln Pro Pro Xaa7 Lys Gly Leu Glu Trp Ile Gly Glu Ile Asn His Ser Gly Xaa8 Thr Asn Tyr Asn Pro Ser Leu Lys Ser Arg Val Thr Ile Ser Val Asp Thr Ser Lys Asn Gln Xaa9 Ser Leu Lys Leu Xaa10 Xaa11 Val Thr Ala Ala Asp Thr Ala Val Tyr Tyr Cys Xaa12 Arg Glu Gly Xaa13 Tyr Gly Asp Tyr Asp Tyr Trp Gly Gln Gly Thr Leu Val Thr Val Ser Ser(SEQ ID NO:35),其中(a)Xaa1是精氨酸(Arg)或甘氨酸(Gly),(b)Xaa2是苏氨酸(Thr)或异亮氨酸(Ile),(c)Xaa3是苏氨酸(Thr)或丙氨酸(Ala),(d)Xaa4是丝氨酸(Ser)或苯丙氨酸(Phe),(e)Xaa5是丝氨酸(Ser)或苯丙氨酸(Phe),(f)Xaa6是丝氨酸(Ser)或异亮氨酸(Ile),(g)Xaa7是甘氨酸(Gly)或精氨酸(Arg),(h)Xaa8是丝氨酸(Ser)或天冬酰胺(Asn),(i)Xaa9是苯丙氨酸(Phe)或亮氨酸(Leu),(j)Xaa10是天冬酰胺(Asn)或丝氨酸(Ser),(k)Xaa11是丝氨酸(Ser)或苯丙氨酸(Phe),(l)Xaa12是丙氨酸(Ala)或缬氨酸(Val),以及(m)Xaa13是天冬氨酸(Asp)或天冬酰胺(Asn)。

[0012] 本发明还提供了一种分离的免疫球蛋白重链多肽,其包括SEQ ID NO:190或191。

[0013] 本发明提供了一种分离的免疫球蛋白轻链多肽,其包括以下氨基酸序列:Asp Xaa1 Val Met Thr Gln Thr Pro Leu Ser Leu Ser Val Thr Pro Gly Gln Pro Ala Ser Ile Ser Cys Arg Xaa2 Ser Gln Ser Leu Val His Ser Asp Xaa3 Xaa4 Thr Tyr Leu His Trp Tyr Leu Gln Lys Pro Gly Gln Ser Pro Gln Leu Leu Ile Tyr Xaa5 Xaa6 Ser Asn Arg Phe Ser Gly Val Pro Asp Arg Phe Ser Gly Ser Gly Ser Gly Thr Asp Phe Thr Leu Lys Ile Ser Arg Val Glu Ala Glu Asp Val Gly Val Tyr Phe Cys Xaa7 Gln Ser Thr Xaa8 Val Pro Tyr Ala Phe Gly Gly Gly Thr Lys Val Glu Ile Lys Arg Thr (SEQ ID NO:57),其中(a)Xaa1是缬氨酸(Val)或异亮氨酸(Ile),(b)Xaa2是半胱氨酸(Cys)

或丝氨酸(Ser)，(c)Xaa3是甘氨酸(Gly)或丝氨酸(Ser)，(d)Xaa4是天冬酰胺(Asn)或天冬氨酸(Asp)，(e)Xaa5是赖氨酸(Lys)、甘氨酸(Gly)、天冬酰胺(Asn)、丝氨酸(Ser)或亮氨酸(Leu)，(f)Xaa6是缬氨酸(Val)或异亮氨酸(Ile)，(g)Xaa7是丝氨酸(Ser)、丙氨酸(Ala)或甘氨酸(Gly)，以及(h)Xaa8是组氨酸(His)或酪氨酸(Tyr)。

[0014] 本发明提供了一种分离的免疫球蛋白轻链多肽，其包括以下氨基酸序列：Asp Ile Gln Met Thr Gln Ser Pro Ser Ser Leu Ser Ala Ser Val Gly Asp Arg Val Thr Ile Thr Cys Gln Ala Ser Gln Asp Ile Ser Asn Tyr Leu Asn Trp Tyr Gln Gln Lys Pro Gly Lys Ala Pro Lys Leu Leu Ile Xaa1 Xaa2 Xaa3 Xaa4 Xaa5 Leu Glu Thr Gly Val Pro Ser Arg Phe Ser Gly Ser Gly Ser Gly Thr Asp Phe Thr Phe Thr Ile Ser Ser Leu Gln Pro Glu Asp Ile Ala Val Tyr Tyr Cys Gln Gln Ser Tyr Ser Xaa6 Leu Ile Thr Phe Gly Gln Gly Thr Arg Leu Glu Ile Lys Arg Thr Val Ala Ala Pro Ser Val (SEQ ID NO:89)，其中(a)所述子序列Xaa1Xaa2Xaa3Xaa4Xaa5缺失或者是Tyr-Asp-Ala-Ser-Asn，以及(b)Xaa6是苏氨酸(Thr)或异亮氨酸(Ile)。

[0015] 本发明还提供了分离的免疫球蛋白轻链多肽，其包括SEQ ID NO:196或197。

[0016] 此外，本发明提供了分离的或纯化的编码前述免疫球蛋白多肽的核酸序列，包含此类核酸序列的载体，包含前述免疫球蛋白多肽的LAG-3结合剂，编码此类LAG-3结合剂的核酸序列，包含此类核酸序列的载体，包含此类载体的分离的细胞，包含此类LAG-3结合剂的或者此类载体及药学上可接受的运载体的组合物，以及通过向哺乳动物施用有效量的此类组合物在哺乳动物中治疗癌症或感染性疾病的方法。

[0017] 附图简述

[0018] 图1A是植入了Colon26结肠腺癌细胞并且注射了所标识抗体的小鼠中平均肿瘤体积随时间的变化图。图中每个数据点代表所标识的处理组。

[0019] 图1B是植入了Colon26结肠腺癌细胞并且注射了所标识抗体的三个处理组的小鼠中各个动物的肿瘤体积随时间的变化图。图中每个数据点代表所述处理组中的各个动物。

[0020] 图2A显示了在不同浓度的抗PD-1或抗LAG-3抗体的条件下，在混合淋巴细胞反应(MLR)测定法中CD4+T细胞分泌IL-2的量。

[0021] 图2B显示了在暴露于树突状细胞之前(初始)或之后(24、48和72小时之后)，CD4+T细胞上LAG-3和PD-1的表达量。

[0022] 发明详述

[0023] 本发明提供了一种分离的免疫球蛋白重链多肽和/或一种分离的免疫球蛋白轻链多肽，或其片段(例如，其抗原结合片段)。本文所用的术语“免疫球蛋白”或“抗体”是指发现于脊椎动物的血液或其他体液中的蛋白质，其被免疫系统用来识别和中和异物(如，细菌和病毒)。所述多肽是“分离的”，是指将其移出其天然环境。在优选的实施方案中，免疫球蛋白或抗体是包括至少一个互补决定区(CDR)的蛋白质。所述CDR形成抗体的“高变区”，其负责结合抗原(下文做进一步讨论)。整个免疫球蛋白通常由四个多肽组成：两个相同的重(H)链多肽拷贝和两个相同的轻(L)链多肽拷贝。每条重链含有一个N端可变(V_H)区和三个C端恒定(C_H1、C_H2和C_H3)区，并且每条轻链含有一个N端可变(V_L)区和一个C端恒定(C_L)区。基于其恒定结构域的氨基酸序列，抗体的轻链可以被归属于或为kappa(κ)或为lambda(λ)的两种不同类型中的一种。在典型的免疫球蛋白中，每条轻链通过二硫键与重链连接，并且两条重

链通过二硫键相互连接。轻链可变区与重链的可变区对齐，并且轻链恒定区与重链的第一恒定区对齐，重链剩下的恒定区彼此对齐。

[0024] 每个轻链和重链对的可变区形成抗体的抗原结合位点。V_H和V_L区具有相同的基本结构，每个区包括四个框架(FW或FR)区。本文所用的术语“框架区”是指可变区内相对保守的氨基酸序列，其位于所述的高变或互补决定区(CDRs)之间。每个可变结构域中有四个框架区，分别命名为FR1、FR2、FR3和FR4。框架区形成提供可变区的结构框架的β片层(参见，例如，C.A.Janeway等(eds.)，Immunobiology，第5版，Garland Publishing，New York，NY(2001))。

[0025] 多个框架区经由三个互补决定区(CDR)连接。如上所述，被称为CDR1、CDR2和CDR3的三个CDR形成抗体的“高变区”，负责抗原结合。多个CDR形成连接由框架区形成的β-片层结构的或者在某些情况下包括部分由框架区形成的β-片层结构的环。虽然轻链和重链的恒定区不直接参与抗体与抗原的结合，但是恒定区可以影响可变区的排布(orientation)。恒定区还表现出多种效应子功能，例如通过与效应分子和细胞的相互作用参与抗体依赖的补体介导的裂解或者抗体依赖的细胞毒性。

[0026] 本发明所述分离的免疫球蛋白重链多肽和分离的免疫球蛋白轻链多肽理想地能结合由淋巴细胞活化基因-3(LAG-3)编码的蛋白质(本文中也称为“LAG-3蛋白”)。如上所述，LAG-3是由498个氨基酸组成的能负调节T细胞功能和稳态的蛋白(Triebel等，J.Exp.Med.，171(5):1393-1405(1990)；和Triebel F.，Trends Immunol.，24(12):619-22(2003))。LAG-3是免疫球蛋白超基因家族的成员并且在结构和遗传学上与CD4相关。已证明LAG-3的胞质内区域与被称为LAP的蛋白质相互作用，LAP被认为是参与下调CD3/TCR活化通路的信号转导分子(Iouzalen等，Eur.J.Immunol.，31:2885-2891(2001))。此外，已证明不管在体外还是在体内，CD4+CD25+调节性T细胞(Treg)在激活时都表达LAG-3并且抗LAG-3的抗体都抑制了经诱导的Treg细胞的压制作用，表明LAG-3有助于Treg细胞的抑制子活性(Huang等，Immunity，21:503-513(2004)))。然而，最近的研究表明，CD4+T细胞上的LAG-3表达会使其更易被Treg抑制，而并非使Treg更具抑制性(参见Durham等，PLoS ONE，9(11):e109080(2014))。在某些情况下，LAG-3也被证明具有免疫刺激效应(参见，例如，Prigent等，Eur.J.Immunol.，29:3867-3876(1999))；El Mir和Triebel，J.Immunol.，164:5583-5589(2000))；以及Casati等，Cancer Res.，66:4450-4460(2006))。本发明的分离的免疫球蛋白重链多肽和本发明的分离的免疫球蛋白轻链多肽可以形成结合LAG-3和另一抗原的试剂，获得“双反应性”的结合剂(例如，双反应性抗体)。例如，所述试剂可以结合LAG-3和免疫系统的另一负调节子，例如，程序性死亡因子1(PD-1)和/或T细胞免疫球蛋白结构域和粘蛋白结构域3蛋白(TIM-3)。

[0027] 结合LAG-3的抗体，及其组分，是本领域已知的(参见，例如，美国专利申请公开号2010/0233183、2011/0150892和2014/0093511)。抗LAG-3的抗体也可以从诸如Abcam(剑桥，美国马萨诸塞州)和R&D Systems，Inc.(明尼阿波利斯，美国明尼苏达州)等来源商购获得。

[0028] 本发明提供了一种分离的免疫球蛋白重链多肽，其包括以下氨基酸序列：Glu Val Gln Leu Val Gln Ser Gly Ala Glu Val Lys Lys Pro Gly Ala Thr Val Lys Ile Ser Cys Lys Ala Ser Gly Phe Xaa1 Ile Xaa2 Asp Asp Tyr Ile His Trp Val Xaa3 Gln Ala Pro Gly Lys Gly Leu Glu Trp Xaa4 Gly Trp Ile Asp Xaa5 Xaa6 Asn Xaa7 Asp

Ser Xaa8 Tyr Xaa9 Ser Lys Phe Xaa10 Gly Arg Val Thr Ile Thr Val Asp Thr Ser Thr Xaa11 Thr Ala Tyr Met Xaa12 Leu Ser Ser Leu Arg Ser Glu Asp Thr Ala Val Tyr Tyr Cys Thr Tyr Ala Phe Gly Gly Tyr Trp Gly Gln Gly Thr Thr Val Thr Val Ser Ser(SEQ ID NO:181)，其中(a)Xaa1是天冬酰胺(Asn)或丝氨酸(Ser)，(b)Xaa2是赖氨酸(Lys)、酪氨酸(Tyr)或天冬酰胺(Asn)，(c)Xaa3是赖氨酸(Lys)或谷氨酰胺(Gln)，(d)Xaa4是异亮氨酸(Ile)或甲硫氨酸(Met)，(e)Xaa5是丙氨酸(Ala)或脯氨酸(Pro)，(f)Xaa6是谷氨酸(Glu)或甲硫氨酸(Met)，(g)Xaa6是甘氨酸(Gly)、天冬酰胺(Asn)或天冬氨酸(Asp)，(h)Xaa8是谷氨酸(Glu)或谷氨酰胺(Q)，(i)Xaa9是丙氨酸(Ala)或丝氨酸(Ser)，(j)Xaa10是谷氨酰胺(Gln)或精氨酸(Arg)，(k)Xaa11是天冬氨酸(Asp)或天冬酰胺(Asn)，以及(l)Xaa12是谷氨酰胺(Gln)或赖氨酸(Lys)。

[0029] 在另一方面，所述免疫球蛋白重链多肽包括以下氨基酸序列，由以下氨基酸序列组成，或者基本上由以下氨基酸序列组成：Glu Val Gln Leu Val Gln Ser Gly Ala Glu Val Lys Lys Pro Gly Ala Thr Val Lys Ile Ser Cys Lys Ala Ser Gly Phe Xaa1 Ile Xaa2 Asp Asp Tyr Ile His Trp Val Xaa3 Gln Ala Pro Gly Lys Gly Leu Glu Trp Xaa4 Gly Trp Ile Asp Xaa5 Glu Asn Xaa6 Asp Ser Glu Tyr Xaa7 Ser Lys Phe Xaa8 Gly Arg Val Thr Ile Thr Val Asp Thr Ser Thr Xaa9 Thr Ala Tyr Met Glu Leu Ser Ser Leu Arg Ser Glu Asp Thr Ala Val Tyr Tyr Cys Thr Tyr Ala Phe Gly Gly Tyr Trp Gly Gln Gly Thr Thr Val Thr Val Ser Ser(SEQ ID NO:1)，其中(a)Xaa1是天冬酰胺(Asn)或丝氨酸(Ser)，(b)Xaa2是赖氨酸(Lys)，酪氨酸(Tyr)或天冬酰胺(Asn)，(c)Xaa3是赖氨酸(Lys)或谷氨酰胺(Gln)，(d)Xaa4是异亮氨酸(Ile)或甲硫氨酸(Met)，(e)Xaa5是丙氨酸(Ala)或脯氨酸(Pro)，(f)Xaa6是甘氨酸(Gly)、天冬酰胺(Asn)或天冬氨酸(Asp)，(g)Xaa7是丙氨酸(Ala)或丝氨酸(Ser)，(h)Xaa8是谷氨酰胺(Gln)或精氨酸(Arg)，以及(i)Xaa9是天冬氨酸(Asp)或天冬酰胺(Asn)。

[0030] 在一个实施方案中，所述分离的免疫球蛋白重链多肽包括以下任一项所示的氨基酸序列，由以下任一项所示的氨基酸序列组成，或者基本上由以下任一项所示的氨基酸序列组成：SEQ ID NO:2、SEQ ID NO:3、SEQ ID NO:4、SEQ ID NO:5、SEQ ID NO:6、SEQ ID NO:7、SEQ ID NO:8、SEQ ID NO:9、SEQ ID NO:10、SEQ ID NO:11、SEQ ID NO:12、SEQ ID NO:13、SEQ ID NO:14、SEQ ID NO:15、SEQ ID NO:16、SEQ ID NO:17、SEQ ID NO:18、SEQ ID NO:19、SEQ ID NO:20、SEQ ID NO:21、SEQ ID NO:22、SEQ ID NO:23、SEQ ID NO:24、SEQ ID NO:25、SEQ ID NO:26、SEQ ID NO:27、SEQ ID NO:28、SEQ ID NO:29、SEQ ID NO:30、SEQ ID NO:31、SEQ ID NO:32、SEQ ID NO:33、SEQ ID NO:34、SEQ ID NO:182、SEQ ID NO:183、SEQ ID NO:184、SEQ ID NO:185、或SEQ ID NO:186。

[0031] 本发明还提供了一种免疫球蛋白重链多肽，其包括以下氨基酸序列，由以下氨基酸序列组成，或者基本上由以下氨基酸序列组成：Gln Val Gln Leu Gln Gln Trp Gly Ala Xaa1 Leu Leu Lys Pro Ser Glu Thr Leu Ser Leu Xaa2 Cys Xaa3 Val Tyr Gly Gly Xaa4 Phe Xaa5 Gly Tyr Tyr Trp Xaa6 Trp Ile Arg Gln Pro Pro Xaa7 Lys Gly Leu Glu Trp Ile Gly Glu Ile Asn His Ser Gly Xaa8 Thr Asn Tyr Asn Pro Ser Leu Lys Ser Arg Val Thr Ile Ser Val Asp Thr Ser Lys Asn Gln Xaa9 Ser Leu Lys Leu Xaa10 Xaa11 Val Thr Ala Ala Asp Thr Ala Val Tyr Tyr Cys Xaa12 Arg Glu Gly

Xaa13 Tyr Gly Asp Tyr Asp Tyr Trp Gly Gln Gly Thr Leu Val Thr Val Ser Ser(SEQ ID NO:35)，其中(a)Xaa1是精氨酸(Arg)或甘氨酸(Gly)，(b)Xaa2是苏氨酸(Thr)或异亮氨酸(Ile)，(c)Xaa3是苏氨酸(Thr)或丙氨酸(Ala)，(d)Xaa4是丝氨酸(Ser)或苯丙氨酸(Phe)，(e)Xaa5是丝氨酸(Ser)或苯丙氨酸(Phe)，(f)Xaa6是丝氨酸(Ser)或异亮氨酸(Ile)，(g)Xaa7是甘氨酸(Gly)或精氨酸(Arg)，(h)Xaa8是丝氨酸(Ser)或天冬酰胺(Asn)，(i)Xaa9是苯丙氨酸(Phe)或亮氨酸(Leu)，(j)Xaa10是天冬酰胺(Asn)或丝氨酸(Ser)，(k)Xaa11是丝氨酸(Ser)或苯丙氨酸(Phe)，(l)Xaa12是丙氨酸(Ala)或缬氨酸(Val)，以及(m)Xaa13是天冬氨酸(Asp)或天冬酰胺(Asn)。在一个实施方案中，所述分离的免疫球蛋白重链多肽包括以下任一项所示的氨基酸序列，由以下任一项所示的氨基酸序列组成，或者基本上由以下任一项所示的氨基酸序列组成：SEQ ID NO:36、SEQ ID NO:37、SEQ ID NO:38、SEQ ID NO:39、SEQ ID NO:40、SEQ ID NO:41、SEQ ID NO:42、SEQ ID NO:43、SEQ ID NO:44、SEQ ID NO:45、SEQ ID NO:46、SEQ ID NO:47、SEQ ID NO:48、SEQ ID NO:49、SEQ ID NO:50、SEQ ID NO:51、SEQ ID NO:52、SEQ ID NO:53、SEQ ID NO:54、SEQ ID NO:55、或SEQ ID NO:56。

[0032] 在另一实施方案中，提供了一种分离的免疫球蛋白重链多肽，其包括SEQ ID NO:190或191。这种多肽的实例包括包含SEQ ID NO:192-195中任一项的多肽。

[0033] 当本发明的免疫球蛋白重链多肽基本上由SEQ ID NO:1-SEQ ID NO:56、SEQ ID NO:182-186、或SEQ ID NO:190-195中的任一项所示的氨基酸序列组成时，所述多肽可以包括另外的在本质上不影响所述多肽的组分(例如，蛋白质部分，如辅助纯化或分离的生物素)。当本发明的免疫球蛋白重链多肽由SEQ ID NO:1-SEQ ID NO:56中的任一项所示的氨基酸序列组成时，所述多肽不包括任何另外的组分(即，对于本发明的免疫球蛋白重链多肽来说不是内源性的组分)。

[0034] 本发明提供了一种分离的免疫球蛋白重链多肽，其包括与SEQ ID NO:1-56中的任一项具有至少90％同一性(例如，至少91％％、至少92％％、至少93％％、至少94％％、至少95％％、至少96％％、至少97％％、至少98％％、至少99％％或100％％同一性)的氨基酸序列。本文所述的核酸或氨基酸序列的“同一性”可以通过将目的核酸或氨基酸的序列与参考核酸或氨基酸的序列进行比较来确定。百分比同一性是用目的序列和参照序列之间同一的(即，一样的)核苷酸或氨基酸残基的数目除以最长序列的长度(即，目的序列或参照序列的长度，以较长者为准)。用于获得两个或多个序列之间的最优比对以及计算同一性的许多数学算法是已知的并且并入了多个可用的软件程序中。这些程序的实例包括CLUSTAL-W、T-Coffee和ALIGN(用于比对核酸和氨基酸序列)、BLAST程序(例如，BLAST 2.1、BL2SEQ及其更新版本)和FASTA程序(例如，FASTA3x、FASTM和SSEARCH)(用于序列比对和序列相似性搜索)。在例如，Altschul等，J.Molecular Biol.，215(3)：403-410(1990)，Beigert等，Proc.Natl.Acad.Sci.USA，106(10)：3770-3775(2009)，Durbin等，eds.，Biological Sequence Analysis:Probalistic Models of Proteins and Nucleic Acids，Cambridge University Press，Cambridge，UK(2009)，Soding，Bioinformatics，21(7)：951-960(2005)，Altschul 等，Nucleic Acids Res.，25(17)：3389-3402(1997)，以及Gusfield，Algorithms on Strings，Trees and Sequences，Cambridge University Press，Cambridge UK(1997))中，也公开了序列比对算法。

[0035] 在另一实施方案中，本发明提供了一种免疫球蛋白轻链多肽，其包括以下氨基酸

序列,由以下氨基酸序列组成,或者基本上由以下氨基酸序列组成:Asp Xaa1 Val Met Thr Gln Thr Pro Leu Ser Leu Ser Val Thr Pro Gly Gln Pro Ala Ser Ile Ser Cys Arg Xaa2 Ser Gln Ser Leu Val His Ser Asp Xaa3 Xaa4 Thr Tyr Leu His Trp Tyr Leu Gln Lys Pro Gly Gln Ser Pro Gln Leu Leu Ile Tyr Xaa5 Xaa6 Ser Asn Arg Phe Ser Gly Val Pro Asp Arg Phe Ser Gly Ser Gly Ser Gly Thr Asp Phe Thr Leu Lys Ile Ser Arg Val Glu Ala Glu Asp Val Gly Val Tyr Phe Cys Xaa7 Gln Ser Thr Xaa8 Val Pro Tyr Ala Phe Gly Gly Gly Thr Lys Val Glu Ile Lys Arg Thr(SEQ ID NO:57),其中(a)Xaa1是缬氨酸(Val)或异亮氨酸(Ile),(b)Xaa2是半胱氨酸(Cys)或丝氨酸(Ser),(c)Xaa3是甘氨酸(Gly)或丝氨酸(Ser),(d)Xaa4是天冬酰胺(Asn)或天冬氨酸(Asp),(e)Xaa5是赖氨酸(Lys)、甘氨酸(Gly)、天冬酰胺(Asn)、丝氨酸(Ser)或亮氨酸(Leu),(f)Xaa6是缬氨酸(Val)或异亮氨酸(Ile),(g)Xaa7是丝氨酸(Ser)、丙氨酸(Ala)或甘氨酸(Gly),以及(h)Xaa8是组氨酸(His)或酪氨酸(Tyr)。

[0036] 在一个实施方案中,所述分离的免疫球蛋白轻链多肽包括以下任一项所示的氨基酸序列,由以下任一项所示的氨基酸序列组成,或者基本上由以下任一项所示的氨基酸序列组成:SEQ ID NO:58、SEQ ID NO:59、SEQ ID NO:60、SEQ ID NO:61、SEQ ID NO:62、SEQ ID NO:63、SEQ ID NO:64、SEQ ID NO:65、SEQ ID NO:66、SEQ ID NO:67、SEQ ID NO:68、SEQ ID NO:69、SEQ ID NO:70、SEQ ID NO:71、SEQ ID NO:72、SEQ ID NO:73、SEQ ID NO:74、SEQ ID NO:75、SEQ ID NO:76、SEQ ID NO:77、SEQ ID NO:78、SEQ ID NO:79、SEQ ID NO:80、SEQ ID NO:81、SEQ ID NO:82、SEQ ID NO:83、SEQ ID NO:84、SEQ ID NO:85、SEQ ID NO:86、SEQ ID NO:87、SEQ ID NO:88、SEQ ID NO:187、SEQ ID NO:188、或SEQ ID NO:189。

[0037] 本发明提供了一种分离的免疫球蛋白轻链多肽,其包括以下氨基酸序列,基本上由以下氨基酸序列组成,或者由以下氨基酸序列组成:Asp Ile Gln Met Thr Gln Ser Pro Ser Ser Leu Ser Ala Ser Val Gly Asp Arg Val Thr Ile Thr Cys Gln Ala Ser Gln Asp Ile Ser Asn Tyr Leu Asn Trp Tyr Gln Gln Lys Pro Gly Lys Ala Pro Lys Leu Leu Ile Xaa1 Xaa2 Xaa3 Xaa4 Xaa5 Leu Glu Thr Gly Val Pro Ser Arg Phe Ser Gly Ser Gly Ser Gly Thr Asp Phe Thr Phe Thr Ile Ser Ser Leu Gln Pro Glu Asp Ile Ala Val Tyr Tyr Cys Gln Gln Ser Tyr Ser Xaa6 Leu Ile Thr Phe Gly Gln Gly Thr Arg Leu Glu Ile Lys Arg Thr Val Ala Ala Pro Ser Val(SEQ ID NO:89),其中(a)子序列Xaa1 Xaa2 Xaa3 Xaa4 Xaa5缺失或者是Tyr-Asp-Ala-Ser-Asn,以及(b)Xaa6是苏氨酸(Thr)或异亮氨酸(Ile)。

[0038] 本发明的免疫球蛋白轻链多肽当Xaa6是苏氨酸(Thr)或异亮氨酸(Ile)时,可以包括或缺失SEQ ID NO:89的49-53位点处的子序列Xaa1 Xaa2 Xaa3 Xaa4 Xaa5。当本发明所述免疫球蛋白轻链多肽包括所述子序列Xaa1 Xaa2 Xaa3 Xaa4 Xaa5时,Xaa1、Xaa2、Xaa3、Xaa4和Xaa5各自可以是任意合适的氨基酸残基。优选地,Xaa1是酪氨酸(Tyr),Xaa2是天冬氨酸(Asp),Xaa3是丙氨酸(Ala),Xaa4是丝氨酸(Ser),以及Xaa5是天冬酰胺(Asn)。免疫球蛋白轻链多肽的优选的包括Xaa1 Xaa2 Xaa3 Xaa4 Xaa5子序列的氨基酸序列包括SEQ ID NO:90。当免疫球蛋白轻链多肽缺少子序列Xaa1 Xaa2 Xaa3 Xaa4 Xaa5时,所述免疫球蛋白轻链多肽优选地包括SEQ ID NO:91或SEQ ID NO:92所示的氨基酸序列。

[0039] 在另一实施方案中,提供了一种分离的免疫球蛋白轻链多肽,其包括SEQ ID NO:

196或197。这种多肽的实例包括包含SEQ ID NO:198-200中的任一项的多肽。

[0040] 当本发明的免疫球蛋白轻链多肽基本上由SEQ ID NO:57-SEQ ID NO:92、SEQ ID NO:187-189、或SEQ ID NO:196-200中的任一项所示的氨基酸序列组成时，所述多肽可以包括另外的不会在本质上影响所述多肽的组分(例如，蛋白质部分，如辅助纯化或分离的生物素)。当本发明的免疫球蛋白轻链多肽由SEQ ID NO:57-SEQ ID NO:92中的任一项所示的氨基酸序列组成时，所述多肽不包括任何另外的组分(即，对于本发明所述免疫球蛋白轻链多肽来说不是内源性的组分)。

[0041] 本发明提供了一种分离的免疫球蛋白轻链多肽，其包括与SEQ ID NO:57-SEQ ID NO:92中的任一项具有至少90％同一性(例如，至少91％、至少92％、至少93％、至少94％、至少95％、至少96％、至少97％、至少98％、至少99％、或者100％同一性)的氨基酸序列。核酸或氨基酸序列的“同一性”可以通过使用本文所描述的方法来确定。

[0042] 上述免疫球蛋白重链多肽和/或轻链多肽的一个或多个氨基酸可以被不同的氨基酸替代或取代。氨基酸的“替代”或“取代”是指多肽序列内在给定位置或残基处的一个氨基酸被另一氨基酸在相同位置或残基处替代。

[0043] 氨基酸大致分为“芳香族”或“脂肪族”。芳香族氨基酸包括芳环。“芳香族”氨基酸的实例包括组氨酸(H或His)、苯丙氨酸(F或Phe)、酪氨酸(Y或Tyr)和色氨酸(W或Trp)。非芳香族氨基酸大致分为“脂肪族”。“脂肪族”氨基酸的实例包括甘氨酸(G或Gly)、丙氨酸(A或Ala)、缬氨酸(V或Val)、亮氨酸(L或Leu)、异亮氨酸(I或Ile)、甲硫氨酸(M或Met)、丝氨酸(S或Ser)、苏氨酸(T或Thr)、半胱氨酸(C或Cys)、脯氨酸(P或Pro)、谷氨酸(E或Glu)、天冬氨酸(A或Asp)、天冬酰胺(N或Asn)、谷氨酰胺(Q或Gln)、赖氨酸(K或Lys)和精氨酸(R或Arg)。

[0044] 脂肪族氨基酸可以细分为四个亚组。“大脂肪族非极性亚组”由缬氨酸、亮氨酸和异亮氨酸组成。“脂肪族微极性亚组”由甲硫氨酸、丝氨酸、苏氨酸和半胱氨酸组成。“脂肪族极性/带电亚组”由谷氨酸、天冬氨酸、天冬酰胺、谷氨酰胺、赖氨酸和精氨酸组成。“小残基亚组”由甘氨酸和丙氨酸组成。带电/极性氨基酸组可以细分为三个亚组：由赖氨酸和精氨酸组成的“带正电荷亚组”、由谷氨酸和天冬氨酸组成的“带负电荷亚组”、以及由天冬酰胺和谷氨酰胺组成的“极性亚组”。

[0045] 芳香族氨基酸可以细分为两个亚组：由组氨酸和色氨酸组成的“氮环亚组”以及由苯丙氨酸和酪氨酸组成的“苯基亚组”。

[0046] 氨基酸的替代或取代可以是保守的、半保守的或者不保守的。术语“保守的氨基酸取代”或“保守的突变”是指用另一个具有共同特性的氨基酸替代一个氨基酸。确定单个氨基酸之间共同特性的一种功能性方法是分析相应的同源生物体蛋白之间氨基酸变化的归一化的频率(Schulz和Schirmer,Principles of Protein Structure,Springer-Verlag,New York(1979))。根据这种分析，可以如下定义氨基酸组：当组内的氨基酸优选地彼此间交换，并且因此在其对整个蛋白质结构的影响方面彼此最为相似(Schulz和Schirmer,同上)。

[0047] 保守的氨基酸取代的实例包括上述的亚组内氨基酸间的取代，例如精氨酸取代赖氨酸(反之亦然)从而使得可以保持正电荷，天冬氨酸取代谷氨酸(反之亦然)使得可以保持负电荷，苏氨酸取代丝氨酸使得可以保留自由的-OH，以及天冬酰胺取代谷氨酰胺使得可以保留自由的-NH_2。

[0048] “半保守突变”包括上面列出的在相同组内但不在相同亚组内的氨基酸间的氨基酸取代。例如，天冬酰胺取代天冬氨酸或者赖氨酸取代天冬酰胺涉及同一组但不同亚组内的氨基酸。“不保守突变”涉及不同组之间的氨基酸取代，例如色氨酸取代赖氨酸，或者丝氨酸取代苯丙氨酸等。

[0049] 此外，可将一个或多个氨基酸插入上述免疫球蛋白重链多肽和/或轻链多肽中。可以将任意数量的任何合适的氨基酸插入到所述免疫球蛋白重链多肽和/或轻链多肽的氨基酸序列中。就这方面而言，可以将至少一个氨基酸(例如，2个或更多、5个或更多、或10个或更多氨基酸)但不超过20个氨基酸(例如18个或更少、15个或更少、或12个或更少氨基酸)插入到所述免疫球蛋白重链多肽和/或轻链多肽的氨基酸序列中。优选将1-10个氨基酸(例如，1个、2个、3个、4个、5个、6个、7个、8个、9个或10个氨基酸)插入到所述免疫球蛋白重链多肽和/或轻链多肽的氨基酸序列中。就这方面而言，可以将氨基酸插入到任一上述免疫球蛋白重链多肽和/或轻链多肽的任何合适的位置。优选地，将氨基酸插入免疫球蛋白重链多肽和/或轻链多肽的CDR(例如，CDR1、CDR2或CDR3)中。

[0050] 本发明的分离的免疫球蛋白重链多肽和轻链多肽不限于包括本文所述特定氨基酸序列的多肽。实际上，所述免疫球蛋白重链多肽或轻链多肽可以是与本发明的免疫球蛋白重链多肽或轻链多肽竞争结合LAG-3的任何重链多肽或轻链多肽。就这方面而言，例如，所述免疫球蛋白重链多肽或轻链多肽可以是与本发明的重链多肽或轻链多肽所识别的LAG-3表位相同的表位结合的任意重链多肽或轻链多肽。可以用利用ELISA、Western印迹或免疫组化方法的常规的肽竞争测定法来测定抗体竞争(参见，例如，美国专利号4,828,981和8,568,992；以及Braitbard等，Proteome Sci.，4:12(2006))。

[0051] 本发明提供了一种分离的LAG-3结合剂，其包括本文所述的本发明的分离的氨基酸序列中的一个或多个，基本上由其组成，或者由其组成。“LAG-3结合剂”是指与LAG-3蛋白特异性结合的分子，优选地为蛋白质类分子。优选地，所述LAG-3结合剂是抗体或其片段(例如，其免疫原性片段)。本发明的LAG-3结合剂包括本发明的分离的免疫球蛋白重链多肽和/或本发明分离的免疫球蛋白轻链多肽、基本上由其组成、或者由其组成。在一个实施方案中，LAG-3结合剂包括本发明的免疫球蛋白重链多肽或本发明的免疫球蛋白轻链多肽、基本上由其组成、或者由其组成。在另一实施方案中，LAG-3结合剂包括本发明的免疫球蛋白重链多肽和本发明的免疫球蛋白轻链多肽、基本上由其组成、或者由其组成。

[0052] 本发明的免疫球蛋白重链多肽和/或本发明的免疫球蛋白轻链多肽的任意氨基酸残基可以以任意组合的形式，被不同的氨基酸残基替换，或者缺失或者被插入，只要所述氨基酸替代、插入和/或缺失能够导致增强或改善所述LAG-3结合剂的生物活性即可。LAG-3结合剂的“生物学活性”是指，例如，对特定LAG-3表位的结合亲和力，对LAG-3与其受体结合的中和作用或抑制作用，在体内对LAG-3活性的中和作用或抑制作用(例如，IC_{50})，药代动力学和交叉反应性(例如，与LAG-3蛋白的非人同源物或直系同源物的交叉反应性，或者与其他蛋白或组织的交叉反应性)。本领域认可的抗原结合剂的其它生物学特性或特征包括，例如，亲合力、选择性、可溶性、折叠、免疫毒性、表达和制剂。可以使用标准技术来观察、测量和/或评估上述特性或特征，包括但不限于ELISA、竞争性ELISA、表面等离子体共振分析(BIACORE™)或KINEXA™、体外或体内中和测定法、受体-配体结合测定法、细胞因子或生长因子生成和/或分泌测定法、以及信号转导和免疫组织化学测定法。

[0053] 本文中所使用的关于LAG-3结合剂的活性的术语“抑制”或“中和”是指能够基本上拮抗、禁止、预防、限制、减缓、破坏、改变、消除、停止或逆转(例如,LAG-3的生物活性或者与LAG-3相关的疾病或病症的)进展或严重程度。本发明的分离的LAG-3结合剂优选地对LAG-3的活性抑制或中和至少约20％、约30％、约40％、约50％、约60％、约70％、约80％、约90％、约95％、约100％,或者由上述任意两个值限定的范围。

[0054] 本发明的分离的LAG-3结合剂可以是如本文所述完整的抗体或者是抗体片段。术语“抗体的片段”、“抗体片段”和“抗体的功能性片段”在本文中可互换使用,以表示保留特异性结合抗原的能力的一个或多个抗体片段(大体参见,Holliger等,Nat.Biotech.,23(9):1126-1129(2005))。所述分离的LAG-3结合剂可以含有结合LAG-3的任意抗体片段。理想地,所述抗体片段包括,例如,一个或多个CDR、可变区(或其部分)、恒定区(或其部分)、或其组合。抗体片段的实例包括,但不限于,(i)Fab片段,其是由V_L、V_H、C_L和CH_1结构域组成的单价片段,(ii)$F(ab')_2$片段,其是包括在铰链区通过二硫键连接的两个Fab片段的二价片段,(iii)由抗体单臂的V_L和V_H结构域组成的Fv片段,(iv)Fab'片段,其通过用温和的还原条件破坏$F(ab')_2$片段的二硫桥而生成,(v)经二硫键稳定的Fv片段(dsFv),和(vi)结构域抗体(dAb),其是特异性结合抗原的抗体单可变区结构域(VH或VL)多肽。

[0055] 在分离的LAG-3结合剂包括免疫球蛋白重链或轻链多肽的片段的实施方案中,只要所述片段与LAG结合并且优选地抑制LAG-3的活性,那么所述片段可以是任意的大小。从这方面来说,免疫球蛋白重链多肽的片段理想地包括约5个到18个之间的(例如,约5个、6个、7个、8个、9个、10个、11个、12个、13个、14个、15个、16个、17个、18个,或者由任意两个前述值定义的范围的)氨基酸。类似地,免疫球蛋白轻链多肽的片段理想地包括约5个到18个之间的(例如,约5个、6个、7个、8个、9个、10个、11个、12个、13个、14个、15个、16个、17个、18个,或者由任意两个前述值定义的范围的)氨基酸。

[0056] 当LAG-3结合剂是抗体或抗体片段时,所述抗体或抗体片段理想地包括任意合适类别的重链恒定区(F_c)。优选地,所述抗体或抗体片段包括基于野生型IgG1、IgG2或IgG4抗体或其变体的重链恒定区。在一些实施方案中,所述LAG-3结合剂包括Fc区,所述Fc区被改造成减少或消除所述抗体的效应子功能。如同用于改造Fc区以减少或消除效应子功能的技术一样,具有降低或废除的效应子功能的改造过的Fc区也是本领域已知的并且可以商购获得,其中任何一种都可以与本发明结合使用。

[0057] LAG-3结合剂也可以是单链抗体片段。单链抗体片段的实例包括,但不限于,(i)单链Fv(scFv),其是由Fv片段的两个结构域(即,V_L和V_H)通过合成的接头连接组成的单价分子,所述接头使得两个结构域能够被合成为单个多肽链(参见,例如,Bird等,Science,242:423-426(1988);Huston等,Proc.Natl.Acad.Sci.USA,85:5879-5883(1988);和Osbourn等,Nat.Biotechnol.,16:778(1998))和(ii)双价抗体,其是多肽链的二聚体,其中每个多肽链包括V_H通过肽接头连接V_L,所述接头太短以至于不能让同一条多肽链上的V_H和V_L之间发生配对,从而驱使不同的V_H-V_L多肽链上的互补结构域之间发生配对,以产生具有两个功能性抗原结合位点的二聚体分子。抗体片段是本领域已知的并且在例如美国专利申请公开号2009/0093024A1中有更详细的描述。

[0058] 分离的LAG-3结合剂还可以是胞内抗体(intrabody)或其片段。胞内抗体是在细胞内表达的并且在细胞内起作用的抗体。胞内抗体通常缺乏二硫键并且能通过其特异性结合

活性来调节靶基因的表达或活性。胞内抗体包括单结构域片段，如分离的V_H和V_L结构域和scFv。胞内抗体可以包括连在胞内抗体的N末端或C末端的亚细胞运送信号，以允许其在靶蛋白所处的亚细胞区室中高浓度表达。在与靶基因相互作用时，胞内抗体调节靶蛋白的功能和/或通过例如封存于非生理性的亚细胞隔室中的机制来实现表型/功能性的敲除。胞内抗体介导的基因失活的其他机制可以依赖于胞内抗体所靶向的表位，例如与靶蛋白上的催化位点或参与蛋白-蛋白、蛋白-DNA或蛋白-RNA的相互作用的表位的结合。

[0059] 分离的LAG-3结合剂也可以是抗体缀合物。就这方面而言，所述分离的LAG-3结合剂可以是(1)抗体、替代支架、或其片段的缀合物，和(2)包括所述LAG-3结合剂的蛋白质或非蛋白质部分的缀合物。例如，LAG-3结合剂可以是与肽、荧光分子或化学治疗剂缀合的抗体的全部或部分。

[0060] 分离的LAG-3结合剂可以是人抗体、非人抗体或嵌合抗体，或者可以从人抗体、非人抗体或嵌合抗体获得。“嵌合的”是指包括人和非人区域的抗体或其片段。优选地，分离的LAG-3结合剂是人源化抗体。“人源化”抗体是包括人抗体支架和至少一个获自或来源于非人抗体的CDR的单克隆抗体。非人抗体包括分离自任何非人动物比如例如啮齿动物(例如，小鼠或大鼠)的抗体。人源化抗体可以包括一个、两个或三个获自或来源于非人抗体的CDR。在本发明的一个实施方案中，本发明的LAG-3结合剂的CDRH3获自或来源于小鼠单克隆抗体，而本发明的LAG-3结合剂的其他可变区和恒定区获自或来源于人单克隆抗体。

[0061] 可以通过任意方式获得人抗体、非人抗体、嵌合抗体或人源化抗体，包括通过体外来源(例如，以重组的方式生产抗体的杂交瘤或细胞系)和体内来源(例如，啮齿动物)的方式获得。用于产生抗体的方法是本领域已知的并且在，例如，Köhler和Milstein，Eur.J.Immunol.，5:511-519(1976)；Harlow和Lane(eds.)，Antibodies:A Laboratory Manual，CSH Press(1988)；以及Janeway等(eds.)，Immunobiology，5th Ed.，Garland Publishing，New York，NY(2001))中有描述。在某些实施方案中，可以使用其中一个或多个内源性免疫球蛋白基因被一个或多个人免疫球蛋白基因替换的转基因动物(例如，小鼠)产生人抗体或嵌合抗体。内源性抗体基因被人抗体基因有效替换的转基因小鼠的实例包括，但不限于，Medarex HUMAB-MOUSE™、Kirin TC MOUSE™，和Kyowa Kirin KM-MOUSE™(参见，例如，Lonberg，Nat.Biotechnol.，23(9):1117-25(2005)和Lonberg，Handb.Exp.Pharmacol.，181:69-97(2008))。可以使用本领域已知的任何合适的方法生成人源化抗体(参见，例如，An，Z.(ed.)，Therapeutic Monoclonal Antibodies:From Bench to Clinic，John Wiley&Sons，Inc.，Hoboken，New Jersey(2009))，包括，例如，将非人CDR移植到人抗体支架上(参见，例如，Kashmiri等，Methods，36(1):25-34(2005)；和Hou等，J.Biochem.，144(1):115-120(2008))。在一个实施方案中，可以使用例如美国专利申请公开号2011/0287485A1中所描述的方法制备人源化抗体。

[0062] 在一个实施方案中，通过使用蛋白质化学或重组DNA技术，可以将本文所述免疫球蛋白重链多肽和/或免疫球蛋白轻链多肽的CDR(例如，CDR1、CDR2或CDR3)或可变区移植(即，嫁接(grafted))到另一个分子(例如，抗体或非抗体多肽)上。就这方面而言，本发明提供了一种分离的LAG-3结合剂，其包括本文所述的免疫球蛋白重链和/或轻链多肽的至少一个CDR。如本文所述，分离的LAG-3结合剂可以包括免疫球蛋白重链和/或轻链可变区的一个、两个或三个CDR。

[0063] 在优选的实施方案中,LAG-3结合剂结合LAG-3的表位,其阻断了LAG-3与MHC II类分子的结合并抑制了LAG-3介导的信号传导。例如,所述LAG-3结合剂可以与LAG-3蛋白的四个Ig样胞外结构域(D1-D4)中的一个或多个结合(参见,例如,Triebel等,J.Exp.Med.,171(5):1393-1405(1990);和Bruniquel等,Immunogenetics,47:96-98(1997))。优选地,所述LAG-3结合剂结合LAG-3蛋白的结构域1(D1)和/或结构域2(D2)。本发明还提供了分离的或纯化的LAG-3表位,其以间接或变构的方式阻断LAG-3与MHC II类分子的结合。

[0064] 本发明还提供了一种或多种分离的或纯化的核酸序列,所述序列编码本发明的免疫球蛋白重链多肽、本发明的免疫球蛋白轻链多肽和本发明的LAG-3结合剂。

[0065] 术语“核酸序列”旨在涵盖DNA或RNA的聚合物,即多核苷酸,其可以是单链或双链并且其可以含有非天然的或改变的核苷酸。本文所用的术语“核酸”和“多核苷酸”是指任意长度的核苷酸(核糖核苷酸(RNA)或脱氧核糖核苷酸(DNA))聚合形式。这些术语是指所述分子的一级结构,并且因此包括双链和单链DNA,以及双链和单链RNA。作为等同物,所述术语包括从核苷酸类似物和经修饰的多核苷酸(例如,但不限于,甲基化的和/或封端的多核苷酸)制得的RNA或DNA的类似物。尽管本领域中已知许多其它连接(例如,硫代磷酸酯phosphorothioate、硼烷磷酸酯boranophosphate等),但是核酸通常通过磷酸键连接以形成核酸序列或多核苷酸。

[0066] 本发明还提供了一种载体,所述载体包括一种或多种编码本发明的免疫球蛋白重链多肽、本发明的免疫球蛋白轻链多肽和/或本发明的LAG-3结合剂的核酸序列。所述载体可以是,例如,质粒、附加体(episome)、粘粒、病毒载体(例如,逆转录病毒或腺病毒)或噬菌体。合适的载体和载体制备方法是本领域熟知的(参见,例如,Sambrook等,Molecular Cloning,a Laboratory Manual,3rd edition,Cold Spring Harbor Press,Cold Spring Harbor,N.Y.(2001),和Ausubel等,Current Protocols in Molecular Biology,Greene Publishing Associates and John Wiley&Sons,New York,N.Y.(1994))。

[0067] 除了编码本发明的免疫球蛋白重多肽、本发明的免疫球蛋白轻链多肽和/或本发明的LAG-3-结合剂的核酸序列之外,所述载体优选地还包括表达控制序列,例如启动子、增强子、多聚腺苷酸化信号、转录终止子、信号肽(例如,骨粘连蛋白信号肽)、内部核糖体进入位点(IRES)等,所述表达控制序列使得编码序列能够在宿主细胞中表达。示例性的表达控制序列是本领域已知的并且在,例如,Goeddel,Gene Expression Technology:Methods in Enzymology,Vol.185,Academic出版社,San Diego,Calif.(1990)中有描述。

[0068] 来自多种不同来源的大量启动子,包括组成型启动子、诱导型启动子和阻抑性启动子是本领域熟知的。启动子的代表性来源包括例如病毒、哺乳动物、昆虫、植物、酵母和细菌,并且来自这些来源的合适的启动子较易获得或者可以基于可公开获得的序列(例如,从诸如ATCC等文库以及其他商业或个人来源公开获得的序列)合成制得。启动子可以是单向的(即,在一个方向上启动转录)或者双向的(即,在或3’或5’方向上启动转录)。启动子的非限制性实例包括,例如,T7细菌表达系统、pBAD(araA)细菌表达系统、巨细胞病毒(CMV)启动子、SV40启动子、RSV启动子。诱导型启动子包括,例如,Tet系统(美国专利号5,464,758和5,814,618)、Ecdysone诱导系统(No等,Proc.Natl.Acad.Sci.,93:3346-3351(1996))、T-REX™系统(Invitrogen,卡尔斯巴德,美国加利福尼亚州)、LACSWITCH™系统(Stratagene,San Diego,CA)和Cre-ERT他莫昔芬可诱导的重组酶系统(Indra等,Nuc.Acid.Res.,27:4324-

4327(1999);Nuc.Acid.Res.,28:e99(2000);美国专利号7,112,715;以及Kramer&Fussenegger,Methods Mol.Biol.,308:123-144(2005))。

[0069] 本文使用的术语“增强子”是指这样的DNA序列:其提高(例如,其以可操作的方式所连接的核酸序列)的转录。增强子可以处于远离所述核酸序列的编码区几千碱基的地方,并且可以介导调节因子的结合、DNA甲基化的模式或者DNA结构的变化。来自各种不同来源的大量的增强子是本领域公知的,并且可以(从,例如,诸如ATCC等的文库以及其他商业或个人来源)以克隆的多核苷酸形式获得或者以存在于克隆的多核苷酸内的形式获得。一些包括启动子(例如,常用的CMV启动子)的多核苷酸还包括增强子序列。增强子可以位于编码序列的上游、内部或下游。

[0070] 载体也可以包括“选择性标记基因”。本文使用的术语“选择性标记基因”是指这样的核酸序列:在存在相应的选择剂的条件下,其允许特异性地选择或剔除表达所述核酸序列的细胞。合适的选择性标记基因是本领域已知的,并且在,例如,国际专利申请公开WO 1992/008796和WO 1994/028143;Wigler等,Proc.Natl.Acad.Sci.USA,77:3567-3570(1980);O'Hare等,Proc.Natl.Acad.Sci.USA,78:1527-1531(1981);Mulligan&Berg,Proc.Natl.Acad.Sci.USA,78:2072-2076(1981);Colberre-Garapin等,J.Mol.Biol.,150:1-14(1981);Santerre等,Gene,30:147-156(1984);Kent等,Science,237:901-903(1987);Wigler等,Cell,11:223-232(1977);Szybalska&Szybalski,Proc.Natl.Acad.Sci.USA,48:2026-2034(1962);Lowy等,Cell,22:817-823(1980);以及美国专利号5,122,464和5,770,359中有描述。

[0071] 在一些实施方案中,所述载体是“附加型表达载体”或“附加体”,其能够在宿主细胞中复制,并且在存在适当的选择压力的条件下作为DNA的染色体外段在所述宿主细胞内持续存在(参见,例如,Conese等,Gene Therapy,11:1735-1742(2004))。代表性的可商购获得的附加型表达载体包括,但不限于,利用爱泼斯坦巴尔核抗原1(EBNA1)和爱泼斯坦巴尔病毒(EBV)复制起点(oriP)的附加型质粒。来自Invitrogen(卡尔斯巴德,美国加利福尼亚州)的pREP4、pCEP4、pREP7和pcDNA3.1载体以及来自Stratagene(拉由拉市,美国加利福尼亚州)的pBK-CMV载体代表了使用T-抗原和SV40复制起点来代替EBNA1和oriP的附加型载体的非限制性实例。

[0072] 其它合适的载体包括整合型表达载体,其可以随机整合到宿主细胞的DNA中,或者可以包括重组位点从而使得所述表达载体和所述宿主细胞的染色体之间能够进行特异性重组。这种整合型表达载体可以利用所述宿主细胞染色体的内源性表达控制序列来影响目标蛋白的表达。以位点特异性的方式整合的载体的实例包括,例如,Invitrogen(卡尔斯巴德,美国加利福尼亚州)的flp-in系统的组分(例如,pcDNA™5/FRT),或者cre-lox系统,例如在来自Stratagene(拉由拉市,美国加利福尼亚州)的pExchange-6核心载体中存在的cre-lox系统。可以随机整合进宿主细胞染色体中的载体的实例包括,例如,Life Technologies(卡尔斯巴德,美国加利福尼亚州)的pcDNA3.1(当在不存在T抗原的情况下引入时),Millipore公司(比尔里卡,美国马萨诸塞州)的UCOE,以及Promega公司(麦迪逊,美国威斯康星州)的pCI或pFN10A(ACT)FLEXI™。

[0073] 也可以使用病毒载体。代表性的可商购获得的病毒表达载体包括,但不限于,从Crucell公司(莱顿,荷兰)购得的基于腺病毒的Per.C6系统,来自Invitrogen(卡尔斯巴德,

美国加利福尼亚州)的基于慢病毒的pLP1,以及来自Stratagene(拉由拉市,美国加利福尼亚州)的pFB-ERV+pCFB-EGSH逆转录病毒载体。

[0074] 可以将编码本发明的多个氨基酸序列的多个核酸序列放在同一个载体上提供给细胞(即,以顺式的方式)。单向启动子可被用于控制每个核酸序列的表达。在另一实施方案中,可以使用双向和单向启动子的组合来控制多个核酸序列的表达。或者,可以将编码本发明的多个氨基酸序列的多个核酸序列各自放在单独的载体(即,以反式的方式)上提供给细胞群。每个所述在单独载体中的核酸序列可以包括相同或不同的表达控制序列。可以将各单独的载体同时或依次提供给细胞。

[0075] 可以将包含编码本发明的氨基酸序列的核酸的载体导入能够就此表达被编码的多肽的宿主细胞中,所述宿主细胞包括任何合适的原核细胞或真核细胞。因此,本发明提供了包括本发明的载体的分离的细胞。优选的宿主细胞是这样的细胞:其可以容易且稳定地(reliably)生长,具有还算快的生长速率,具有良好表征的表达系统,并且可以容易且有效地进行转化或转染。

[0076] 合适的原核细胞的实例包括,但不限于,来自芽孢杆菌属(例如,枯草芽孢杆菌和短芽孢杆菌)、大肠杆菌属(例如,E.coli)、假单胞菌属、链霉菌属、沙门氏菌属和欧文氏菌属的细胞。特别有用的原核细胞包括多种大肠杆菌菌株(例如,K12、HB101(ATCC No.33694)、DH5α、DH10、MC1061(ATCC No.53338)和CC102)。

[0077] 优选地,将载体导入真核细胞。合适的真核细胞是本领域已知的并且包括,例如,酵母细胞、昆虫细胞和哺乳动物细胞。合适的酵母细胞的实例包括来自克鲁维酵母属(Kluyveromyce)、毕赤酵母属(Pichia)、鼻孢子菌属(Rhino-sporidium)、酿酒酵母属(Saccharomyces)和裂殖酵母属(Schizosaccharomyces)的细胞。优选的酵母细胞包括例如酿酒酵母(Saccharomyces cerivisae)和巴斯德毕赤酵母(Pichia pastoris)。

[0078] 合适的昆虫细胞在,例如,Kitts等,Biotechniques,14:810-817(1993);Lucklow,Curr.Opin.Biotechnol.,4:564-572(1993);和Lucklow等,J.Virol.,67:4566-4579(1993)中有描述。优选的昆虫细胞包括Sf-9和HI5(Invitrogen,卡尔斯巴德,美国加利福尼亚州)。

[0079] 本发明优选地使用哺乳动物细胞。许多合适的哺乳动物宿主细胞是本领域已知的,并且很多可从美国典型培养物保藏中心(ATCC,马纳萨斯,美国弗吉尼亚州)获得。合适的哺乳动物细胞的实例包括,但不限于,中国仓鼠卵巢细胞(CHO)(ATCC No.CCL61)、CHO DHFR细胞(Urlaub等,Proc.Natl.Acad.Sci.USA,97:4216-4220(1980))、人类胚胎肾(HEK)293或293T细胞(ATCC No.CRL1573)和3T3细胞(ATCC No.CCL92)。其它合适的哺乳动物细胞系是猴COS-1(ATCC No.CRL1650)和COS-7细胞系(ATCC No.CRL1651)以及CV-1细胞系(ATCC No.CCL70)。其它示例性哺乳动物宿主细胞包括灵长类动物细胞系和啮齿动物细胞系,包括转化的细胞系。正常二倍体细胞、源自原代组织的体外培养的细胞株、以及原代外植体也是合适的。其他合适的哺乳动物细胞系包括,但不限于,小鼠成神经细胞瘤N2A细胞、HeLa、小鼠L-929细胞以及BHK或HaK仓鼠细胞系,所有这些都可从ATCC获得。用于选择合适的哺乳动物宿主细胞的方法以及用于转化、培养、扩增、筛选和纯化细胞的方法是本领域已知的。

[0080] 在一个实施方案中,所述哺乳动物细胞是人细胞。例如,所述哺乳动物细胞可以是人淋巴样细胞或从淋巴样细胞衍生出来的细胞系,例如具有前B淋巴细胞起源的细胞系。人淋巴样细胞系的实例包括,但不限于,RAMOS(CRL-1596)、Daudi(CCL-213)、EB-3(CCL-85)、

DT40(CRL-2111)、18-81(Jack等,Proc.Natl.Acad.Sci.USA,85:1581-1585(1988))、Raji细胞(CCL-86)、PER.C6细胞(Crucell Holland B.V.,莱顿,荷兰)、及其衍生物。

[0081] 可以通过“转染”、“转化”或“转导”将编码本发明的氨基酸序列的核酸序列引入细胞。本文所用的“转染”、“转化”或“转导”是指通过使用物理或化学方法将一种或多种外源性多核苷酸引入宿主细胞。很多转染技术是本领域已知的并且包括,例如,磷酸钙DNA共沉淀法(参见,例如,Murray E.J.(ed.),Methods in Molecular Biology,Vol.7,Gene Transfer and Expression Protocols,Humana Press(1991));DEAE-葡聚糖法;电穿孔法;阳离子脂质体介导的转染法;钨颗粒促进的微粒轰击法(Johnston,Nature,346:776-777(1990));以及磷酸锶DNA共沉淀法(Brash等,Mol.Cell Biol.,7:2031-2034(1987))。在感染性颗粒在合适的包装细胞(其有很多可以通过商购获得)中生长了之后,可将噬菌体或病毒载体导入宿主细胞。

[0082] 本发明提供了一种组合物,所述组合物包括有效量的本发明的免疫球蛋白重链多肽、本发明的免疫球蛋白轻链多肽、本发明的LAG-3结合剂、本发明的编码前述任何一种的核酸序列、或者包括本发明的核酸序列的本发明的载体。优选地,所述组合物是药学上可接受的(例如,生理上可接受的)组合物,其包括运载体,优选地药学上可接受的(例如,生理上可接受的)运载体,以及本发明的氨基酸序列、抗原结合剂或载体。任何合适的运载体都可以在本发明的背景下使用,并且这种运载体是本领域熟知的。运载体的选择部分取决于组合物可以施用的特定部位和用于施用组合物的特定方法。任选地,组合物可以是无菌的。可以将组合物冷冻或冻干储存并且于使用前在合适的无菌运载体中重构。可以根据在,例如,Remington:The Science and Practice of Pharmacy,第21版,Lippincott Williams& Wilkins,Philadelphia,PA(2001)中所描述的常规技术来生成所述组合物。

[0083] 本发明还提供了一种在哺乳动物中治疗对LAG-3的抑制或中和有应答的疾病的方法。所述方法包括向患有对LAG-3的抑制或中和有应答的疾病的哺乳动物施用前述组合物,从而在该哺乳动物中治疗所述疾病。“对LAG-3的抑制有应答的”或者“对LAG-3的中和有应答”的疾病是指任意以下疾病或紊乱:其中哺乳动物(优选人)中LAG-3水平或活性的降低具有治疗益处,或者LAG-3的不正常表达(例如,过表达)或增加的活性导致或促成所述疾病或紊乱的病理效应。对LAG-3的抑制有应答的疾病包括,例如,癌症和感染性疾病。本发明的方法可用于治疗本领域已知的任何类型的癌症,比如例如,黑色素瘤、肾细胞癌、肺癌、膀胱癌、乳腺癌、子宫颈癌、结肠癌、胆囊癌、喉癌、肝癌、甲状腺癌、胃癌、唾液腺癌、前列腺癌、胰腺癌或默克尔细胞癌(参见,例如,Bhatia等,Curr.Oncol.Rep.,13(6):488-497(2011))。本发明的方法可用于治疗任何类型的感染性疾病(即,由细菌、病毒、真菌或寄生虫引起的疾病或紊乱)。可以通过本发明的方法治疗的感染性疾病的实例包括,但不限于,由人类免疫缺陷病毒(HIV)、呼吸道合胞病毒(RSV)、流感病毒、登革热病毒、乙型肝炎病毒(HBV,或丙型肝炎病毒(HCV))引起的疾病。施用包括本发明的免疫球蛋白重链多肽、本发明的免疫球蛋白轻链多肽、本发明的LAG-3结合剂、编码前述任何一种的本发明的核酸序列、或者包括本发明的核酸序列的本发明的载体的组合物,能在哺乳动物中诱导针对癌症或感染性疾病的免疫应答。“免疫应答”可能涉及,例如,抗体的产生和/或免疫效应细胞(例如,T细胞)的激活。

[0084] 本文使用的术语“治疗(treatment)”、“治疗(treating)”等是指获得所想要的药

理学和/或生理学效应。优选地，所述效应是治疗性的，即，所述效应部分地或完全地治愈疾病和/或可归因于所述疾病的不利症状。为此，本发明的方法包括施用“治疗有效量的”LAG-3结合剂。“治疗有效量”是指在必要的时间段内以各种剂型有效达到所想要的治疗结果的量。治疗有效量可以根据多种因素而变化，例如个体的疾病状态、年龄、性别和体重以及LAG-3结合剂在个体中引发所需应答的能力。例如，本发明的LAG-3结合剂的治疗有效量是降低人体中LAG-3生物活性的量。

[0085] 或者，所述药理学和/或生理学效应可以是预防性的，即，所述效应完全地或部分地防止疾病或其症状。就这方面而言，本发明的方法包括施用“预防性有效量的”LAG-3结合剂。“预防性有效量”是指在必要的时间段内以各种剂型有效达到所想要的预防结果(例如，预防疾病发作)的量。

[0086] 典型的剂量可以是，例如，以动物或人体重计算1pg/kg到20mg/kg的范围；然而，低于或高于该示例性范围的剂量也在本发明范围内。每日肠胃外剂量可以是以总体重计算约0.00001μg/kg到约20mg/kg(例如，约0.001μg/kg、约0.1μg/kg、约1μg/kg、约5μg/kg、约10μg/kg、约100μg/kg、约500μg/kg、约1mg/kg、约5mg/kg、约10mg/kg，或由前述值中的任意两个定义的范围)，优选以总体重计算从约0.1μg/kg到约10mg/kg(例如，约0.5μg/kg、约1μg/kg、约50μg/kg、约150μg/kg、约300μg/kg、约750μg/kg、约1.5mg/kg、约5mg/kg，或由前述值中的任意两个定义的范围)，更优选以总体重计算从约1μg/kg到5mg/kg(例如，约3μg/kg、约15μg/kg、约75μg/kg、约300μg/kg、约900μg/kg、约2mg/kg、约4mg/kg、或由前述值中的任意两个定义的范围)，以及甚至更优选以体重计算从约0.5到15mg/kg/天(例如，约1mg/kg、约2.5mg/kg、约3mg/kg、约6mg/kg、约9mg/kg、约11mg/kg、约13mg/kg、或由前述值中的任意两个定义的范围)。可以通过定期评估所治疗的患者来监测治疗或预防功效。对于数天或更长时间的重复施用，可以根据病情，重复所述治疗直到发生所想要的对疾病症状的抑制。然而，其它剂量方案也可能有用并且在本发明范围内。可以通过单次推注施用组合物、通过多次推注施用组合物、或者通过连续输注施用组合物来递送所需剂量。

[0087] 可以使用标准的施用技术，包括口服、静脉内、腹膜内、皮下、经肺、经皮、肌内、鼻内、口腔、舌下或栓剂将组合物施用给哺乳动物，所述组合物包括有效量的本发明的免疫球蛋白重链多肽、本发明的免疫球蛋白轻链多肽、本发明的LAG-3结合剂、本发明的编码前述任意一种的核酸序列、或者本发明所述包括本发明的核酸序列的载体。组合物优选适用于肠胃外施用。本文使用的术语“肠胃外”包括静脉内施用、肌内施用、皮下施用、直肠施用、阴道施用和腹膜内施用。更优选地，使用通过静脉内注射、腹膜内注射或皮下注射的外周全身性递送将所述组合物施用给哺乳动物。

[0088] 一旦施用给了哺乳动物(例如，具有交叉反应性的人)，就可以通过本领域已知的任何合适的方法来测量本发明的LAG-3结合剂的生物学活性。例如，可以通过确定特定LAG-3结合剂的稳定性来评估生物学活性。在本发明的一个实施方案中，LAG-3结合剂(例如，抗体)具有在约30分钟和45天之间(例如，约30分钟、约45分钟、约1小时、约2小时、约4小时、约6小时、约10小时、约12小时、约1天、约5天、约10天、约15天、约25天、约35天、约40天、约45天、或者由任意两个前述值定义的范围)的体内半衰期。在另一实施方案中，LAG-3结合剂具有在约2小时和20天之间(例如，约5小时、约10小时、约15小时、约20小时、约2天、约3天、约7天、约12天、约14天、约17天、约19天、或者由任意两个前述值定义的范围)的体内半衰期。在

另一实施方案中，LAG-3结合剂具有在约10天和约40天之间(例如，约10天、约13天、约16天、约18天、约20天、约23天、约26天、约29天、约30天、约33天、约37天、约38天、约39天、约40天、或者由任意两个前述值定义的范围)的体内半衰期。

[0089] 也可以通过确定特定的LAG-3结合剂与LAG-3或其表位的结合亲和力来评估其生物学活性。术语“亲和力”是指两种试剂可逆性结合的平衡常数并且以解离常数(K_D)表示。结合剂对配体的亲和力(例如，抗体对表位的亲和力)可以是，例如，约1皮摩尔(pM)到约100微摩尔(μM)(例如，从约1皮摩尔(pM)到约1纳摩尔(nM)，从约1nM到约1微摩尔(μM)，或者从约1μM到约100μM)。在一个实施方案中，LAG-3结合剂可以以小于或等于1纳摩尔的K_D结合LAG-3蛋白(例如，0.9nM、0.8nM、0.7nM、0.6nM、0.5nM、0.4nM、0.3nM、0.2nM、0.1nM、0.05nM、0.025nM、0.01nM、0.001nM、或者由任意两个前述值定义的范围)。在另一实施方案中，LAG-3结合剂可以以小于或等于200pM的K_D结合LAG-3(例如，190pM、175pM、150pM、125pM、110pM、100pM、90pM、80pM、75pM、60pM、50pM、40pM、30pM、25pM、20pM、15pM、10pM、5pM、1pM、或者由任意两个前述值定义的范围)。可以使用任何本领域认可的测定法来测量对目的抗原或表位的免疫球蛋白亲和力。这样的方法包括，例如，荧光激活细胞分选法(FACS)、可分离珠(例如，磁珠)法、表面等离子体共振法(SPR)、液相竞争法(KINEXA™)、抗原淘选法和/或ELISA(参见，例如，Janeway等(eds.)，Immunobiology，5th ed.，Garland Publishing，New York，NY，2001)。

[0090] 可以单独施用或者与其它药物(例如，作为佐剂的药物)组合施用本发明的LAG-3结合剂。例如，可以将LAG-3结合剂与其它药物组合施用，用于治疗或预防本文中所公开的疾病。就这方面而言，可以将LAG-3结合剂与至少一种其他抗癌剂(包括，例如，本领域已知的任何化学治疗剂)、电离辐射、小分子抗癌剂、癌症疫苗、生物疗法(例如，其他单克隆抗体、杀癌病毒、基因疗法和过继性T细胞转移)和/或手术组合使用。当用本发明的方法治疗感染性疾病时，可以将LAG-3结合剂与至少一种抗菌剂或至少一种抗病毒剂组合施用。就这方面而言，所述抗菌剂可以是本领域已知的任何合适的抗生素。所述抗病毒剂可以是特异性靶向特定病毒的任何合适类别的任意疫苗(例如，活减毒疫苗、亚单位疫苗、重组载体疫苗和小分子抗病毒疗法(例如，病毒复制抑制剂和核苷类似物))。

[0091] 在另一实施方案中，可以将本发明的LAG-3结合剂与抑制免疫检查点通路的其它试剂组合施用。例如，可以将本发明的LAG-3结合剂与抑制或拮抗程序性死亡1(PD-1)通路、T细胞免疫球蛋白结构域和粘蛋白结构域3蛋白(TIM-3)通路和细胞毒性T淋巴细胞相关蛋白4(CTLA-4)通路的试剂组合施用。已经证明，同时靶向这些免疫检查点通路中的两种或多种的组合治疗具有改善的并且潜在协同性的抗肿瘤活性(参见，例如，Sakuishi等，J.Exp.Med.，207:2187-2194(2010)；Ngiow等，Cancer Res.，71:3540-3551(2011)；和Woo等，Cancer Res.，72:917-927(2012))。在一个实施方案中，将本发明的LAG-3结合剂与结合TIM-3的抗体和/或结合PD-1的抗体组合施用。就这方面而言，本发明的治疗哺乳动物的癌症或感染性疾病的方法还可以包括向所述哺乳动物施用包括(i)结合TIM-3蛋白的抗体和(ii)药学上可接受的运载体的组合物，或者包括(i)结合PD-1蛋白的抗体和(ii)药学上可接受的运载体的组合物。

[0092] 除治疗用途外，本文的LAG-3结合剂可应用于诊断或研究。就这方面而言，LAG-3结合剂可用在紊乱或疾病的诊断方法中，其中在所述紊乱或疾病中，LAG-3的不适当表达(例

如,过表达)或者升高的活性会导致或有助于产生所述疾病或紊乱的病理学效应。在被用来测试对LAG-3的抑制有应答的疾病或紊乱的受试者中,可以以类似的方式,在监测受试者中LAG-3蛋白水平的测定法中使用LAG-3结合剂。研究上的应用包括,例如,利用LAG-3结合剂和标记物来检测样品中(例如,在人的体液中或者在细胞或组织提取物中)LAG-3蛋白的方法。可以对LAG-3结合剂进行修饰或者不做修饰(例如,被可检测部分共价地或非共价地标记),进行使用。例如,所述可检测部分可以是放射性同位素(例如,^{3}H、^{14}C、^{32}P、^{35}S或^{125}I)、荧光化合物或化学发光化合物(例如,异硫氰酸荧光素、罗丹明或荧光素)、酶(例如,碱性磷酸酶、β-半乳糖苷酶或辣根过氧化物酶)、或者假体基团。本领域已知的用于将抗原结合剂(例如,抗体)单独缀合到可检测部分上的任何方法都可以在本发明的背景下使用(参见,例如,Hunter等,Nature,194:495-496(1962);David等,Biochemistry,13:1014-1021(1974);Pain等,J.Immunol.Meth.,40:219-230(1981);和Nygren,J.Histochem.and Cytochem.,30:407-412(1982))。

[0093] 可以使用本发明的LAG-3结合剂,通过本领域已知的任何合适的方法来测量LAG-3的蛋白水平。这些方法包括,例如,放射免疫测定法(RIA)和FACS。可以使用任何合适的技术,例如,通过在适合于形成抗原-抗体复合物的条件下将包括(或者疑似包括)LAG-3的样品与LAG-3特异性抗体组合,来确立LAG-3的正常表达值或标准表达值。将所述抗体用可检测物质直接或间接标记,以便于检测结合的或者未结合的抗体。合适的可检测物质包括各种酶、假体基团、荧光材料、发光材料和放射性材料(参见,例如,Zola,Monoclonal Antibodies:A Manual of Techniques,CRC Press,Inc.(1987))。然后将样品中表达的LAG-3多肽的量与标准值进行比较。

[0094] 可以在试剂盒(即,预定量的试剂的包装组合并且带有用于执行诊断测定法的说明书)中提供LAG-3结合剂。如果用酶标记所述LAG-3结合剂,则所述试剂盒理想地包括底物和所述酶所需的辅因子(例如,提供可检测的发色团或荧光团的底物前体)。此外,试剂盒中可以包括其它添加物,例如稳定剂、缓冲液(例如,封闭缓冲液或裂解缓冲液)等。可以改变各种试剂的相对量,使得试剂的溶液浓度基本上能优化测定法的灵敏度。提供的试剂可以是干粉形式(通常是冻干形式),包括一旦溶解将会提供具有适当浓度的试剂溶液的赋形剂。

[0095] 以下实施例进一步说明本发明,但是当然不应被理解成以任何方式限制其范围。

[0096] 实施例1

[0097] 本实施例展示了一种产生针对人LAG-3的单克隆抗体的方法。

[0098] 将编码人LAG-3的胞外结构域(ECD)的基因与小鼠IgG2a(人LAG-3 mIgG2a Fc)或失效形式的芥末绿(wasabi)荧光蛋白(dWFP人LAG-3)融合以产生抗原,用于小鼠免疫和杂交瘤筛选。具体地,雌性瑞士韦伯斯特(Swiss Webster,SWR)小鼠购自Harlan Laboratories公司(印第安纳波利斯,美国印第安纳州)并且分成两组。经过6天的环境适应后,使用弗氏完全佐剂(CFA)或弗氏不完全佐剂(IFA),对一组小鼠以50μg/小鼠的剂量以三到四周的间隔施用四到六次纯化的人LAG-3 mIgG2a Fc进行免疫。第二组动物以三到四周的间隔交替注射四到六次人LAG-3 mIgG2a Fc或dWFP人LAG-3 ECD。在第二组中也将CFA或IFA用作佐剂。将动物放血,用于评估针对人LAG-3的血清滴度,其中通过测量与细胞表面人LAG-3的结合来进行评估。用与H-2Kk跨膜结构域融合的全长人LAG-3胞外结构域转染CHO-S

细胞(CHO-S huLAG-3 ECD细胞)。以1:1,000-1:1,000,000稀释血清,并在4℃下与CHO-S huLAG-3 ECD细胞一起孵育30分钟。将细胞离心,用PBS/1%BSA洗涤一次,并与PE缀合的(Southern Biotech,伯明翰,美国阿拉巴马州)或者ALEXAFLUOR™647-(Jackson Immunoresearch,西格罗夫,美国宾夕法尼亚州)标记的山羊抗小鼠IgG(H+L)在4℃下共孵育30分钟。将细胞在PBS/1%BSA中洗涤两次,重悬于PBS/1%BSA中,并在BD FACSARRAY™生物分析仪(BD Biosciences,Franklin Lakes,NJ)上分析。根据滴度读数,每组有1只动物在收集脾脏前3天进行免疫加强。从脾脏组织制备单细胞悬浮液,并且用于使用标准技术通过细胞融合产生杂交瘤。使用两种不同的骨髓瘤细胞系进行融合,F0(描述于de St.Groth和Scheidegger,J.Immunol.Methods,35:1-21(1980)中)和P3X63Ag8.653(描述于Kearney等,J.Immunol.,123:1548-1550(1979)中)。

[0099] 如上所述,筛选了杂交瘤上清液与CHO-S huLAG-3 ECD细胞的结合情况并且与其和未转染的CHO-S细胞的结合情况进行比较。在结合CHO-S huLAG-3 ECD细胞的基础上,将杂交瘤转移到48孔板并扩增。

[0100] 然后测试了上清液对DyL650标记的人LAG-3 mIgG2a Fc(人LAG-3 mIgG2a Fc DyL650)与Daudi细胞结合的阻断能力,其中Daudi细胞是内源性表达高水平MHC II(LAG-3受体)的B细胞系。简言之,在加入Daudi细胞之前,将人LAG-3 mIgG2a Fc DyL650与对照IgG或抗人LAG-3的候选单克隆抗体预孵育。使用BD FACSARRAY™生物分析仪,通过Daudi细胞的荧光降低来测量阻断作用。然后将这些杂交瘤进一步克隆并扩增到平板上用于产生排液上清(exhaust supernatant)。随后纯化并重新测试抗体以确认与CHO-S huLAG-3 ECD细胞的结合和在Daudi测定法中的阻断能力。

[0101] 本实施例的结果证实使用杂交瘤细胞技术能生产抗LAG-3的单克隆抗体。

[0102] 实施例2

[0103] 本实施例描述了CDR移植和嵌合的抗LAG-3单克隆抗体的设计和生成。

[0104] 对来自实施例1中所描述的杂交瘤的抗体进行同种型检测,进行RT-PCR以克隆抗体重链可变区(V_H)和轻链可变区(V_L)并对其进行测序。具体地,使用RNEASY™试剂盒(Qiagen,芬洛,荷兰)从杂交瘤克隆的细胞团($1x10^6$细胞/团)中分离RNA,并使用以oligo-dT为引物的SUPERSCRIPT™III第一链合成系统(Life Technologies,卡尔斯巴德,美国加利福尼亚州)制备cDNA。V_L的PCR扩增利用了简并的小鼠V_L正向引物池(参见Kontermann和Dubel,eds.,Antibody Engineering,Springer-Verlag,Berlin(2001))和小鼠κ恒定区反向引物。V_H的PCR扩增利用了简并的小鼠V_H正向引物池(Kontermann和Dubel,同上)和小鼠γ1或γ2a恒定区反向引物(基于来自每个克隆的纯化抗体测定的同种型),使用了SUPERSCRIPT™III第一链合成系统(Life Technologies,卡尔斯巴德,美国加利福尼亚州)中推荐的方案进行。将PCR产物纯化并克隆到pcDNA3.3-TOPO(Life Technologies,卡尔斯巴德,美国加利福尼亚州)中。

[0105] 从每个细胞团中选择独立菌落并使用标准的Sanger测序法(Genewiz,Inc.,South Plainfield,NJ)进行测序。检测可变区序列并将其与最接近的人重链或轻链V区种系序列进行比对。选择三个抗体用于CDR移植,分别命名为(1)5.B11、(2)5.D7和(3)1.E10。

[0106] 通过将来自上述每种小鼠抗体的CDR残基克隆到最接近的人种系同源物中来设计CDR移植的抗体序列。合成CDR移植的抗体可变区,并与人IgG1/κ恒定区一起表达用于分析。

另外，使用上述小鼠抗体的可变区与人IgG1/κ恒定区连接来构建小鼠：人嵌合抗体。对于嵌合的和CDR移植的抗体，就其与CHO-S huLAG-3 ECD细胞的结合以及在上述人LAG-3 ECD/Daudi阻断测定法中的活性方面，对其进行了表征。

[0107] 还在人$CD4^+$T细胞：树突细胞混合的淋巴细胞反应(MLR)测定法中测试了嵌合的和CDR移植的抗体的功能拮抗剂活性，其中在所述MLR测定法中，在存在抗LAG-3抗体的情况下通过测量IL-2的分泌来评估$CD4^+$T细胞的活化。由于LAG-3是T细胞功能的负调节因子，所以预期对LAG-3的拮抗作用可以导致T细胞活化的升高，其中T细胞活化的升高可以通过IL-2产量的升高而测得。通过测得IL-2活性的升高发现，移植了5.B11、5.D7和1.E10的CDR的抗体在MLR测定法中显示出了拮抗活性。

[0108] 本实施例的结果展示了一种生成嵌合的和CDR移植的单克隆抗体的方法，所述单克隆抗体特异性结合并抑制LAG-3。

[0109] 实施例3

[0110] 本实施例展示了针对人LAG-3的人源化单克隆抗体的亲和力成熟。

[0111] 通过生物信息学预测(in silico)体细胞高频突变(iSHM)来对衍生自实施例2所述的5.D7和1.E10两种原始的鼠单克隆抗体的CDR移植抗体进行亲和力成熟。该方法集合了通过计算分析比较了从NCBI下载的体内成熟的抗体序列，并且将其与人种系IGHV、IGKV和IGLV序列及其等位基因形式进行比较(如Bowers等，J.Biol.Chem.，288(11):7688-7696(2013)中所述)所预测的突变。使用表面等离子体共振法(SPR)测定了所得抗体针对LAG-3的结合特性以及测定了如前所述的与CHO-S huLAG-3 ECD细胞结合的能力。还使用KINEXA™3000测定法(Sapidyne Instruments，博伊西，美国爱达荷州)执行了基于溶液的亲和力分析，并使用3.2.6版本的KINEXA™Pro软件分析结果。对实验参数进行了选择使得在0.8和1.2V之间抗体单独存在时达到最大信号，同时将单独存在缓冲液时的非特异性结合信号限制到小于最大信号的10％。通过使用溶解在50mM Na_2CO_3中的人或食蟹猴WFP-LAG-3(1mL中50μg/mL)溶液稀释吖内酯小珠(azlactone beads)(50mg)来使其被抗原包被。将所述溶液在室温下旋转2小时，在picofuge离心机中沉淀小珠并用封闭液(10mg/mL BSA，1M Tris-HCl，pH 8.0)洗涤两次。将小珠重新悬浮在封闭液(1mL)中，在室温下旋转1小时，并在25份体积的PBS/0.02％NaN_3中稀释。用于亲和力测定的二抗是ALEXFLUOR™647染料标记的抗人IgG(500ng/mL)。使样品抗体的浓度保持不变(50pM或75pM)，但是对人或食蟹猴WFP-LAG-3抗原用从1μM到17pM的三倍倍比稀释进行了滴定。所有样品在PBS、0.2％NaN_3、1mg/mL BSA中稀释，并使其在室温下平衡30小时。另外，测试了仅含有抗体和仅含有缓冲液的样品，以分别确定最大信号和非特异性结合信号。

[0112] 使用如McConnell等，Protein Eng.Des.Sel.，26:151(2013)中所描述的Thermofluor测定法来评估所选抗体的热稳定性。该测定法通过疏水性荧光染料结合蛋白质表面上的随蛋白质解折叠而暴露出来的疏水性补丁(patches)的能力评估稳定性。确定50％的蛋白质解折叠时的温度(Tm)以测量热稳定性。该测定法证明，5.D7单克隆抗体变体具有适合于药物开发的可接受的熔解温度(T_m)(即，大于70℃)。

[0113] 通过评估与阴性靶细胞的非特异性结合来消除与所测抗体的体内药物动力学相关的潜在问题带来的风险(参见，例如，Hotzel等，mAbs，4:753-760(2012))。使用基于流式细胞术的测定法测试了抗体与HEK 293f细胞的结合。结果表明，对于5.D7来说非特异性结

合较低并且可以通过第二步纯化进一步消除。

[0114] 本实施例的结果证实了一种针对LAG-3的人源化单克隆抗体的亲和性成熟方法。

[0115] 实施例4

[0116] 本实施例展示了一种从可进化文库中鉴定针对人LAG-3的抗体的方法。

[0117] 根据Bowers等.Proc.Natl.Acad.Sci.USA,108(51):20455-20460(2011)中的描述,构建了基于种系序列V基因区段连接衍生自人供体的重组(D)J区的IgG可进化文库。将IgG重链(HC)和轻链(LC)克隆进单独的附加型载体(Horlick等,Gene,243(1-2):187-194(2000))中,并且每个载体编码不同的抗生素选择标记。格式化HC载体使得抗体既能递呈于细胞表面上也能分泌到组织培养基中(Horlick等,J.Biol.Chem.,288(27):19861-19869(2013))。将不同的HC和LC组合共转染进HEK293细胞中并扩增至约10^9个细胞。然后针对单独的链霉亲和素(SA)偶联磁珠(目录号11047,Life Technologies,卡尔斯巴德,美国加利福尼亚州)和经SA偶联磁珠包被的不相关的生物素化抗原,分别对细胞文库进行两轮阴性筛选。然后使用被人LAG-3 mIgG2a Fc直接包被的磁珠或者用被生物素化的LAG-3 ECDmIgG1Fc包被的SA偶联磁珠执行一轮阳性筛选。将通过阳性筛选出来的细胞稀释并以1-10个细胞/孔的近似密度进行96孔铺板。将所得克隆在子板上扩增,并且通过FACSARRAY™分析测试每个群落中的一部分与LAG-3 ECD mIgG1Fc DyL650的结合情况。对于分泌到上清液中的抗体,也通过BIACORE™测试其与LAG-3 ECD mIgG1Fc结合的能力。

[0118] 对通过FACSARRAY™分析显示出针对人LAG-3 mIgG2a Fc DyL650具有特异性染色的和/或通过BIACORE™显示出结合人LAG-3 mIgG2a Fc DyL650的细胞,对其进行扩增以用于分选并将其提交用于测序,以复原能够结合人LAG-3的特定的HC/LC组合。通过PCR来获取(rescue)编码所述细胞群中发现的抗体的HC和LC的开放阅读框(ORF)。通常来说,通过测序发现多个HC/LC序列。在一些情况下,通过使用人LAG-3 mIgG2a Fc DyL650进行首轮FACS分选来富集表达目标单克隆抗体的细胞,从而鉴定所需的HC/LC组合。将呈现出高抗体表达并与人LAG-3 mIgG2a Fc DyL650的结合呈阳性的细胞群分离出来,并进行随后的序列分析。总体来说,12个不同的HC/LC对被鉴定为潜在的适合进一步表征的特异性抗LAG-3抗体。将这些配对策略(strategies)被标记为A1/A14、A2、A3/A17、A4/A19、A5/A16、A6、A8/A20、A9、A10/A15、A11、A12和A13。

[0119] 还对抗体结合食蟹猴LAG-3蛋白(cyno LAG-3)的能力进行了表征。由于从所述文库中鉴定出的这些种系抗体太弱以至于不能结合表达在细胞表面的抗原,因此用DyL650标记了与人抗原相似的可溶性抗原(cyno LAG-3 mIgG2a Fc DyL650),然后与HEK293细胞孵育,所述HEK293细胞在细胞表面陈列抗体配对策略。测试了从所述可进化文库中鉴定出的八种抗体配对策略,并且证明其具有结合cyno LAG-3 ECD mIgG1Fc的能力。

[0120] 本实施例的结果证实,可以使用可进化文库来鉴定针对人和非人LAG-3的单克隆抗体。

[0121] 实施例5

[0122] 本实施例展示了使用可进化文库鉴定出的针对人LAG-3的抗体的亲和力成熟。

[0123] 用活化诱导的胞苷脱氨酶(AID)转染共表达从实施例4中所描述的可进化文库中鉴定出的每种抗体的HC和LC的稳定细胞系,以引发体外SHM。还将AID直接转染入了从文库筛选扩增出来的原始混合细胞群中。在所有情况下,对细胞群体的IgG表达和抗原结合进行

了染色，通过流式细胞术将其收集成大细胞群，然后对其进行扩增以通过下一代测序法(NGS)进行序列分析。对于每种配对策略，迭代重复该过程，使得对于每种配对策略，积累重链和轻链可变区及其衍生物中源自SHM的突变。通过(1)SPR，(2)结合CHO-S huLAG-3 ECD细胞的能力和(3)在MLR测定法中的活性，来监测亲和力的改善。随着每种抗体亲和性的改善，提高了选择严格性，直到通过鉴定和重新组合新的突变来实现亲和性的目标。

[0124] 使用如上所述的Thermofluor测定法来评估所选抗体的热稳定性。该测定法证明，从所述配对策略A17选择出来的单克隆抗体具有适合于药物开发的可接受的Tm。还使用了基于流式细胞术的测定法测试了抗体与HEK 293f细胞的结合。结果表明，所选的候选物A17的非特异性结合较低。

[0125] 如上所述，测试了所选抗体阻断DyL650标记的人LAG-3 mIgG2a Fc(人LAG-3 mIgG2a Fc DyL650)与Daudi细胞结合的能力。将一定剂量范围的中和性抗体与可溶性LAG-3预孵育并用流式细胞术进行分析。某些亲和力成熟的抗LAG-3抗体完全抑制了可溶性LAG-3与MHC II的相互作用。

[0126] 本实施例的结果证实了一种使得经可进化文库鉴定出的针对LAG-3的单克隆抗体的亲和力成熟的方法。

[0127] 实施例6

[0128] 本实施例证明本发明所述抗LAG-3的单克隆抗体可以在体内单独地以及以与抗PD-1抗体或抗TIM-3抗体组合地抑制LAG-3信号传导并且增强T细胞活化。

[0129] 为了建立用于抗LAG-3和抗PD-1组合研究的参数，在上述人CD4+T细胞MLR测定法中以剂量-应答的方式滴定了抗PD-1的抗体APE02058。在多次MLR测定中滴定所述抗PD-1抗体的结果的基础上，选择了133pM(近似EC50)和13pM(近似EC10)用于在拮抗剂研究中测试其与抗LAG-3单克隆抗体的组合。当与133pM或13.3pM的抗PD-1组合时，抗LAG-3单克隆抗体的EC50从690pM(仅存在抗LAG-3)下降到40pM(+133pM抗PD-1)或200pM(+13.3pM抗PD-1)，效力分别升高了17倍和3倍。

[0130] 为了建立用于抗LAG-3和抗TIM-3组合研究的参数，在上述人CD4+T细胞MLR测定法中以剂量-应答的方式滴定了抗LAG-3的抗体APE05505。在多次MLR测定中滴定所述抗LAG-3抗体的结果的基础上，选择了2nM(近似EC50)和0.2nM(近似EC10)用于在拮抗剂研究中测试其与抗TIM-3单克隆抗体的组合。当与2nM或0.2nM抗LAG-3组合时，抗LAG-3 mAb的EC50从11nM(仅存在抗LAG-3)下降到6nM(+0.2nM抗TIM-3)或3nM(+2nM抗TIM-3)，效力分别升高了1.8倍和3.6倍。

[0131] 本实施例的结果证明，本发明的LAG-3结合剂可以单独地以及与免疫系统其它负调节因子的拮抗剂组合来抑制LAG-3的生物学活性。

[0132] 实施例7

[0133] 本实施例证明本发明的抗LAG-3单克隆抗体可以在体内与抗PD-1抗体组合来抑制LAG-3信号传导并且增强T细胞活化。

[0134] 在MC38同系肿瘤模型中单独测试了抗小鼠LAG-3替代型单克隆抗体(mAb C9B7W，BioXcell，西黎巴嫩，美国新罕布什尔州)的活性，或者其与抗小鼠PD-1替代型单克隆抗体(mAb RMP1-14，BioXcell，西黎巴嫩，美国新罕布什尔州)组合时的活性。对所有组(10只动物/组)皮下注射$1x10^6$个MC38细胞。接种后10天，将动物按肿瘤大小随机分组。在第1天、第4

天、第8天和第11天，用5mg/kg的抗PD-1单克隆抗体和/或10mg/kg的抗LAG-3单克隆抗体治疗小鼠，每种抗体或抗体组合共给药4次。每周测量两次肿瘤，以评估对治疗的应答。抗PD-1+抗LAG-3的组合在降低肿瘤生长方面比单独使用单一药物更有效。在组合治疗组中观察到所有10只动物都有完全应答，相比之下，在仅给予PD-1的组中观察到7只动物具有完全应答以及在仅给于抗LAG-3的组中没有动物具有完全应答。然后通过用$4x10^6$个MC38细胞进行皮下接种，对来自组合组的显示完全应答的9只动物进行了再接种(rechallenged)。在再接种组中，没有动物发展出可测量的肿瘤，但是注射有相同量细胞的所有对照初始小鼠都生长出了明显的肿瘤。

[0135] 还在Colon26同系肿瘤模型中单独地或以组合的形式测试了上文描述的替代型单克隆抗体的活性。所有组(12只动物/组)皮下注射$5x\ 10^5$个Colon26细胞。在第4天、第7天、第11天和第14天，用10mg/kg抗PD-1抗体和/或10mg/kg抗LAG-3抗体治疗小鼠，每种抗体或抗体组合共给药4次。每周测量两次肿瘤以评估对治疗的应答。抗PD-1+抗LAG-3的组合在肿瘤生长方面比单独的每种单一药物更有效。在组合治疗组中观察到12只动物中有10只具有完全应答，相比之下，在仅给予PD-1的组中观察到三只动物具有完全应答以及在仅给予抗LAG-3的组中有一只动物具有完全应答。然后用$5x10^5$个Colon26细胞对来自组合组的显示完全应答的九只动物进行了再接种(rechallenged)。在再接种组中没有动物发展出可测量的肿瘤，但是注射有相同量细胞的所有对照初始小鼠都生长出了明显的肿瘤。

[0136] 本实施例的结果证明，本发明的LAG-3结合剂在与免疫系统的其他负调节因子的拮抗剂组合时，能够在体内抑制LAG-3的生物学活性。

[0137] 实施例8

[0138] 本实施例证明，抗体同种型在单独的抗LAG-3抗体的抗肿瘤活性或者在抗LAG-3抗体与抗PD-1抗体组合时在同系小鼠肿瘤模型中的抗肿瘤活性的效应。

[0139] 从大鼠杂交瘤细胞系中测序和克隆抗小鼠LAG-3中和性抗体(mAb C9B7W，BioXcell，西黎巴嫩，美国新罕布什尔州)的可变区之后，将其克隆进小鼠IgG1或小鼠IgG2a表达载体，产生识别小鼠LAG-3的IgG1(D265A)和IgG2a同种型替代抗体。然后测试这些抗体在单独存在时以及与识别小鼠PD-1的小鼠IgG1(D265A)替代抗体组合时的功效，所述小鼠IgG1(D265A)替代抗体以类似的方式从购自BioXcel(mAb RMP1-14，西黎巴嫩，美国新罕布什尔州)的大鼠抗体产生。具体地，将Colon26结肠腺癌细胞($5x10^5$s.c.)植入Balb/c小鼠中并使其生长3天。将小鼠随机分为7组(12只动物/组)，并如表1所示在第4天、第7天、第11天和第14天施予每种抗体或抗体组合。注射了匹配的同种型抗体的小鼠被用作对照。每周两次测量肿瘤体积，直到研究结束。

[0140] 表1

[0141]

组别	治疗	剂量
1	同种型 IgG2a +同种型 IgG1(D265A)	10 mg/kg，1 mg/kg
2	同种型 IgG1 (D265A)	10 mg/kg
3	抗 mPD-1 IgG1(D265A)	1 mg/kg
4	抗 mLAG-3 IgG2a	10 mg/kg
5	抗 mLAG-3 IgG1(D265A)	10 mg/kg
6	抗 mPD-1 IgG1(D265A) + 抗 mLAG-3 IgG2a	1 mg/kg，10 mg/kg
7	抗 mPD-1 IgG1(D265A)+ 抗 mLAG-3 IgG1(D265A)	1 mg/kg，10 mg/kg

[0142] 该实验的结果示于图1A和1B中，其显示与具有效应子功能的抗小鼠LAG-3抗体(即，IgG2a)(其对肿瘤生长没有明显的效应)相比，具有最低效应子功能的单一试剂的抗小鼠LAG-3抗体(即，IgG1(D265A))具有抗肿瘤功效。

[0143] 另外，图1A显示了与单独的抗小鼠PD-1 IgG1(D265A)抗体相比，具有最低效应子功能的抗小鼠LAG-3抗体(即，IgG1(D265A))与抗小鼠PD-1 IgG1(D265A)抗体的治疗方案的组合显示出升高的抗肿瘤活性。然而，具有完整效应子功能(IgG2a)的抗小鼠LAG-3抗体与抗小鼠PD-1抗体的组合不如单独的抗小鼠PD-1 IgG1(D265A)有效，表明抗体的效应子功能可能干扰了抗小鼠PD-1介导的功效。

[0144] 图1B提供了来自第3治疗组(用抗小鼠PD-1 IgG1(D265A)抗体治疗的动物组)、第7组(用抗小鼠PD-1 IgG1(D265A)抗体与抗小鼠LAG-3IgG1(D265A)抗体的组合治疗的动物组)和第6组(用抗小鼠PD-1 IgG1(D265A)抗体与抗小鼠LAG-3IgG2抗体的组合治疗的动物组)的动物个体的肿瘤体积随时间的变化图。在第7组(抗小鼠PD-1 IgG1(D265A)抗体与抗小鼠LAG-3IgG1(D265A)的组合组)中，12只动物中的8只在研究结束时未见肿瘤生长。相比之下，在第6组(抗小鼠PD-1 IgG1(D265A)抗体与抗小鼠LAG-3IgG2抗体的组合组)中/12只动物中仅有3只在研究结束时未见肿瘤。在第3组(单独使用抗小鼠PD-1 IgG1(D265A)的组)中，12只动物中的6只在研究结束时无肿瘤，表明当与抗小鼠PD-1 IgG1(D265A)抗体组合施予时，抗小鼠LAG-3IgG2抗体的效应子功能可能造成干扰。

[0145] 本实施例的结果证明，没有效应子功能的抗小鼠LAG-3和抗小鼠PD-1抗体在单独和以组合的形式下都可以在小鼠同系肿瘤模型中抑制肿瘤生长。当使用具有效应子功能的抗小鼠LAG-3抗体时未观察到功效，并且还可能干扰抗PD-1介导的功效。

[0146] 实施例9

[0147] 本实施例证明，在混合淋巴细胞反应中，可以基于收获时间将本发明所述抗LAG-3单克隆抗体的抑制活性与抗PD-1单克隆抗体的抑制活性区分开来，并且与PD-1和LAG-3的表达相关。

[0148] 在人CD4+T细胞的混合淋巴细胞反应(MLR)测定法中测试了功能性LAG-3拮抗剂抗体，其中在所述测定法中通过测量IL-2的分泌评估了在抗LAG-3抗体存在的条件下CD4+T细胞的活化。抗LAG-3抗体与拮抗性抗PD-1抗体被一起测试，其中在不同时间点添加和/或收获所述抗体。具体地，从人供体分离得到的外周血单核细胞被分化成树突状细胞(DC)，然后与从第二供体分离得到的CD4+T细胞混合。在共培养开始时或者在共培养开始后24小时时

加入抑制性抗体。在抗体加入后24和48小时测量IL-2的水平。

[0149] 预期对LAG-3和PD-1的拮抗作用可以导致T细胞活化升高，其中T细胞活化升高可以通过IL-2产量的升高而测得。当在检测开始时加入，抗PD-1抗体在抗体加入后24和48小时提升了IL-2的分泌量，而抗LAG-3抗体在48小时时的MLR测定中提升了IL-2的分泌量，但在24小时没有提升IL-2的分泌量。当在共培养开始后24小时加入抑制性抗LAG-3或抗PD-1抗体并在72小时收获时，两种抗体都有活性并且EC50似乎相当(图2A)。这与表达相关，因为在24-72小时观察到了升高的PD-1的表达，而LAG-3似乎在48和72小时的测定中更晚表达(图2B)。

[0150] 本实施例的结果证明，LAG-3抑制的效果与靶点表达相关，并且LAG-3的表达在时间上比PD-1更晚发生。

[0151] 本文引用的所有参考文献，包括出版物、专利申请和专利，在此通过引用并入本文，其程度如同每个参考文献被单独和具体地指明通过引用并入并且全部在此列出。

[0152] 除非本文另有说明或者与上下文明显矛盾，否则在描述本发明的上下文中(特别是在所附权利要求的上下文中)使用的术语“一”和“一个”以及“所述”和“至少一个”及相似的指示词应被解释成涵盖单数和复数。除非本文另有说明或者与上下文明显矛盾，否则使用的术语“至少一个”后跟一个或多个项目列表(例如，“A和B”中的至少一个)应被解释成表示从列出的项目中选出的一个项目(A或B)或者所列项目中的两个或多个的任意组合(A和B)。除非另有说明，否则术语“包含”、“具有”、“包括”和“含有”将被解释成开放式术语(即表示“包括，但不限于，”)。除非本文另有说明，否则本文中关于值的范围的描述仅旨在用作单独提及落在所述范围内的每个单独值的简便方法，并且将每个单独值并入本说明书中，如同在本文中单独列举一样。除非本文另有说明或者与上下文明显矛盾，否则本文所述的所有方法可以以任何合适的顺序执行。对本文提供的任何及所有的实施例或示例性语言(例如，“例如”)的使用，仅旨在更好地说明本发明，并且除非另有说明，否则不对本发明的范围构成限制。本说明书中的语言不应被解释成表示任何未被要求保护的元件对于本发明的实践是必需的。

[0153] 本文描述了本发明的优选实施方案，包括发明人已知的用于实施本发明的最佳模式。在阅读了前面的描述之后，对于本领域普通技术人员来说，这些优选实施方案的变化形式会变得显而易见。发明人可以预期本领域技术人员可适当地应用这些变化形式，并且发明人预期到以不同于本文具体描述的方式来实施本发明。因此，本发明包括适用法律所允许的所附权利要求中列举主题的所有修改和等同物。此外，除非本文另有说明或者与上下文明显矛盾，否则本发明涵盖了上述元件的所有可能的变化形式的任何组合。

序列表

<110> 安奈普泰斯生物有限公司

<120> 针对淋巴细胞活化基因3(LAG-3)的抗体

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<221> MISC_FEATURE

<222> (28)..(28)

<223> Xaa1 是 天冬酰胺(Asn) 或 丝氨酸(Ser)

<220>

<221> MISC_FEATURE

<222> (30)..(30)

<223> Xaa2 是 赖氨酸(Lys)、酪氨酸(Tyr)、或 天冬酰胺(Asn)

<220>

<221> MISC_FEATURE

<222> (38)..(38)

<223> Xaa3 是 赖氨酸(Lys) 或 谷氨酰胺(Gln)

<220>

<221> MISC_FEATURE

<222> (48)..(48)

<223> Xaa4 是 异亮氨酸(Ile) 或 甲硫氨酸(Met)

<220>

<221> MISC_FEATURE

<222> (53)..(53)

<223> Xaa5 是 丙氨酸(Ala) 或 脯氨酸(Pro)

<220>

<221> MISC_FEATURE

<222> (56)..(56)

<223> Xaa6 是 甘氨酸(Gly)、天冬酰胺(Asn)、或 天冬氨酸(Asp)

<220>
<221> MISC_FEATURE
<222> (61)..(61)
<223> Xaa7 是 丙氨酸(Ala) 或 丝氨酸(Ser)
<220>
<221> MISC_FEATURE
<222> (65)..(65)
<223> Xaa8 是 谷氨酰胺(Gln) 或 精氨酸(Arg)
<220>
<221> MISC_FEATURE
<222> (77)..(77)
<223> Xaa9 是 天冬氨酸(Asp) 或 天冬酰胺(Asn)
<400> 1

Glu Val Gln Leu Val Gln Ser Gly Ala Glu Val Lys Lys Pro Gly Ala
1 5 10 15
Thr Val Lys Ile Ser Cys Lys Ala Ser Gly Phe Xaa Ile Xaa Asp Asp
20 25 30
Tyr Ile His Trp Val Xaa Gln Ala Pro Gly Lys Gly Leu Glu Trp Xaa
35 40 45
Gly Trp Ile Asp Xaa Glu Asn Xaa Asp Ser Glu Tyr Xaa Ser Lys Phe
50 55 60
Xaa Gly Arg Val Thr Ile Thr Val Asp Thr Ser Thr Xaa Thr Ala Tyr
65 70 75 80
Met Glu Leu Ser Ser Leu Arg Ser Glu Asp Thr Ala Val Tyr Tyr Cys
85 90 95
Thr Tyr Ala Phe Gly Gly Tyr Trp Gly Gln Gly Thr Thr Val Thr Val
100 105 110
Ser Ser

<210> 2
<211> 114
<212> PRT
<213> 人工序列
<220>
<223> 合成序列
<400> 2

Glu Val Gln Leu Val Gln Ser Gly Ala Glu Val Lys Lys Pro Gly Ala
1 5 10 15
Thr Val Lys Ile Ser Cys Lys Ala Ser Gly Phe Asn Ile Lys Asp Asp
20 25 30

Tyr Ile His Trp Val Lys Gln Ala Pro Gly Lys Gly Leu Glu Trp Ile
35 40 45
Gly Trp Ile Asp Pro Glu Asn Gly Asp Ser Glu Tyr Ala Ser Lys Phe
50 55 60
Gln Gly Arg Val Thr Ile Thr Val Asp Thr Ser Thr Asn Thr Ala Tyr
65 70 75 80
Met Glu Leu Ser Ser Leu Arg Ser Glu Asp Thr Ala Val Tyr Tyr Cys
85 90 95
Thr Tyr Ala Phe Gly Gly Tyr Trp Gly Gln Gly Thr Thr Val Thr Val
100 105 110
Ser Ser

<210> 3
<211> 114
<212> PRT
<213> 人工序列
<220>
<223> 合成序列
<400> 3

Glu Val Gln Leu Val Gln Ser Gly Ala Glu Val Lys Lys Pro Gly Ala
1 5 10 15
Thr Val Lys Ile Ser Cys Lys Ala Ser Gly Phe Ser Ile Lys Asp Asp
20 25 30
Tyr Ile His Trp Val Lys Gln Ala Pro Gly Lys Gly Leu Glu Trp Ile
35 40 45
Gly Trp Ile Asp Pro Glu Asn Gly Asp Ser Glu Tyr Ala Ser Lys Phe
50 55 60
Gln Gly Arg Val Thr Ile Thr Val Asp Thr Ser Thr Asn Thr Ala Tyr
65 70 75 80
Met Glu Leu Ser Ser Leu Arg Ser Glu Asp Thr Ala Val Tyr Tyr Cys
85 90 95
Thr Tyr Ala Phe Gly Gly Tyr Trp Gly Gln Gly Thr Thr Val Thr Val
100 105 110
Ser Ser

<210> 4
<211> 114
<212> PRT
<213> 人工序列
<220>
<223> 合成序列

<400> 4

Glu Val Gln Leu Val Gln Ser Gly Ala Glu Val Lys Lys Pro Gly Ala
1 5 10 15
Thr Val Lys Ile Ser Cys Lys Ala Ser Gly Phe Asn Ile Lys Asp Asp
20 25 30
Tyr Ile His Trp Val Lys Gln Ala Pro Gly Lys Gly Leu Glu Trp Ile
35 40 45
Gly Trp Ile Asp Pro Glu Asn Asn Asp Ser Glu Tyr Ala Ser Lys Phe
50 55 60
Gln Gly Arg Val Thr Ile Thr Val Asp Thr Ser Thr Asn Thr Ala Tyr
65 70 75 80
Met Glu Leu Ser Ser Leu Arg Ser Glu Asp Thr Ala Val Tyr Tyr Cys
85 90 95
Thr Tyr Ala Phe Gly Gly Tyr Trp Gly Gln Gly Thr Thr Val Thr Val
100 105 110
Ser Ser

<210> 5

<211> 114

<212> PRT

<213> 人工序列

<220>

<223> 合成序列

<400> 5

Glu Val Gln Leu Val Gln Ser Gly Ala Glu Val Lys Lys Pro Gly Ala
1 5 10 15
Thr Val Lys Ile Ser Cys Lys Ala Ser Gly Phe Asn Ile Lys Asp Asp
20 25 30
Tyr Ile His Trp Val Lys Gln Ala Pro Gly Lys Gly Leu Glu Trp Ile
35 40 45
Gly Trp Ile Asp Pro Glu Asn Gly Asp Ser Glu Tyr Ser Ser Lys Phe
50 55 60
Gln Gly Arg Val Thr Ile Thr Val Asp Thr Ser Thr Asn Thr Ala Tyr
65 70 75 80
Met Glu Leu Ser Ser Leu Arg Ser Glu Asp Thr Ala Val Tyr Tyr Cys
85 90 95
Thr Tyr Ala Phe Gly Gly Tyr Trp Gly Gln Gly Thr Thr Val Thr Val
100 105 110
Ser Ser

<210> 6

<211> 114
<212> PRT
<213> 人工序列
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<223> 合成序列
<400> 6

Glu Val Gln Leu Val Gln Ser Gly Ala Glu Val Lys Lys Pro Gly Ala
1 5 10 15
Thr Val Lys Ile Ser Cys Lys Ala Ser Gly Phe Asn Ile Lys Asp Asp
20 25 30
Tyr Ile His Trp Val Gln Gln Ala Pro Gly Lys Gly Leu Glu Trp Met
35 40 45
Gly Trp Ile Asp Pro Glu Asn Gly Asp Ser Glu Tyr Ala Ser Lys Phe
50 55 60
Gln Gly Arg Val Thr Ile Thr Val Asp Thr Ser Thr Asn Thr Ala Tyr
65 70 75 80
Met Glu Leu Ser Ser Leu Arg Ser Glu Asp Thr Ala Val Tyr Tyr Cys
85 90 95
Thr Tyr Ala Phe Gly Gly Tyr Trp Gly Gln Gly Thr Thr Val Thr Val
100 105 110
Ser Ser

<210> 7
<211> 114
<212> PRT
<213> 人工序列
<220>
<223> 合成序列
<400> 7

Glu Val Gln Leu Val Gln Ser Gly Ala Glu Val Lys Lys Pro Gly Ala
1 5 10 15
Thr Val Lys Ile Ser Cys Lys Ala Ser Gly Phe Asn Ile Lys Asp Asp
20 25 30
Tyr Ile His Trp Val Lys Gln Ala Pro Gly Lys Gly Leu Glu Trp Ile
35 40 45
Gly Trp Ile Asp Pro Glu Asn Asp Asp Ser Glu Tyr Ala Ser Lys Phe
50 55 60
Gln Gly Arg Val Thr Ile Thr Val Asp Thr Ser Thr Asn Thr Ala Tyr
65 70 75 80
Met Glu Leu Ser Ser Leu Arg Ser Glu Asp Thr Ala Val Tyr Tyr Cys

85 90 95
Thr Tyr Ala Phe Gly Gly Tyr Trp Gly Gln Gly Thr Thr Val Thr Val
100 105 110
Ser Ser
<210> 8
<211> 114
<212> PRT
<213> 人工序列
<220>
<223> 合成序列
<400> 8
Glu Val Gln Leu Val Gln Ser Gly Ala Glu Val Lys Lys Pro Gly Ala
1 5 10 15
Thr Val Lys Ile Ser Cys Lys Ala Ser Gly Phe Asn Ile Tyr Asp Asp
20 25 30
Tyr Ile His Trp Val Gln Gln Ala Pro Gly Lys Gly Leu Glu Trp Met
35 40 45
Gly Trp Ile Asp Pro Glu Asn Gly Asp Ser Glu Tyr Ala Ser Lys Phe
50 55 60
Gln Gly Arg Val Thr Ile Thr Val Asp Thr Ser Thr Asn Thr Ala Tyr
65 70 75 80
Met Glu Leu Ser Ser Leu Arg Ser Glu Asp Thr Ala Val Tyr Tyr Cys
85 90 95
Thr Tyr Ala Phe Gly Gly Tyr Trp Gly Gln Gly Thr Thr Val Thr Val
100 105 110
Ser Ser
<210> 9
<211> 114
<212> PRT
<213> 人工序列
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<223> 合成序列
<400> 9
Glu Val Gln Leu Val Gln Ser Gly Ala Glu Val Lys Lys Pro Gly Ala
1 5 10 15
Thr Val Lys Ile Ser Cys Lys Ala Ser Gly Phe Ser Ile Tyr Asp Asp
20 25 30
Tyr Ile His Trp Val Gln Gln Ala Pro Gly Lys Gly Leu Glu Trp Met
35 40 45

Gly Trp Ile Asp Pro Glu Asn Gly Asp Ser Glu Tyr Ala Ser Lys Phe
50 55 60
Gln Gly Arg Val Thr Ile Thr Val Asp Thr Ser Thr Asn Thr Ala Tyr
65 70 75 80
Met Glu Leu Ser Ser Leu Arg Ser Glu Asp Thr Ala Val Tyr Tyr Cys
85 90 95
Thr Tyr Ala Phe Gly Gly Tyr Trp Gly Gln Gly Thr Thr Val Thr Val
100 105 110
Ser Ser

<210> 10
<211> 114
<212> PRT
<213> 人工序列
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<223> 合成序列
<400> 10

Glu Val Gln Leu Val Gln Ser Gly Ala Glu Val Lys Lys Pro Gly Ala
1 5 10 15
Thr Val Lys Ile Ser Cys Lys Ala Ser Gly Phe Ser Ile Lys Asp Asp
20 25 30
Tyr Ile His Trp Val Gln Gln Ala Pro Gly Lys Gly Leu Glu Trp Met
35 40 45
Gly Trp Ile Asp Pro Glu Asn Gly Asp Ser Glu Tyr Ala Ser Lys Phe
50 55 60
Gln Gly Arg Val Thr Ile Thr Val Asp Thr Ser Thr Asn Thr Ala Tyr
65 70 75 80
Met Glu Leu Ser Ser Leu Arg Ser Glu Asp Thr Ala Val Tyr Tyr Cys
85 90 95
Thr Tyr Ala Phe Gly Gly Tyr Trp Gly Gln Gly Thr Thr Val Thr Val
100 105 110
Ser Ser

<210> 11
<211> 114
<212> PRT
<213> 人工序列
<220>
<223> 合成序列
<400> 11

Glu Val Gln Leu Val Gln Ser Gly Ala Glu Val Lys Lys Pro Gly Ala

1 5 10 15
Thr Val Lys Ile Ser Cys Lys Ala Ser Gly Phe Ser Ile Asn Asp Asp
20 25 30
Tyr Ile His Trp Val Gln Gln Ala Pro Gly Lys Gly Leu Glu Trp Met
35 40 45
Gly Trp Ile Asp Pro Glu Asn Gly Asp Ser Glu Tyr Ala Ser Lys Phe
50 55 60
Gln Gly Arg Val Thr Ile Thr Val Asp Thr Ser Thr Asn Thr Ala Tyr
65 70 75 80
Met Glu Leu Ser Ser Leu Arg Ser Glu Asp Thr Ala Val Tyr Tyr Cys
85 90 95
Thr Tyr Ala Phe Gly Gly Tyr Trp Gly Gln Gly Thr Thr Val Thr Val
100 105 110
Ser Ser

<210> 12
<211> 114
<212> PRT
<213> 人工序列
<220>
<223> 合成序列
<400> 12

Glu Val Gln Leu Val Gln Ser Gly Ala Glu Val Lys Lys Pro Gly Ala
1 5 10 15
Thr Val Lys Ile Ser Cys Lys Ala Ser Gly Phe Asn Ile Lys Asp Asp
20 25 30
Tyr Ile His Trp Val Gln Gln Ala Pro Gly Lys Gly Leu Glu Trp Met
35 40 45
Gly Trp Ile Asp Pro Glu Asn Asn Asp Ser Glu Tyr Ser Ser Lys Phe
50 55 60
Gln Gly Arg Val Thr Ile Thr Val Asp Thr Ser Thr Asn Thr Ala Tyr
65 70 75 80
Met Glu Leu Ser Ser Leu Arg Ser Glu Asp Thr Ala Val Tyr Tyr Cys
85 90 95
Thr Tyr Ala Phe Gly Gly Tyr Trp Gly Gln Gly Thr Thr Val Thr Val
100 105 110
Ser Ser

<210> 13
<211> 114
<212> PRT

<213> 人工序列
<220>
<223> 合成序列
<400> 13

Glu Val Gln Leu Val Gln Ser Gly Ala Glu Val Lys Lys Pro Gly Ala
1 5 10 15
Thr Val Lys Ile Ser Cys Lys Ala Ser Gly Phe Asn Ile Tyr Asp Asp
20 25 30
Tyr Ile His Trp Val Gln Gln Ala Pro Gly Lys Gly Leu Glu Trp Met
35 40 45
Gly Trp Ile Asp Pro Glu Asn Asn Asp Ser Glu Tyr Ala Ser Lys Phe
50 55 60
Gln Gly Arg Val Thr Ile Thr Val Asp Thr Ser Thr Asn Thr Ala Tyr
65 70 75 80
Met Glu Leu Ser Ser Leu Arg Ser Glu Asp Thr Ala Val Tyr Tyr Cys
85 90 95
Thr Tyr Ala Phe Gly Gly Tyr Trp Gly Gln Gly Thr Thr Val Thr Val
100 105 110
Ser Ser

<210> 14
<211> 114
<212> PRT
<213> 人工序列
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<223> 合成序列
<400> 14

Glu Val Gln Leu Val Gln Ser Gly Ala Glu Val Lys Lys Pro Gly Ala
1 5 10 15
Thr Val Lys Ile Ser Cys Lys Ala Ser Gly Phe Asn Ile Lys Asp Asp
20 25 30
Tyr Ile His Trp Val Gln Gln Ala Pro Gly Lys Gly Leu Glu Trp Met
35 40 45
Gly Trp Ile Asp Pro Glu Asn Asn Asp Ser Glu Tyr Ala Ser Lys Phe
50 55 60
Gln Gly Arg Val Thr Ile Thr Val Asp Thr Ser Thr Asn Thr Ala Tyr
65 70 75 80
Met Glu Leu Ser Ser Leu Arg Ser Glu Asp Thr Ala Val Tyr Tyr Cys
85 90 95
Thr Tyr Ala Phe Gly Gly Tyr Trp Gly Gln Gly Thr Thr Val Thr Val

100 105 110

Ser Ser

<210> 15

<211> 114

<212> PRT

<213> 人工序列

<220>

<223> 合成序列

<400> 15

Glu Val Gln Leu Val Gln Ser Gly Ala Glu Val Lys Lys Pro Gly Ala
1 5 10 15
Thr Val Lys Ile Ser Cys Lys Ala Ser Gly Phe Asn Ile Lys Asp Asp
20 25 30
Tyr Ile His Trp Val Gln Gln Ala Pro Gly Lys Gly Leu Glu Trp Met
35 40 45
Gly Trp Ile Asp Pro Glu Asn Gly Asp Ser Glu Tyr Ala Ser Lys Phe
50 55 60
Arg Gly Arg Val Thr Ile Thr Val Asp Thr Ser Thr Asn Thr Ala Tyr
65 70 75 80
Met Glu Leu Ser Ser Leu Arg Ser Glu Asp Thr Ala Val Tyr Tyr Cys
85 90 95
Thr Tyr Ala Phe Gly Gly Tyr Trp Gly Gln Gly Thr Thr Val Thr Val
100 105 110

Ser Ser

<210> 16

<211> 114

<212> PRT

<213> 人工序列

<220>

<223> 合成序列

<400> 16

Glu Val Gln Leu Val Gln Ser Gly Ala Glu Val Lys Lys Pro Gly Ala
1 5 10 15
Thr Val Lys Ile Ser Cys Lys Ala Ser Gly Phe Asn Ile Lys Asp Asp
20 25 30
Tyr Ile His Trp Val Gln Gln Ala Pro Gly Lys Gly Leu Glu Trp Met
35 40 45
Gly Trp Ile Asp Pro Glu Asn Gly Asp Ser Glu Tyr Ser Ser Lys Phe
50 55 60

Gln Gly Arg Val Thr Ile Thr Val Asp Thr Ser Thr Asn Thr Ala Tyr
65 70 75 80
Met Glu Leu Ser Ser Leu Arg Ser Glu Asp Thr Ala Val Tyr Tyr Cys
85 90 95
Thr Tyr Ala Phe Gly Gly Tyr Trp Gly Gln Gly Thr Thr Val Thr Val
100 105 110
Ser Ser

<210> 17
<211> 114
<212> PRT
<213> 人工序列
<220>
<223> 合成序列
<400> 17

Glu Val Gln Leu Val Gln Ser Gly Ala Glu Val Lys Lys Pro Gly Ala
1 5 10 15
Thr Val Lys Ile Ser Cys Lys Ala Ser Gly Phe Asn Ile Lys Asp Asp
20 25 30
Tyr Ile His Trp Val Gln Gln Ala Pro Gly Lys Gly Leu Glu Trp Met
35 40 45
Gly Trp Ile Asp Pro Glu Asn Asn Asp Ser Glu Tyr Ser Ser Lys Phe
50 55 60
Gln Gly Arg Val Thr Ile Thr Val Asp Thr Ser Thr Asn Thr Ala Tyr
65 70 75 80
Met Glu Leu Ser Ser Leu Arg Ser Glu Asp Thr Ala Val Tyr Tyr Cys
85 90 95
Thr Tyr Ala Phe Gly Gly Tyr Trp Gly Gln Gly Thr Thr Val Thr Val
100 105 110
Ser Ser

<210> 18
<211> 114
<212> PRT
<213> 人工序列
<220>
<223> 合成序列
<400> 18

Glu Val Gln Leu Val Gln Ser Gly Ala Glu Val Lys Lys Pro Gly Ala
1 5 10 15
Thr Val Lys Ile Ser Cys Lys Ala Ser Gly Phe Ser Ile Tyr Asp Asp

20 25 30
Tyr Ile His Trp Val Gln Gln Ala Pro Gly Lys Gly Leu Glu Trp Met
35 40 45
Gly Trp Ile Asp Pro Glu Asn Asn Asp Ser Glu Tyr Ser Ser Lys Phe
50 55 60
Gln Gly Arg Val Thr Ile Thr Val Asp Thr Ser Thr Asn Thr Ala Tyr
65 70 75 80
Met Glu Leu Ser Ser Leu Arg Ser Glu Asp Thr Ala Val Tyr Tyr Cys
85 90 95
Thr Tyr Ala Phe Gly Gly Tyr Trp Gly Gln Gly Thr Thr Val Thr Val
100 105 110
Ser Ser

<210> 19
<211> 114
<212> PRT
<213> 人工序列
<220>
<223> 合成序列
<400> 19
Glu Val Gln Leu Val Gln Ser Gly Ala Glu Val Lys Lys Pro Gly Ala
1 5 10 15
Thr Val Lys Ile Ser Cys Lys Ala Ser Gly Phe Ser Ile Tyr Asp Asp
20 25 30
Tyr Ile His Trp Val Gln Gln Ala Pro Gly Lys Gly Leu Glu Trp Met
35 40 45
Gly Trp Ile Asp Pro Glu Asn Gly Asp Ser Glu Tyr Ser Ser Lys Phe
50 55 60
Gln Gly Arg Val Thr Ile Thr Val Asp Thr Ser Thr Asn Thr Ala Tyr
65 70 75 80
Met Glu Leu Ser Ser Leu Arg Ser Glu Asp Thr Ala Val Tyr Tyr Cys
85 90 95
Thr Tyr Ala Phe Gly Gly Tyr Trp Gly Gln Gly Thr Thr Val Thr Val
100 105 110
Ser Ser

<210> 20
<211> 114
<212> PRT
<213> 人工序列
<220>

<223> 合成序列

<400> 20

Glu Val Gln Leu Val Gln Ser Gly Ala Glu Val Lys Lys Pro Gly Ala
1 5 10 15
Thr Val Lys Ile Ser Cys Lys Ala Ser Gly Phe Asn Ile Tyr Asp Asp
20 25 30
Tyr Ile His Trp Val Gln Gln Ala Pro Gly Lys Gly Leu Glu Trp Met
35 40 45
Gly Trp Ile Asp Pro Glu Asn Gly Asp Ser Glu Tyr Ser Ser Lys Phe
50 55 60
Gln Gly Arg Val Thr Ile Thr Val Asp Thr Ser Thr Asn Thr Ala Tyr
65 70 75 80
Met Glu Leu Ser Ser Leu Arg Ser Glu Asp Thr Ala Val Tyr Tyr Cys
85 90 95
Thr Tyr Ala Phe Gly Gly Tyr Trp Gly Gln Gly Thr Thr Val Thr Val
100 105 110
Ser Ser

<210> 21

<211> 114

<212> PRT

<213> 人工序列

<220>

<223> 合成序列

<400> 21

Glu Val Gln Leu Val Gln Ser Gly Ala Glu Val Lys Lys Pro Gly Ala
1 5 10 15
Thr Val Lys Ile Ser Cys Lys Ala Ser Gly Phe Ser Ile Lys Asp Asp
20 25 30
Tyr Ile His Trp Val Gln Gln Ala Pro Gly Lys Gly Leu Glu Trp Met
35 40 45
Gly Trp Ile Asp Pro Glu Asn Gly Asp Ser Glu Tyr Ser Ser Lys Phe
50 55 60
Gln Gly Arg Val Thr Ile Thr Val Asp Thr Ser Thr Asn Thr Ala Tyr
65 70 75 80
Met Glu Leu Ser Ser Leu Arg Ser Glu Asp Thr Ala Val Tyr Tyr Cys
85 90 95
Thr Tyr Ala Phe Gly Gly Tyr Trp Gly Gln Gly Thr Thr Val Thr Val
100 105 110
Ser Ser

<210> 22
<211> 114
<212> PRT
<213> 人工序列
<220>
<223> 合成序列
<400> 22

Glu Val Gln Leu Val Gln Ser Gly Ala Glu Val Lys Lys Pro Gly Ala
1 5 10 15
Thr Val Lys Ile Ser Cys Lys Ala Ser Gly Phe Asn Ile Lys Asp Asp
20 25 30
Tyr Ile His Trp Val Gln Gln Ala Pro Gly Lys Gly Leu Glu Trp Met
35 40 45
Gly Trp Ile Asp Pro Glu Asn Asn Asp Ser Glu Tyr Ala Ser Lys Phe
50 55 60
Arg Gly Arg Val Thr Ile Thr Val Asp Thr Ser Thr Asn Thr Ala Tyr
65 70 75 80
Met Glu Leu Ser Ser Leu Arg Ser Glu Asp Thr Ala Val Tyr Tyr Cys
85 90 95
Thr Tyr Ala Phe Gly Gly Tyr Trp Gly Gln Gly Thr Thr Val Thr Val
100 105 110
Ser Ser

<210> 23
<211> 114
<212> PRT
<213> 人工序列
<220>
<223> 合成序列
<400> 23

Glu Val Gln Leu Val Gln Ser Gly Ala Glu Val Lys Lys Pro Gly Ala
1 5 10 15
Thr Val Lys Ile Ser Cys Lys Ala Ser Gly Phe Ser Ile Tyr Asp Asp
20 25 30
Tyr Ile His Trp Val Gln Gln Ala Pro Gly Lys Gly Leu Glu Trp Met
35 40 45
Gly Trp Ile Asp Pro Glu Asn Gly Asp Ser Glu Tyr Ala Ser Lys Phe
50 55 60
Arg Gly Arg Val Thr Ile Thr Val Asp Thr Ser Thr Asn Thr Ala Tyr
65 70 75 80

Met Glu Leu Ser Ser Leu Arg Ser Glu Asp Thr Ala Val Tyr Tyr Cys
85 90 95
Thr Tyr Ala Phe Gly Gly Tyr Trp Gly Gln Gly Thr Thr Val Thr Val
100 105 110
Ser Ser
<210> 24
<211> 114
<212> PRT
<213> 人工序列
<220>
<223> 合成序列
<400> 24
Glu Val Gln Leu Val Gln Ser Gly Ala Glu Val Lys Lys Pro Gly Ala
1 5 10 15
Thr Val Lys Ile Ser Cys Lys Ala Ser Gly Phe Asn Ile Tyr Asp Asp
20 25 30
Tyr Ile His Trp Val Gln Gln Ala Pro Gly Lys Gly Leu Glu Trp Met
35 40 45
Gly Trp Ile Asp Pro Glu Asn Asn Asp Ser Glu Tyr Ser Ser Lys Phe
50 55 60
Gln Gly Arg Val Thr Ile Thr Val Asp Thr Ser Thr Asn Thr Ala Tyr
65 70 75 80
Met Glu Leu Ser Ser Leu Arg Ser Glu Asp Thr Ala Val Tyr Tyr Cys
85 90 95
Thr Tyr Ala Phe Gly Gly Tyr Trp Gly Gln Gly Thr Thr Val Thr Val
100 105 110
Ser Ser
<210> 25
<211> 114
<212> PRT
<213> 人工序列
<220>
<223> 合成序列
<400> 25
Glu Val Gln Leu Val Gln Ser Gly Ala Glu Val Lys Lys Pro Gly Ala
1 5 10 15
Thr Val Lys Ile Ser Cys Lys Ala Ser Gly Phe Ser Ile Lys Asp Asp
20 25 30
Tyr Ile His Trp Val Gln Gln Ala Pro Gly Lys Gly Leu Glu Trp Met

35 40 45

Gly Trp Ile Asp Pro Glu Asn Asn Asp Ser Glu Tyr Ala Ser Lys Phe
50 55 60

Gln Gly Arg Val Thr Ile Thr Val Asp Thr Ser Thr Asn Thr Ala Tyr
65 70 75 80

Met Glu Leu Ser Ser Leu Arg Ser Glu Asp Thr Ala Val Tyr Tyr Cys
85 90 95

Thr Tyr Ala Phe Gly Gly Tyr Trp Gly Gln Gly Thr Thr Val Thr Val
100 105 110

Ser Ser

<210> 26

<211> 114

<212> PRT

<213> 人工序列

<220>

<223> 合成序列

<400> 26

Glu Val Gln Leu Val Gln Ser Gly Ala Glu Val Lys Lys Pro Gly Ala
1 5 10 15

Thr Val Lys Ile Ser Cys Lys Ala Ser Gly Phe Ser Ile Lys Asp Asp
20 25 30

Tyr Ile His Trp Val Gln Gln Ala Pro Gly Lys Gly Leu Glu Trp Met
35 40 45

Gly Trp Ile Asp Pro Glu Asn Gly Asp Ser Glu Tyr Ala Ser Lys Phe
50 55 60

Arg Gly Arg Val Thr Ile Thr Val Asp Thr Ser Thr Asn Thr Ala Tyr
65 70 75 80

Met Glu Leu Ser Ser Leu Arg Ser Glu Asp Thr Ala Val Tyr Tyr Cys
85 90 95

Thr Tyr Ala Phe Gly Gly Tyr Trp Gly Gln Gly Thr Thr Val Thr Val
100 105 110

Ser Ser

<210> 27

<211> 114

<212> PRT

<213> 人工序列

<220>

<223> 合成序列

<400> 27

Glu Val Gln Leu Val Gln Ser Gly Ala Glu Val Lys Lys Pro Gly Ala
1 5 10 15
Thr Val Lys Ile Ser Cys Lys Ala Ser Gly Phe Ser Ile Lys Asp Asp
20 25 30
Tyr Ile His Trp Val Gln Gln Ala Pro Gly Lys Gly Leu Glu Trp Met
35 40 45
Gly Trp Ile Asp Pro Glu Asn Asn Asp Ser Glu Tyr Ser Ser Lys Phe
50 55 60
Gln Gly Arg Val Thr Ile Thr Val Asp Thr Ser Thr Asn Thr Ala Tyr
65 70 75 80
Met Glu Leu Ser Ser Leu Arg Ser Glu Asp Thr Ala Val Tyr Tyr Cys
85 90 95
Thr Tyr Ala Phe Gly Gly Tyr Trp Gly Gln Gly Thr Thr Val Thr Val
100 105 110
Ser Ser

<210> 28
<211> 114
<212> PRT
<213> 人工序列
<220>
<223> 合成序列
<400> 28

Glu Val Gln Leu Val Gln Ser Gly Ala Glu Val Lys Lys Pro Gly Ala
1 5 10 15
Thr Val Lys Ile Ser Cys Lys Ala Ser Gly Phe Asn Ile Tyr Asp Asp
20 25 30
Tyr Ile His Trp Val Gln Gln Ala Pro Gly Lys Gly Leu Glu Trp Met
35 40 45
Gly Trp Ile Asp Pro Glu Asn Asn Asp Ser Glu Tyr Ser Ser Lys Phe
50 55 60
Gln Gly Arg Val Thr Ile Thr Val Asp Thr Ser Thr Asn Thr Ala Tyr
65 70 75 80
Met Glu Leu Ser Ser Leu Arg Ser Glu Asp Thr Ala Val Tyr Tyr Cys
85 90 95
Thr Tyr Ala Phe Gly Gly Tyr Trp Gly Gln Gly Thr Thr Val Thr Val
100 105 110
Ser Ser

<210> 29
<211> 114

<212> PRT
<213> 人工序列
<220>
<223> 合成序列
<400> 29

Glu Val Gln Leu Val Gln Ser Gly Ala Glu Val Lys Lys Pro Gly Ala
1 5 10 15
Thr Val Lys Ile Ser Cys Lys Ala Ser Gly Phe Ser Ile Lys Asp Asp
20 25 30
Tyr Ile His Trp Val Gln Gln Ala Pro Gly Lys Gly Leu Glu Trp Met
35 40 45
Gly Trp Ile Asp Ala Glu Asn Asn Asp Ser Glu Tyr Ser Ser Lys Phe
50 55 60
Gln Gly Arg Val Thr Ile Thr Val Asp Thr Ser Thr Asn Thr Ala Tyr
65 70 75 80
Met Glu Leu Ser Ser Leu Arg Ser Glu Asp Thr Ala Val Tyr Tyr Cys
85 90 95
Thr Tyr Ala Phe Gly Gly Tyr Trp Gly Gln Gly Thr Thr Val Thr Val
100 105 110
Ser Ser

<210> 30
<211> 114
<212> PRT
<213> 人工序列
<220>
<223> 合成序列
<400> 30

Glu Val Gln Leu Val Gln Ser Gly Ala Glu Val Lys Lys Pro Gly Ala
1 5 10 15
Thr Val Lys Ile Ser Cys Lys Ala Ser Gly Phe Ser Ile Tyr Asp Asp
20 25 30
Tyr Ile His Trp Val Gln Gln Ala Pro Gly Lys Gly Leu Glu Trp Met
35 40 45
Gly Trp Ile Asp Ala Glu Asn Asn Asp Ser Glu Tyr Ser Ser Lys Phe
50 55 60
Gln Gly Arg Val Thr Ile Thr Val Asp Thr Ser Thr Asn Thr Ala Tyr
65 70 75 80
Met Glu Leu Ser Ser Leu Arg Ser Glu Asp Thr Ala Val Tyr Tyr Cys
85 90 95

Thr Tyr Ala Phe Gly Gly Tyr Trp Gly Gln Gly Thr Thr Val Thr Val
100 105 110

Ser Ser

<210> 31

<211> 114

<212> PRT

<213> 人工序列

<220>

<223> 合成序列

<400> 31

Glu Val Gln Leu Val Gln Ser Gly Ala Glu Val Lys Lys Pro Gly Ala
1 5 10 15

Thr Val Lys Ile Ser Cys Lys Ala Ser Gly Phe Ser Ile Lys Asp Asp
20 25 30

Tyr Ile His Trp Val Gln Gln Ala Pro Gly Lys Gly Leu Glu Trp Met
35 40 45

Gly Trp Ile Asp Ala Glu Asn Asp Asp Ser Glu Tyr Ser Ser Lys Phe
50 55 60

Gln Gly Arg Val Thr Ile Thr Val Asp Thr Ser Thr Asn Thr Ala Tyr
65 70 75 80

Met Glu Leu Ser Ser Leu Arg Ser Glu Asp Thr Ala Val Tyr Tyr Cys
85 90 95

Thr Tyr Ala Phe Gly Gly Tyr Trp Gly Gln Gly Thr Thr Val Thr Val
100 105 110

Ser Ser

<210> 32

<211> 114

<212> PRT

<213> 人工序列

<220>

<223> 合成序列

<400> 32

Glu Val Gln Leu Val Gln Ser Gly Ala Glu Val Lys Lys Pro Gly Ala
1 5 10 15

Thr Val Lys Ile Ser Cys Lys Ala Ser Gly Phe Ser Ile Tyr Asp Asp
20 25 30

Tyr Ile His Trp Val Gln Gln Ala Pro Gly Lys Gly Leu Glu Trp Met
35 40 45

Gly Trp Ile Asp Ala Glu Asn Asp Asp Ser Glu Tyr Ser Ser Lys Phe

50 55 60
Gln Gly Arg Val Thr Ile Thr Val Asp Thr Ser Thr Asn Thr Ala Tyr
65 70 75 80
Met Glu Leu Ser Ser Leu Arg Ser Glu Asp Thr Ala Val Tyr Tyr Cys
85 90 95
Thr Tyr Ala Phe Gly Gly Tyr Trp Gly Gln Gly Thr Thr Val Thr Val
100 105 110
Ser Ser

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Glu Val Gln Leu Val Gln Ser Gly Ala Glu Val Lys Lys Pro Gly Ala
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Thr Val Lys Ile Ser Cys Lys Ala Ser Gly Phe Ser Ile Lys Asp Asp
20 25 30
Tyr Ile His Trp Val Gln Gln Ala Pro Gly Lys Gly Leu Glu Trp Met
35 40 45
Gly Trp Ile Asp Ala Glu Asn Asp Asp Ser Glu Tyr Ser Ser Lys Phe
50 55 60
Gln Gly Arg Val Thr Ile Thr Val Asp Thr Ser Thr Asp Thr Ala Tyr
65 70 75 80
Met Glu Leu Ser Ser Leu Arg Ser Glu Asp Thr Ala Val Tyr Tyr Cys
85 90 95
Thr Tyr Ala Phe Gly Gly Tyr Trp Gly Gln Gly Thr Thr Val Thr Val
100 105 110
Ser Ser

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Glu Val Gln Leu Val Gln Ser Gly Ala Glu Val Lys Lys Pro Gly Ala
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Thr Val Lys Ile Ser Cys Lys Ala Ser Gly Phe Ser Ile Tyr Asp Asp
20 25 30
Tyr Ile His Trp Val Gln Gln Ala Pro Gly Lys Gly Leu Glu Trp Met
35 40 45
Gly Trp Ile Asp Ala Glu Asn Asp Asp Ser Glu Tyr Ser Ser Lys Phe
50 55 60
Gln Gly Arg Val Thr Ile Thr Val Asp Thr Ser Thr Asp Thr Ala Tyr
65 70 75 80
Met Glu Leu Ser Ser Leu Arg Ser Glu Asp Thr Ala Val Tyr Tyr Cys
85 90 95
Thr Tyr Ala Phe Gly Gly Tyr Trp Gly Gln Gly Thr Thr Val Thr Val
100 105 110
Ser Ser

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<223> Xaa8 是 丝氨酸(Ser) 或 天冬酰胺(Asn)
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<223> Xaa9 是 苯丙氨酸(Phe) 或 亮氨酸(Leu)
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<223> Xaa10 是 天冬酰胺(Asn) 或 丝氨酸(Ser)
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<400> 35

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Gln Val Gln Leu Gln Gln Trp Gly Ala Xaa Leu Leu Lys Pro Ser Glu
1               5                  10                  15
Thr Leu Ser Leu Xaa Cys Xaa Val Tyr Gly Gly Xaa Phe Xaa Gly Tyr
            20                  25                  30
Tyr Trp Xaa Trp Ile Arg Gln Pro Pro Xaa Lys Gly Leu Glu Trp Ile
        35                  40                  45
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Gly Glu Ile Asn His Ser Gly Xaa Thr Asn Tyr Asn Pro Ser Leu Lys
50 55 60
Ser Arg Val Thr Ile Ser Val Asp Thr Ser Lys Asn Gln Xaa Ser Leu
65 70 75 80
Lys Leu Xaa Xaa Val Thr Ala Ala Asp Thr Ala Val Tyr Tyr Cys Xaa
85 90 95
Arg Glu Gly Xaa Tyr Gly Asp Tyr Asp Tyr Trp Gly Gln Gly Thr Leu
100 105 110
Val Thr Val Ser Ser
115

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Gln Val Gln Leu Gln Gln Trp Gly Ala Gly Leu Leu Lys Pro Ser Glu
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Thr Leu Ser Leu Thr Cys Ala Val Tyr Gly Gly Ser Phe Ser Gly Tyr
20 25 30
Tyr Trp Ser Trp Ile Arg Gln Pro Pro Gly Lys Gly Leu Glu Trp Ile
35 40 45
Gly Glu Ile Asn His Ser Gly Ser Thr Asn Tyr Asn Pro Ser Leu Lys
50 55 60
Ser Arg Val Thr Ile Ser Val Asp Thr Ser Lys Asn Gln Phe Ser Leu
65 70 75 80
Lys Leu Ser Ser Val Thr Ala Ala Asp Thr Ala Val Tyr Tyr Cys Ala
85 90 95
Arg Glu Gly Asp Tyr Gly Asp Tyr Asp Tyr Trp Gly Gln Gly Thr Leu
100 105 110
Val Thr Val Ser Ser
115

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<400> 37

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Thr Leu Ser Leu Thr Cys Ala Val Tyr Gly Gly Ser Phe Ser Gly Tyr
20 25 30
Tyr Trp Ser Trp Ile Arg Gln Pro Pro Gly Lys Gly Leu Glu Trp Ile
35 40 45
Gly Glu Ile Asn His Ser Gly Ser Thr Asn Tyr Asn Pro Ser Leu Lys
50 55 60
Ser Arg Val Thr Ile Ser Val Asp Thr Ser Lys Asn Gln Phe Ser Leu
65 70 75 80
Lys Leu Ser Ser Val Thr Ala Ala Asp Thr Ala Val Tyr Tyr Cys Val
85 90 95
Arg Glu Gly Asp Tyr Gly Asp Tyr Asp Tyr Trp Gly Gln Gly Thr Leu
100 105 110
Val Thr Val Ser Ser
115

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Gln Val Gln Leu Gln Gln Trp Gly Ala Gly Leu Leu Lys Pro Ser Glu
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Thr Leu Ser Leu Thr Cys Ala Val Tyr Gly Gly Ser Phe Ser Gly Tyr
20 25 30
Tyr Trp Ser Trp Ile Arg Gln Pro Pro Gly Lys Gly Leu Glu Trp Ile
35 40 45
Gly Glu Ile Asn His Ser Gly Ser Thr Asn Tyr Asn Pro Ser Leu Lys
50 55 60
Ser Arg Val Thr Ile Ser Val Asp Thr Ser Lys Asn Gln Phe Ser Leu
65 70 75 80
Lys Leu Asn Ser Val Thr Ala Ala Asp Thr Ala Val Tyr Tyr Cys Ala
85 90 95
Arg Glu Gly Asp Tyr Gly Asp Tyr Asp Tyr Trp Gly Gln Gly Thr Leu
100 105 110
Val Thr Val Ser Ser

115

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Gln Val Gln Leu Gln Gln Trp Gly Ala Arg Leu Leu Lys Pro Ser Glu
1 5 10 15
Thr Leu Ser Leu Thr Cys Ala Val Tyr Gly Gly Ser Phe Ser Gly Tyr
20 25 30
Tyr Trp Ser Trp Ile Arg Gln Pro Pro Gly Lys Gly Leu Glu Trp Ile
35 40 45
Gly Glu Ile Asn His Ser Gly Ser Thr Asn Tyr Asn Pro Ser Leu Lys
50 55 60
Ser Arg Val Thr Ile Ser Val Asp Thr Ser Lys Asn Gln Phe Ser Leu
65 70 75 80
Lys Leu Ser Ser Val Thr Ala Ala Asp Thr Ala Val Tyr Tyr Cys Val
85 90 95
Arg Glu Gly Asp Tyr Gly Asp Tyr Asp Tyr Trp Gly Gln Gly Thr Leu
100 105 110
Val Thr Val Ser Ser
115

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Gln Val Gln Leu Gln Gln Trp Gly Ala Gly Leu Leu Lys Pro Ser Glu
1 5 10 15
Thr Leu Ser Leu Thr Cys Ala Val Tyr Gly Gly Ser Phe Ser Gly Tyr
20 25 30
Tyr Trp Ser Trp Ile Arg Gln Pro Pro Gly Lys Gly Leu Glu Trp Ile
35 40 45
Gly Glu Ile Asn His Ser Gly Asn Thr Asn Tyr Asn Pro Ser Leu Lys
50 55 60

Ser Arg Val Thr Ile Ser Val Asp Thr Ser Lys Asn Gln Phe Ser Leu
65 70 75 80
Lys Leu Ser Ser Val Thr Ala Ala Asp Thr Ala Val Tyr Tyr Cys Val
85 90 95
Arg Glu Gly Asp Tyr Gly Asp Tyr Asp Tyr Trp Gly Gln Gly Thr Leu
100 105 110
Val Thr Val Ser Ser
115

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Gln Val Gln Leu Gln Gln Trp Gly Ala Gly Leu Leu Lys Pro Ser Glu
1 5 10 15
Thr Leu Ser Leu Ile Cys Ala Val Tyr Gly Gly Ser Phe Ser Gly Tyr
20 25 30
Tyr Trp Ser Trp Ile Arg Gln Pro Pro Gly Lys Gly Leu Glu Trp Ile
35 40 45
Gly Glu Ile Asn His Ser Gly Asn Thr Asn Tyr Asn Pro Ser Leu Lys
50 55 60
Ser Arg Val Thr Ile Ser Val Asp Thr Ser Lys Asn Gln Phe Ser Leu
65 70 75 80
Lys Leu Ser Ser Val Thr Ala Ala Asp Thr Ala Val Tyr Tyr Cys Val
85 90 95
Arg Glu Gly Asp Tyr Gly Asp Tyr Asp Tyr Trp Gly Gln Gly Thr Leu
100 105 110
Val Thr Val Ser Ser
115

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Gln Val Gln Leu Gln Gln Trp Gly Ala Arg Leu Leu Lys Pro Ser Glu

1 5 10 15
Thr Leu Ser Leu Ile Cys Ala Val Tyr Gly Gly Ser Phe Ser Gly Tyr
20 25 30
Tyr Trp Ser Trp Ile Arg Gln Pro Pro Gly Lys Gly Leu Glu Trp Ile
35 40 45
Gly Glu Ile Asn His Ser Gly Ser Thr Asn Tyr Asn Pro Ser Leu Lys
50 55 60
Ser Arg Val Thr Ile Ser Val Asp Thr Ser Lys Asn Gln Phe Ser Leu
65 70 75 80
Lys Leu Ser Ser Val Thr Ala Ala Asp Thr Ala Val Tyr Tyr Cys Val
85 90 95
Arg Glu Gly Asp Tyr Gly Asp Tyr Asp Tyr Trp Gly Gln Gly Thr Leu
100 105 110
Val Thr Val Ser Ser
115

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Gln Val Gln Leu Gln Gln Trp Gly Ala Gly Leu Leu Lys Pro Ser Glu
1 5 10 15
Thr Leu Ser Leu Thr Cys Ala Val Tyr Gly Gly Ser Phe Ser Gly Tyr
20 25 30
Tyr Trp Ser Trp Ile Arg Gln Pro Pro Gly Lys Gly Leu Glu Trp Ile
35 40 45
Gly Glu Ile Asn His Ser Gly Asn Thr Asn Tyr Asn Pro Ser Leu Lys
50 55 60
Ser Arg Val Thr Ile Ser Val Asp Thr Ser Lys Asn Gln Phe Ser Leu
65 70 75 80
Lys Leu Ser Ser Val Thr Ala Ala Asp Thr Ala Val Tyr Tyr Cys Val
85 90 95
Arg Glu Gly Asp Tyr Gly Asp Tyr Asp Tyr Trp Gly Gln Gly Thr Leu
100 105 110
Val Thr Val Ser Ser
115

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Gln Val Gln Leu Gln Gln Trp Gly Ala Arg Leu Leu Lys Pro Ser Glu
1 5 10 15
Thr Leu Ser Leu Thr Cys Ala Val Tyr Gly Gly Ser Phe Ser Gly Tyr
20 25 30
Tyr Trp Ser Trp Ile Arg Gln Pro Pro Arg Lys Gly Leu Glu Trp Ile
35 40 45
Gly Glu Ile Asn His Ser Gly Ser Thr Asn Tyr Asn Pro Ser Leu Lys
50 55 60
Ser Arg Val Thr Ile Ser Val Asp Thr Ser Lys Asn Gln Phe Ser Leu
65 70 75 80
Lys Leu Ser Ser Val Thr Ala Ala Asp Thr Ala Val Tyr Tyr Cys Val
85 90 95
Arg Glu Gly Asp Tyr Gly Asp Tyr Asp Tyr Trp Gly Gln Gly Thr Leu
100 105 110
Val Thr Val Ser Ser
115

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Gln Val Gln Leu Gln Gln Trp Gly Ala Gly Leu Leu Lys Pro Ser Glu
1 5 10 15
Thr Leu Ser Leu Thr Cys Ala Val Tyr Gly Gly Ser Phe Ser Gly Tyr
20 25 30
Tyr Trp Ser Trp Ile Arg Gln Pro Pro Gly Lys Gly Leu Glu Trp Ile
35 40 45
Gly Glu Ile Asn His Ser Gly Asn Thr Asn Tyr Asn Pro Ser Leu Lys
50 55 60
Ser Arg Val Thr Ile Ser Val Asp Thr Ser Lys Asn Gln Phe Ser Leu
65 70 75 80

Lys Leu Ser Phe Val Thr Ala Ala Asp Thr Ala Val Tyr Tyr Cys Val
85 90 95
Arg Glu Gly Asp Tyr Gly Asp Tyr Asp Tyr Trp Gly Gln Gly Thr Leu
100 105 110
Val Thr Val Ser Ser
115

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Gln Val Gln Leu Gln Gln Trp Gly Ala Arg Leu Leu Lys Pro Ser Glu
1 5 10 15
Thr Leu Ser Leu Thr Cys Ala Val Tyr Gly Gly Ser Phe Ser Gly Tyr
20 25 30
Tyr Trp Ser Trp Ile Arg Gln Pro Pro Gly Lys Gly Leu Glu Trp Ile
35 40 45
Gly Glu Ile Asn His Ser Gly Ser Thr Asn Tyr Asn Pro Ser Leu Lys
50 55 60
Ser Arg Val Thr Ile Ser Val Asp Thr Ser Lys Asn Gln Phe Ser Leu
65 70 75 80
Lys Leu Ser Phe Val Thr Ala Ala Asp Thr Ala Val Tyr Tyr Cys Val
85 90 95
Arg Glu Gly Asp Tyr Gly Asp Tyr Asp Tyr Trp Gly Gln Gly Thr Leu
100 105 110
Val Thr Val Ser Ser
115

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Gln Val Gln Leu Gln Gln Trp Gly Ala Arg Leu Leu Lys Pro Ser Glu
1 5 10 15
Thr Leu Ser Leu Thr Cys Ala Val Tyr Gly Gly Ser Phe Ser Gly Tyr

20 25 30
Tyr Trp Ser Trp Ile Arg Gln Pro Pro Gly Lys Gly Leu Glu Trp Ile
35 40 45
Gly Glu Ile Asn His Ser Gly Asn Thr Asn Tyr Asn Pro Ser Leu Lys
50 55 60
Ser Arg Val Thr Ile Ser Val Asp Thr Ser Lys Asn Gln Phe Ser Leu
65 70 75 80
Lys Leu Ser Ser Val Thr Ala Ala Asp Thr Ala Val Tyr Tyr Cys Val
85 90 95
Arg Glu Gly Asp Tyr Gly Asp Tyr Asp Tyr Trp Gly Gln Gly Thr Leu
100 105 110
Val Thr Val Ser Ser
115

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1 5 10 15
Thr Leu Ser Leu Thr Cys Thr Val Tyr Gly Gly Ser Phe Ser Gly Tyr
20 25 30
Tyr Trp Ser Trp Ile Arg Gln Pro Pro Gly Lys Gly Leu Glu Trp Ile
35 40 45
Gly Glu Ile Asn His Ser Gly Ser Thr Asn Tyr Asn Pro Ser Leu Lys
50 55 60
Ser Arg Val Thr Ile Ser Val Asp Thr Ser Lys Asn Gln Phe Ser Leu
65 70 75 80
Lys Leu Asn Ser Val Thr Ala Ala Asp Thr Ala Val Tyr Tyr Cys Ala
85 90 95
Arg Glu Gly Asp Tyr Gly Asp Tyr Asp Tyr Trp Gly Gln Gly Thr Leu
100 105 110
Val Thr Val Ser Ser
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Gln Val Gln Leu Gln Gln Trp Gly Ala Gly Leu Leu Lys Pro Ser Glu
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Thr Leu Ser Leu Thr Cys Ala Val Tyr Gly Gly Ser Phe Ser Gly Tyr
20 25 30
Tyr Trp Ser Trp Ile Arg Gln Pro Pro Gly Lys Gly Leu Glu Trp Ile
35 40 45
Gly Glu Ile Asn His Ser Gly Ser Thr Asn Tyr Asn Pro Ser Leu Lys
50 55 60
Ser Arg Val Thr Ile Ser Val Asp Thr Ser Lys Asn Gln Leu Ser Leu
65 70 75 80
Lys Leu Asn Ser Val Thr Ala Ala Asp Thr Ala Val Tyr Tyr Cys Ala
85 90 95
Arg Glu Gly Asp Tyr Gly Asp Tyr Asp Tyr Trp Gly Gln Gly Thr Leu
100 105 110
Val Thr Val Ser Ser
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Gln Val Gln Leu Gln Gln Trp Gly Ala Gly Leu Leu Lys Pro Ser Glu
1 5 10 15
Thr Leu Ser Leu Thr Cys Ala Val Tyr Gly Gly Phe Phe Ser Gly Tyr
20 25 30
Tyr Trp Ser Trp Ile Arg Gln Pro Pro Gly Lys Gly Leu Glu Trp Ile
35 40 45
Gly Glu Ile Asn His Ser Gly Ser Thr Asn Tyr Asn Pro Ser Leu Lys
50 55 60
Ser Arg Val Thr Ile Ser Val Asp Thr Ser Lys Asn Gln Phe Ser Leu
65 70 75 80
Lys Leu Ser Ser Val Thr Ala Ala Asp Thr Ala Val Tyr Tyr Cys Val
85 90 95

Arg Glu Gly Asp Tyr Gly Asp Tyr Asp Tyr Trp Gly Gln Gly Thr Leu
100 105 110
Val Thr Val Ser Ser
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Gln Val Gln Leu Gln Gln Trp Gly Ala Gly Leu Leu Lys Pro Ser Glu
1 5 10 15
Thr Leu Ser Leu Thr Cys Ala Val Tyr Gly Gly Ser Phe Ser Gly Tyr
20 25 30
Tyr Trp Ser Trp Ile Arg Gln Pro Pro Gly Lys Gly Leu Glu Trp Ile
35 40 45
Gly Glu Ile Asn His Ser Gly Ser Thr Asn Tyr Asn Pro Ser Leu Lys
50 55 60
Ser Arg Val Thr Ile Ser Val Asp Thr Ser Lys Asn Gln Phe Ser Leu
65 70 75 80
Lys Leu Ser Ser Val Thr Ala Ala Asp Thr Ala Val Tyr Tyr Cys Val
85 90 95
Arg Glu Gly Asn Tyr Gly Asp Tyr Asp Tyr Trp Gly Gln Gly Thr Leu
100 105 110
Val Thr Val Ser Ser
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Gln Val Gln Leu Gln Gln Trp Gly Ala Gly Leu Leu Lys Pro Ser Glu
1 5 10 15
Thr Leu Ser Leu Thr Cys Ala Val Tyr Gly Gly Ser Phe Ser Gly Tyr
20 25 30
Tyr Trp Ile Trp Ile Arg Gln Pro Pro Gly Lys Gly Leu Glu Trp Ile

35 40 45

Gly Glu Ile Asn His Ser Gly Ser Thr Asn Tyr Asn Pro Ser Leu Lys
50 55 60

Ser Arg Val Thr Ile Ser Val Asp Thr Ser Lys Asn Gln Phe Ser Leu
65 70 75 80

Lys Leu Ser Ser Val Thr Ala Ala Asp Thr Ala Val Tyr Tyr Cys Val
85 90 95

Arg Glu Gly Asp Tyr Gly Asp Tyr Asp Tyr Trp Gly Gln Gly Thr Leu
100 105 110

Val Thr Val Ser Ser
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Gln Val Gln Leu Gln Gln Trp Gly Ala Gly Leu Leu Lys Pro Ser Glu
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Thr Leu Ser Leu Thr Cys Ala Val Tyr Gly Gly Ser Phe Ser Gly Tyr
20 25 30

Tyr Trp Ile Trp Ile Arg Gln Pro Pro Gly Lys Gly Leu Glu Trp Ile
35 40 45

Gly Glu Ile Asn His Ser Gly Ser Thr Asn Tyr Asn Pro Ser Leu Lys
50 55 60

Ser Arg Val Thr Ile Ser Val Asp Thr Ser Lys Asn Gln Phe Ser Leu
65 70 75 80

Lys Leu Asn Ser Val Thr Ala Ala Asp Thr Ala Val Tyr Tyr Cys Val
85 90 95

Arg Glu Gly Asp Tyr Gly Asp Tyr Asp Tyr Trp Gly Gln Gly Thr Leu
100 105 110

Val Thr Val Ser Ser
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<400> 54

Gln Val Gln Leu Gln Gln Trp Gly Ala Gly Leu Leu Lys Pro Ser Glu
1 5 10 15
Thr Leu Ser Leu Thr Cys Ala Val Tyr Gly Gly Ser Phe Phe Gly Tyr
20 25 30
Tyr Trp Ile Trp Ile Arg Gln Pro Pro Gly Lys Gly Leu Glu Trp Ile
35 40 45
Gly Glu Ile Asn His Ser Gly Ser Thr Asn Tyr Asn Pro Ser Leu Lys
50 55 60
Ser Arg Val Thr Ile Ser Val Asp Thr Ser Lys Asn Gln Phe Ser Leu
65 70 75 80
Lys Leu Asn Ser Val Thr Ala Ala Asp Thr Ala Val Tyr Tyr Cys Val
85 90 95
Arg Glu Gly Asn Tyr Gly Asp Tyr Asp Tyr Trp Gly Gln Gly Thr Leu
100 105 110
Val Thr Val Ser Ser
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Gln Val Gln Leu Gln Gln Trp Gly Ala Gly Leu Leu Lys Pro Ser Glu
1 5 10 15
Thr Leu Ser Leu Thr Cys Ala Val Tyr Gly Gly Ser Phe Phe Gly Tyr
20 25 30
Tyr Trp Ile Trp Ile Arg Gln Pro Pro Gly Lys Gly Leu Glu Trp Ile
35 40 45
Gly Glu Ile Asn His Ser Gly Ser Thr Asn Tyr Asn Pro Ser Leu Lys
50 55 60
Ser Arg Val Thr Ile Ser Val Asp Thr Ser Lys Asn Gln Phe Ser Leu
65 70 75 80
Lys Leu Asn Ser Val Thr Ala Ala Asp Thr Ala Val Tyr Tyr Cys Val
85 90 95
Arg Glu Gly Asp Tyr Gly Asp Tyr Asp Tyr Trp Gly Gln Gly Thr Leu
100 105 110

Val Thr Val Ser Ser
115

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Gln Val Gln Leu Gln Gln Trp Gly Ala Gly Leu Leu Lys Pro Ser Glu
1 5 10 15
Thr Leu Ser Leu Thr Cys Ala Val Tyr Gly Gly Ser Phe Ser Gly Tyr
20 25 30
Tyr Trp Ser Trp Ile Arg Gln Pro Pro Gly Lys Gly Leu Glu Trp Ile
35 40 45
Gly Glu Ile Asn His Ser Gly Ser Thr Asn Tyr Asn Pro Ser Leu Lys
50 55 60
Ser Arg Val Thr Ile Ser Val Asp Thr Ser Lys Asn Gln Phe Ser Leu
65 70 75 80
Lys Leu Asn Ser Val Thr Ala Ala Asp Thr Ala Val Tyr Tyr Cys Val
85 90 95
Arg Glu Gly Asn Tyr Gly Asp Tyr Asp Tyr Trp Gly Gln Gly Thr Leu
100 105 110
Val Thr Val Ser Ser
115

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<222> (25)..(25)
<223> Xaa2 是 半胱氨酸(Cys) 或 丝氨酸(Ser)

<220>
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<222> (34)..(34)
<223> Xaa3 是 甘氨酸(Gly) 或 丝氨酸(Ser)
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<222> (35)..(35)
<223> Xaa4 是 天冬酰胺(Asn) 或 天冬氨酸(Asp)
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<222> (55)..(55)
<223> Xaa5 是 赖氨酸(Lys)、甘氨酸(Gly)、天冬酰胺(Asn)、丝氨酸(Ser)、或 亮氨酸(Leu)
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<222> (56)..(56)
<223> Xaa6 是 缬氨酸(Val) 或 异亮氨酸(Ile)
<220>
<221> MISC_FEATURE
<222> (94)..(94)
<223> Xaa7 是 丝氨酸(Ser)、丙氨酸(Ala)、或 甘氨酸(Gly)
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<223> Xaa8 是 组氨酸(His) 或 酪氨酸(Tyr)
<400> 57

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Asp Xaa Val Met Thr Gln Thr Pro Leu Ser Leu Ser Val Thr Pro Gly
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Gln Pro Ala Ser Ile Ser Cys Arg Xaa Ser Gln Ser Leu Val His Ser
            20                  25                  30
Asp Xaa Xaa Thr Tyr Leu His Trp Tyr Leu Gln Lys Pro Gly Gln Ser
        35                  40                  45
Pro Gln Leu Leu Ile Tyr Xaa Xaa Ser Asn Arg Phe Ser Gly Val Pro
    50                  55                  60
Asp Arg Phe Ser Gly Ser Gly Ser Gly Thr Asp Phe Thr Leu Lys Ile
65                  70                  75                  80
Ser Arg Val Glu Ala Glu Asp Val Gly Val Tyr Phe Cys Xaa Gln Ser
                85                  90                  95
Thr Xaa Val Pro Tyr Ala Phe Gly Gly Gly Thr Lys Val Glu Ile Lys
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100 105 110

Arg Thr

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<211> 114

<212> PRT

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<220>

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<400> 58

Asp Val Val Met Thr Gln Thr Pro Leu Ser Leu Ser Val Thr Pro Gly
1 5 10 15
Gln Pro Ala Ser Ile Ser Cys Arg Cys Ser Gln Ser Leu Val His Ser
20 25 30
Asp Gly Asn Thr Tyr Leu His Trp Tyr Leu Gln Lys Pro Gly Gln Ser
35 40 45
Pro Gln Leu Leu Ile Tyr Lys Val Ser Asn Arg Phe Ser Gly Val Pro
50 55 60
Asp Arg Phe Ser Gly Ser Gly Ser Gly Thr Asp Phe Thr Leu Lys Ile
65 70 75 80
Ser Arg Val Glu Ala Glu Asp Val Gly Val Tyr Phe Cys Ser Gln Ser
85 90 95
Thr His Val Pro Tyr Ala Phe Gly Gly Gly Thr Lys Val Glu Ile Lys
100 105 110

Arg Thr

<210> 59

<211> 114

<212> PRT

<213> 人工序列

<220>

<223> 合成序列

<400> 59

Asp Val Val Met Thr Gln Thr Pro Leu Ser Leu Ser Val Thr Pro Gly
1 5 10 15
Gln Pro Ala Ser Ile Ser Cys Arg Cys Ser Gln Ser Leu Val His Ser
20 25 30
Asp Ser Asn Thr Tyr Leu His Trp Tyr Leu Gln Lys Pro Gly Gln Ser
35 40 45
Pro Gln Leu Leu Ile Tyr Lys Val Ser Asn Arg Phe Ser Gly Val Pro
50 55 60

Asp Arg Phe Ser Gly Ser Gly Ser Gly Thr Asp Phe Thr Leu Lys Ile
65 70 75 80
Ser Arg Val Glu Ala Glu Asp Val Gly Val Tyr Phe Cys Ser Gln Ser
85 90 95
Thr His Val Pro Tyr Ala Phe Gly Gly Gly Thr Lys Val Glu Ile Lys
100 105 110
Arg Thr

<210> 60
<211> 114
<212> PRT
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<400> 60

Asp Val Val Met Thr Gln Thr Pro Leu Ser Leu Ser Val Thr Pro Gly
1 5 10 15
Gln Pro Ala Ser Ile Ser Cys Arg Ser Ser Gln Ser Leu Val His Ser
20 25 30
Asp Gly Asn Thr Tyr Leu His Trp Tyr Leu Gln Lys Pro Gly Gln Ser
35 40 45
Pro Gln Leu Leu Ile Tyr Lys Val Ser Asn Arg Phe Ser Gly Val Pro
50 55 60
Asp Arg Phe Ser Gly Ser Gly Ser Gly Thr Asp Phe Thr Leu Lys Ile
65 70 75 80
Ser Arg Val Glu Ala Glu Asp Val Gly Val Tyr Phe Cys Ser Gln Ser
85 90 95
Thr His Val Pro Tyr Ala Phe Gly Gly Gly Thr Lys Val Glu Ile Lys
100 105 110
Arg Thr

<210> 61
<211> 114
<212> PRT
<213> 人工序列
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<223> 合成序列
<400> 61

Asp Val Val Met Thr Gln Thr Pro Leu Ser Leu Ser Val Thr Pro Gly
1 5 10 15
Gln Pro Ala Ser Ile Ser Cys Arg Cys Ser Gln Ser Leu Val His Ser

20 25 30
Asp Gly Asn Thr Tyr Leu His Trp Tyr Leu Gln Lys Pro Gly Gln Ser
35 40 45
Pro Gln Leu Leu Ile Tyr Gly Val Ser Asn Arg Phe Ser Gly Val Pro
50 55 60
Asp Arg Phe Ser Gly Ser Gly Ser Gly Thr Asp Phe Thr Leu Lys Ile
65 70 75 80
Ser Arg Val Glu Ala Glu Asp Val Gly Val Tyr Phe Cys Ser Gln Ser
85 90 95
Thr His Val Pro Tyr Ala Phe Gly Gly Gly Thr Lys Val Glu Ile Lys
100 105 110
Arg Thr

<210> 62
<211> 114
<212> PRT
<213> 人工序列
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<400> 62

Asp Val Val Met Thr Gln Thr Pro Leu Ser Leu Ser Val Thr Pro Gly
1 5 10 15
Gln Pro Ala Ser Ile Ser Cys Arg Cys Ser Gln Ser Leu Val His Ser
20 25 30
Asp Gly Asn Thr Tyr Leu His Trp Tyr Leu Gln Lys Pro Gly Gln Ser
35 40 45
Pro Gln Leu Leu Ile Tyr Asn Val Ser Asn Arg Phe Ser Gly Val Pro
50 55 60
Asp Arg Phe Ser Gly Ser Gly Ser Gly Thr Asp Phe Thr Leu Lys Ile
65 70 75 80
Ser Arg Val Glu Ala Glu Asp Val Gly Val Tyr Phe Cys Ser Gln Ser
85 90 95
Thr His Val Pro Tyr Ala Phe Gly Gly Gly Thr Lys Val Glu Ile Lys
100 105 110
Arg Thr

<210> 63
<211> 114
<212> PRT
<213> 人工序列
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<223> 合成序列
<400> 63

Asp Val Val Met Thr Gln Thr Pro Leu Ser Leu Ser Val Thr Pro Gly
1 5 10 15
Gln Pro Ala Ser Ile Ser Cys Arg Cys Ser Gln Ser Leu Val His Ser
20 25 30
Asp Gly Asn Thr Tyr Leu His Trp Tyr Leu Gln Lys Pro Gly Gln Ser
35 40 45
Pro Gln Leu Leu Ile Tyr Ser Val Ser Asn Arg Phe Ser Gly Val Pro
50 55 60
Asp Arg Phe Ser Gly Ser Gly Ser Gly Thr Asp Phe Thr Leu Lys Ile
65 70 75 80
Ser Arg Val Glu Ala Glu Asp Val Gly Val Tyr Phe Cys Ser Gln Ser
85 90 95
Thr His Val Pro Tyr Ala Phe Gly Gly Gly Thr Lys Val Glu Ile Lys
100 105 110
Arg Thr

<210> 64
<211> 114
<212> PRT
<213> 人工序列
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<223> 合成序列
<400> 64

Asp Val Val Met Thr Gln Thr Pro Leu Ser Leu Ser Val Thr Pro Gly
1 5 10 15
Gln Pro Ala Ser Ile Ser Cys Arg Cys Ser Gln Ser Leu Val His Ser
20 25 30
Asp Gly Asp Thr Tyr Leu His Trp Tyr Leu Gln Lys Pro Gly Gln Ser
35 40 45
Pro Gln Leu Leu Ile Tyr Lys Val Ser Asn Arg Phe Ser Gly Val Pro
50 55 60
Asp Arg Phe Ser Gly Ser Gly Ser Gly Thr Asp Phe Thr Leu Lys Ile
65 70 75 80
Ser Arg Val Glu Ala Glu Asp Val Gly Val Tyr Phe Cys Ser Gln Ser
85 90 95
Thr His Val Pro Tyr Ala Phe Gly Gly Gly Thr Lys Val Glu Ile Lys
100 105 110
Arg Thr

<210> 65
<211> 114
<212> PRT
<213> 人工序列
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<223> 合成序列
<400> 65

Asp Val Val Met Thr Gln Thr Pro Leu Ser Leu Ser Val Thr Pro Gly
1 5 10 15
Gln Pro Ala Ser Ile Ser Cys Arg Cys Ser Gln Ser Leu Val His Ser
20 25 30
Asp Gly Asn Thr Tyr Leu His Trp Tyr Leu Gln Lys Pro Gly Gln Ser
35 40 45
Pro Gln Leu Leu Ile Tyr Lys Val Ser Asn Arg Phe Ser Gly Val Pro
50 55 60
Asp Arg Phe Ser Gly Ser Gly Ser Gly Thr Asp Phe Thr Leu Lys Ile
65 70 75 80
Ser Arg Val Glu Ala Glu Asp Val Gly Val Tyr Phe Cys Ala Gln Ser
85 90 95
Thr His Val Pro Tyr Ala Phe Gly Gly Gly Thr Lys Val Glu Ile Lys
100 105 110
Arg Thr

<210> 66
<211> 114
<212> PRT
<213> 人工序列
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<223> 合成序列
<400> 66

Asp Val Val Met Thr Gln Thr Pro Leu Ser Leu Ser Val Thr Pro Gly
1 5 10 15
Gln Pro Ala Ser Ile Ser Cys Arg Cys Ser Gln Ser Leu Val His Ser
20 25 30
Asp Gly Asn Thr Tyr Leu His Trp Tyr Leu Gln Lys Pro Gly Gln Ser
35 40 45
Pro Gln Leu Leu Ile Tyr Lys Val Ser Asn Arg Phe Ser Gly Val Pro
50 55 60
Asp Arg Phe Ser Gly Ser Gly Ser Gly Thr Asp Phe Thr Leu Lys Ile
65 70 75 80

Ser Arg Val Glu Ala Glu Asp Val Gly Val Tyr Phe Cys Gly Gln Ser
85 90 95
Thr His Val Pro Tyr Ala Phe Gly Gly Gly Thr Lys Val Glu Ile Lys
100 105 110
Arg Thr
<210> 67
<211> 114
<212> PRT
<213> 人工序列
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<223> 合成序列
<400> 67
Asp Val Val Met Thr Gln Thr Pro Leu Ser Leu Ser Val Thr Pro Gly
1 5 10 15
Gln Pro Ala Ser Ile Ser Cys Arg Cys Ser Gln Ser Leu Val His Ser
20 25 30
Asp Gly Asn Thr Tyr Leu His Trp Tyr Leu Gln Lys Pro Gly Gln Ser
35 40 45
Pro Gln Leu Leu Ile Tyr Lys Val Ser Asn Arg Phe Ser Gly Val Pro
50 55 60
Asp Arg Phe Ser Gly Ser Gly Ser Gly Thr Asp Phe Thr Leu Lys Ile
65 70 75 80
Ser Arg Val Glu Ala Glu Asp Val Gly Val Tyr Phe Cys Ser Gln Ser
85 90 95
Thr Tyr Val Pro Tyr Ala Phe Gly Gly Gly Thr Lys Val Glu Ile Lys
100 105 110
Arg Thr
<210> 68
<211> 114
<212> PRT
<213> 人工序列
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<223> 合成序列
<400> 68
Asp Val Val Met Thr Gln Thr Pro Leu Ser Leu Ser Val Thr Pro Gly
1 5 10 15
Gln Pro Ala Ser Ile Ser Cys Arg Cys Ser Gln Ser Leu Val His Ser
20 25 30
Asp Gly Asn Thr Tyr Leu His Trp Tyr Leu Gln Lys Pro Gly Gln Ser

35 40 45

Pro Gln Leu Leu Ile Tyr Lys Ile Ser Asn Arg Phe Ser Gly Val Pro
50 55 60

Asp Arg Phe Ser Gly Ser Gly Ser Gly Thr Asp Phe Thr Leu Lys Ile
65 70 75 80

Ser Arg Val Glu Ala Glu Asp Val Gly Val Tyr Phe Cys Ser Gln Ser
85 90 95

Thr His Val Pro Tyr Ala Phe Gly Gly Gly Thr Lys Val Glu Ile Lys
100 105 110

Arg Thr

<210> 69

<211> 114

<212> PRT

<213> 人工序列

<220>

<223> 合成序列

<400> 69

Asp Val Val Met Thr Gln Thr Pro Leu Ser Leu Ser Val Thr Pro Gly
1 5 10 15

Gln Pro Ala Ser Ile Ser Cys Arg Ser Ser Gln Ser Leu Val His Ser
20 25 30

Asp Ser Asn Thr Tyr Leu His Trp Tyr Leu Gln Lys Pro Gly Gln Ser
35 40 45

Pro Gln Leu Leu Ile Tyr Leu Val Ser Asn Arg Phe Ser Gly Val Pro
50 55 60

Asp Arg Phe Ser Gly Ser Gly Ser Gly Thr Asp Phe Thr Leu Lys Ile
65 70 75 80

Ser Arg Val Glu Ala Glu Asp Val Gly Val Tyr Phe Cys Gly Gln Ser
85 90 95

Thr His Val Pro Tyr Ala Phe Gly Gly Gly Thr Lys Val Glu Ile Lys
100 105 110

Arg Thr

<210> 70

<211> 114

<212> PRT

<213> 人工序列

<220>

<223> 合成序列

<400> 70

Asp Val Val Met Thr Gln Thr Pro Leu Ser Leu Ser Val Thr Pro Gly
1 5 10 15
Gln Pro Ala Ser Ile Ser Cys Arg Ser Ser Gln Ser Leu Val His Ser
20 25 30
Asp Ser Asn Thr Tyr Leu His Trp Tyr Leu Gln Lys Pro Gly Gln Ser
35 40 45
Pro Gln Leu Leu Ile Tyr Lys Val Ser Asn Arg Phe Ser Gly Val Pro
50 55 60
Asp Arg Phe Ser Gly Ser Gly Ser Gly Thr Asp Phe Thr Leu Lys Ile
65 70 75 80
Ser Arg Val Glu Ala Glu Asp Val Gly Val Tyr Phe Cys Gly Gln Ser
85 90 95
Thr His Val Pro Tyr Ala Phe Gly Gly Gly Thr Lys Val Glu Ile Lys
100 105 110
Arg Thr

<210> 71
<211> 114
<212> PRT
<213> 人工序列
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<223> 合成序列
<400> 71

Asp Val Val Met Thr Gln Thr Pro Leu Ser Leu Ser Val Thr Pro Gly
1 5 10 15
Gln Pro Ala Ser Ile Ser Cys Arg Ser Ser Gln Ser Leu Val His Ser
20 25 30
Asp Gly Asn Thr Tyr Leu His Trp Tyr Leu Gln Lys Pro Gly Gln Ser
35 40 45
Pro Gln Leu Leu Ile Tyr Lys Val Ser Asn Arg Phe Ser Gly Val Pro
50 55 60
Asp Arg Phe Ser Gly Ser Gly Ser Gly Thr Asp Phe Thr Leu Lys Ile
65 70 75 80
Ser Arg Val Glu Ala Glu Asp Val Gly Val Tyr Phe Cys Gly Gln Ser
85 90 95
Thr His Val Pro Tyr Ala Phe Gly Gly Gly Thr Lys Val Glu Ile Lys
100 105 110
Arg Thr

<210> 72
<211> 114

<212> PRT
<213> 人工序列
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<223> 合成序列
<400> 72

Asp Val Val Met Thr Gln Thr Pro Leu Ser Leu Ser Val Thr Pro Gly
1 5 10 15
Gln Pro Ala Ser Ile Ser Cys Arg Ser Ser Gln Ser Leu Val His Ser
20 25 30
Asp Ser Asn Thr Tyr Leu His Trp Tyr Leu Gln Lys Pro Gly Gln Ser
35 40 45
Pro Gln Leu Leu Ile Tyr Leu Val Ser Asn Arg Phe Ser Gly Val Pro
50 55 60
Asp Arg Phe Ser Gly Ser Gly Ser Gly Thr Asp Phe Thr Leu Lys Ile
65 70 75 80
Ser Arg Val Glu Ala Glu Asp Val Gly Val Tyr Phe Cys Ser Gln Ser
85 90 95
Thr His Val Pro Tyr Ala Phe Gly Gly Gly Thr Lys Val Glu Ile Lys
100 105 110
Arg Thr

<210> 73
<211> 114
<212> PRT
<213> 人工序列
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<223> 合成序列
<400> 73

Asp Val Val Met Thr Gln Thr Pro Leu Ser Leu Ser Val Thr Pro Gly
1 5 10 15
Gln Pro Ala Ser Ile Ser Cys Arg Ser Ser Gln Ser Leu Val His Ser
20 25 30
Asp Gly Asn Thr Tyr Leu His Trp Tyr Leu Gln Lys Pro Gly Gln Ser
35 40 45
Pro Gln Leu Leu Ile Tyr Leu Val Ser Asn Arg Phe Ser Gly Val Pro
50 55 60
Asp Arg Phe Ser Gly Ser Gly Ser Gly Thr Asp Phe Thr Leu Lys Ile
65 70 75 80
Ser Arg Val Glu Ala Glu Asp Val Gly Val Tyr Phe Cys Gly Gln Ser
85 90 95

Thr His Val Pro Tyr Ala Phe Gly Gly Gly Thr Lys Val Glu Ile Lys
100 105 110

Arg Thr

<210> 74

<211> 114

<212> PRT

<213> 人工序列

<220>

<223> 合成序列

<400> 74

Asp Val Val Met Thr Gln Thr Pro Leu Ser Leu Ser Val Thr Pro Gly
1 5 10 15

Gln Pro Ala Ser Ile Ser Cys Arg Ser Ser Gln Ser Leu Val His Ser
20 25 30

Asp Gly Asn Thr Tyr Leu His Trp Tyr Leu Gln Lys Pro Gly Gln Ser
35 40 45

Pro Gln Leu Leu Ile Tyr Leu Ile Ser Asn Arg Phe Ser Gly Val Pro
50 55 60

Asp Arg Phe Ser Gly Ser Gly Ser Gly Thr Asp Phe Thr Leu Lys Ile
65 70 75 80

Ser Arg Val Glu Ala Glu Asp Val Gly Val Tyr Phe Cys Gly Gln Ser
85 90 95

Thr His Val Pro Tyr Ala Phe Gly Gly Gly Thr Lys Val Glu Ile Lys
100 105 110

Arg Thr

<210> 75

<211> 114

<212> PRT

<213> 人工序列

<220>

<223> 合成序列

<400> 75

Asp Val Val Met Thr Gln Thr Pro Leu Ser Leu Ser Val Thr Pro Gly
1 5 10 15

Gln Pro Ala Ser Ile Ser Cys Arg Ser Ser Gln Ser Leu Val His Ser
20 25 30

Asp Gly Asn Thr Tyr Leu His Trp Tyr Leu Gln Lys Pro Gly Gln Ser
35 40 45

Pro Gln Leu Leu Ile Tyr Leu Val Ser Asn Arg Phe Ser Gly Val Pro

50 55 60
Asp Arg Phe Ser Gly Ser Gly Ser Gly Thr Asp Phe Thr Leu Lys Ile
65 70 75 80
Ser Arg Val Glu Ala Glu Asp Val Gly Val Tyr Phe Cys Ser Gln Ser
85 90 95
Thr His Val Pro Tyr Ala Phe Gly Gly Gly Thr Lys Val Glu Ile Lys
100 105 110
Arg Thr

<210> 76
<211> 114
<212> PRT
<213> 人工序列
<220>
<223> 合成序列
<400> 76
Asp Val Val Met Thr Gln Thr Pro Leu Ser Leu Ser Val Thr Pro Gly
1 5 10 15
Gln Pro Ala Ser Ile Ser Cys Arg Ser Ser Gln Ser Leu Val His Ser
20 25 30
Asp Ser Asn Thr Tyr Leu His Trp Tyr Leu Gln Lys Pro Gly Gln Ser
35 40 45
Pro Gln Leu Leu Ile Tyr Lys Ile Ser Asn Arg Phe Ser Gly Val Pro
50 55 60
Asp Arg Phe Ser Gly Ser Gly Ser Gly Thr Asp Phe Thr Leu Lys Ile
65 70 75 80
Ser Arg Val Glu Ala Glu Asp Val Gly Val Tyr Phe Cys Ser Gln Ser
85 90 95
Thr His Val Pro Tyr Ala Phe Gly Gly Gly Thr Lys Val Glu Ile Lys
100 105 110
Arg Thr

<210> 77
<211> 114
<212> PRT
<213> 人工序列
<220>
<223> 合成序列
<400> 77
Asp Val Val Met Thr Gln Thr Pro Leu Ser Leu Ser Val Thr Pro Gly
1 5 10 15

Gln Pro Ala Ser Ile Ser Cys Arg Ser Ser Gln Ser Leu Val His Ser
20 25 30
Asp Ser Asn Thr Tyr Leu His Trp Tyr Leu Gln Lys Pro Gly Gln Ser
35 40 45
Pro Gln Leu Leu Ile Tyr Lys Ile Ser Asn Arg Phe Ser Gly Val Pro
50 55 60
Asp Arg Phe Ser Gly Ser Gly Ser Gly Thr Asp Phe Thr Leu Lys Ile
65 70 75 80
Ser Arg Val Glu Ala Glu Asp Val Gly Val Tyr Phe Cys Gly Gln Ser
85 90 95
Thr His Val Pro Tyr Ala Phe Gly Gly Gly Thr Lys Val Glu Ile Lys
100 105 110
Arg Thr

<210> 78
<211> 114
<212> PRT
<213> 人工序列
<220>
<223> 合成序列
<400> 78

Asp Val Val Met Thr Gln Thr Pro Leu Ser Leu Ser Val Thr Pro Gly
1 5 10 15
Gln Pro Ala Ser Ile Ser Cys Arg Ser Ser Gln Ser Leu Val His Ser
20 25 30
Asp Gly Asn Thr Tyr Leu His Trp Tyr Leu Gln Lys Pro Gly Gln Ser
35 40 45
Pro Gln Leu Leu Ile Tyr Lys Val Ser Asn Arg Phe Ser Gly Val Pro
50 55 60
Asp Arg Phe Ser Gly Ser Gly Ser Gly Thr Asp Phe Thr Leu Lys Ile
65 70 75 80
Ser Arg Val Glu Ala Glu Asp Val Gly Val Tyr Phe Cys Ser Gln Ser
85 90 95
Thr Tyr Val Pro Tyr Ala Phe Gly Gly Gly Thr Lys Val Glu Ile Lys
100 105 110
Arg Thr

<210> 79
<211> 114
<212> PRT
<213> 人工序列

<220>
<223> 合成序列
<400> 79

Asp Val Val Met Thr Gln Thr Pro Leu Ser Leu Ser Val Thr Pro Gly
1 5 10 15
Gln Pro Ala Ser Ile Ser Cys Arg Ser Ser Gln Ser Leu Val His Ser
20 25 30
Asp Ser Asn Thr Tyr Leu His Trp Tyr Leu Gln Lys Pro Gly Gln Ser
35 40 45
Pro Gln Leu Leu Ile Tyr Leu Ile Ser Asn Arg Phe Ser Gly Val Pro
50 55 60
Asp Arg Phe Ser Gly Ser Gly Ser Gly Thr Asp Phe Thr Leu Lys Ile
65 70 75 80
Ser Arg Val Glu Ala Glu Asp Val Gly Val Tyr Phe Cys Gly Gln Ser
85 90 95
Thr His Val Pro Tyr Ala Phe Gly Gly Gly Thr Lys Val Glu Ile Lys
100 105 110
Arg Thr

<210> 80
<211> 114
<212> PRT
<213> 人工序列
<220>
<223> 合成序列
<400> 80

Asp Val Val Met Thr Gln Thr Pro Leu Ser Leu Ser Val Thr Pro Gly
1 5 10 15
Gln Pro Ala Ser Ile Ser Cys Arg Ser Ser Gln Ser Leu Val His Ser
20 25 30
Asp Ser Asn Thr Tyr Leu His Trp Tyr Leu Gln Lys Pro Gly Gln Ser
35 40 45
Pro Gln Leu Leu Ile Tyr Leu Val Ser Asn Arg Phe Ser Gly Val Pro
50 55 60
Asp Arg Phe Ser Gly Ser Gly Ser Gly Thr Asp Phe Thr Leu Lys Ile
65 70 75 80
Ser Arg Val Glu Ala Glu Asp Val Gly Val Tyr Phe Cys Gly Gln Ser
85 90 95
Thr Tyr Val Pro Tyr Ala Phe Gly Gly Gly Thr Lys Val Glu Ile Lys
100 105 110

Arg Thr
<210> 81
<211> 114
<212> PRT
<213> 人工序列
<220>
<223> 合成序列
<400> 81
Asp Val Val Met Thr Gln Thr Pro Leu Ser Leu Ser Val Thr Pro Gly
1 5 10 15
Gln Pro Ala Ser Ile Ser Cys Arg Ser Ser Gln Ser Leu Val His Ser
20 25 30
Asp Ser Asn Thr Tyr Leu His Trp Tyr Leu Gln Lys Pro Gly Gln Ser
35 40 45
Pro Gln Leu Leu Ile Tyr Leu Ile Ser Asn Arg Phe Ser Gly Val Pro
50 55 60
Asp Arg Phe Ser Gly Ser Gly Ser Gly Thr Asp Phe Thr Leu Lys Ile
65 70 75 80
Ser Arg Val Glu Ala Glu Asp Val Gly Val Tyr Phe Cys Gly Gln Ser
85 90 95
Thr Tyr Val Pro Tyr Ala Phe Gly Gly Gly Thr Lys Val Glu Ile Lys
100 105 110
Arg Thr
<210> 82
<211> 114
<212> PRT
<213> 人工序列
<220>
<223> 合成序列
<400> 82
Asp Val Val Met Thr Gln Thr Pro Leu Ser Leu Ser Val Thr Pro Gly
1 5 10 15
Gln Pro Ala Ser Ile Ser Cys Arg Ser Ser Gln Ser Leu Val His Ser
20 25 30
Asp Ser Asn Thr Tyr Leu His Trp Tyr Leu Gln Lys Pro Gly Gln Ser
35 40 45
Pro Gln Leu Leu Ile Tyr Leu Val Ser Asn Arg Phe Ser Gly Val Pro
50 55 60
Asp Arg Phe Ser Gly Ser Gly Ser Gly Thr Asp Phe Thr Leu Lys Ile

65 70 75 80
Ser Arg Val Glu Ala Glu Asp Val Gly Val Tyr Phe Cys Gly Gln Ser
85 90 95
Thr Tyr Val Pro Tyr Ala Phe Gly Gly Gly Thr Lys Val Glu Ile Lys
100 105 110
Arg Thr
<210> 83
<211> 114
<212> PRT
<213> 人工序列
<220>
<223> 合成序列
<400> 83
Asp Val Val Met Thr Gln Thr Pro Leu Ser Leu Ser Val Thr Pro Gly
1 5 10 15
Gln Pro Ala Ser Ile Ser Cys Arg Ser Ser Gln Ser Leu Val His Ser
20 25 30
Asp Ser Asn Thr Tyr Leu His Trp Tyr Leu Gln Lys Pro Gly Gln Ser
35 40 45
Pro Gln Leu Leu Ile Tyr Leu Val Ser Asn Arg Phe Ser Gly Val Pro
50 55 60
Asp Arg Phe Ser Gly Ser Gly Ser Gly Thr Asp Phe Thr Leu Lys Ile
65 70 75 80
Ser Arg Val Glu Ala Glu Asp Val Gly Val Tyr Phe Cys Gly Gln Ser
85 90 95
Thr His Val Pro Tyr Ala Phe Gly Gly Gly Thr Lys Val Glu Ile Lys
100 105 110
Arg Thr
<210> 84
<211> 114
<212> PRT
<213> 人工序列
<220>
<223> 合成序列
<400> 84
Asp Val Val Met Thr Gln Thr Pro Leu Ser Leu Ser Val Thr Pro Gly
1 5 10 15
Gln Pro Ala Ser Ile Ser Cys Arg Ser Ser Gln Ser Leu Val His Ser
20 25 30

Asp Ser Asn Thr Tyr Leu His Trp Tyr Leu Gln Lys Pro Gly Gln Ser
35 40 45
Pro Gln Leu Leu Ile Tyr Lys Val Ser Asn Arg Phe Ser Gly Val Pro
50 55 60
Asp Arg Phe Ser Gly Ser Gly Ser Gly Thr Asp Phe Thr Leu Lys Ile
65 70 75 80
Ser Arg Val Glu Ala Glu Asp Val Gly Val Tyr Phe Cys Gly Gln Ser
85 90 95
Thr His Val Pro Tyr Ala Phe Gly Gly Gly Thr Lys Val Glu Ile Lys
100 105 110
Arg Thr

<210> 85
<211> 114
<212> PRT
<213> 人工序列
<220>
<223> 合成序列
<400> 85

Asp Val Val Met Thr Gln Thr Pro Leu Ser Leu Ser Val Thr Pro Gly
1 5 10 15
Gln Pro Ala Ser Ile Ser Cys Arg Ser Ser Gln Ser Leu Val His Ser
20 25 30
Asp Ser Asn Thr Tyr Leu His Trp Tyr Leu Gln Lys Pro Gly Gln Ser
35 40 45
Pro Gln Leu Leu Ile Tyr Leu Val Ser Asn Arg Phe Ser Gly Val Pro
50 55 60
Asp Arg Phe Ser Gly Ser Gly Ser Gly Thr Asp Phe Thr Leu Lys Ile
65 70 75 80
Ser Arg Val Glu Ala Glu Asp Val Gly Val Tyr Phe Cys Ser Gln Ser
85 90 95
Thr His Val Pro Tyr Ala Phe Gly Gly Gly Thr Lys Val Glu Ile Lys
100 105 110
Arg Thr

<210> 86
<211> 114
<212> PRT
<213> 人工序列
<220>
<223> 合成序列

<400> 86

Asp Val Val Met Thr Gln Thr Pro Leu Ser Leu Ser Val Thr Pro Gly
1 5 10 15
Gln Pro Ala Ser Ile Ser Cys Arg Ser Ser Gln Ser Leu Val His Ser
20 25 30
Asp Ser Asn Thr Tyr Leu His Trp Tyr Leu Gln Lys Pro Gly Gln Ser
35 40 45
Pro Gln Leu Leu Ile Tyr Leu Val Ser Asn Arg Phe Ser Gly Val Pro
50 55 60
Asp Arg Phe Ser Gly Ser Gly Ser Gly Thr Asp Phe Thr Leu Lys Ile
65 70 75 80
Ser Arg Val Glu Ala Glu Asp Val Gly Val Tyr Phe Cys Gly Gln Ser
85 90 95
Thr His Val Pro Tyr Ala Phe Gly Gly Gly Thr Lys Val Glu Ile Lys
100 105 110
Arg Thr

<210> 87

<211> 114

<212> PRT

<213> 人工序列

<220>

<223> 合成序列

<400> 87

Asp Ile Val Met Thr Gln Thr Pro Leu Ser Leu Ser Val Thr Pro Gly
1 5 10 15
Gln Pro Ala Ser Ile Ser Cys Arg Ser Ser Gln Ser Leu Val His Ser
20 25 30
Asp Ser Asn Thr Tyr Leu His Trp Tyr Leu Gln Lys Pro Gly Gln Ser
35 40 45
Pro Gln Leu Leu Ile Tyr Lys Val Ser Asn Arg Phe Ser Gly Val Pro
50 55 60
Asp Arg Phe Ser Gly Ser Gly Ser Gly Thr Asp Phe Thr Leu Lys Ile
65 70 75 80
Ser Arg Val Glu Ala Glu Asp Val Gly Val Tyr Phe Cys Gly Gln Ser
85 90 95
Thr His Val Pro Tyr Ala Phe Gly Gly Gly Thr Lys Val Glu Ile Lys
100 105 110
Arg Thr

<210> 88

<211> 114
<212> PRT
<213> 人工序列
<220>
<223> 合成序列
<400> 88

Asp Ile Val Met Thr Gln Thr Pro Leu Ser Leu Ser Val Thr Pro Gly
1 5 10 15
Gln Pro Ala Ser Ile Ser Cys Arg Ser Ser Gln Ser Leu Val His Ser
20 25 30
Asp Ser Asn Thr Tyr Leu His Trp Tyr Leu Gln Lys Pro Gly Gln Ser
35 40 45
Pro Gln Leu Leu Ile Tyr Leu Val Ser Asn Arg Phe Ser Gly Val Pro
50 55 60
Asp Arg Phe Ser Gly Ser Gly Ser Gly Thr Asp Phe Thr Leu Lys Ile
65 70 75 80
Ser Arg Val Glu Ala Glu Asp Val Gly Val Tyr Phe Cys Gly Gln Ser
85 90 95
Thr His Val Pro Tyr Ala Phe Gly Gly Gly Thr Lys Val Glu Ile Lys
100 105 110
Arg Thr

<210> 89
<211> 115
<212> PRT
<213> 人工序列
<220>
<223> 合成序列
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<221> MISC_FEATURE
<222> (49)..(53)
<223> 子序列 Xaa1 Xaa2 Xaa3 Xaa4 Xaa5 缺失或是Tyr-Asp-Ala-Ser-Asn
<220>
<221> MISC_FEATURE
<222> (94)..(94)
<223> Xaa6 是 苏氨酸(Thr) 或 异亮氨酸(Ile)
<400> 89

Asp Ile Gln Met Thr Gln Ser Pro Ser Ser Leu Ser Ala Ser Val Gly
1 5 10 15
Asp Arg Val Thr Ile Thr Cys Gln Ala Ser Gln Asp Ile Ser Asn Tyr

20 25 30
Leu Asn Trp Tyr Gln Gln Lys Pro Gly Lys Ala Pro Lys Leu Leu Ile
35 40 45
Xaa Xaa Xaa Xaa Xaa Leu Glu Thr Gly Val Pro Ser Arg Phe Ser Gly
50 55 60
Ser Gly Ser Gly Thr Asp Phe Thr Phe Thr Ile Ser Ser Leu Gln Pro
65 70 75 80
Glu Asp Ile Ala Val Tyr Tyr Cys Gln Gln Ser Tyr Ser Xaa Leu Ile
85 90 95
Thr Phe Gly Gln Gly Thr Arg Leu Glu Ile Lys Arg Thr Val Ala Ala
100 105 110
Pro Ser Val
115

<210> 90
<211> 115
<212> PRT
<213> 人工序列
<220>
<223> 合成序列
<400> 90
Asp Ile Gln Met Thr Gln Ser Pro Ser Ser Leu Ser Ala Ser Val Gly
1 5 10 15
Asp Arg Val Thr Ile Thr Cys Gln Ala Ser Gln Asp Ile Ser Asn Tyr
20 25 30
Leu Asn Trp Tyr Gln Gln Lys Pro Gly Lys Ala Pro Lys Leu Leu Ile
35 40 45
Tyr Asp Ala Ser Asn Leu Glu Thr Gly Val Pro Ser Arg Phe Ser Gly
50 55 60
Ser Gly Ser Gly Thr Asp Phe Thr Phe Thr Ile Ser Ser Leu Gln Pro
65 70 75 80
Glu Asp Ile Ala Val Tyr Tyr Cys Gln Gln Ser Tyr Ser Thr Leu Ile
85 90 95
Thr Phe Gly Gln Gly Thr Arg Leu Glu Ile Lys Arg Thr Val Ala Ala
100 105 110
Pro Ser Val
115

<210> 91
<211> 110
<212> PRT

<213> 人工序列
<220>
<223> 合成序列
<400> 91

Asp Ile Gln Met Thr Gln Ser Pro Ser Ser Leu Ser Ala Ser Val Gly
1 5 10 15
Asp Arg Val Thr Ile Thr Cys Gln Ala Ser Gln Asp Ile Ser Asn Tyr
20 25 30
Leu Asn Trp Tyr Gln Gln Lys Pro Gly Lys Ala Pro Lys Leu Leu Ile
35 40 45
Leu Glu Thr Gly Val Pro Ser Arg Phe Ser Gly Ser Gly Ser Gly Thr
50 55 60
Asp Phe Thr Phe Thr Ile Ser Ser Leu Gln Pro Glu Asp Ile Ala Val
65 70 75 80
Tyr Tyr Cys Gln Gln Ser Tyr Ser Thr Leu Ile Thr Phe Gly Gln Gly
85 90 95
Thr Arg Leu Glu Ile Lys Arg Thr Val Ala Ala Pro Ser Val
100 105 110

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Asp Arg Val Thr Ile Thr Cys Gln Ala Ser Gln Asp Ile Ser Asn Tyr
20 25 30
Leu Asn Trp Tyr Gln Gln Lys Pro Gly Lys Ala Pro Lys Leu Leu Ile
35 40 45
Leu Glu Thr Gly Val Pro Ser Arg Phe Ser Gly Ser Gly Ser Gly Thr
50 55 60
Asp Phe Thr Phe Thr Ile Ser Ser Leu Gln Pro Glu Asp Ile Ala Val
65 70 75 80
Tyr Tyr Cys Gln Gln Ser Tyr Ser Ile Leu Ile Thr Phe Gly Gln Gly
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Thr Arg Leu Glu Ile Lys Arg Thr Val Ala Ala Pro Ser Val
100 105 110

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tctggagtgc cagataggtt cagtggcagc ggatcaggga cagatttcac actgaaaatc 240
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<400> 156
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<213> 人工序列
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tacctgcaga agccaggcca gtctccacag ctcctgatct atctagtttc caaccgattt 180
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tctggagtgc cagataggtt cagtggcagc ggatcaggga cagatttcac actgaaaatc 240
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tctggagtgc cagataggtt cagtggcagc ggatcaggga cagatttcac actgaaaatc 240
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tacgcgttcg gcggagggac caaggtggag atcaaacgga ct 342

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<212> DNA
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<210> 177
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<400> 177
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<212> DNA
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<210> 180
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<223> 合成序列
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<210> 181
<211> 114
<212> PRT
<213> 人工序列
<220>
<223> 合成序列
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<221> MISC_FEATURE
<222> (28)..(28)
<223> Xaa1 是 天冬酰胺(Asn) 或 丝氨酸(Ser)
<220>
<221> MISC_FEATURE
<222> (30)..(30)
<223> Xaa2 是 赖氨酸(Lys)、酪氨酸(Tyr)、或 天冬酰胺(Asn)
<220>
<221> MISC_FEATURE
<222> (38)..(38)
<223> Xaa3 是 赖氨酸(Lys) 或 谷氨酰胺(Gln)
<220>
<221> MISC_FEATURE
<222> (48)..(48)
<223> Xaa4 是 异亮氨酸(Ile) 或 甲硫氨酸(Met)
<220>
<221> MISC_FEATURE
<222> (53)..(54)
<223> Xaa5 是 丙氨酸(Ala) 或 脯氨酸(Pro) Xaa6 是 谷氨酸(Glu) 或 甲硫氨酸

(Met)

<220>

<221> MISC_FEATURE

<222> (56)..(56)

<223> Xaa7 是 甘氨酸(Gly)、天冬酰胺(Asn)、或 天冬氨酸(Asp)

<220>

<221> MISC_FEATURE

<222> (59)..(59)

<223> Xaa8 是 谷氨酸(Glu) 或 谷氨酰胺(Gln)

<220>

<221> MISC_FEATURE

<222> (61)..(61)

<223> Xaa9 是 丙氨酸(Ala) 或 丝氨酸(Ser)

<220>

<221> MISC_FEATURE

<222> (65)..(65)

<223> Xaa10 是 谷氨酰胺(Gln) 或 精氨酸(Arg)

<220>

<221> MISC_FEATURE

<222> (77)..(77)

<223> Xaa11 是 天冬氨酸(Asp) 或 天冬酰胺(Asn)

<220>

<221> MISC_FEATURE

<222> (82)..(82)

<223> Xaa12 是 谷氨酸(Glu) 或 赖氨酸(Lys)

<400> 181

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1 5 10 15

Thr Val Lys Ile Ser Cys Lys Ala Ser Gly Phe Xaa Ile Xaa Asp Asp
20 25 30

Tyr Ile His Trp Val Xaa Gln Ala Pro Gly Lys Gly Leu Glu Trp Xaa
35 40 45

Gly Trp Ile Asp Xaa Xaa Asn Xaa Asp Ser Xaa Tyr Xaa Ser Lys Phe
50 55 60

Xaa Gly Arg Val Thr Ile Thr Val Asp Thr Ser Thr Xaa Thr Ala Tyr
65 70 75 80

Met Xaa Leu Ser Ser Leu Arg Ser Glu Asp Thr Ala Val Tyr Tyr Cys
85 90 95

Thr Tyr Ala Phe Gly Gly Tyr Trp Gly Gln Gly Thr Thr Val Thr Val

100 105 110

Ser Ser

<210> 182

<211> 114

<212> PRT

<213> 人工序列

<220>

<223> 合成序列

<400> 182

Glu Val Gln Leu Val Gln Ser Gly Ala Glu Val Lys Lys Pro Gly Ala
1 5 10 15
Thr Val Lys Ile Ser Cys Lys Ala Ser Gly Phe Ser Ile Lys Asp Asp
20 25 30
Tyr Ile His Trp Val Gln Gln Ala Pro Gly Lys Gly Leu Glu Trp Met
35 40 45
Gly Trp Ile Asp Ala Met Asn Asp Asp Ser Gln Tyr Ser Ser Lys Phe
50 55 60
Gln Gly Arg Val Thr Ile Thr Val Asp Thr Ser Thr Asn Thr Ala Tyr
65 70 75 80
Met Lys Leu Ser Ser Leu Arg Ser Glu Asp Thr Ala Val Tyr Tyr Cys
85 90 95
Thr Tyr Ala Phe Gly Gly Tyr Trp Gly Gln Gly Thr Thr Val Thr Val
100 105 110

Ser Ser

<210> 183

<211> 114

<212> PRT

<213> 人工序列

<220>

<223> 合成序列

<400> 183

Glu Val Gln Leu Val Gln Ser Gly Ala Glu Val Lys Lys Pro Gly Ala
1 5 10 15
Thr Val Lys Ile Ser Cys Lys Ala Ser Gly Phe Ser Ile Lys Asp Asp
20 25 30
Tyr Ile His Trp Val Gln Gln Ala Pro Gly Lys Gly Leu Glu Trp Met
35 40 45
Gly Trp Ile Asp Ala Glu Asn Asp Asp Ser Gln Tyr Ser Ser Lys Phe
50 55 60

Gln Gly Arg Val Thr Ile Thr Val Asp Thr Ser Thr Asn Thr Ala Tyr
65 70 75 80
Met Lys Leu Ser Ser Leu Arg Ser Glu Asp Thr Ala Val Tyr Tyr Cys
85 90 95
Thr Tyr Ala Phe Gly Gly Tyr Trp Gly Gln Gly Thr Thr Val Thr Val
100 105 110
Ser Ser

<210> 184
<211> 114
<212> PRT
<213> 人工序列
<220>
<223> 合成序列
<400> 184

Glu Val Gln Leu Val Gln Ser Gly Ala Glu Val Lys Lys Pro Gly Ala
1 5 10 15
Thr Val Lys Ile Ser Cys Lys Ala Ser Gly Phe Ser Ile Lys Asp Asp
20 25 30
Tyr Ile His Trp Val Gln Gln Ala Pro Gly Lys Gly Leu Glu Trp Met
35 40 45
Gly Trp Ile Asp Ala Glu Asn Asp Asp Ser Gln Tyr Ser Ser Lys Phe
50 55 60
Gln Gly Arg Val Thr Ile Thr Val Asp Thr Ser Thr Asn Thr Ala Tyr
65 70 75 80
Met Glu Leu Ser Ser Leu Arg Ser Glu Asp Thr Ala Val Tyr Tyr Cys
85 90 95
Thr Tyr Ala Phe Gly Gly Tyr Trp Gly Gln Gly Thr Thr Val Thr Val
100 105 110
Ser Ser

<210> 185
<211> 114
<212> PRT
<213> 人工序列
<220>
<223> 合成序列
<400> 185

Glu Val Gln Leu Val Gln Ser Gly Ala Glu Val Lys Lys Pro Gly Ala
1 5 10 15
Thr Val Lys Ile Ser Cys Lys Ala Ser Gly Phe Ser Ile Lys Asp Asp

20 25 30
Tyr Ile His Trp Val Gln Gln Ala Pro Gly Lys Gly Leu Glu Trp Met
35 40 45
Gly Trp Ile Asp Ala Glu Asn Asp Asp Ser Glu Tyr Ser Ser Lys Phe
50 55 60
Gln Gly Arg Val Thr Ile Thr Val Asp Thr Ser Thr Asp Thr Ala Tyr
65 70 75 80
Met Glu Leu Ser Ser Leu Arg Ser Glu Asp Thr Ala Val Tyr Tyr Cys
85 90 95
Thr Tyr Ala Phe Gly Gly Tyr Trp Gly Gln Gly Thr Thr Val Thr Val
100 105 110
Ser Ser

<210> 186
<211> 114
<212> PRT
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Glu Val Gln Leu Val Gln Ser Gly Ala Glu Val Lys Lys Pro Gly Ala
1 5 10 15
Thr Val Lys Ile Ser Cys Lys Ala Ser Gly Phe Asn Ile Lys Asp Asp
20 25 30
Tyr Ile His Trp Val Gln Gln Ala Pro Gly Lys Gly Leu Glu Trp Met
35 40 45
Gly Trp Ile Asp Ala Glu Asn Asp Asp Ser Glu Tyr Ser Ser Lys Phe
50 55 60
Gln Gly Arg Val Thr Ile Thr Val Asp Thr Ser Thr Asn Thr Ala Tyr
65 70 75 80
Met Glu Leu Ser Ser Leu Arg Ser Glu Asp Thr Ala Val Tyr Tyr Cys
85 90 95
Thr Tyr Ala Phe Gly Gly Tyr Trp Gly Gln Gly Thr Thr Val Thr Val
100 105 110
Ser Ser

<210> 187
<211> 120
<212> PRT
<213> 人工序列
<220>

<223> 合成序列

<400> 187

Asp Val Val Met Thr Gln Thr Pro Leu Ser Leu Ser Val Thr Pro Gly
1 5 10 15
Gln Pro Ala Ser Ile Ser Cys Arg Ser Ser Gln Ser Leu Val His Ser
20 25 30
Asp Ser Asn Thr Tyr Leu His Trp Tyr Leu Gln Lys Pro Gly Gln Ser
35 40 45
Pro Gln Leu Leu Ile Tyr Leu Ile Ser Asn Arg Phe Ser Gly Val Pro
50 55 60
Asp Arg Phe Ser Gly Ser Gly Ser Gly Thr Asp Phe Thr Leu Lys Ile
65 70 75 80
Ser Arg Val Glu Ala Glu Asp Val Gly Val Tyr Phe Cys Ala Gln Ser
85 90 95
Thr His Val Pro Tyr Ala Phe Gly Gly Gly Thr Lys Val Glu Ile Lys
100 105 110
Arg Thr Val Ala Ala Pro Ser Val
115 120

<210> 188

<211> 120

<212> PRT

<213> 人工序列

<220>

<223> 合成序列

<400> 188

Asp Val Val Met Thr Gln Thr Pro Leu Ser Leu Ser Val Thr Pro Gly
1 5 10 15
Gln Pro Ala Ser Ile Ser Cys Arg Ser Ser Gln Ser Leu Val His Ser
20 25 30
Asp Gly Asn Thr Tyr Leu His Trp Tyr Leu Gln Lys Pro Gly Gln Ser
35 40 45
Pro Gln Leu Leu Ile Tyr Leu Ile Ser Asn Arg Phe Ser Gly Val Pro
50 55 60
Asp Arg Phe Ser Gly Ser Gly Ser Gly Thr Asp Phe Thr Leu Lys Ile
65 70 75 80
Ser Arg Val Glu Ala Glu Asp Val Gly Val Tyr Phe Cys Gly Gln Ser
85 90 95
Thr His Val Pro Tyr Ala Phe Gly Gly Gly Thr Lys Val Glu Ile Lys
100 105 110

Arg Thr Val Ala Ala Pro Ser Val
115 120

<210> 189
<211> 120
<212> PRT
<213> 人工序列
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<400> 189

Asp Ile Val Met Thr Gln Thr Pro Leu Ser Leu Ser Val Thr Pro Gly
1 5 10 15
Gln Pro Ala Ser Ile Ser Cys Arg Ser Ser Gln Ser Leu Val His Ser
20 25 30
Asp Ser Asn Thr Tyr Leu His Trp Tyr Leu Gln Lys Pro Gly Gln Ser
35 40 45
Pro Gln Leu Leu Ile Tyr Leu Ile Ser Asn Arg Phe Ser Gly Val Pro
50 55 60
Asp Arg Phe Ser Gly Ser Gly Ser Gly Thr Asp Phe Thr Leu Lys Ile
65 70 75 80
Ser Arg Val Glu Ala Glu Asp Val Gly Val Tyr Phe Cys Gly Gln Ser
85 90 95
Thr His Val Pro Tyr Ala Phe Gly Gly Gly Thr Lys Val Glu Ile Lys
100 105 110
Arg Thr Val Ala Ala Pro Ser Val
115 120

<210> 190
<211> 119
<212> PRT
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<223> Xaa1 是 Q、V
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<222> (11)..(11)
<223> Xaa2 是 L、V

<220>
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<222> (12)..(12)
<223> Xaa3 是 V、K
<220>
<221> MISC_FEATURE
<222> (13)..(13)
<223> Xaa4 是 R、K
<220>
<221> MISC_FEATURE
<222> (17)..(17)
<223> Xaa5 是 S、T
<220>
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<223> Xaa6 是 L、I
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<223> Xaa7 是 T、K
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<223> Xaa8 是 K、Q
<220>
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<223> Xaa9 是 R、A
<220>
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<223> Xaa10 是 E、G
<220>
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<222> (43)..(43)
<223> Xaa11 是 Q、K
<220>
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<223> Xaa12 是 I、M
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<223> Xaa15 是 A、V
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<223> Xaa16 是 L、I
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<223> Xaa17 是 A、T
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<223> Xaa20 是 V、A
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<223> Xaa21 是 L、M
<220>
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<223> Xaa22 是 H、E
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<223> Xaa23 是 F、L
<220>
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<223> Xaa24 是 T、R
<220>
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<223> Xaa29 是 Y 或 没有
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<223> Xaa30 是 W 或 没有
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<221> MISC_FEATURE
<222> (103)..(103)
<223> Xaa31 是 G 或 没有
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<223> Xaa32 是 T、L
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<223> Xaa34 是 L、V
<220>
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<222> (115)..(115)
<223> Xaa35 是 I、T
<400> 190

Glu Val Gln Leu Xaa Gln Ser Gly Ala Glu Xaa Xaa Xaa Pro Gly Ala
1 5 10 15
Xaa Val Lys Xaa Ser Cys Xaa Val Ser Gly Phe Asn Ile Lys Asp Asp
20 25 30
Tyr Met Phe Trp Val Xaa Gln Xaa Pro Xaa Xaa Gly Leu Glu Trp Xaa
35 40 45
Gly Trp Ile Asp Pro Glu Xaa Gly Asp Thr Glu Tyr Ala Ser Lys Phe
50 55 60
Gln Asp Xaa Xaa Thr Xaa Thr Ala Asp Thr Ser Xaa Xaa Xaa Xaa Tyr
65 70 75 80
Xaa Xaa Xaa Ser Ser Leu Xaa Ser Glu Asp Thr Ala Val Tyr Tyr Cys
85 90 95
Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa Phe Ala Tyr Trp Gly Gln Gly Thr
100 105 110
Xaa Xaa Xaa Val Ser Ser Ala
115

<210> 191
<211> 119
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<220>
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<223> Xaa4 是 L 或 没有
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<221> MISC_FEATURE
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<223> Xaa5 是 F 或 没有
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<221> MISC_FEATURE
<222> (100)..(100)
<223> Xaa6 是 A 或 没有
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<222> (101)..(101)
<223> Xaa7 是 Y 或 没有
<220>
<221> MISC_FEATURE
<222> (102)..(102)
<223> Xaa8 是 W 或 没有
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<221> MISC_FEATURE
<222> (103)..(103)
<223> Xaa9 是 G 或 没有
<220>
<221> MISC_FEATURE
<222> (104)..(104)
<223> Xaa10 是 T、L

<400> 191

Glu Val Gln Leu Val Gln Ser Gly Ala Glu Val Lys Lys Pro Gly Ala
1 5 10 15
Thr Val Lys Ile Ser Cys Lys Val Ser Gly Phe Asn Ile Lys Asp Asp
20 25 30
Tyr Met Phe Trp Val Gln Gln Ala Pro Gly Lys Gly Leu Glu Trp Met
35 40 45
Gly Trp Ile Asp Pro Glu Xaa Gly Asp Thr Glu Tyr Ala Ser Lys Phe
50 55 60
Gln Asp Arg Val Thr Ile Thr Ala Asp Thr Ser Thr Xaa Thr Ala Tyr
65 70 75 80
Met Glu Leu Ser Ser Leu Arg Ser Glu Asp Thr Ala Val Tyr Tyr Cys
85 90 95
Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa Phe Ala Tyr Trp Gly Gln Gly Thr
100 105 110
Thr Val Thr Val Ser Ser Ala
115

<210> 192

<211> 113

<212> PRT

<213> 人工序列

<220>

<223> 合成序列

<400> 192

Glu Val Gln Leu Gln Gln Ser Gly Ala Glu Leu Val Arg Pro Gly Ala
1 5 10 15
Ser Val Lys Leu Ser Cys Thr Val Ser Gly Phe Asn Ile Lys Asp Asp
20 25 30
Tyr Met Phe Trp Val Lys Gln Arg Pro Glu Gln Gly Leu Glu Trp Ile
35 40 45
Gly Trp Ile Asp Pro Glu Asn Gly Asp Thr Glu Tyr Ala Ser Lys Phe
50 55 60
Gln Asp Lys Ala Thr Leu Thr Ala Asp Thr Ser Ala Asn Ile Val Tyr
65 70 75 80
Leu His Phe Ser Ser Leu Thr Ser Glu Asp Thr Ala Val Tyr Tyr Cys
85 90 95
Thr Leu Phe Ala Tyr Trp Gly Gln Gly Thr Ser Leu Ile Val Ser Ser
100 105 110
Ala

<210> 193
<211> 113
<212> PRT
<213> 人工序列
<220>
<223> 合成序列
<400> 193

Glu Val Gln Leu Val Gln Ser Gly Ala Glu Val Lys Lys Pro Gly Ala
1 5 10 15
Thr Val Lys Ile Ser Cys Lys Val Ser Gly Phe Asn Ile Lys Asp Asp
20 25 30
Tyr Met Phe Trp Val Gln Gln Ala Pro Gly Lys Gly Leu Glu Trp Met
35 40 45
Gly Trp Ile Asp Pro Glu Asn Gly Asp Thr Glu Tyr Ala Ser Lys Phe
50 55 60
Gln Asp Arg Val Thr Ile Thr Ala Asp Thr Ser Thr Asp Thr Ala Tyr
65 70 75 80
Met Glu Leu Ser Ser Leu Arg Ser Glu Asp Thr Ala Val Tyr Tyr Cys
85 90 95
Ala Thr Phe Ala Tyr Trp Gly Gln Gly Thr Thr Val Thr Val Ser Ser
100 105 110
Ala

<210> 194
<211> 113
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<400> 194

Glu Val Gln Leu Val Gln Ser Gly Ala Glu Val Lys Lys Pro Gly Ala
1 5 10 15
Thr Val Lys Ile Ser Cys Lys Val Ser Gly Phe Asn Ile Lys Asp Asp
20 25 30
Tyr Met Phe Trp Val Gln Gln Ala Pro Gly Lys Gly Leu Glu Trp Met
35 40 45
Gly Trp Ile Asp Pro Glu Asn Gly Asp Thr Glu Tyr Ala Ser Lys Phe
50 55 60
Gln Asp Arg Val Thr Ile Thr Ala Asp Thr Ser Thr Asn Thr Ala Tyr
65 70 75 80

Met Glu Leu Ser Ser Leu Arg Ser Glu Asp Thr Ala Val Tyr Tyr Cys
85 90 95
Thr Leu Phe Ala Tyr Trp Gly Gln Gly Thr Thr Val Thr Val Ser Ser
100 105 110
Ala

<210> 195
<211> 119
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<223> 合成序列
<400> 195
Glu Val Gln Leu Val Gln Ser Gly Ala Glu Val Lys Lys Pro Gly Ala
1 5 10 15
Thr Val Lys Ile Ser Cys Lys Val Ser Gly Phe Asn Ile Lys Asp Asp
20 25 30
Tyr Met Phe Trp Val Gln Gln Ala Pro Gly Lys Gly Leu Glu Trp Met
35 40 45
Gly Trp Ile Asp Pro Glu Gln Gly Asp Thr Glu Tyr Ala Ser Lys Phe
50 55 60
Gln Asp Arg Val Thr Ile Thr Ala Asp Thr Ser Thr Asn Thr Ala Tyr
65 70 75 80
Met Glu Leu Ser Ser Leu Arg Ser Glu Asp Thr Ala Val Tyr Tyr Cys
85 90 95
Thr Leu Phe Ala Tyr Trp Gly Leu Phe Ala Tyr Trp Gly Gln Gly Thr
100 105 110
Thr Val Thr Val Ser Ser Ala
115

<210> 196
<211> 113
<212> PRT
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<223> Xaa4 是 L、F
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<222> (42)..(42)
<223> Xaa5 是 Q、L
<220>
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<222> (50)..(50)
<223> Xaa6 是 R、K
<220>
<221> MISC_FEATURE
<222> (68)..(68)
<223> Xaa7 是 A、S
<220>
<221> MISC_FEATURE
<222> (88)..(88)
<223> Xaa8 是 V、L
<220>
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<222> (109)..(109)
<223> Xaa9 是 L、V
<400> 196

Asp Val Val Met Thr Gln Xaa Pro Leu Xaa Leu Xaa Val Thr Leu Gly
1 5 10 15
Gln Pro Ala Ser Ile Ser Cys Lys Ser Ser Gln Ser Leu Leu Asp Ser
20 25 30
Asp Gly Lys Thr Tyr Leu Asn Trp Xaa Xaa Gln Arg Pro Gly Gln Ser
35 40 45
Pro Xaa Arg Leu Ile Tyr Leu Val Ser Lys Leu Asp Ser Gly Val Pro

50 55 60

Asp Arg Phe Xaa Gly Ser Gly Ser Gly Thr Asp Phe Thr Leu Lys Ile
65 70 75 80
Ser Arg Val Glu Ala Glu Asp Xaa Gly Val Tyr Tyr Cys Trp Gln Gly
85 90 95
Ala His Phe Pro Gln Thr Phe Gly Gly Gly Thr Lys Xaa Glu Ile Lys
100 105 110
Arg

<210> 197
<211> 108
<212> PRT
<213> 人工序列
<220>
<223> 合成序列
<400> 197

Asp Ile Gln Met Thr Gln Ser Pro Ser Ser Leu Ser Ala Ser Val Gly
1 5 10 15
Asp Arg Val Thr Ile Thr Cys Arg Ala Ser Gln Ser Ile Ser Ser Tyr
20 25 30
Leu Asn Trp Tyr Gln Gln Lys Pro Gly Lys Ala Pro Lys Leu Leu Ile
35 40 45
Tyr Ala Ala Ser Ser Leu Gln Ser Gly Val Pro Ser Arg Phe Ser Gly
50 55 60
Ser Gly Ser Gly Thr Asp Phe Thr Leu Thr Ile Ser Ser Leu Gln Pro
65 70 75 80
Glu Asp Phe Ala Val Tyr Tyr Cys Gln Gln Ser Tyr Ser Thr Pro Leu
85 90 95
Thr Phe Gly Gly Gly Thr Lys Val Glu Ile Lys Arg
100 105

<210> 198
<211> 113
<212> PRT
<213> 人工序列
<220>
<223> 合成序列
<400> 198

Asp Val Val Met Thr Gln Thr Pro Leu Thr Leu Ser Val Thr Leu Gly
1 5 10 15
Gln Pro Ala Ser Ile Ser Cys Lys Ser Ser Gln Ser Leu Leu Asp Ser

20 25 30
Asp Gly Lys Thr Tyr Leu Asn Trp Leu Leu Gln Arg Pro Gly Gln Ser
35 40 45
Pro Lys Arg Leu Ile Tyr Leu Val Ser Lys Leu Asp Ser Gly Val Pro
50 55 60
Asp Arg Phe Ala Gly Ser Gly Ser Gly Thr Asp Phe Thr Leu Lys Ile
65 70 75 80
Ser Arg Val Glu Ala Glu Asp Leu Gly Val Tyr Tyr Cys Trp Gln Gly
85 90 95
Ala His Phe Pro Gln Thr Phe Gly Gly Gly Thr Lys Leu Glu Ile Lys
100 105 110
Arg

<210> 199
<211> 113
<212> PRT
<213> 人工序列
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<223> 合成序列
<400> 199
Asp Val Val Met Thr Gln Ser Pro Leu Ser Leu Pro Val Thr Leu Gly
1 5 10 15
Gln Pro Ala Ser Ile Ser Cys Lys Ser Ser Gln Ser Leu Leu Asp Ser
20 25 30
Asp Gly Lys Thr Tyr Leu Asn Trp Phe Gln Gln Arg Pro Gly Gln Ser
35 40 45
Pro Arg Arg Leu Ile Tyr Leu Val Ser Lys Leu Asp Ser Gly Val Pro
50 55 60
Asp Arg Phe Ser Gly Ser Gly Ser Gly Thr Asp Phe Thr Leu Lys Ile
65 70 75 80
Ser Arg Val Glu Ala Glu Asp Val Gly Val Tyr Tyr Cys Trp Gln Gly
85 90 95
Ala His Phe Pro Gln Thr Phe Gly Gly Gly Thr Lys Val Glu Ile Lys
100 105 110
Arg

<210> 200
<211> 113
<212> PRT
<213> 人工序列
<220>

<223> 合成序列

<400> 200

Asp Val Val Met Thr Gln Ser Pro Leu Ser Leu Pro Val Thr Leu Gly
1 5 10 15
Gln Pro Ala Ser Ile Ser Cys Lys Ser Ser Gln Ser Leu Leu Asp Ser
20 25 30
Asp Gly Lys Thr Tyr Leu Asn Trp Leu Gln Gln Arg Pro Gly Gln Ser
35 40 45
Pro Lys Arg Leu Ile Tyr Leu Val Ser Lys Leu Asp Ser Gly Val Pro
50 55 60
Asp Arg Phe Ser Gly Ser Gly Ser Gly Thr Asp Phe Thr Leu Lys Ile
65 70 75 80
Ser Arg Val Glu Ala Glu Asp Val Gly Val Tyr Tyr Cys Trp Gln Gly
85 90 95
Ala His Phe Pro Gln Thr Phe Gly Gly Gly Thr Lys Val Glu Ile Lys
100 105 110
Arg

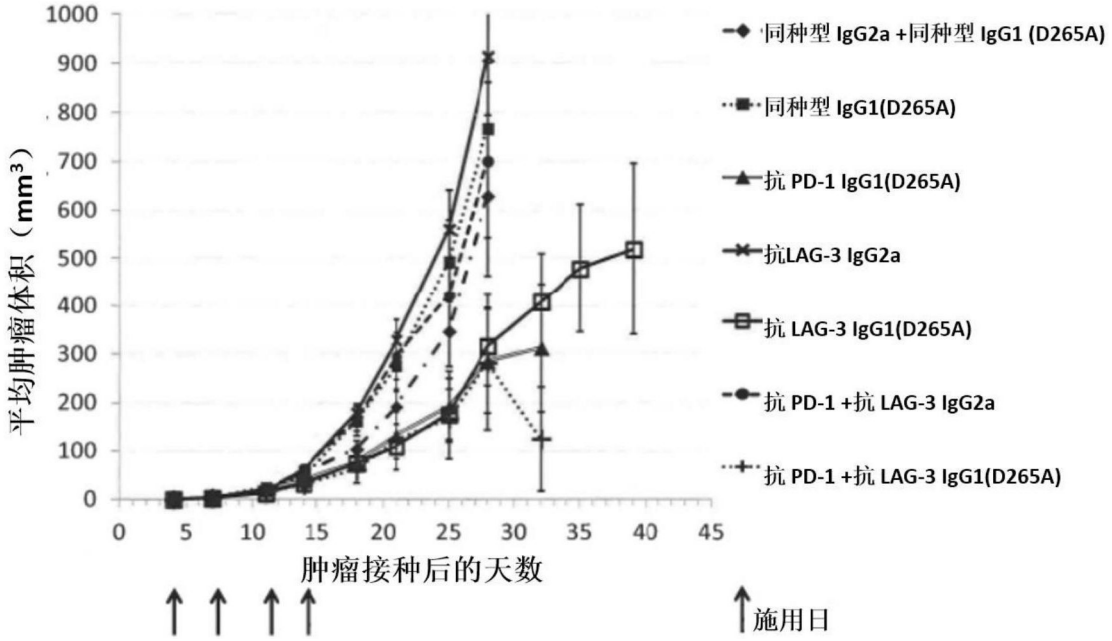

平均肿瘤体积（mm³）
肿瘤接种后的天数
施用日
同种型 IgG2a +同种型 IgG1 (D265A)
同种型 IgG1(D265A)
抗 PD-1 IgG1(D265A)
抗LAG-3 IgG2a
抗 LAG-3 IgG1(D265A)
抗 PD-1 +抗 LAG-3 IgG2a
抗 PD-1 +抗 LAG-3 IgG1(D265A)

图1A

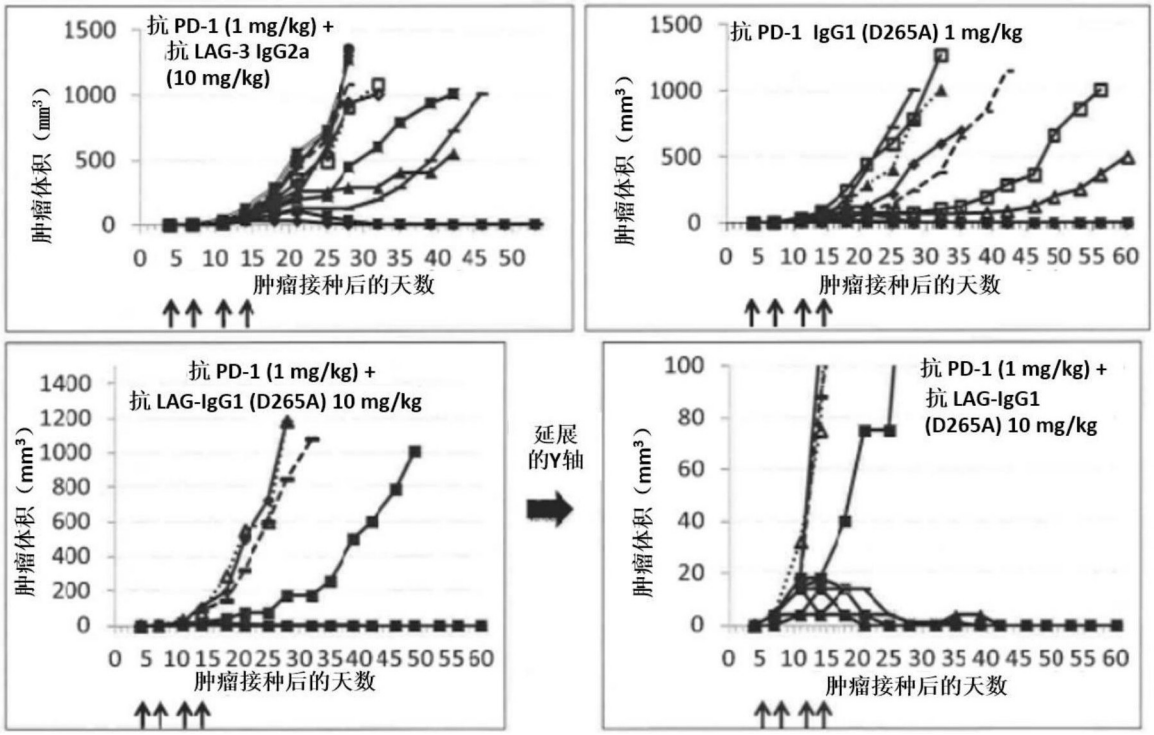
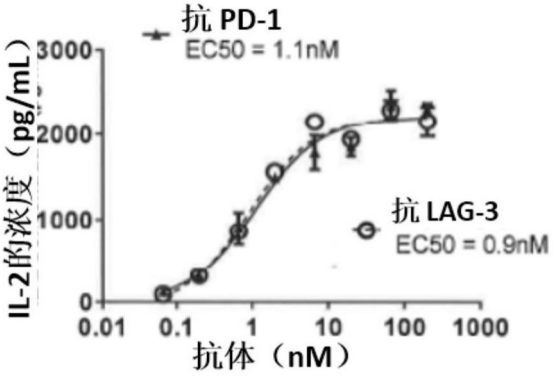
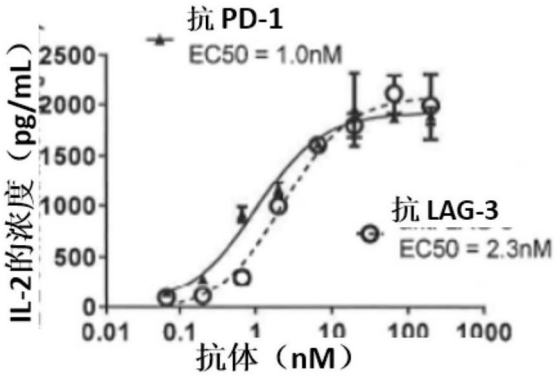

抗 PD-1 (1 mg/kg) +
抗 LAG-3 IgG2a
(10 mg/kg)
抗 PD-1 IgG1 (D265A) 1 mg/kg
抗 PD-1 (1 mg/kg) +
抗 LAG-IgG1 (D265A) 10 mg/kg
延展
的Y轴
抗 PD-1 (1 mg/kg) +
抗 LAG-IgG1
(D265A) 10 mg/kg
肿瘤体积（mm³）
肿瘤接种后的天数

图1B

在t = 24小时添加
在72小时收获



在t = 0添加
在48小时收获



在t = 0添加
在24小时收获

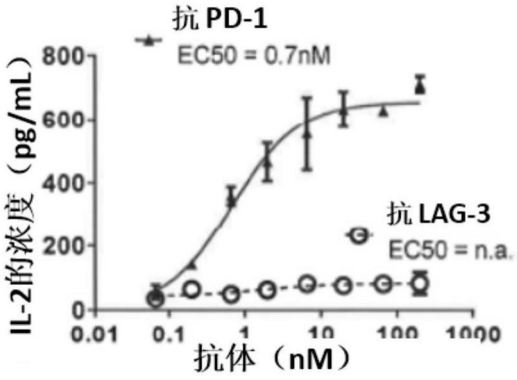


图2A

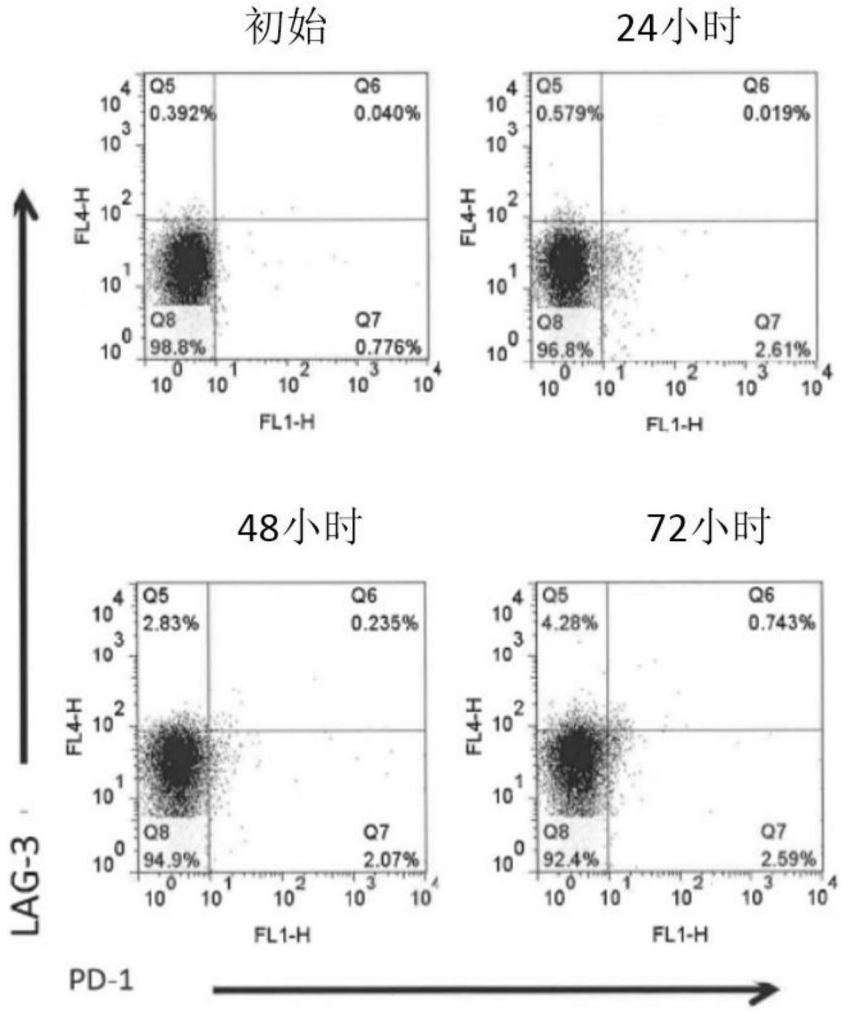

初始
Q5
0.392%
Q6
0.040%
Q8
98.8%
Q7
0.776%
24小时
Q5
0.579%
Q6
0.019%
Q8
96.8%
Q7
2.61%
48小时
Q5
2.83%
Q6
0.235%
Q8
94.9%
Q7
2.07%
72小时
Q5
4.28%
Q6
0.743%
Q8
92.4%
Q7
2.59%
FL4-H
FL1-H
LAG-3
PD-1

图2B