

ANTI-LAG-3 ANTIBODIES AND METHODS OF USE THEREOF

1. RELATED APPLICATIONS

[0001] This application claims the benefit of U.S. Provisional Application Nos.: 62/406,766, filed October 11, 2016; and 62/420,280, filed November 10, 2016, each of which is incorporated by reference herein in its entirety.

2. FIELD

[0002] The instant disclosure relates to antibodies that specifically bind to LAG-3 (*e.g.*, human LAG-3) and methods of using the same.

3. BACKGROUND

[0003] Lymphocyte activation gene 3 (LAG-3), also known as CD223, is a type I membrane protein in the immunoglobulin (Ig) superfamily that is composed of four extracellular Ig domains and a cytoplasmic domain containing a conserved repeated EP motif and a single conserved KIEELE motif (Triebel *et al.*, (1990) J Exp Med, 171: 1393-405; Workman *et al.*, (2002) J Immunol, 169: 5392-5). LAG-3 is expressed on activated effector T lymphocytes (Teff), activated regulatory T lymphocytes (Treg), activated B lymphocytes, a subset of resting natural killer (NK) cells, and resting plasmacytoid dendritic cells (PDC) (Huang *et al.*, (2004) Immunity, 21: 503-13; Workman *et al.*, (2009) J Immunol, 182: 1885-91; Kisielow *et al.*, (2005) Eur J Immunol, 35: 2081-8; Baixeras *et al.*, (1992) J Exp Med, 176: 327-37; Workman *et al.*, (2002) Eur J Immunol, 32: 2255-63). Under conditions of persistent antigenic exposure, such as in chronic pathogenic infections or within the tumor microenvironment (TME), LAG-3 expression is sustained on T regulatory type 1 cells (Tr1) and so-called exhausted antigen-specific T cells (Park *et al.*, (2012) Cell Immunol, 278: 76-83; Gagliani *et al.*, (2013) Nat Med, 19: 739-46; Blackburn *et al.*, (2009) Nat Immunol, 10: 29-37).

[0004] LAG-3 functions to negatively regulate activated T cells. The ligand for LAG-3 is MHC class II, expressed on antigen presenting cells (APC) and activated T cells (Roche and Furuta (2015) Nat Rev Immunol, 15: 203-16). The interaction between LAG-3 and its ligand inhibits proliferation and cytokine secretion of CD4⁺ and CD8⁺ Teff cells (Macon-Lemaitre and Triebel (2005) Immunology, 115: 170-8; Huard *et al.*, (1997) Proc Natl Acad Sci U S A, 94: 5744-9). LAG-3 in Tregs and PDCs contributes to the negative regulation of T cell function (Huang *et al.*, (2004) Immunity, 21: 503-13; Workman *et al.*, (2009) J Immunol, 182: 1885-91). Consistent with its role in maintaining immune homeostasis, LAG-3 deficiency induced lethal myocarditis in mice also genetically deficient in PD-1 (Okazaki *et*

al., (2011) J Exp Med, 208: 395-407). Furthermore, *in vivo* blockade with a monoclonal antibody against mouse LAG-3 in combination with PD-1 blockade synergized to potentiate anti-tumor immunity in syngeneic mouse tumor models (Woo *et al.*, (2012) Cancer Res, 72: 917-27).

5 [0005] Given LAG-3's role in modulating immune responses, therapeutic agents designed to antagonize LAG-3 signaling hold great promise for the treatment of diseases that involve LAG-3-mediated immune suppression.

4. SUMMARY

[0006] The instant disclosure provides antibodies that specifically bind to LAG-3 (*e.g.*,
10 human LAG-3) and antagonize LAG-3 function, *e.g.*, LAG-3-mediated immune suppression. Also provided are pharmaceutical compositions comprising these antibodies, nucleic acids encoding these antibodies, expression vectors and host cells for making these antibodies, and methods of treating a subject using these antibodies. The antibodies disclosed herein are particularly useful for increasing T cell activation in response to an antigen (*e.g.*, a tumor
15 antigen or an infectious disease antigen) and/or decreasing Treg-mediated immune suppression, and hence for treating cancer in a subject or treating or preventing an infectious disease in a subject.

[0007] Accordingly, in one aspect, the instant disclosure provides an antibody or isolated antibody comprising a heavy chain variable region comprising complementarity determining
20 regions CDRH1, CDRH2 and CDRH3 and a light chain variable region comprising complementarity determining regions CDRL1, CDRL2 and CDRL3, wherein:

(a) CDRH1 comprises the amino acid sequence of DX₁YX₂X₃ (SEQ ID NO: 140),
wherein

X₁ is T or N,
25 X₂ is I or M, and
X₃ is H, Y or D;

(b) CDRH2 comprises the amino acid sequence of X₁IDPANX₂X₃X₄X₅X₆X₇PX₈X₉QX₁₀
(SEQ ID NO: 142), wherein

X₁ is E, R, S, or K,
30 X₂ is D or G,
X₃ is N or H,
X₄ is T or S,
X₅ is K or H,
X₆ is Y or F,

X₇ is D or A,

X₈ is K or R,

X₉ is F or L, and

X₁₀ is G or D;

5 (c) CDRH3 comprises the amino acid sequence of YX₁X₂X₃YX₄VGGX₅DY (SEQ ID NO: 144), wherein

X₁ is Y, F, or S,

X₂ is Y or D,

X₃ is K or R,

10 X₄ is D or E, and

X₅ is F or C;

(d) CDRL1 comprises the amino acid sequence of SVSSX₁ISSSX₂LX₃ (SEQ ID NO: 147), wherein

X₁ is S or G,

15 X₂ is N or T, and

X₃ is H or Y;

(e) CDRL2 comprises the amino acid sequence of GTSNLAS (SEQ ID NO: 104); and

(f) CDRL3 comprises the amino acid sequence of QQWX₁X₂YPX₃T (SEQ ID NO: 149), wherein

20 X₁ is S, N, or R,

X₂ is S, T or R, and

X₃ is F, L, H, or W.

[0008] In another aspect, the instant disclosure provides an antibody or isolated antibody that specifically binds to human LAG-3, the antibody comprising a heavy chain variable
25 region comprising complementarity determining regions CDRH1, CDRH2 and CDRH3 and a light chain variable region comprising complementarity determining regions CDRL1, CDRL2 and CDRL3, wherein:

(a) CDRH1 comprises the amino acid sequence of DX₁YX₂X₃ (SEQ ID NO: 140), wherein

30 X₁ is T or N,

X₂ is I or M, and

X₃ is H, Y or D;

(b) CDRH2 comprises the amino acid sequence of X₁IDPANX₂X₃X₄X₅X₆X₇PX₈X₉QX₁₀ (SEQ ID NO: 142), wherein

- X₁ is E, R, S, or K,
 X₂ is D or G,
 X₃ is N or H,
 X₄ is T or S,
 5 X₅ is K or H,
 X₆ is Y or F,
 X₇ is D or A,
 X₈ is K or R,
 X₉ is F or L, and
 10 X₁₀ is G or D;
 (c) CDRH3 comprises the amino acid sequence of YX₁X₂X₃YX₄VGGX₅DY (SEQ ID NO: 144), wherein
 X₁ is Y, F, or S,
 X₂ is Y or D,
 15 X₃ is K or R,
 X₄ is D or E, and
 X₅ is F or C;
 (d) CDRL1 comprises the amino acid sequence of SVSSX₁ISSSX₂LX₃ (SEQ ID NO: 147), wherein
 20 X₁ is S or G,
 X₂ is N or T, and
 X₃ is H or Y;
 (e) CDRL2 comprises the amino acid sequence of GTSNLAS (SEQ ID NO: 104); and
 (f) CDRL3 comprises the amino acid sequence of QQWX₁X₂YPX₃T (SEQ ID NO: 149),
 25 wherein
 X₁ is S, N, or R,
 X₂ is S, T or R, and
 X₃ is F, L, H, or W.

[0009] In certain embodiments, CDRH1 comprises the amino acid sequence of DX₁YX₂X₃ (SEQ ID NO: 141), wherein: X₁ is T or N; X₂ is I or M; and X₃ is H or Y. In certain embodiments, CDRH2 comprises the amino acid sequence of X₁IDPANX₂X₃X₄KX₅X₆PX₇FQX₈ (SEQ ID NO: 143), wherein: X₁ is E, R, or S; X₂ is D or G; X₃ is N or H; X₄ is T or S; X₅ is Y or F; X₆ is D or A; X₇ is K or R; and X₈ is G or D. In certain embodiments, CDRH3 comprises the amino acid sequence of

YX₁X₂X₃YDVGGX₄DY (SEQ ID NO: 145), wherein: X₁ is Y, F, or S; X₂ is Y or D; X₃ is K or R; and X₄ is F or C. In certain embodiments, CDRH3 comprises the amino acid sequence of YYYX₁YX₂VGGFDY (SEQ ID NO: 146), wherein: X₁ is K or R; and X₂ is D or E. In certain embodiments, CDRL1 comprises the amino acid sequence of SVSSSISSSNLX₁ (SEQ ID NO: 148), wherein: X₁ is H or Y. In certain embodiments, CDRL3 comprises the amino acid sequence of QQWX₁SYPX₂T (SEQ ID NO: 150), wherein: X₁ is S, N, or R; and X₂ is F, L, or H.

[0010] In certain embodiments:

- (a) CDRH1 comprises the amino acid sequence of DTYIH (SEQ ID NO: 79);
- (b) CDRH2 comprises the amino acid sequence of EIDPANDNTKYDPKFQG (SEQ ID NO: 90);
- (c) CDRH3 comprises the amino acid sequence of YYYX₁YX₂VGGFDY (SEQ ID NO: 146), wherein: X₁ is K or R; and X₂ is D or E;
- (d) CDRL1 comprises the amino acid sequence of SVSSSISSSNLH (SEQ ID NO: 100);
- (e) CDRL2 comprises the amino acid sequence of GTSNLAS (SEQ ID NO: 104); and
- (f) CDRL3 comprises the amino acid sequence of QQWSSYPFT (SEQ ID NO: 105).

[0011] In certain embodiments, CDRH1, CDRH2, and CDRH3 comprise the amino acid sequences set forth in SEQ ID NOs: 79, 90, and 98, respectively. In certain embodiments, the heavy chain variable region comprises an amino acid sequence that is at least 75%, 80%, 85%, 90%, 95%, 97%, 98%, or 99% identical to the amino acid sequence of SEQ ID NO: 65 or 220. In certain embodiments, the heavy chain variable region comprises an amino acid sequence that is at least 98% identical to the amino acid sequence of SEQ ID NO: 65 or 220. In certain embodiments, the heavy chain variable region comprises the amino acid sequence of SEQ ID NO: 220. In certain embodiments, the heavy chain variable region comprises the amino acid sequence of SEQ ID NO: 65. In certain embodiments, the heavy chain variable region comprises the amino acid sequence of SEQ ID NO: 220. In certain embodiments, the antibody comprises a heavy chain comprising the amino acid sequence of SEQ ID NO: 226. In certain embodiments, the antibody comprises a heavy chain comprising the amino acid sequence of SEQ ID NO: 169.

[0012] In certain embodiments, CDRL1, CDRL2, and CDRL3 comprise the amino acid sequences set forth in SEQ ID NOs: 100, 104, and 105, respectively. In certain embodiments, the light chain variable region comprises an amino acid sequence that is at least 75%, 80%, 85%, 90%, 95%, 97%, 98%, or 99% identical to the amino acid sequence of SEQ ID NO: 73 or 221. In certain embodiments, the light chain variable region comprises an

amino acid sequence that is at least 98% identical to the amino acid sequence of SEQ ID NO: 73 or 221. In certain embodiments, the light chain variable region comprises the amino acid sequence of SEQ ID NO: 221. In certain embodiments, the light chain variable region comprises the amino acid sequence of SEQ ID NO: 73. In certain embodiments, the antibody
 5 comprises a light chain comprising the amino acid sequence of SEQ ID NO: 228. In certain embodiments, the antibody comprises a light chain comprising the amino acid sequence of SEQ ID NO: 187.

[0013] In certain embodiments, CDRH1 comprises an amino acid sequence selected from the group consisting of SEQ ID NOs: 78-82. In certain embodiments, CDRH2 comprises an amino acid sequence selected from the group consisting of SEQ ID NOs: 83-93. In certain
 10 embodiments, CDRH3 comprises an amino acid sequence selected from the group consisting of SEQ ID NOs: 94-99. In certain embodiments, CDRL1 comprises an amino acid sequence selected from the group consisting of SEQ ID NOs: 100-103. In certain embodiments, CDRL3 comprises an amino acid sequence selected from the group consisting of SEQ ID
 15 NOs: 105-112.

[0014] In certain embodiments, CDRH1, CDRH2 and CDRH3 comprise the CDRH1, CDRH2 and CDRH3 amino acid sequences, respectively, set forth in SEQ ID NOs: 78, 83, and 94; 78, 85, and 95; 78, 86, and 96; 78, 86, and 97; 78, 91, and 94; 78, 92, and 96; 79, 84, and 95; 79, 88, and 95; 79, 89, and 95; 79, 90, and 95; 79, 90, and 98; 79, 90, and 99; 80, 85, and 96; 81, 87, and 96; or, 82, 93, and 95.
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[0015] In certain embodiments, CDRL1, CDRL2 and CDRL3 comprise the CDRL1, CDRL2 and CDRL3 amino acid sequences, respectively, set forth in SEQ ID NOs: 100, 104, and 105; 100, 104, and 106; 100, 104, and 107; 100, 104, and 109; 100, 104, and 110; 101, 104, and 108; 102, 104, and 105; 102, 104, and 112; or, 103, 104, and 111.

[0016] In certain embodiments, CDRH1, CDRH2, CDRH3, CDRL1, CDRL2, and CDRL3 comprise the amino acid sequences set forth in SEQ ID NOs: 78, 83, 94, 100, 104, and 105; 78, 85, 95, 100, 104, and 105; 78, 86, 96, 100, 104, and 105; 78, 86, 96, 100, 104, and 109; 78, 86, 96, 100, 104, and 110; 78, 86, 96, 101, 104, and 108; 78, 86, 96, 103, 104, and 111; 78, 86, 97, 102, 104, and 112; 78, 91, 94, 100, 104, and 107; 78, 92, 96, 100, 104, and 105; 78, 92, 96, 100, 104, and 109; 79, 84, 95, 100, 104, and 105; 79, 84, 95, 100, 104, and 106; 79, 84, 95, 102, 104, and 105; 79, 88, 95, 100, 104, and 105; 79, 89, 95, 100, 104, and 105; 79, 90, 95, 100, 104, and 105; 79, 90, 98, 100, 104, and 105; 79, 90, 99, 100, 104, and 105; 80, 85, 96, 100, 104, and 105; 81, 87, 96, 100, 104, and 105; 81, 87, 96, 100, 104, and 107; or, 82, 93, 95, 100, 104, and 105, respectively.
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[0017] In another aspect, the instant disclosure provides an antibody or isolated antibody comprising a heavy chain variable region comprising complementarity determining regions CDRH1, CDRH2 and CDRH3, and a light chain variable region comprising complementarity determining regions CDRL1, CDRL2 and CDRL3, wherein CDRH1, CDRH2, CDRH3, CDRL1, CDRL2, and CDRL3 comprise the amino acid sequences set forth in SEQ ID NOs: 79, 90, 95, 100, 104, and 105, respectively.

[0018] In another aspect, the instant disclosure provides an antibody or isolated antibody that specifically binds to human LAG-3, comprising a heavy chain variable region comprising complementarity determining regions CDRH1, CDRH2 and CDRH3, and a light chain variable region comprising complementarity determining regions CDRL1, CDRL2 and CDRL3, wherein CDRH1, CDRH2, CDRH3, CDRL1, CDRL2, and CDRL3 comprise the amino acid sequences set forth in SEQ ID NOs: 79, 90, 95, 100, 104, and 105, respectively.

[0019] In another aspect, the instant disclosure provides an antibody or isolated antibody comprising a heavy chain variable region comprising complementarity determining regions CDRH1, CDRH2 and CDRH3, and a light chain variable region comprising complementarity determining regions CDRL1, CDRL2 and CDRL3, wherein CDRH1, CDRH2, CDRH3, CDRL1, CDRL2, and CDRL3 comprise the amino acid sequences set forth in SEQ ID NOs: 79, 90, 98, 100, 104, and 105, respectively.

[0020] In another aspect, the instant disclosure provides an antibody or isolated antibody that specifically binds to human LAG-3, comprising a heavy chain variable region comprising complementarity determining regions CDRH1, CDRH2 and CDRH3, and a light chain variable region comprising complementarity determining regions CDRL1, CDRL2 and CDRL3, wherein CDRH1, CDRH2, CDRH3, CDRL1, CDRL2, and CDRL3 comprise the amino acid sequences set forth in SEQ ID NOs: 79, 90, 98, 100, 104, and 105, respectively.

[0021] In certain embodiments, the heavy chain variable region comprises an amino acid sequence that is at least 75%, 80%, 85%, 90%, 95%, 97%, 98%, or 99% identical to the amino acid sequence of SEQ ID NO: 65 or 220. In certain embodiments, the heavy chain variable region comprises an amino acid sequence that is at least 98% identical to the amino acid sequence of SEQ ID NO: 65 or 220. In certain embodiments, the heavy chain variable region comprises the amino acid sequence of SEQ ID NO: 220. In certain embodiments, the heavy chain variable region comprises the amino acid sequence of SEQ ID NO: 65. In certain embodiments, the antibody comprises a heavy chain comprising the amino acid sequence of SEQ ID NO: 226. In certain embodiments, the antibody comprises a heavy chain comprising the amino acid sequence of SEQ ID NO: 169.

[0022] In certain embodiments, the light chain variable region comprises an amino acid sequence that is at least 75%, 80%, 85%, 90%, 95%, 97%, 98%, or 99% identical to the amino acid sequence of SEQ ID NO: 73 or 221. In certain embodiments, the light chain variable region comprises an amino acid sequence that is at least 98% identical to the amino acid sequence of SEQ ID NO: 73 or 221. In certain embodiments, the light chain variable region comprises the amino acid sequence of SEQ ID NO: 221. In certain embodiments, the light chain variable region comprises the amino acid sequence of SEQ ID NO: 73. In certain embodiments, the antibody comprises a light chain comprising the amino acid sequence of SEQ ID NO: 228. In certain embodiments, the antibody comprises a light chain comprising the amino acid sequence of SEQ ID NO: 228. In certain embodiments, the antibody comprises a light chain comprising the amino acid sequence of SEQ ID NO: 187.

[0023] In certain embodiments, the heavy chain variable region comprises an amino acid sequence that is at least 75%, 80%, 85%, 90%, 95%, 97%, 98%, or 99% identical to the amino acid sequence of SEQ ID NO: 65 or 220, and the light chain variable region comprises an amino acid sequence that is at least 75%, 80%, 85%, 90%, 95%, 97%, 98%, or 99% identical to the amino acid sequence of SEQ ID NO: 73 or 221. In certain embodiments, the heavy chain variable region comprises an amino acid sequence that is at least 98% identical to the amino acid sequence of SEQ ID NO: 65 or 220, and the light chain variable region comprises an amino acid sequence that is at least 98% identical to the amino acid sequence of SEQ ID NO: 73 or 221. In certain embodiments, the heavy chain variable region comprises the amino acid sequence of SEQ ID NO: 220. In certain embodiments, the heavy chain variable region comprises the amino acid sequence of SEQ ID NO: 65. In certain embodiments, the light chain variable region comprises the amino acid sequence of SEQ ID NO: 73 or 221. In certain embodiments, the light chain variable region comprises the amino acid sequence of SEQ ID NO: 73.

[0024] In another aspect, the instant disclosure provides an isolated antibody that specifically binds to human LAG-3, comprising a heavy chain variable region comprising the amino acid sequence of SEQ ID NO: 65 or 220. In certain embodiments, the antibody comprises a light chain variable region comprising an amino acid sequence that is at least 75%, 80%, 85%, 90%, 95%, 97%, 98%, or 99% identical to the amino acid sequence of SEQ ID NO: 73 or 221. In certain embodiments, the antibody comprises a light chain variable region comprising an amino acid sequence that is at least 98% identical to the amino acid sequence of SEQ ID NO: 73 or 221.

[0025] In another aspect, the instant disclosure provides an isolated antibody that

specifically binds to human LAG-3, comprising a light chain variable region comprising the amino acid sequence of SEQ ID NO: 73 or 221. In certain embodiments, the antibody comprises a heavy chain variable region comprising an amino acid sequence that is at least 75%, 80%, 85%, 90%, 95%, 97%, 98%, or 99% identical to the amino acid sequence of SEQ ID NO: 65 or 220. In certain embodiments, the antibody comprises a heavy chain variable region comprising an amino acid sequence that is at least 98% identical to the amino acid sequence of SEQ ID NO: 65 or 220.

[0026] In certain embodiments, the antibody comprises a heavy chain variable region comprising the framework regions of the heavy chain variable region sequence of SEQ ID NO: 151 or 222. In certain embodiments, the antibody comprises a heavy chain variable region comprising the amino acid sequence of SEQ ID NO: 151 or 222. In certain embodiments, the antibody comprises a heavy chain variable region comprising the framework regions of the heavy chain variable region sequence of SEQ ID NO: 218 or 223. In certain embodiments, the antibody comprises a heavy chain variable region comprising the amino acid sequence of SEQ ID NO: 218 or 223. In certain embodiments, the antibody comprises a heavy chain variable region comprising an amino acid sequence which is at least 75%, 80%, 85%, 90%, 95%, or 100% identical to an amino acid sequence selected from the group consisting of SEQ ID NOs: 56-72 and 220. In certain embodiments, the heavy chain variable region comprises an amino acid sequence selected from the group consisting of SEQ ID NOs: 56-72 and 220. In certain embodiments, the heavy chain variable region comprises the amino acid sequence of SEQ ID NO: 220. In certain embodiments, the heavy chain variable region comprises the amino acid sequence of SEQ ID NO: 65. In certain embodiments, the antibody comprises a heavy chain comprising an amino acid sequence selected from the group consisting of SEQ ID NOs: 168-186 and 225-227. In certain embodiments, the antibody comprises a heavy chain variable region having human derived framework regions. In certain embodiments, the antibody comprises a heavy chain variable framework region that is or is derived from an amino acid sequence encoded by a human gene, wherein said amino acid sequence is selected from the group consisting of IGHV1-46*01 (SEQ ID NO: 153), IGHV1-69-2*01 (SEQ ID NO: 154), IGHV1-3*01 (SEQ ID NO: 155), IGHV1-24*01 (SEQ ID NO: 156), IGHV1-2*01 (SEQ ID NO: 157), IGHV1-45*01 (SEQ ID NO: 158), and IGHV1-18*01 (SEQ ID NO: 159). In certain embodiments, the antibody comprises a heavy chain variable framework region that is derived from the amino acid sequence IGHV1-46*01 (SEQ ID NO: 153), wherein at least one amino acid in the amino acid sequence IGHV1-46*01 (SEQ ID NO: 153) is substituted with an amino acid in

an analogous position in a corresponding non-human heavy chain variable framework region. In certain embodiments, the amino acid substitution is at an amino acid position selected from the group consisting of 4, 5, 12, 23, 27, 28, 29, 30, 48, 69, 71, 75, 76, 80, 81, and 94, wherein the amino acid position is indicated according to the Kabat numbering system. In

5 certain embodiments, the amino acid substitution is selected from the group consisting of 4M, 5K, 12V, 23T, 27F, 28N, 29I, 30K, 48I, 69I, 71A, 75S, 76N, 80L, 81Q, and 94T, wherein the position of the amino acid substitution is indicated according to the Kabat numbering system. In certain embodiments, the amino acid substitution is at an amino acid position selected from the group consisting of 4, 27, 28, 29, 30, 69, 71, and 94, wherein the amino acid

10 position is indicated according to the Kabat numbering system. In certain embodiments, the amino acid substitution is selected from the group consisting of 4M, 27F, 28N, 29I, 30K, 69I, 71A, and 94T, wherein the position of the amino acid substitution is indicated according to the Kabat numbering system.

[0027] In certain embodiments, the antibody comprises a light chain variable region

15 comprising the framework regions of the light chain variable region sequence of SEQ ID NO: 152 or 224. In certain embodiments, the antibody comprises a light chain variable region comprising the amino acid sequence of SEQ ID NO: 152 or 224. In certain embodiments, the antibody comprises a light chain variable region comprising an amino acid sequence which is at least 75%, 80%, 85%, 90%, 95%, or 100% identical to an amino acid sequence selected

20 from the group consisting of SEQ ID NOs: 73-77 and 221. In certain embodiments, the light chain variable region comprises an amino acid sequence selected from the group consisting of SEQ ID NOs: 73-77 and 221. In certain embodiments, the light chain variable region comprises the amino acid sequence of SEQ ID NO: 221. In certain embodiments, the light chain variable region comprises the amino acid sequence of SEQ ID NO: 73. In certain

25 embodiments, the antibody comprises a light chain comprising an amino acid sequence selected from the group consisting of SEQ ID NOs: 187-191. In certain embodiments, the antibody comprises a light chain variable region having human derived framework regions. In certain embodiments, the antibody comprises a light chain variable framework region that is or is derived from an amino acid sequence encoded by a human gene, wherein said amino

30 acid sequence is selected from the group consisting of IGKV3-20*01 (SEQ ID NO: 160), IGKV3D-15*01 (SEQ ID NO: 161), IGKV3-15*01 (SEQ ID NO: 161), IGKV3D-20*01 (SEQ ID NO: 162), IGKV3D-7*01 (SEQ ID NO: 163), IGKV1-9*01 (SEQ ID NO: 164), and IGKV3-11*01 (SEQ ID NO: 165). In certain embodiments, the antibody comprises a light chain variable framework region that is from the amino acid sequence IGKV3-20*01 (SEQ

ID NO: 160). In certain embodiments, the antibody comprises a light chain variable framework region that is derived from the amino acid sequence IGKV3-20*01 (SEQ ID NO: 160), wherein at least one amino acid in the amino acid sequence IGKV3-20*01 (SEQ ID NO: 160) is substituted with an amino acid in an analogous position in a corresponding non-human light chain variable framework region. In certain embodiments, the amino acid substitution is at an amino acid position selected from the group consisting of 3, 22, 36, 43, 47, 58, 70, and 71, wherein the amino acid position is indicated according to the Kabat numbering system. In certain embodiments, the amino acid substitution is selected from the group consisting of 3L, 22T, 36F, 43S, 47W, 58V, 70S, and 71Y, wherein the position of the amino acid substitution is indicated according to the Kabat numbering system.

[0028] In another aspect, the instant disclosure provides an antibody or isolated antibody comprising a heavy chain variable region comprising an amino acid sequence selected from the group consisting of SEQ ID NOs: 56-72 and 220. In certain embodiments, the heavy chain variable region comprises the amino acid sequence of SEQ ID NO: 220. In certain embodiments, the heavy chain variable region comprises the amino acid sequence of SEQ ID NO: 65. In certain embodiments, the antibody comprises a heavy chain comprising an amino acid sequence selected from the group consisting of SEQ ID NOs: 168-186 and 225-227. In certain embodiments, the antibody comprises a heavy chain comprising the amino acid sequence of SEQ ID NO: 225. In certain embodiments, the antibody comprises a heavy chain comprising the amino acid sequence of SEQ ID NO: 168. In certain embodiments, the antibody comprises a heavy chain comprising the amino acid sequence of SEQ ID NO: 226. In certain embodiments, the antibody comprises a heavy chain comprising the amino acid sequence of SEQ ID NO: 169. In certain embodiments, the antibody comprises a heavy chain comprising the amino acid sequence of SEQ ID NO: 227. In certain embodiments, the antibody comprises a heavy chain comprising the amino acid sequence of SEQ ID NO: 170.

[0029] In another aspect, the instant disclosure provides an antibody or isolated antibody that specifically binds to human LAG-3, the antibody comprising a heavy chain variable region comprising an amino acid sequence selected from the group consisting of SEQ ID NOs: 56-72 and 220. In certain embodiments, the heavy chain variable region comprises the amino acid sequence of SEQ ID NO: 220. In certain embodiments, the heavy chain variable region comprises the amino acid sequence of SEQ ID NO: 65. In certain embodiments, the antibody comprises a heavy chain comprising an amino acid sequence selected from the group consisting of SEQ ID NOs: 168-186 and 225-227. In certain embodiments, the antibody comprises a heavy chain comprising the amino acid sequence of SEQ ID NO: 225.

In certain embodiments, the antibody comprises a heavy chain comprising the amino acid sequence of SEQ ID NO: 168. In certain embodiments, the antibody comprises a heavy chain comprising the amino acid sequence of SEQ ID NO: 226. In certain embodiments, the antibody comprises a heavy chain comprising the amino acid sequence of SEQ ID NO: 169.

5 In certain embodiments, the antibody comprises a heavy chain comprising the amino acid sequence of SEQ ID NO: 227. In certain embodiments, the antibody comprises a heavy chain comprising the amino acid sequence of SEQ ID NO: 170.

[0030] In another aspect, the instant disclosure provides an antibody or isolated antibody comprising a light chain variable region comprising an amino acid sequence selected from the group consisting of SEQ ID NOs: 73-77 and 221. In certain embodiments, the light chain variable region comprises the amino acid sequence of SEQ ID NO: 221. In certain
10 embodiments, the light chain variable region comprises the amino acid sequence of SEQ ID NO: 73. In certain embodiments, the antibody comprises a light chain comprising an amino acid sequence selected from the group consisting of SEQ ID NOs: 187-191, and 228. In
15 certain embodiments, the antibody comprises a light chain comprising the amino acid sequence of SEQ ID NO: 228. In certain embodiments, the antibody comprises a light chain comprising the amino acid sequence of SEQ ID NO: 187.

[0031] In another aspect, the instant disclosure provides an antibody or isolated antibody that specifically binds to human LAG-3, the antibody comprising a light chain variable region
20 comprising an amino acid sequence selected from the group consisting of SEQ ID NOs: 73-77 and 221. In certain embodiments, the light chain variable region comprises the amino acid sequence of SEQ ID NO: 221. In certain embodiments, the light chain variable region comprises the amino acid sequence of SEQ ID NO: 73. In certain embodiments, the antibody comprises a light chain comprising an amino acid sequence selected from the group
25 consisting of SEQ ID NOs: 187-191, and 228. In certain embodiments, the antibody comprises a light chain comprising the amino acid sequence of SEQ ID NO: 228. In certain embodiments, the antibody comprises a light chain comprising the amino acid sequence of SEQ ID NO: 187.

[0032] In another aspect, the instant disclosure provides an antibody or isolated antibody comprising a heavy chain variable region and a light chain variable region, wherein the heavy chain variable region and the light chain variable region, respectively, comprise the amino acid sequences set forth in SEQ ID NOs: 56 and 73; 56 and 74; 56 and 75; 56 and 76; 56 and
30 77; 57 and 73; 57 and 74; 57 and 75; 57 and 76; 57 and 77; 58 and 73; 58 and 74; 58 and 75; 58 and 76; 58 and 77; 59 and 73; 59 and 74; 59 and 75; 59 and 76; 59 and 77; 60 and 73; 60

and 74; 60 and 75; 60 and 76; 60 and 77; 61 and 77; 62 and 77; 63 and 73; 64 and 73; 65 and 73; 220 and 73; 65 and 221; 220 and 221; 66 and 73; 67 and 73; 68 and 73; 69 and 73; 70 and 73; 71 and 73; or 72 and 73. In certain embodiments, the heavy chain variable region and the light chain variable region, respectively, comprise the amino acid sequences set forth in SEQ ID NOs: 65 and 73. In certain embodiments, the heavy chain variable region and the light chain variable region, respectively, comprise the amino acid sequences set forth in SEQ ID NOs: 220 and 73; 65 and 221; or 220 and 221.

[0033] In another aspect, the instant disclosure provides an antibody or isolated antibody comprising a heavy chain variable region and a light chain variable region, wherein the amino acid sequences of the heavy chain variable region and the light chain variable region, respectively, consist of the amino acid sequences set forth in SEQ ID NOs: 56 and 73; 56 and 74; 56 and 75; 56 and 76; 56 and 77; 57 and 73; 57 and 74; 57 and 75; 57 and 76; 57 and 77; 58 and 73; 58 and 74; 58 and 75; 58 and 76; 58 and 77; 59 and 73; 59 and 74; 59 and 75; 59 and 76; 59 and 77; 60 and 73; 60 and 74; 60 and 75; 60 and 76; 60 and 77; 61 and 77; 62 and 77; 63 and 73; 64 and 73; 65 and 73; 220 and 73; 65 and 221; 220 and 221; 66 and 73; 67 and 73; 68 and 73; 69 and 73; 70 and 73; 71 and 73; or 72 and 73. In certain embodiments, the amino acid sequences of the heavy chain variable region and the light chain variable region, respectively, consist of the amino acid sequences set forth in SEQ ID NOs: 65 and 73. In certain embodiments, the amino acid sequences of the heavy chain variable region and the light chain variable region, respectively, consist of the amino acid sequences set forth in SEQ ID NOs: 220 and 73; 65 and 221; or 220 and 221.

[0034] In another aspect, the instant disclosure provides an antibody or isolated antibody that specifically binds to human LAG-3, the antibody comprising a heavy chain variable region and a light chain variable region, wherein the heavy chain variable region and the light chain variable region, respectively, comprise the amino acid sequences set forth in SEQ ID NOs: 56 and 73; 56 and 74; 56 and 75; 56 and 76; 56 and 77; 57 and 73; 57 and 74; 57 and 75; 57 and 76; 57 and 77; 58 and 73; 58 and 74; 58 and 75; 58 and 76; 58 and 77; 59 and 73; 59 and 74; 59 and 75; 59 and 76; 59 and 77; 60 and 73; 60 and 74; 60 and 75; 60 and 76; 60 and 77; 61 and 77; 62 and 77; 63 and 73; 64 and 73; 65 and 73; 220 and 73; 65 and 221; 220 and 221; 66 and 73; 67 and 73; 68 and 73; 69 and 73; 70 and 73; 71 and 73; or 72 and 73. In certain embodiments, the heavy chain variable region and the light chain variable region, respectively, comprise the amino acid sequences set forth in SEQ ID NOs: 65 and 73. In certain embodiments, the heavy chain variable region and the light chain variable region, respectively, comprise the amino acid sequences set forth in SEQ ID NOs: 220 and 73; 65

and 221; or 220 and 221.

[0035] In another aspect, the instant disclosure provides an antibody or isolated antibody that specifically binds to human LAG-3, the antibody comprising a heavy chain variable region and a light chain variable region, wherein the amino acid sequences of the heavy chain variable region and the light chain variable region, respectively, consist of the amino acid sequences set forth in SEQ ID NOs: 56 and 73; 56 and 74; 56 and 75; 56 and 76; 56 and 77; 57 and 73; 57 and 74; 57 and 75; 57 and 76; 57 and 77; 58 and 73; 58 and 74; 58 and 75; 58 and 76; 58 and 77; 59 and 73; 59 and 74; 59 and 75; 59 and 76; 59 and 77; 60 and 73; 60 and 74; 60 and 75; 60 and 76; 60 and 77; 61 and 77; 62 and 77; 63 and 73; 64 and 73; 65 and 73; 220 and 73; 65 and 221; 220 and 221; 66 and 73; 67 and 73; 68 and 73; 69 and 73; 70 and 73; 71 and 73; or 72 and 73. In certain embodiments, the amino acid sequences of the heavy chain variable region and the light chain variable region, respectively, consist of the amino acid sequences set forth in SEQ ID NOs: 65 and 73. In certain embodiments, the amino acid sequences of the heavy chain variable region and the light chain variable region, respectively, consist of the amino acid sequences set forth in SEQ ID NOs: 220 and 73; 65 and 221; or 220 and 221.

[0036] In certain embodiments of any one of the foregoing aspects where applicable, the X in SEQ ID NO: 220 is Q. In certain embodiments of any one of the foregoing aspects where applicable, the X in SEQ ID NO: 220 is pyroglutamate. In certain embodiments of any one of the foregoing aspects where applicable, the X in SEQ ID NO: 221 is E. In certain embodiments of any one of the foregoing aspects where applicable, the X in SEQ ID NO: 221 is pyroglutamate. In certain embodiments of any one of the foregoing aspects where applicable, the X in SEQ ID NO: 220 is Q, and the X in SEQ ID NO: 221 is E. In certain embodiments of any one of the foregoing aspects where applicable, the X in SEQ ID NO: 220 is Q, and the X in SEQ ID NO: 221 is pyroglutamate. In certain embodiments of any one of the foregoing aspects where applicable, the X in SEQ ID NO: 220 is pyroglutamate, and the X in SEQ ID NO: 221 is E. In certain embodiments of any one of the foregoing aspects where applicable, the X in SEQ ID NO: 220 is pyroglutamate, and the X in SEQ ID NO: 221 is pyroglutamate.

[0037] In another aspect, the instant disclosure provides an antibody or isolated antibody comprising a heavy chain comprising the amino acid sequence of SEQ ID NO: 168 or 225, and a light chain comprising the amino acid sequence of SEQ ID NO: 187 or 228.

[0038] In another aspect, the instant disclosure provides an antibody or isolated antibody comprising a heavy chain comprising the amino acid sequence of SEQ ID NO: 168, and a

light chain comprising the amino acid sequence of SEQ ID NO: 187.

[0039] In another aspect, the instant disclosure provides an antibody or isolated antibody that specifically binds to human LAG-3, the antibody comprising a heavy chain comprising the amino acid sequence of SEQ ID NO: 168 or 225, and a light chain comprising the amino acid sequence of SEQ ID NO: 187 or 228.

[0040] In another aspect, the instant disclosure provides an antibody or isolated antibody that specifically binds to human LAG-3, the antibody comprising a heavy chain comprising the amino acid sequence of SEQ ID NO: 168, and a light chain comprising the amino acid sequence of SEQ ID NO: 187.

[0041] In another aspect, the instant disclosure provides an antibody or isolated antibody comprising a heavy chain comprising the amino acid sequence of SEQ ID NO: 169 or 226, and a light chain comprising the amino acid sequence of SEQ ID NO: 187 or 228.

[0042] In another aspect, the instant disclosure provides an antibody or isolated antibody comprising a heavy chain comprising the amino acid sequence of SEQ ID NO: 169, and a light chain comprising the amino acid sequence of SEQ ID NO: 187.

[0043] In another aspect, the instant disclosure provides an antibody or isolated antibody that specifically binds to human LAG-3, the antibody comprising a heavy chain comprising the amino acid sequence of SEQ ID NO: 169 or 226, and a light chain comprising the amino acid sequence of SEQ ID NO: 187 or 228.

[0044] In another aspect, the instant disclosure provides an antibody or isolated antibody that specifically binds to human LAG-3, the antibody comprising a heavy chain comprising the amino acid sequence of SEQ ID NO: 169, and a light chain comprising the amino acid sequence of SEQ ID NO: 187.

[0045] In another aspect, the instant disclosure provides an antibody or isolated antibody comprising a heavy chain comprising the amino acid sequence of SEQ ID NO: 170 or 227, and a light chain comprising the amino acid sequence of SEQ ID NO: 187 or 228.

[0046] In another aspect, the instant disclosure provides an antibody or isolated antibody comprising a heavy chain comprising the amino acid sequence of SEQ ID NO: 170, and a light chain comprising the amino acid sequence of SEQ ID NO: 187.

[0047] In another aspect, the instant disclosure provides an antibody or isolated antibody that specifically binds to human LAG-3, the antibody comprising a heavy chain comprising the amino acid sequence of SEQ ID NO: 170 or 227, and a light chain comprising the amino acid sequence of SEQ ID NO: 187 or 228.

[0048] In another aspect, the instant disclosure provides an antibody or isolated antibody

that specifically binds to human LAG-3, the antibody comprising a heavy chain comprising the amino acid sequence of SEQ ID NO: 170, and a light chain comprising the amino acid sequence of SEQ ID NO: 187.

[0049] In another aspect, the instant disclosure provides an antibody or isolated antibody comprising a heavy chain and a light chain, wherein the amino acid sequence of the heavy chain consists of the amino acid sequence set forth in SEQ ID NO: 168 or 225, and the amino acid sequence of the light chain consists of the amino acid sequence set forth in SEQ ID NO: 187 or 228.

[0050] In another aspect, the instant disclosure provides an antibody or isolated antibody comprising a heavy chain and a light chain, wherein the amino acid sequences of the heavy chain and the light chain, respectively, consist of the amino acid sequences set forth in SEQ ID NOs: 168 and 187.

[0051] In another aspect, the instant disclosure provides an antibody or isolated antibody that specifically binds to human LAG-3, the antibody comprising a heavy chain and a light chain, wherein the amino acid sequence of the heavy chain consists of the amino acid sequence set forth in SEQ ID NO: 168 or 225, and the amino acid sequence of the light chain consists of the amino acid sequence set forth in SEQ ID NO: 187 or 228.

[0052] In another aspect, the instant disclosure provides an antibody or isolated antibody that specifically binds to human LAG-3, the antibody comprising a heavy chain and a light chain, wherein the amino acid sequences of the heavy chain and the light chain, respectively, consist of the amino acid sequences set forth in SEQ ID NOs: 168 and 187.

[0053] In another aspect, the instant disclosure provides an antibody or isolated antibody comprising a heavy chain and a light chain, wherein the amino acid sequence of the heavy chain consists of the amino acid sequence set forth in SEQ ID NO: 169 or 226, and the amino acid sequence of the light chain consists of the amino acid sequence set forth in SEQ ID NO: 187 or 228.

[0054] In another aspect, the instant disclosure provides an antibody or isolated antibody comprising a heavy chain and a light chain, wherein the amino acid sequences of the heavy chain and the light chain, respectively, consist of the amino acid sequences set forth in SEQ ID NOs: 169 and 187.

[0055] In another aspect, the instant disclosure provides an antibody or isolated antibody that specifically binds to human LAG-3, the antibody comprising a heavy chain and a light chain, wherein the amino acid sequence of the heavy chain consists of the amino acid sequence set forth in SEQ ID NO: 169 or 226, and the amino acid sequence of the light chain

consists of the amino acid sequence set forth in SEQ ID NO: 187 or 228.

[0056] In another aspect, the instant disclosure provides an antibody or isolated antibody that specifically binds to human LAG-3, the antibody comprising a heavy chain and a light chain, wherein the amino acid sequences of the heavy chain and the light chain, respectively, consist of the amino acid sequences set forth in SEQ ID NOs: 169 and 187.

[0057] In another aspect, the instant disclosure provides an antibody or isolated antibody comprising a heavy chain and a light chain, wherein the amino acid sequence of the heavy chain consists of the amino acid sequence set forth in SEQ ID NO: 170 or 227, and the amino acid sequence of the light chain consists of the amino acid sequence set forth in SEQ ID NO: 187 or 228.

[0058] In another aspect, the instant disclosure provides an antibody or isolated antibody comprising a heavy chain and a light chain, wherein the amino acid sequences of the heavy chain and the light chain, respectively, consist of the amino acid sequences set forth in SEQ ID NOs: 170 and 187.

[0059] In another aspect, the instant disclosure provides an antibody or isolated antibody that specifically binds to human LAG-3, the antibody comprising a heavy chain and a light chain, wherein the amino acid sequence of the heavy chain consists of the amino acid sequence set forth in SEQ ID NO: 170 or 227, and the amino acid sequence of the light chain consists of the amino acid sequence set forth in SEQ ID NO: 187 or 228.

[0060] In another aspect, the instant disclosure provides an antibody or isolated antibody that specifically binds to human LAG-3, the antibody comprising a heavy chain and a light chain, wherein the amino acid sequences of the heavy chain and the light chain, respectively, consist of the amino acid sequences set forth in SEQ ID NOs: 170 and 187.

[0061] In certain embodiments of any one of the foregoing aspects where applicable, the X in SEQ ID NO: 225 is Q. In certain embodiments of any one of the foregoing aspects where applicable, the X in SEQ ID NO: 225 is pyroglutamate. In certain embodiments of any one of the foregoing aspects where applicable, the X in SEQ ID NO: 228 is E. In certain embodiments of any one of the foregoing aspects where applicable, the X in SEQ ID NO: 228 is pyroglutamate. In certain embodiments of any one of the foregoing aspects where applicable, the X in SEQ ID NO: 225 is Q, and the X in SEQ ID NO: 228 is E. In certain embodiments of any one of the foregoing aspects where applicable, the X in SEQ ID NO: 225 is Q, and the X in SEQ ID NO: 228 is pyroglutamate. In certain embodiments of any one of the foregoing aspects where applicable, the X in SEQ ID NO: 225 is pyroglutamate, and the X in SEQ ID NO: 228 is E. In certain embodiments of any one of the foregoing aspects

where applicable, the X in SEQ ID NO: 225 is pyroglutamate, and the X in SEQ ID NO: 228 is pyroglutamate.

[0062] In certain embodiments of any one of the foregoing aspects where applicable, the X in SEQ ID NO: 226 is Q. In certain embodiments of any one of the foregoing aspects where applicable, the X in SEQ ID NO: 226 is pyroglutamate. In certain embodiments of any one of the foregoing aspects where applicable, the X in SEQ ID NO: 228 is E. In certain embodiments of any one of the foregoing aspects where applicable, the X in SEQ ID NO: 228 is pyroglutamate. In certain embodiments of any one of the foregoing aspects where applicable, the X in SEQ ID NO: 226 is Q, and the X in SEQ ID NO: 228 is E. In certain embodiments of any one of the foregoing aspects where applicable, the X in SEQ ID NO: 226 is Q, and the X in SEQ ID NO: 228 is pyroglutamate. In certain embodiments of any one of the foregoing aspects where applicable, the X in SEQ ID NO: 226 is pyroglutamate, and the X in SEQ ID NO: 228 is E. In certain embodiments of any one of the foregoing aspects where applicable, the X in SEQ ID NO: 226 is pyroglutamate, and the X in SEQ ID NO: 228 is pyroglutamate.

[0063] In certain embodiments of any one of the foregoing aspects where applicable, the X in SEQ ID NO: 227 is Q. In certain embodiments of any one of the foregoing aspects where applicable, the X in SEQ ID NO: 227 is pyroglutamate. In certain embodiments of any one of the foregoing aspects where applicable, the X in SEQ ID NO: 228 is E. In certain embodiments of any one of the foregoing aspects where applicable, the X in SEQ ID NO: 228 is pyroglutamate. In certain embodiments of any one of the foregoing aspects where applicable, the X in SEQ ID NO: 227 is Q, and the X in SEQ ID NO: 228 is E. In certain embodiments of any one of the foregoing aspects where applicable, the X in SEQ ID NO: 227 is Q, and the X in SEQ ID NO: 228 is pyroglutamate. In certain embodiments of any one of the foregoing aspects where applicable, the X in SEQ ID NO: 227 is pyroglutamate, and the X in SEQ ID NO: 228 is E. In certain embodiments of any one of the foregoing aspects where applicable, the X in SEQ ID NO: 227 is pyroglutamate, and the X in SEQ ID NO: 228 is pyroglutamate.

[0064] In certain embodiments, the antibody comprises a heavy chain constant region selected from the group consisting of human IgG₁, IgG₂, IgG₃, IgG₄, IgA₁, and IgA₂. In certain embodiments, the heavy chain constant region is IgG₁. In certain embodiments, the amino acid sequence of IgG₁ comprises a N297A mutation, numbered according to the EU numbering system. In certain embodiments, the antibody comprises a heavy chain constant region comprising the amino acid sequence of SEQ ID NO: 194. In certain embodiments, the

amino acid sequence of IgG₁ comprises a N297Q mutation, numbered according to the EU numbering system. In certain embodiments, the IgG₁ is afucosylated IgG₁. In certain embodiments, the heavy chain constant region is IgG₄. In certain embodiments, the amino acid sequence of IgG₄ comprises a S228P mutation, numbered according to the EU numbering system. In certain embodiments, the antibody comprises a heavy chain constant region comprising the amino acid sequence of SEQ ID NO: 196.

[0065] In certain embodiments, the antibody comprises a light chain constant region selected from the group consisting of human IgGκ and IgGλ. In certain embodiments, the light chain constant region is IgGκ. In certain embodiments, the antibody comprises a light chain constant region comprising the amino acid sequence of SEQ ID NO: 198. In certain embodiments, the antibody comprises a light chain constant region comprising the amino acid sequence of SEQ ID NO: 219. In certain embodiments, the light chain constant region is IgGλ.

[0066] In another aspect, the instant disclosure provides an antibody or isolated antibody that cross-competes for binding to human LAG-3 with an antibody as disclosed herein. In certain embodiments, the instant disclosure provides an antibody or isolated antibody that cross-competes for binding to human LAG-3 with an antibody comprising the heavy and light chain variable region amino acid sequences set forth in SEQ ID NOs: 15 and 16, respectively. In certain embodiments, the instant disclosure provides an antibody or isolated antibody that cross-competes for binding to human LAG-3 with an antibody comprising the heavy and light chain variable region amino acid sequences set forth in SEQ ID NOs: 65 and 73; 220 and 73; 65 and 221; or 220 and 221, respectively.

[0067] In another aspect, the instant disclosure provides an antibody or isolated antibody that binds to the same epitope of human LAG-3 as an antibody disclosed herein. In certain embodiments, the instant disclosure provides an antibody or isolated antibody that binds to the same epitope of human LAG-3 as an antibody comprising the heavy and light chain variable region amino acid sequences set forth in SEQ ID NOs: 15 and 16, respectively. In certain embodiments, the instant disclosure provides an antibody or isolated antibody that binds to the same epitope of human LAG-3 as an antibody comprising the heavy and light chain variable region amino acid sequences set forth in SEQ ID NOs: 65 and 73; 220 and 73; 65 and 221; or 220 and 221, respectively.

[0068] In another aspect, the instant disclosure provides an antibody or isolated antibody that binds, *e.g.*, specifically binds, to an epitope of human LAG-3. In certain embodiments, the antibody binds to an epitope located within a region of human LAG-3 consisting of the

amino acid sequence of SEQ ID NO: 216. In certain embodiments, the antibody binds to an epitope located within a region of human LAG-3 consisting of the amino acid sequence of SEQ ID NO: 215. In certain embodiments, the antibody binds to an epitope located within a region of human LAG-3 consisting of the amino acid sequence of SEQ ID NO: 214. In certain embodiments, the antibody binds to an epitope located within a region of human LAG-3 consisting of the amino acid sequence of SEQ ID NO: 213. In certain embodiments, the antibody binds to an epitope located within a region of human LAG-3 consisting of the amino acid sequence of SEQ ID NO: 212. In certain embodiments, the antibody binds to an epitope located within a region of human LAG-3 consisting of the amino acid sequence of SEQ ID NO: 211.

[0069] In another aspect, the instant disclosure provides an antibody or isolated antibody that specifically binds to the same epitope of human LAG-3 as any antibody of the present invention. In certain embodiments, the antibody binds to an epitope located within a region of human LAG-3 consisting of the amino acid sequence of SEQ ID NO: 216. In certain embodiments, the antibody binds to an epitope located within a region of human LAG-3 consisting of the amino acid sequence of SEQ ID NO: 215. In certain embodiments, the antibody binds to an epitope located within a region of human LAG-3 consisting of the amino acid sequence of SEQ ID NO: 214. In certain embodiments, the antibody binds to an epitope located within a region of human LAG-3 consisting of the amino acid sequence of SEQ ID NO: 213. In certain embodiments, the antibody binds to an epitope located within a region of human LAG-3 consisting of the amino acid sequence of SEQ ID NO: 212. In certain embodiments, the antibody binds to an epitope located within a region of human LAG-3 consisting of the amino acid sequence of SEQ ID NO: 211.

[0070] In another aspect, the instant disclosure provides an antibody that, when bound to a human LAG-3 protein or fragment thereof comprising the amino acid sequence of SEQ ID NO: 217, reduces hydrogen/deuterium exchange in a region consisting of the amino acid sequence set forth in SEQ ID NO: 216 relative to hydrogen/deuterium exchange in the region consisting of the amino acid sequence set forth in SEQ ID NO: 216 in the absence of the antibody, as determined by a hydrogen/deuterium assay. In another aspect, the instant disclosure provides an antibody that, when bound to a human LAG-3 protein or fragment thereof comprising the amino acid sequence of SEQ ID NO: 217, reduces hydrogen/deuterium exchange in a region consisting of the amino acid sequence set forth in SEQ ID NO: 215 relative to hydrogen/deuterium exchange in the region consisting of the amino acid sequence set forth in SEQ ID NO: 215 in the absence of the antibody, as

determined by a hydrogen/deuterium assay. In another aspect, the instant disclosure provides an antibody that, when bound to a human LAG-3 protein or fragment thereof comprising the amino acid sequence of SEQ ID NO: 217, reduces hydrogen/deuterium exchange in a region consisting of the amino acid sequence set forth in SEQ ID NO: 214 relative to hydrogen/deuterium exchange in the region consisting of the amino acid sequence set forth in SEQ ID NO: 214 in the absence of the antibody, as determined by a hydrogen/deuterium assay. In another aspect, the instant disclosure provides an antibody that, when bound to a human LAG-3 protein or fragment thereof comprising the amino acid sequence of SEQ ID NO: 217, reduces hydrogen/deuterium exchange in a region consisting of the amino acid sequence set forth in SEQ ID NO: 213 relative to hydrogen/deuterium exchange in the region consisting of the amino acid sequence set forth in SEQ ID NO: 213 in the absence of the antibody, as determined by a hydrogen/deuterium assay. In another aspect, the instant disclosure provides an antibody that, when bound to a human LAG-3 protein or fragment thereof comprising the amino acid sequence of SEQ ID NO: 217, reduces hydrogen/deuterium exchange in a region consisting of the amino acid sequence set forth in SEQ ID NO: 212 relative to hydrogen/deuterium exchange in the region consisting of the amino acid sequence set forth in SEQ ID NO: 212 in the absence of the antibody, as determined by a hydrogen/deuterium assay. In another aspect, the instant disclosure provides an antibody that, when bound to a human LAG-3 protein or fragment thereof comprising the amino acid sequence of SEQ ID NO: 217, reduces hydrogen/deuterium exchange in a region consisting of the amino acid sequence set forth in SEQ ID NO: 211 relative to hydrogen/deuterium exchange in the region consisting of the amino acid sequence set forth in SEQ ID NO: 211 in the absence of the antibody, as determined by a hydrogen/deuterium assay. In some embodiments, the reduction in hydrogen/deuterium exchange is measured using hydrogen-deuterium exchange (HDX), for example as described herein in the examples.

[0071] In another aspect, the instant disclosure provides an antibody or isolated antibody that specifically binds to the same epitope of human LAG-3 as any antibody of the present invention. In certain embodiments, the antibody, when bound to a human LAG-3 protein or fragment thereof comprising the amino acid sequence of SEQ ID NO: 217, reduces hydrogen/deuterium exchange in a region consisting of the amino acid sequence set forth in SEQ ID NO: 216 relative to hydrogen/deuterium exchange in the region consisting of the amino acid sequence set forth in SEQ ID NO: 216 in the absence of the antibody, as determined by a hydrogen/deuterium assay. In certain embodiments, the antibody, when

bound to a human LAG-3 protein or fragment thereof comprising the amino acid sequence of SEQ ID NO: 217, reduces hydrogen/deuterium exchange in a region consisting of the amino acid sequence set forth in SEQ ID NO: 215 relative to hydrogen/deuterium exchange in the region consisting of the amino acid sequence set forth in SEQ ID NO: 215 in the absence of the antibody, as determined by a hydrogen/deuterium assay. In certain embodiments, the antibody, when bound to a human LAG-3 protein or fragment thereof comprising the amino acid sequence of SEQ ID NO: 217, reduces hydrogen/deuterium exchange in a region consisting of the amino acid sequence set forth in SEQ ID NO: 214 relative to hydrogen/deuterium exchange in the region consisting of the amino acid sequence set forth in SEQ ID NO: 214 in the absence of the antibody, as determined by a hydrogen/deuterium assay. In certain embodiments, the antibody, when bound to a human LAG-3 protein or fragment thereof comprising the amino acid sequence of SEQ ID NO: 217, reduces hydrogen/deuterium exchange in a region consisting of the amino acid sequence set forth in SEQ ID NO: 213 relative to hydrogen/deuterium exchange in the region consisting of the amino acid sequence set forth in SEQ ID NO: 213 in the absence of the antibody, as determined by a hydrogen/deuterium assay. In certain embodiments, the antibody, when bound to a human LAG-3 protein or fragment thereof comprising the amino acid sequence of SEQ ID NO: 217, reduces hydrogen/deuterium exchange in a region consisting of the amino acid sequence set forth in SEQ ID NO: 212 relative to hydrogen/deuterium exchange in the region consisting of the amino acid sequence set forth in SEQ ID NO: 212 in the absence of the antibody, as determined by a hydrogen/deuterium assay. In certain embodiments, the antibody, when bound to a human LAG-3 protein or fragment thereof comprising the amino acid sequence of SEQ ID NO: 217, reduces hydrogen/deuterium exchange in a region consisting of the amino acid sequence set forth in SEQ ID NO: 211 relative to hydrogen/deuterium exchange in the region consisting of the amino acid sequence set forth in SEQ ID NO: 211 in the absence of the antibody, as determined by a hydrogen/deuterium assay. In some embodiments, the reduction in hydrogen/deuterium exchange is measured using hydrogen-deuterium exchange (HDX), for example as described herein in the examples.

[0072] In certain embodiments, the antibody is a humanized antibody. In certain embodiments, the antibody is a murine antibody. In certain embodiments, the antibody is a chimeric antibody. In certain embodiments, the antibody is antagonistic to human LAG-3. In certain embodiments, the antibody deactivates, reduces, or inhibits an activity of human LAG-3. In certain embodiments, the antibody inhibits binding of human LAG-3 to MHC

class II. In certain embodiments, the antibody induces IL-2 production by peripheral blood mononuclear cells (PBMCs) stimulated with staphylococcal enterotoxin A (SEA). In certain embodiments, the antibody induces TNF α production by tumor infiltrating lymphocytes (TILs) stimulated with anti-CD3 and anti-CD28 antibodies.

5 [0073] In another aspect, the instant disclosure provides an antibody or isolated antibody as disclosed herein conjugated to a cytotoxic agent.

[0074] In another aspect, the instant disclosure provides an antibody or isolated antibody as disclosed herein conjugated to a cytostatic agent.

10 [0075] In another aspect, the instant disclosure provides an antibody or isolated antibody as disclosed herein conjugated to a toxin.

[0076] In another aspect, the instant disclosure provides an antibody or isolated antibody as disclosed herein conjugated to a radionuclide.

[0077] In another aspect, the instant disclosure provides an antibody or isolated antibody as disclosed herein conjugated to a detectable label.

15 [0078] In another aspect, the instant disclosure provides an antibody or isolated antibody as disclosed herein, wherein the N-terminal amino acid residue of the heavy chain variable region is pyroglutamate (e.g., as a result of post-translational cyclization of the free amino group of the N-terminal E or Q residue of the heavy chain variable region). In another aspect, the instant disclosure provides an antibody or isolated antibody as disclosed herein, 20 wherein the N-terminal amino acid residue of the heavy chain is pyroglutamate (e.g., as a result of post-translational cyclization of the free amino group of the N-terminal E or Q residue of the heavy chain).

[0079] In another aspect, the instant disclosure provides an antibody or isolated antibody as disclosed herein, wherein the N-terminal amino acid residue of the light chain variable 25 region is pyroglutamate (e.g., as a result of post-translational cyclization of the free amino group of the N-terminal E or Q residue the light chain variable region). In another aspect, the instant disclosure provides an antibody or isolated antibody as disclosed herein, wherein the N-terminal amino acid residue of the light chain is pyroglutamate (e.g., as a result of post-translational cyclization of the free amino group of the N-terminal E or Q residue the light 30 chain).

[0080] In another aspect, the instant disclosure provides an antibody or isolated antibody as disclosed herein, in which the heavy chain is aglycosylated.

[0081] In another aspect, the instant disclosure provides a pharmaceutical composition comprising an antibody as disclosed herein and a pharmaceutically acceptable carrier or

excipient.

[0082] In another aspect, the instant disclosure provides a polynucleotide isolated polynucleotide encoding a heavy and/or light chain of an antibody as disclosed herein. In another aspect, the instant disclosure provides a vector comprising the polynucleotide. In
5 another aspect, the instant disclosure provides a recombinant host cell comprising the polynucleotide. In another aspect, the instant disclosure provides a recombinant host cell comprising the vector. In another aspect, the instant disclosure provides a method of producing an antibody as disclosed herein, the method comprising culturing the host cell so that the polynucleotide is expressed and the antibody is produced. In one embodiment, the
10 method is an *in vitro* method.

[0083] In one embodiment, the present invention relates to an antibody of the invention, or a pharmaceutical composition of the invention, or a polynucleotide of the invention, or a vector of the invention, or a recombinant host cell of the invention for use as a medicament.

[0084] In one embodiment, the present invention relates to an antibody of the invention, or a pharmaceutical composition of the invention, or a polynucleotide of the invention, or a
15 vector of the invention, or a recombinant host cell of the invention for use as a diagnostic.

[0085] In another aspect, the instant disclosure provides a method of increasing T cell activation in response to an antigen in a subject, the method comprising administering to the subject an effective amount of an antibody or pharmaceutical composition as disclosed
20 herein. In another aspect, the instant disclosure provides a method of treating cancer in a subject, the method comprising administering to the subject an effective amount of an antibody or pharmaceutical composition as disclosed herein. In certain embodiments of the foregoing methods, the antibody or pharmaceutical composition is administered subcutaneously. In certain embodiments of the foregoing methods, the antibody or
25 pharmaceutical composition is administered intravenously. In certain embodiments of the foregoing methods, the antibody or pharmaceutical composition is administered intratumorally. In certain embodiments of the foregoing methods, the antibody or pharmaceutical composition is delivered to a tumor draining lymph node. In certain embodiments of the foregoing methods, the antibody or pharmaceutical composition is
30 administered intra-arterially. In certain embodiments of the foregoing methods, the antibody or pharmaceutical composition is administered intranasally.

[0086] In one aspect, the present invention relates to an antibody, polynucleotide, vector, recombinant host cell, and/or pharmaceutical composition of the present invention for use in a method for increasing T cell activation in response to an antigen.

[0087] In one aspect, the present invention relates to an antibody, polynucleotide, vector, recombinant host cell, and/or pharmaceutical composition of the present invention for use in a method for increasing T cell activation in response to an antigen in a subject.

[0088] In one aspect, the present invention relates to an antibody, polynucleotide, vector, recombinant host cell, and/or pharmaceutical composition of the present invention for use in a method for increasing T cell activation in response to an antigen in a subject comprising administering to the subject an effective amount of an antibody, polynucleotide, vector, recombinant host cell, and/or pharmaceutical composition of the invention.

[0089] In one aspect, the present invention relates to an antibody, polynucleotide, vector, recombinant host cell, and/or pharmaceutical composition of the present invention for use in a method for the treatment of cancer.

[0090] In one aspect, the present invention relates to an antibody, polynucleotide, vector, recombinant host cell, and/or pharmaceutical composition of the present invention for use in a method for the treatment of cancer in a subject.

[0091] In one aspect, the present invention relates to an antibody, polynucleotide, vector, recombinant host cell, and/or pharmaceutical composition of the present invention for use in a method for the treatment of cancer in a subject comprising administering to the subject an effective amount of an antibody, polynucleotide, vector, recombinant host cell, and/or pharmaceutical composition of the invention.

[0092] In one embodiment of an antibody, polynucleotide, vector, recombinant host cell, and/or pharmaceutical composition for use of the present invention, the antibody, polynucleotide, vector, recombinant host cell, and/or pharmaceutical composition is administered subcutaneously or intravenously. In one embodiment of an antibody, polynucleotide, vector, recombinant host cell, and/or pharmaceutical composition for use of the present invention, the antibody, polynucleotide, vector, recombinant host cell, and/or pharmaceutical composition is administered intratumorally or intra-arterially. In one embodiment of an antibody, polynucleotide, vector, recombinant host cell, and/or pharmaceutical composition for use of the present invention, the antibody, polynucleotide, vector, recombinant host cell, and/or pharmaceutical composition is administered intranasally.

[0093] In certain embodiments, the foregoing methods further comprise administering an additional therapeutic agent to the subject. Therefore, in one embodiment of an antibody, polynucleotide, vector, recombinant host cell, and/or pharmaceutical composition for use in a method of the present invention, the method further comprises administering an additional

therapeutic agent to the subject.

[0094] In one aspect, the present invention relates to (a) an antibody, polynucleotide, vector, recombinant host cell, and/or pharmaceutical composition of the present invention and (b) an additional therapeutic agent for use as a medicament.

5 [0095] In one aspect, the present invention relates to (a) an antibody, polynucleotide, vector, recombinant host cell, and/or pharmaceutical composition of the present invention and (b) an additional therapeutic agent for use in a method for the treatment of cancer.

[0096] In one aspect, the present invention relates to a pharmaceutical composition, kit or kit-of-parts comprising (a) an antibody, polynucleotide, vector, recombinant host cell, and/or
10 pharmaceutical composition of the present invention and (b) an additional therapeutic agent.

[0097] In certain embodiments, the additional therapeutic agent is a chemotherapeutic. In certain embodiments, the additional therapeutic agent is a radiotherapeutic.

[0098] In certain embodiments, the additional therapeutic agent is a checkpoint targeting agent. In certain embodiments, the checkpoint targeting agent is selected from the group
15 consisting of an antagonist anti-PD-1 antibody, an antagonist anti-PD-L1 antibody, an antagonist anti-PD-L2 antibody, an antagonist anti-CTLA-4 antibody, an antagonist anti-TIM-3 antibody, an antagonist anti-LAG-3 antibody, an antagonist anti-CEACAM1 antibody, an agonist anti-GITR antibody, an agonist anti-OX40 antibody, an antagonist anti-TIGIT antibody, an agonist anti-CD137 antibody, an antagonist anti-VISTA antibody, an antagonist
20 anti-CD73 antibody, and an antagonist anti-CD96 antibody. In certain embodiments, the additional therapeutic agent is an anti-PD-1 antibody. In certain embodiments, the anti-PD-1 antibody is pembrolizumab. In certain embodiments, the anti-PD-1 antibody is nivolumab. In certain embodiments, the additional therapeutic agent is an anti-PD-L1 antibody. In certain embodiments, the additional therapeutic agent is an anti-CTLA-4 antibody.

25 [0099] In certain embodiments, the additional therapeutic agent comprises a small molecule. In certain embodiments, the additional therapeutic agent is a small molecule inhibitor of the PD-1 pathway. In certain embodiments, the additional therapeutic agent is a small molecule inhibitor of PD-1 or PD-L1.

[00100] In certain embodiments, the additional therapeutic agent is an inhibitor of
30 indoleamine-2,3-dioxygenase (IDO). In certain embodiments, the inhibitor is selected from the group consisting of epacadostat, BMS-986205 (also known as F001287, see Example 19 of WO2016/073770, which is incorporated by reference herein in its entirety), indoximod, and NLG919. In certain embodiments, the inhibitor is epacadostat. In certain embodiments, the inhibitor is BMS-986205. In certain embodiments, the inhibitor is indoximod. In certain

embodiments, the inhibitor is NLG919.

[00101] In certain embodiments, the additional therapeutic agent is an inhibitor of ARG, LSD1, CD112, CD112R, or VEGF. In certain embodiments, the additional therapeutic agent is a Stimulator of Interferon Genes (STING) agonist. In certain embodiments, the additional
5 therapeutic agent is a CD80-Fc protein.

[00102] In certain embodiments, the additional therapeutic agent is a vaccine. In certain embodiments, the vaccine comprises a heat shock protein peptide complex (HSPPC) comprising a heat shock protein complexed with an antigenic peptide. In certain
10 embodiments, the heat shock protein is hsc70 and is complexed with a tumor-associated antigenic peptide. In certain embodiments, the heat shock protein is gp96 protein and is complexed with a tumor-associated antigenic peptide, wherein the HSPPC is derived from a tumor obtained from a subject. In certain embodiments, the heat shock protein is gp96
15 protein and is complexed with a tumor-associated antigenic peptide, wherein the HSPPC is derived from a tumor obtained from a subject. In certain embodiments, the additional therapeutic agent comprises a TCR. In certain embodiments, the additional therapeutic agent is a soluble TCR. In certain embodiments, the additional therapeutic agent is a cell
20 expressing a TCR. In certain embodiments, the additional therapeutic agent is a cell expressing a chimeric antigen receptor. In certain embodiments, the additional therapeutic agent is an antibody that specifically binds to a peptide-MHC complex. In certain
25 embodiments, the additional therapeutic agent is an adjuvant. In one aspect, the present invention relates to (a) an antibody, polynucleotide, vector, recombinant host cell, and/or pharmaceutical composition of the present invention and (b) a vaccine for use as a medicament, for example, for use in a method for the treatment of cancer, optionally wherein
30 the vaccine comprises a heat shock protein peptide complex (HSPPC) comprising a heat shock protein complexed with an antigenic peptide. In one aspect, the present invention relates to a pharmaceutical composition, kit or kit-of-parts comprising (a) an antibody, polynucleotide, vector, recombinant host cell, and/or pharmaceutical composition of the
present invention and (b) a vaccine, optionally wherein the vaccine comprises a heat shock protein peptide complex (HSPPC) comprising a heat shock protein complexed with an
antigenic peptide.

5. BRIEF DESCRIPTION OF THE DRAWINGS

[00103] **Figures 1A, 1B, and 1C** are a set of histograms showing the binding of anti-LAG-3 Fabs to wild type Jurkat cells or Jurkat cells engineered to express human LAG-3, as measured by flow cytometry. The anti-LAG-3 Fabs tested in this study include P01A12,

P01C09, P05E01, P13A04, P13A06, P13B01, P13B02, P13B03, P13B11, P13C06, P13C08, P13C10, P13D04, P13D05, P13E02, P13F01, P13F02, P13F06, P13F09, P13G01, P13G04, P13G05, P13H05, P14A04, P14B07, P14C04, P14F01, P14F06, P14G01, P14G03, P15B06, P15C02, P15E06, P15F06, P15G05, P16D04, and P16H05.

5 [00104] **Figures 2A and 2B** are graphs showing results from assays testing the ability of anti-LAG-3 Fabs or a negative control Fab not specific for LAG-3 to block the binding of cross-linked recombinant LAG-3-6His to MHC class II expressing Raji cells. Figure 2A is a bar graph showing the percentage of blocking mediated by the negative control Fab or anti-LAG-3 Fab P13B02, P13C08, P13C10, P13E02, P13F02, P01A12, P13B01, P05E01, or
10 P01C09. Figure 2B is a line graph showing the percentage of LAG-3 binding in the presence of a dose titration of anti-LAG-3 Fab P01A12, P13A06, P13B01, P13B02, P13C06, P13C08, P13C10, P13E02, or P14C04, or the negative control Fab.

[00105] **Figure 3** is a line graph similar to the one shown in Figure 2B, in which the percentage of LAG-3 binding is plotted against a dose titration of full length chimeric anti-
15 LAG-3 antibody P13A06, P13B01, P13B02, P13C06, P13C08, or P13E02, or an isotype control antibody.

[00106] **Figure 4** is a graph showing the production of IL-2 in human peripheral blood mononuclear cells (PBMCs) upon Staphylococcus Enterotoxin A (SEA) stimulation in the absence of any antibody, or in the presence of an isotype control antibody or the chimeric
20 anti-LAG-3 antibody P13B02.

[00107] **Figures 5A and 5B** are sequence alignments of humanized variable regions with corresponding murine sequences and human germline sequences. Figure 5A is a sequence alignment comparing the humanized heavy chain variable regions H0-H4 (SEQ ID NOs: 56-60, respectively), the murine antibody P13B02 heavy chain variable region (SEQ ID NO: 15),
25 and the human germline sequences IGHV1-46*01 (SEQ ID NO: 153) and IGHJ1*01 (SEQ ID NO: 200). Figure 5B is a sequence alignment comparing the humanized light chain variable regions L0-L4 (SEQ ID NOs: 73-77, respectively), the murine antibody P13B02 light chain variable region (SEQ ID NO: 16), and the human germline sequences IGKV3-20*01 (SEQ ID NO: 160) and IGKJ1*01 (SEQ ID NO: 201). Dots represent residues
30 identical to corresponding residues in H0 (Figure 5A) or L0 (Figure 5B). Dashes represent lack of amino acid residues compared with H0 (Figure 5A) or L0 (Figure 5B).

[00108] **Figure 6A and 6B** are graphs showing the binding of anti-LAG-3 antibodies to human T cells activated by Staphylococcus Enterotoxin A (SEA), as measured by flow cytometry. Figure 6A is a set of histograms testing the chimeric antibody P13B02 (IgG₁),

and the humanized antibodies P13B02-06 (IgG₁), P13B02-07 (IgG₁), P13B02-16 (IgG₁), P13B02-25 (IgG₁), P13B02-26 (IgG₁), P13B02-27 (IgG₁), P13B02-30 (IgG₁ G1m17 N297A), and P13B02-30 (IgG₄). Figure 6B is a graph showing the binding of the anti-LAG-3 antibody P13B02-16 (IgG₁) or an isotype control antibody to activated primary human CD4+ T cells. The median fluorescence intensity (MFI) values are plotted against antibody concentrations.

[00109] **Figures 7A and 7B** are line graphs similar to the one shown in Figure 2B, in which the percentage of LAG-3 binding is plotted against different doses of an isotype control antibody, the chimeric antibody P13B02 (IgG₁), the humanized antibody P13B02-06 (IgG₁), P13B02-07 (IgG₁), P13B02-16 (IgG₁), P13B02-26 (IgG₁), or P13B02-27 (IgG₁) (Figure 7A) or the humanized antibody P13B02-30 (IgG₁ G1m17 N297A) (Figure 7B).

[00110] **Figures 8A and 8B** are graphs showing the production of IL-2 induced by anti-LAG-3 antibody or isotype control antibody in human peripheral blood mononuclear cells (PBMCs) upon Staphylococcus Enterotoxin A (SEA) stimulation. In Figure 8A, the anti-LAG-3 antibody tested is P13B02-30 (IgG₁). In Figure 8B, the anti-LAG-3 antibody P13B02-16 (IgG₁) or an isotype control antibody was tested in the presence or absence of anti-PD-1 antibody pembrolizumab (Pembro) or nivolumab (Nivo), anti-PD-L1 antibody #1, #2, or #3, or anti-CTLA-4 antibody ipilimumab (Ipi).

[00111] **Figures 9A and 9B** are graphs showing TNF α production of primary tumor infiltrating lymphocytes (TILs) induced by anti-LAG-3 antibody P13B02-30 (IgG₁ G1m3 N297A) or an isotype control antibody, either alone or in combination with the anti-PD-1 antibody pembrolizumab (Pembro). The TILs were isolated from renal cell carcinoma (Figure 9A) or colorectal cancer (Figure 9B) tumors and activated with anti-CD3/CD28 microbeads.

[00112] **Figures 10A and 10B** are graphs showing that the anti-LAG-3 antibody P13B02-30 (IgG₁ G1m3 N297A) enhanced T cell activation in a LAG-3-mediated cell suppression assay. Jurkat-NFAT-luciferase-LAG-3 cells were incubated in the presence of sextuplet dose titrations of either an anti-LAG-3 antibody (black dots) or an isotype control antibody (white dots), a fixed concentration of Raji cells, and a fixed concentration of Staphylococcal Enterotoxin E (SEE) peptide. In a first experiment, antibody concentrations between 0.2-50 μ g/mL were tested (Figure 10A). In a second experiment, antibody concentrations between 0.1-100 μ g/mL were tested (Figure 10B). RLU = relative light units of luciferase reporter.

6. DETAILED DESCRIPTION

[00113] The instant disclosure provides antibodies that specifically bind to LAG-3 (*e.g.*, human LAG-3) and antagonize LAG-3 function, *e.g.*, LAG-3-mediated immune suppression. Also provided are pharmaceutical compositions comprising these antibodies, nucleic acids
5 encoding these antibodies, expression vectors and host cells for making these antibodies, and methods of treating a subject using these antibodies. The antibodies disclosed herein are particularly useful for increasing T cell activation in response to an antigen (*e.g.*, a tumor antigen or an infectious disease antigen), and hence for treating cancer in a subject or treating or preventing an infectious disease in a subject. All instances of “isolated antibodies”
10 described herein are additionally contemplated as antibodies that may be, but need not be, isolated. All instances of “isolated polynucleotides” described herein are additionally contemplated as polynucleotides that may be, but need not be, isolated. All instances of “antibodies” described herein are additionally contemplated as antibodies that may be, but need not be, isolated. All instances of “polynucleotides” described herein are additionally
15 contemplated as polynucleotides that may be, but need not be, isolated.

6.1 Definitions

[00114] As used herein, the terms “about” and “approximately,” when used to modify a numeric value or numeric range, indicate that deviations of 5% to 10% above (*e.g.*, up to 5% to 10% above) and 5% to 10% below (*e.g.*, up to 5% to 10% below) the value or range
20 remain within the intended meaning of the recited value or range.

[00115] As used herein, the term “LAG-3” refers to Lymphocyte activation gene 3 (also known as CD223). As used herein, the term “human LAG-3” refers to a human LAG-3 protein encoded by a wild type human LAG-3 gene, *e.g.*, GenBank™ accession number NM_002286.5. An exemplary immature amino acid sequence of human LAG-3 is provided
25 as SEQ ID NO: 166. Exemplary mature amino acid sequences of human LAG-3 are provided as SEQ ID NO: 167 and SEQ ID NO: 210.

[00116] As used herein, the terms “antibody” and “antibodies” include full length antibodies, antigen-binding fragments of full length antibodies, and molecules comprising antibody CDRs, VH regions or VL regions. Examples of antibodies include monoclonal
30 antibodies, recombinantly produced antibodies, monospecific antibodies, multispecific antibodies (including bispecific antibodies), human antibodies, humanized antibodies, chimeric antibodies, murine antibodies, immunoglobulins, synthetic antibodies, tetrameric antibodies comprising two heavy chain and two light chain molecules, an antibody light chain monomer, an antibody heavy chain monomer, an antibody light chain dimer, an antibody

heavy chain dimer, an antibody light chain- antibody heavy chain pair, intrabodies, heteroconjugate antibodies, antibody-drug conjugates, single domain antibodies, monovalent antibodies, single chain antibodies or single-chain Fvs (scFv), camelized antibodies, affybodies, Fab fragments, F(ab')₂ fragments, disulfide-linked Fvs (sdFv), anti-idiotypic
 5 (anti-Id) antibodies (including, *e.g.*, anti-anti-Id antibodies), and antigen-binding fragments of any of the above. In certain embodiments, antibodies described herein refer to polyclonal antibody populations. Antibodies can be of any type (*e.g.*, IgG, IgE, IgM, IgD, IgA or IgY), any class (*e.g.*, IgG₁, IgG₂, IgG₃, IgG₄, IgA₁ or IgA₂), or any subclass (*e.g.*, IgG_{2a} or IgG_{2b}) of immunoglobulin molecule. In certain embodiments, antibodies described herein are IgG
 10 antibodies, or a class (*e.g.*, human IgG₁ or IgG₄) or subclass thereof. In a specific embodiment, the antibody is a humanized monoclonal antibody. In another specific embodiment, the antibody is a human monoclonal antibody.

[00117] As used herein, the terms “VH region” and “VL region” refer to single antibody heavy and light chain variable regions, respectively, comprising FR (Framework Regions) 1,
 15 2, 3 and 4 and CDR (Complementarity Determining Regions) 1, 2 and 3 (see Kabat *et al.*, (1991) Sequences of Proteins of Immunological Interest (NIH Publication No. 91-3242, Bethesda), which is herein incorporated by reference in its entirety).

[00118] As used herein, the term “CDR” or “complementarity determining region” means the noncontiguous antigen combining sites found within the variable region of both heavy
 20 and light chain polypeptides. These particular regions have been described by Kabat *et al.*, J. Biol. Chem. 252, 6609-6616 (1977) and Kabat *et al.*, Sequences of protein of immunological interest. (1991), by Chothia *et al.*, J. Mol. Biol. 196:901-917 (1987), and by MacCallum *et al.*, J. Mol. Biol. 262:732-745 (1996), all of which are herein incorporated by reference in their entireties, where the definitions include overlapping or subsets of amino acid residues
 25 when compared against each other. In certain embodiments, the term “CDR” is a CDR as defined by Kabat *et al.*, J. Biol. Chem. 252, 6609-6616 (1977) and Kabat *et al.*, Sequences of protein of immunological interest. (1991). In certain embodiments, the term “CDR” is a CDR as defined by Chothia *et al.*, J. Mol. Biol. 196:901-917 (1987). In certain embodiments, the term “CDR” is a CDR as defined by MacCallum *et al.*, J. Mol. Biol. 262:732-745 (1996)
 30 and Martin A. “Protein Sequence and Structure Analysis of Antibody Variable Domains,” in *Antibody Engineering*, Kontermann and Dübel, eds., Chapter 31, pp. 422-439, Springer-Verlag, Berlin (2001).

[00119] As used herein, the term “framework (FR) amino acid residues” refers to those amino acids in the framework region of an immunoglobulin chain. The term “framework

region” or “FR region” as used herein, includes the amino acid residues that are part of the variable region, but are not part of the CDRs (*e.g.*, using the Kabat or Chothia definition of CDRs).

[00120] As used herein, the terms “variable region” and “variable domain” are used interchangeably and are common in the art. The variable region typically refers to a portion of an antibody, generally, a portion of a light or heavy chain, typically about the amino-terminal 110 to 125 amino acids in the mature heavy chain and about 90 to 115 amino acids in the mature light chain, which differ extensively in sequence among antibodies and are used in the binding and specificity of a particular antibody for its particular antigen. The variability in sequence is concentrated in those regions called complementarity determining regions (CDRs) while the more highly conserved regions in the variable domain are called framework regions (FR). Without wishing to be bound by any particular mechanism or theory, it is believed that the CDRs of the light and heavy chains are primarily responsible for the interaction and specificity of the antibody with antigen. In certain embodiments, the variable region is a human variable region. In certain embodiments, the variable region comprises rodent or murine CDRs and human framework regions (FRs). In particular embodiments, the variable region is a primate (*e.g.*, non-human primate) variable region. In certain embodiments, the variable region comprises rodent or murine CDRs and primate (*e.g.*, non-human primate) framework regions (FRs).

[00121] The terms “VL” and “VL domain” are used interchangeably to refer to the light chain variable region of an antibody.

[00122] The terms “VH” and “VH domain” are used interchangeably to refer to the heavy chain variable region of an antibody.

[00123] As used herein, the terms “constant region” and “constant domain” are interchangeable and are common in the art. The constant region is an antibody portion, *e.g.*, a carboxyl terminal portion of a light and/or heavy chain which is not directly involved in binding of an antibody to antigen but which can exhibit various effector functions, such as interaction with an Fc receptor (*e.g.*, Fc gamma receptor). The constant region of an immunoglobulin molecule generally has a more conserved amino acid sequence relative to an immunoglobulin variable domain.

[00124] As used herein, the term “heavy chain” when used in reference to an antibody can refer to any distinct type, *e.g.*, alpha (α), delta (δ), epsilon (ϵ), gamma (γ), and mu (μ), based on the amino acid sequence of the constant domain, which give rise to IgA, IgD, IgE, IgG, and IgM classes of antibodies, respectively, including subclasses of IgG, *e.g.*, IgG₁, IgG₂,

IgG₃, and IgG₄.

[00125] As used herein, the term “light chain” when used in reference to an antibody can refer to any distinct type, *e.g.*, kappa (κ) or lambda (λ) based on the amino acid sequence of the constant domains. Light chain amino acid sequences are well known in the art.

5 [00126] As used herein, the term “EU numbering system” refers to the EU numbering convention for the constant regions of an antibody, as described in Edelman, G.M. *et al.*, Proc. Natl. Acad. USA, 63, 78-85 (1969) and Kabat *et al.*, Sequences of Proteins of Immunological Interest, U.S. Dept. Health and Human Services, 5th edition, 1991, each of which is herein incorporated by reference in its entirety.

10 [00127] “Binding affinity” generally refers to the strength of the sum total of non-covalent interactions between a single binding site of a molecule (*e.g.*, an antibody) and its binding partner (*e.g.*, an antigen). Unless indicated otherwise, as used herein, “binding affinity” refers to intrinsic binding affinity which reflects a 1:1 interaction between members of a binding pair (*e.g.*, antibody and antigen). The affinity of a molecule X for its partner Y can
15 generally be represented by the dissociation constant (K_D). Affinity can be measured and/or expressed in a number of ways known in the art, including, but not limited to, equilibrium dissociation constant (K_D), and equilibrium association constant (K_A). The K_D is calculated from the quotient of k_{off}/k_{on} , whereas K_A is calculated from the quotient of k_{on}/k_{off} . k_{on} refers to the association rate constant of, *e.g.*, an antibody to an antigen, and k_{off} refers to the
20 dissociation rate constant of, *e.g.*, an antibody to an antigen. The k_{on} and k_{off} can be determined by techniques known to one of ordinary skill in the art, such as BIAcore[®] or KinExA. As used herein, a “lower affinity” refers to a larger K_D .

[00128] As used herein, the terms “specifically binds,” “specifically recognizes,” “immunospecifically binds,” and “immunospecifically recognizes” are analogous terms in the
25 context of antibodies and refer to molecules that bind to an antigen (*e.g.*, epitope or immune complex) as such binding is understood by one skilled in the art. For example, a molecule that specifically binds to an antigen can bind to other peptides or polypeptides, generally with lower affinity as determined by, *e.g.*, immunoassays, BIAcore[®], KinExA 3000 instrument (Sapidyne Instruments, Boise, ID), or other assays known in the art. In a specific
30 embodiment, molecules that specifically bind to an antigen bind to the antigen with a K_A that is at least 2 logs (*i.e.*, factors of 10), 2.5 logs, 3 logs, 4 logs or greater than the K_A when the molecules bind non-specifically to another antigen.

[00129] In another specific embodiment, molecules that specifically bind to an antigen do not cross react with other proteins under similar binding conditions. In another specific

embodiment, molecules that specifically bind to LAG-3 do not cross react with other non-LAG-3 proteins. In a specific embodiment, provided herein is an antibody that binds to LAG-3 (*e.g.*, human LAG-3) with higher affinity than to another unrelated antigen. In certain embodiments, provided herein is an antibody that binds to LAG-3 (*e.g.*, human LAG-3) with
5 a 20%, 25%, 30%, 35%, 40%, 45%, 50%, 55%, 60%, 65%, 70%, 75%, 80%, 85%, 90%, 95% or higher affinity than to another, unrelated antigen as measured by, *e.g.*, a radioimmunoassay, surface plasmon resonance, or kinetic exclusion assay. In a specific embodiment, the extent of binding of an anti-LAG-3 antibody described herein to an unrelated, non-LAG-3 protein is less than 10%, 15%, or 20% of the binding of the antibody
10 to LAG-3 protein as measured by, *e.g.*, a radioimmunoassay.

[00130] As used herein, the term “afucosylation” or “afucosylated” in the context of an Fc refers to a substantial lack of a fucose covalently attached, directly or indirectly, to residue 297 of the human IgG₁ Fc region, numbered according to the EU numbering system, or the corresponding residue in non-IgG₁ or non-human IgG₁ immunoglobulins. Thus, in a
15 composition comprising a plurality of afucosylated antibodies, at least 70% of the antibodies will not be fucosylated, directly or indirectly (*e.g.*, via intervening sugars) at residue 297 of the Fc region of the antibodies, and in some embodiments at least 80%, 85%, 90%, 95%, or 99% will not be fucosylated, directly or indirectly, at residue 297 of the Fc region.

[00131] As used herein, an “epitope” is a term in the art and refers to a localized region of
20 an antigen to which an antibody can specifically bind. An epitope can be, for example, contiguous amino acids of a polypeptide (linear or contiguous epitope) or an epitope can, for example, come together from two or more non-contiguous regions of a polypeptide or polypeptides (conformational, non-linear, discontinuous, or non-contiguous epitope). In certain embodiments, the epitope to which an antibody binds can be determined by, *e.g.*,
25 NMR spectroscopy, X-ray diffraction crystallography studies, ELISA assays, hydrogen/deuterium exchange coupled with mass spectrometry (*e.g.*, liquid chromatography electrospray mass spectrometry), array-based oligo-peptide scanning assays (*e.g.*, constraining peptides using CLIPS (Chemical Linkage of Peptides onto Scaffolds) to map discontinuous or conformational epitopes), and/or mutagenesis mapping (*e.g.*, site-directed
30 mutagenesis mapping). For X-ray crystallography, crystallization may be accomplished using any of the known methods in the art (*e.g.*, Giegé R *et al.*, (1994) *Acta Crystallogr D Biol Crystallogr* 50(Pt 4): 339-350; McPherson A (1990) *Eur J Biochem* 189: 1-23; Chayen NE (1997) *Structure* 5: 1269-1274; McPherson A (1976) *J Biol Chem* 251: 6300-6303, all of which are herein incorporated by reference in their entireties). Antibody:antigen crystals may

be studied using well known X-ray diffraction techniques and may be refined using computer software such as X-PLOR (Yale University, 1992, distributed by Molecular Simulations, Inc.; *see, e.g.*, Meth Enzymol (1985) volumes 114 & 115, eds Wyckoff HW *et al.*; U.S. 2004/0014194), and BUSTER (Bricogne G (1993) Acta Crystallogr D Biol Crystallogr 49(Pt 1): 37-60; Bricogne G (1997) Meth Enzymol 276A: 361-423, ed Carter CW; Roversi P *et al.*, (2000) Acta Crystallogr D Biol Crystallogr 56(Pt 10): 1316-1323), all of which are herein incorporated by reference in their entirety. Mutagenesis mapping studies may be accomplished using any method known to one of skill in the art. *See, e.g.*, Champe M *et al.*, (1995) J Biol Chem 270: 1388-1394 and Cunningham BC & Wells JA (1989) Science 244: 1081-1085, each of which is herein incorporated by reference in its entirety, for a description of mutagenesis techniques, including alanine scanning mutagenesis techniques. CLIPS (Chemical Linkage of Peptides onto Scaffolds) is a technology to present one or more peptides in a structurally constrained configuration to behave as functional mimics of complex protein domains. *See, e.g.*, U.S. Publication Nos. US 2008/0139407 A1 and US 2007/099240 A1, and US Patent No. 7,972,993, each of which is herein incorporated by reference in its entirety. In a specific embodiment, the epitope of an antibody is determined using alanine scanning mutagenesis studies. In a specific embodiment, the epitope of an antibody is determined using hydrogen/deuterium exchange coupled with mass spectrometry. In a specific embodiment, the epitope of an antibody is determined using CLIPS Epitope Mapping Technology from Pepscan Therapeutics.

[00132] As used herein, the term “an epitope located within a region of human LAG-3” consisting of a particular amino acid sequence or a set of amino acid residues refers to an epitope comprising one or more of the amino acid residues of the specified region, wherein the specified region includes the first specified amino acid residue and the last specified amino acid residue of the region of human LAG-3. In certain embodiments, the epitope comprises each one of the amino acid residues located within the specified region. In certain embodiments, one or more additional amino acid residues of human LAG-3 outside the specified region bind to an antibody together with an epitope located within the specified region.

[00133] As used herein, the terms “T cell receptor” and “TCR” are used interchangeably and refer to full length heterodimeric $\alpha\beta$ or $\gamma\delta$ TCRs, antigen-binding fragments of full length TCRs, and molecules comprising TCR CDRs or variable regions. Examples of TCRs include, but are not limited to, full length TCRs, antigen-binding fragments of full length TCRs, soluble TCRs lacking transmembrane and cytoplasmic regions, single-chain TCRs

containing variable regions of TCRs attached by a flexible linker, TCR chains linked by an engineered disulfide bond, monospecific TCRs, multi-specific TCRs (including bispecific TCRs), TCR fusions, human TCRs, humanized TCRs, chimeric TCRs, recombinantly produced TCRs, and synthetic TCRs. The term encompasses wild-type TCRs and genetically engineered TCRs (*e.g.*, a chimeric TCR comprising a chimeric TCR chain which includes a first portion from a TCR of a first species and a second portion from a TCR of a second species).

[00134] As used herein, the terms “major histocompatibility complex” and “MHC” are used interchangeably and refer to an MHC class I molecule and/or an MHC class II molecule.

[00135] As used herein, the term “peptide-MHC complex” refers to an MHC molecule (MHC class I or MHC class II) with a peptide bound in the art-recognized peptide binding pocket of the MHC.

[00136] As used herein, the term “treat,” “treating,” and “treatment” refer to therapeutic or preventative measures described herein. The methods of “treatment” employ administration of an antibody to a subject having a disease or disorder, or predisposed to having such a disease or disorder, in order to prevent, cure, delay, reduce the severity of, or ameliorate one or more symptoms of the disease or disorder or recurring disease or disorder, or in order to prolong the survival of a subject beyond that expected in the absence of such treatment.

[00137] As used herein, the term “effective amount” in the context of the administration of a therapy to a subject refers to the amount of a therapy that achieves a desired prophylactic or therapeutic effect.

[00138] As used herein, the term “subject” includes any human or non-human animal. In one embodiment, the subject is a human or non-human mammal. In one embodiment, the subject is a human.

[00139] The determination of “percent identity” between two sequences (*e.g.*, amino acid sequences or nucleic acid sequences) can be accomplished using a mathematical algorithm. A specific, non-limiting example of a mathematical algorithm utilized for the comparison of two sequences is the algorithm of Karlin S & Altschul SF (1990) PNAS 87: 2264-2268, modified as in Karlin S & Altschul SF (1993) PNAS 90: 5873-5877, each of which is herein incorporated by reference in its entirety. Such an algorithm is incorporated into the NBLAST and XBLAST programs of Altschul SF *et al.*, (1990) J Mol Biol 215: 403, which is herein incorporated by reference in its entirety. BLAST nucleotide searches can be performed with the NBLAST nucleotide program parameters set, *e.g.*, for score=100, wordlength=12 to obtain nucleotide sequences homologous to a nucleic acid molecules described herein.

BLAST protein searches can be performed with the XBLAST program parameters set, *e.g.*, to score 50, wordlength=3 to obtain amino acid sequences homologous to a protein molecule described herein. To obtain gapped alignments for comparison purposes, Gapped BLAST can be utilized as described in Altschul SF *et al.*, (1997) Nuc Acids Res 25: 3389-3402, which is herein incorporated by reference in its entirety. Alternatively, PSI BLAST can be used to perform an iterated search which detects distant relationships between molecules (*Id.*). When utilizing BLAST, Gapped BLAST, and PSI Blast programs, the default parameters of the respective programs (*e.g.*, of XBLAST and NBLAST) can be used (*see, e.g.*, National Center for Biotechnology Information (NCBI) on the worldwide web, ncbi.nlm.nih.gov). Another specific, non-limiting example of a mathematical algorithm utilized for the comparison of sequences is the algorithm of Myers and Miller, 1988, CABIOS 4:11-17, which is herein incorporated by reference in its entirety. Such an algorithm is incorporated in the ALIGN program (version 2.0) which is part of the GCG sequence alignment software package. When utilizing the ALIGN program for comparing amino acid sequences, a PAM120 weight residue table, a gap length penalty of 12, and a gap penalty of 4 can be used.

[00140] The percent identity between two sequences can be determined using techniques similar to those described above, with or without allowing gaps. In calculating percent identity, typically only exact matches are counted.

20 6.2 Anti-LAG-3 Antibodies

[00141] In one aspect the instant disclosure provides antibodies that specifically bind to LAG-3 (*e.g.*, human LAG-3) and antagonize LAG-3 function. The amino acid sequences of exemplary antibodies are set forth in Tables 1-7 herein.

The skilled worker will appreciate that the N-terminal E or Q amino acid residue can, under certain conditions, spontaneously convert to pyroglutamate by post-translational cyclization of the free amino group to form a lactam. Accordingly, in certain embodiments, the instant disclosure provides antibodies comprising an antibody heavy chain variable region or light chain variable region disclosed herein (*e.g.* SEQ ID NOs: 56-72 and 73-77, respectively) or full length heavy chain or light chain disclosed herein (*e.g.* SEQ ID NOs: 168-186 and 187-191, respectively), wherein the N-terminal E or Q amino acid residue has been converted to pyroglutamate (*e.g.*, as a result of post-translational cyclization of the free amino group of the N-terminal E or Q residue).

Table 1. Amino acid sequences of exemplary anti-LAG-3 antibodies.

SEQ ID NO:	Description	Amino acid sequence
1	P01C09 VH	QVQLKQSGAELVKPGASVKLSCTASGFNIKDTYMYWV KQRPEQGLEWIGRIDPANGNTKYDPKLQGKATITADT SSNTVYLQLSSLTSEDVAVFYCVIYSYRYDVGGFDYW GQGTTTLTVS
2	P01C09 VL, P13F01 VL, P13G05 VL, P05E03 VL	EIVLTQSPALMAASPGEKVTITCSVSSSISSSNLHWY QQKSGTSPKLWIYGTSNLASGVPVRFSGSGSGTSYSL TISSMEAEDAATYYCQQWSSYPFTFGSGTKLEIK
3	P05E01 VH	DVQLVESGAELVKPGASVKLSCTASGFTIKDTYIHWV KQRPEQGLEWIGEIDPANGNTKYDPKFQGKATITADT SSNTAYLQLSSLTSEDVAVYYCATYYYRYDVGGFDYW GQGTTTLTVS
4	P05E01 VL	DIVLTQSPALMAASPGEKVTITCSVSSSISSSNLHWF QQKSGTSPKLWIYGTSNLASGVPVRFSGSGSGTSYSL TISNMEAEDAATYYCQQWNSYPLTFGAGTKLELK
5	P01A12 VH	EVQLQQSGAELVKPGASVKLSCTASGFNIKDTYMYWV KQRPEQGLDWIGRIDPANGNTKFDPKFQGKATITADT SSNTAYLQLSSLTSEDVAVYYCSTYYYRYDVGGFDYW GQGTTTLTVS
6	P01A12 VL	DIVLTQSPALMAASPGEKVTITCSVSSSISSSNLHWY QQKSGTSPKLWIYGTSNLASGVPVRFSGSGSGTSYSL TISSMEAEDAATYYCQQWSSYPFTFGSGTKLEIK
7	P13B01 VH	EVQLQQSGAELVKPGASVKLSCTASGFNIKDTYIYWV KQRPERGLEWIGRIDPANGNTKFDPKFQGTATITADT SSNTAYLQLSSLTSEDVAVYYCTTYFYRYDVGGFDYW GQGTTTLTVS
8	P13B01 VL, P14C04 VL, P14B07 VL	ENVLTQSPALMAASPGEKVTITCSVSSSISSSNLHWY QQKSGTSPKLWIYGTSNLASGVPVRFSGSGSGTSYSL TISSMEAEDAATYYCQQWSSYPFTFGSGTKLEIK
9	P13C10 VH, P15C02 VH, P16D04 VH, P13G05 VH, P13F06 VH, P14F01 VH, P16H01 VH	QVQLQQSGAELVKPGASVELSCTASGFNIRDYMYWV KQRPEQGLEWIGRIDPANGNTKFDPKFQDRATMTADT SSNTAYLQLSSLTSEDVAVYYCTTYFYRYDVGGFDYW GQGTTTLTVS
10	P13C10 VL, P13E02 VL, P13F02 VL, P13B03 VL, P13H05 VL, P13G04 VL	ENVLTQSPALMAASPGEKVTITCSVSSSISSSNLHWF QQKSGTSPKLWIYGTSNLASGVPVRFSGSGSGTSYSL TISSMEAEDAATYYCQQWSSYPFTFGSGTKLEIK
11	P13C08 VH	QVQLKQSGAELVKPGASVKLSCTASGFNIKDNYIHWV KQRPEQGLEWIGSIDPANGNTKYDPKFQGKASITADT SSNTAYLQLSSLTSEDVAVYYCASYFYRYDVGGFDYW GQGTTTLTVS
12	P13C08 VL	DVVMQTTPALMAASPGEKVTITCSVSSSISSSNLHWF QQKSGTSPKLWIYGTSNLASGVPVRFSGSGSGTSYSL TISSMEAEDAATYYCQQWSSYPFTFGSGTKLEIK
13	P13E02 VH	QVQLQQPGAELVKPGASVKLSCTVSGFNKDITYIHWV KQRPEQGLEWIGEIDPANGNSKYAPRFQGKATITADT SSNTAYLQLSSLTSEDVAVYYCATYYYRYDVGGFDYW GQGTTTLTVS

SEQ ID NO:	Description	Amino acid sequence
14	P13F02 VH	QVQLQQPGAELVKPGASVKLSCTASGFNIKDTYIHWV KQRPEQGLDWGEIDPANGHTKYDPKFQ GKATITADT SSNTAYLQLSSLTSED TAVYYCATYYYRYDVGGFDYW GQGTTTLTVS
15	P13B02 VH	QVQMKQSGAELVKPGASVKLSCTASGFNIKDTYIHWV KQRPEQGLEWIGEIDPANDNTKYDPKFQ GKATITADT SSNTAYLQLSSLTSED TAVYYCATYYYRYDVGGFDYW GQGTTTLTVS
16	P13B02 VL	EILLTQSPALMAASPGEKVTITCSVSSSISSSNLHWF QQKSGTSPKLWIYGTSNLAGVPVRFSGSGSGTSYSL TISSMEAEDAATYYCQQWSSYPFTFGSGTKLEIK
17	P13A06 VH	QVQLKQSGAELVKPGASVKLSCTASGFNIKDTYMYWV KQRPEQGLEWIGRIDPANGNTKYDPKFQ GKATITADT SSNTVYLQLSSLTSED TAVFYCVIYSYRYDVGGFDYW GQGTTTLTVS
18	P13A06 VL	ENVLTQSPALMAASPGEKVTITCSVSSSISSSNLHWY QQKSGTSPKLWIYGTSNLAGVPVRFSGSGSGTSYSL TISSMEAEDAATYYCQQWSSYPHTFGGSGTKLEIK
19	P14C04 VH	QVQLQQPGAELVKPGASVKLSCTASGFNIKDTYMYWV KQRPEQGLDWIGRIDPANGNTHFDPKFQ GKATITADT SSNTAYLQLSSLTSED TAVYYCSTYFYRYDVGGFDYW GQGTTTLTVS
20	P14A04 VH, P13F01 VH, P15E06 VH	QVQLQQPGAELVKPGASVELSCTASGFNIRDYMYWV KQRPEQGLEWIGRIDPANGNTKFDPKFQDRATMTADT SSNTAYLQLSSLTSED TAVYYCTTYFYRYDVGGFDYW GQGTTTLTVS
21	P14A04 VL, P14G01 VL	DIVLTQSPALMAASPGEKVTITCSVSSSISSSNLHWF QQKSGTSPKLWIYGTSNLAGVPVRFSGSGSGTSYSL TISSMEAEDAATYYCQQWSSYPFTFGSGTKLEIK
22	P15F06 VH	QVQLKQSGAELVKPGASVELSCTASGFNIRDYMYWV KQRPEQGLEWIGRIDPANGNTKFDPKFQDRATMTADT SSNTAYLQLSSLTSED TAVYYCTTYFYRYDVGGFDYW GQGTTTLTVS
23	P15F06 VL	ENVLTQSPALMAASPGEKVTITCSVSSSISSSTLHWF QQKSGTSPKLWIYGTSNLAGVPVRFSGSGSGTSYSL TISSMEAEDAATYYCQQWSSYPWTFGGSGTKLEIK
24	P13B03 VH	EVQLQQSGAELVKPGASVKLSCTASGFNIKDTYIHWV KQRPEQGLEWIGEIDPANGNTKYDPKFQ GKATITADT SSNTAYLQLSSLTSED TAVYYCATYYYRYDVGGFDYW GQGTTTLTVS
25	P15C02 VL	DVVMQTTPALMAASPGEKVTITCSVSSSISSSNLHWY QQKSGTSPKLWIYGTSNLAGVPVRFSGSGSGTSYSL TISSMEAEDAATYYCQQWSSYPFTFGSGTKLEIK
26	P16D04 VL, P16H01 VL	ENVLTQSPALMAASPGEKVTITCSVSSSISSSNLHWY QQKSGTSPKLWIYGTSNLAGVPVRFSGSGSGTSYSL TISSMEAEDAATYYCQQWSSYPLTFGAGTKLEIK

SEQ ID NO:	Description	Amino acid sequence
27	P13A04 VH	QVQLQQPGAELVKPGASVELSCTASGFNIRDITYMYWV KQRPEQGLEWIGRIDPANGNTKFDPKFQDRATMTADT SSNTAYLQLSSLTSADTAVYYCTTYFYRYDVGGFDYW GQGTTTLTVS
28	P13A04 VL	DIVLTQSPALMAASPGEKVTITCSVSSSISSSNLHWY QQKSGTSPKLWIYGTSNLASGVPVRFSGSGSGTSYSL TISNMEAEDAATYYCQQWSSYPFTFGSGTKLEIK
29	P16H05 VH	QVQLQQPGAELVKPGASVKLSCTASGFNIKDNIIHWV KQRPEQGLEWIGSIDPANGNTKYDPKFQGKASITADT SSNTAYLQLSSLTSED TAVYYCASYFYRYDVGGFDYW GQGTTTLTVS
30	P16H05 VL	EIVLTQSPALMAASPGEKVTITCSVSSSISSSNLHWY QQKSGTSPKLWIYGTSNLASGVPVRFSGSGSGTSYSL TISSMEAEDAATYYCQQWSSYPHTFGGGTKLEIK
31	P13F09 VH	QVQLQQSGAELVKPGASVKLSCTASGFNIKDNIIHWV KQRPEQGLEWIGSIDPANGNTKYDPKFQGKASITADT SSNTAYLQLSSLTSEGTAVYYCASYFYRYDVGGFDYW GQGTTTLTVS
32	P13F09 VL, P14F06 VL	EIVLTQSPALMAASPGEKVTITCSVSSSISSSNLHWF QQKSGTSPKLWIYGTSNLASGVPVRFSGSGSGTSYSL TISSMEAEDAATYYCQQWSSYPFTFGSGTKLEIK
33	P13G01 VH, P15G05 VH	EVQLQQSGAELVKPGASVELSCTASGFNIRDITYMYWV KQRPEQGLEWIGRIDPANGNTKFDPKFQDRATMTADT SSNTAYLQLSSLTSED TAVYYCTTYFYRYDVGGFDYW GQGTTTLTVS
34	P13G01 VL	QIVLTQSPALMAASPGEKVTITCSVSSSISSSNLHWY QQKSGTSPKLWIYGTSNLASGVPVRFSGSGSGTSYSL TISSMEAEDAATYYCQQWSSYPFTFGSGTKLEIK
35	P13H05 VH	QVQLQQPGAELVKPGASVKLSCTASGFNIKDTYIHWV KQRPEQGLEWIGEIDPANDNTKYDPKFQGKATITADT SSNTAYLQLSSLTSED TAVYYCATYYYRYDVGGFDYW GQGTTTLTVS
36	P13D04 VH	QVQLQQSGAELVKPGASVKLSCTASGFNIKDNIMDWV KQRPEQGLEWIGKIDPANGNTKYDPKFQGKATITADT SSNTAYLQLSSLTSED TAVYYCATYYYRYDVGGFDYW GQGTTTLTVS
37	P13D04 VL	DIVLTQSPALMAASPGEKVTITCSVSSSISSSNLHWF QQKSGTSPKLWIYGTSNLASGVPVRFSGSGSGTSYSL TISSMEAEDAATYYCQQWSSYPFTFGSGTKLEIK
38	P14G01 VH, P05E03 VH	QVQLKESGAELVKPGASVELSCTASGFNIRDITYMYWV KQRPEQGLEWIGRIDPANGNTKFDPKFQDRATMTADT SSNTAYLQLSSLTSED TAVYYCTTYFYRYDVGGFDYW GQGTTTLTVS
39	P14G03 VH	QVQMKQSGAELVKPGASVKLSCTASGFNIKDTYIHWV KQRPGQGLEWIGEIDPANGNTKYDPKFQGKATITADT SSNTAYLQLSSLTSED TAVYYCATYYYRYDVGGFDYW GQGTTTLTVS

SEQ ID NO:	Description	Amino acid sequence
40	P14G03 VL	DIVLTQSPALMAASPGEKVTITCSVSSSISSSNLYWF QQKSGTSPKLWIYGTSNLASGVPVRFSGSGSGTSYSL TISSMEAEDAATYYCQQWSSYPFTFGSGTKLEIK
41	P13F06 VL	EILLTQSPALMAASPGEKVTITCSVSSSISSSNLHWY QQKSGTSPKLWIYGTSNLASGVPVRFSGSGSGTSYSL TISSMEAEDAATYYCQQWSSYPFTFGSGTKLEIK
42	P13B11 VH	QVQLQQPGAELVKPGASVKLSCTASGFNIKDNMYMDWV KQRPEQGLEWIGKIDPANGNTKYDPKFQGKATITADT SSNTAYLQLSSLTSEDYAVYYCATYYYRYDVGGFDYW GQGTTTLTVS
43	P13B11 VL	QIVLTQSPALMAASPGEKVTITCSVSSSISSSNLHWF QQKSGTSPKLWIYGTSNLASGVPVRFSGSGSGTSYSL TISSMEAEDAATYYCQQWSSYPFTFGSGTKLEIK
44	P14F01 VL	ENVLTQSPALMAASPGEKVTITCSVSSSISSSNLHWY QQKSGTSPKLWIYGTSNLASGVPVRFSGSGSGTSYSL TISSMEAEDAATYYCQQWSTYPFTFGSGTKLEIK
45	P14F06 VH	QVQMKQSGAELVKPGASVKLSCTASGFNIKDNIIHWV KQRPEQGLEWIGSIDPANGNTKYDPKFQGKASITADT SSNTAYLQLSSLTSEDYAVYYCASYFYRYDVGGFDYW GQGTTTLTVS
46	P13D05 VH	QVQLQQPGAELVKPGASVELSCTASGFNIRDYMYWV KQRPEQGLGWIGRIDPANGNTKFDPKFQDRATMTADT SSNTAYLQLSSLTSEDYAVYYCTTYFYRYDVGGFDYW GQGTTTLTVS
47	P13D05 VL	EIVLTQSPALMAASPGEKVTITCSVSSSISSSNLHWF RQKSGTSPKLWIYGTSNLASGVPVRFSGSGSGTSYSL TISSMEAEDAATYYCQQWSSYPFTFGSGTKLEIK
48	P13G04 VH	EVKLMESGAELVKPGASAEELSCTASGFNIRDYMYWV KQRPEQGLEWIGRIDPANGNTKFDPKFQDRATMTADT SSNTAYLQLSSLTSEDYAVYYCTTYFYRYDVGGFDYW GQGTTTLTVS
49	P15E06 VL	ENVLTQSPALMAASPGEKVTITCSVSSGISSSNLHWY QQKSGTSPKLWIYGTSNLASGVPVRFSGSGSGTSYSL TISSMEAEDAATYYCQQWSRYPWTFGGGKLEIK
50	P15G05 VL	EIVLTQSPALMAASPGEKVTITCSVSSSISSSNLHWY QQKSGTSPKLWIYGTSNLASGVPVRFSGSGSGTSYSL TISSMEAEDAATYYCQQWSSYPLTFGAGTKLELK
51	P15B06 VH	EVQLQQSGAELVKPGASVKLSCTASGFNIKDTYMYWV KQRPEQGLDWIGRIDPANGNTHFDPKFQGKATITADT SSNTAYLQLSSLTSEDYAVYYCSTYFYRYDVGGFDYW GQGTTTLTVS
52	P15B06 VL	QILLTQSPALMAASPGEKVTITCSVSSSISSSNLHWY QQKSGTSPKLWIYGTSNLASGVPVRFSGSGSGTSYSL TISSMEAEDAATYYCQQWSSYPLTFGAGTKLELK
53	P14B07 VH	QVQLQQPGAELVKPGASVKLSCTASGFNIKDNIIHWV KQRPEQGLEWIGSIDPANGNTKYDPKFQGKASITADT SSNTAYLQLSSLTSEDYAVYYCASYFYRYDVGGFDYW GQGTTTLTVS

SEQ ID NO:	Description	Amino acid sequence
54	P13C06 VH	QVQMKQSGAELVKPGASVELSCTASGFNITDTYMYWV KQRPEQGLEWIGRIDPANGNTKFDPKFQDRATMTADT SSNTAYLQLSSLTSEDVAVYYCTTYFDKYDVGGCDYW GQGTTTLTVS
55	P13C06 VL	EIVLTQSPALMAASPGEKVTITCSVSSSISSSNLYWF QHKSGTSPKLWIYGTSNLASGVPVRFSGSGSGTSYSL TISSMDAENAATYYCQQWRSYPFTFGSGTKLEIK
56	H0	QVQLVQSGAEVKKPGASVKVSCASGFNIKDTYIHWV RQAPGQGLEWMGEIDPANDNTKYDPKFQGRVTMTTRDT STSTVYMELSSLRSEDVAVYYCARYYYRYDVGGFDYW GQGTLLVTVSS
57	H1	QVQLVQSGAEVKKPGASVKVSCASGFNIKDTYIHWV RQAPGQGLEWMGEIDPANDNTKYDPKFQGRVTITADT STSTVYMELSSLRSEDVAVYYCATYYYRYDVGGFDYW GQGTLLVTVSS
58	H2	QVQLVQSGAEVKKPGASVKVSCASGFNIKDTYIHWV RQAPGQGLEWMGEIDPANDNTKYDPKFQGRVTITADT SSNTVYMELSSLRSEDVAVYYCATYYYRYDVGGFDYW GQGTLLVTVSS
59	H3	QVQMKQSGAEVKKPGASVKVSCASGFNIKDTYIHWV RQAPGQGLEWMGEIDPANDNTKYDPKFQGRVTITADT SSNTVYMELSSLRSEDVAVYYCATYYYRYDVGGFDYW GQGTLLVTVSS
60	H4	QVQMKQSGAEVVKPGASVKVSCASGFNIKDTYIHWV RQAPGQGLEWIGEIDPANDNTKYDPKFQGRVTITADT SSNTVYLQLSSLRSEDVAVYYCATYYYRYDVGGFDYW GQGTLLVTVSS
61	H4_R98K	QVQMKQSGAEVVKPGASVKVSCASGFNIKDTYIHWV RQAPGQGLEWIGEIDPANDNTKYDPKFQGRVTITADT SSNTVYLQLSSLRSEDVAVYYCATYYYKYDVGGFDYW GQGTLLVTVSS
62	H4_D100E	QVQMKQSGAEVVKPGASVKVSCASGFNIKDTYIHWV RQAPGQGLEWIGEIDPANDNTKYDPKFQGRVTITADT SSNTVYLQLSSLRSEDVAVYYCATYYYRYEVGGFDYW GQGTLLVTVSS
63	H1_R98K	QVQLVQSGAEVKKPGASVKVSCASGFNIKDTYIHWV RQAPGQGLEWMGEIDPANDNTKYDPKFQGRVTITADT STSTVYMELSSLRSEDVAVYYCATYYYKYDVGGFDYW GQGTLLVTVSS
64	H1_R98K_K23T	QVQLVQSGAEVKKPGASVKVSCASGFNIKDTYIHWV RQAPGQGLEWMGEIDPANDNTKYDPKFQGRVTITADT STSTVYMELSSLRSEDVAVYYCATYYYKYDVGGFDYW GQGTLLVTVSS
65	H1_R98K_L4M	QVQMVQSGAEVKKPGASVKVSCASGFNIKDTYIHWV RQAPGQGLEWMGEIDPANDNTKYDPKFQGRVTITADT STSTVYMELSSLRSEDVAVYYCATYYYKYDVGGFDYW GQGTLLVTVSS

SEQ ID NO:	Description	Amino acid sequence
220	H1_R98K_L4M	XVQMVQSGAEVKKPGASVKVSCKASGFNIKDTYIHWV RQAPGQGLEWMGEIDPANDNTKYDPKFQGRVTITADT STSTVYMELSSLRSEDTAVYYCATYYYKYDVGGFDYW GQGTLLVTVSS, wherein X = glutamine (Q) or pyroglutamate (pE)
66	H1_R98K_L4M_K23T	QVQMVQSGAEVKKPGASVKVSCTASGFNIKDTYIHWV RQAPGQGLEWMGEIDPANDNTKYDPKFQGRVTITADT STSTVYMELSSLRSEDTAVYYCATYYYKYDVGGFDYW GQGTLLVTVSS
67	H1_R98K_L4M_V5K	QVQMKQSGAEVKKPGASVKVSCKASGFNIKDTYIHWV RQAPGQGLEWMGEIDPANDNTKYDPKFQGRVTITADT STSTVYMELSSLRSEDTAVYYCATYYYKYDVGGFDYW GQGTLLVTVSS
68	H1_R98K_L4M_V5K_K23T	QVQMKQSGAEVKKPGASVKVSCTASGFNIKDTYIHWV RQAPGQGLEWMGEIDPANDNTKYDPKFQGRVTITADT STSTVYMELSSLRSEDTAVYYCATYYYKYDVGGFDYW GQGTLLVTVSS
69	H1_R98K_V5K	QVQLKQSGAEVKKPGASVKVSCKASGFNIKDTYIHWV RQAPGQGLEWMGEIDPANDNTKYDPKFQGRVTITADT STSTVYMELSSLRSEDTAVYYCATYYYKYDVGGFDYW GQGTLLVTVSS
70	H1_R98K_V5K_K23T	QVQLKQSGAEVKKPGASVKVSCTASGFNIKDTYIHWV RQAPGQGLEWMGEIDPANDNTKYDPKFQGRVTITADT STSTVYMELSSLRSEDTAVYYCATYYYKYDVGGFDYW GQGTLLVTVSS
71	H2_R98K	QVQLVQSGAEVKKPGASVKVSCKASGFNIKDTYIHWV RQAPGQGLEWMGEIDPANDNTKYDPKFQGRVTITADT SSNTVYMELSSLRSEDTAVYYCATYYYKYDVGGFDYW GQGTLLVTVSS
72	H3_R98K	QVQMKQSGAEVKKPGASVKVSCTASGFNIKDTYIHWV RQAPGQGLEWMGEIDPANDNTKYDPKFQGRVTITADT SSNTVYMELSSLRSEDTAVYYCATYYYKYDVGGFDYW GQGTLLVTVSS
73	L0	EIVLTQSPGTLSSLSPGERATLSCSVSSSISSSNLHWY QQKPGQAPRLLIYGTSNLAGIPDRFSGSGSGTDFTL TISRLEPEDFAVYYCQQWSSYPFTFGQGTKVEIK
221	L0	XIVLTQSPGTLSSLSPGERATLSCSVSSSISSSNLHWY QQKPGQAPRLLIYGTSNLAGIPDRFSGSGSGTDFTL TISRLEPEDFAVYYCQQWSSYPFTFGQGTKVEIK, wherein X = glutamate (E) or pyroglutamate (pE)
74	L1	EIVLTQSPGTLSSLSPGERATLSCSVSSSISSSNLHWY QQKPGQAPRLLIYGTSNLAGIPDRFSGSGSGTSYTL TISRLEPEDFAVYYCQQWSSYPFTFGQGTKVEIK
75	L2	EILLTQSPGTLSSLSPGERATLSCSVSSSISSSNLHWF QQKPGQAPRLLIYGTSNLAGIPDRFSGSGSGTSYTL TISRLEPEDFAVYYCQQWSSYPFTFGQGTKVEIK
76	L3	EILLTQSPGTLSSLSPGERATLSCSVSSSISSSNLHWF QQKPGQAPRLWIYGTSNLAGVPDRFSGSGSGTSYTL TISRLEPEDFAVYYCQQWSSYPFTFGQGTKVEIK

SEQ ID NO:	Description	Amino acid sequence
77	L4	EILLTQSPGTLSSLSPGERATLTCSVSSSISSSNLHWF QQKPGQSPRLWIYGTSNLAGVDPDRFSGSGSGTSYTL TISRLEPEDFAVYYCQQWSSYPFTFGQGTKVEIK
78	CDRH1	DTYMY
79	CDRH1	DTYIH
80	CDRH1	DTYIY
81	CDRH1	DNYIH
82	CDRH1	DNYMD
83	CDRH2	RIDPANGNTKYDPKLQG
84	CDRH2	EIDPANGNTKYDPKFQG
85	CDRH2	RIDPANGNTKFDPKFQG
86	CDRH2	RIDPANGNTKFDPKFQD
87	CDRH2	SIDPANGNTKYDPKFQG
88	CDRH2	EIDPANGNSKYAPRFQG
89	CDRH2	EIDPANGHTKYDPKFQG
90	CDRH2	EIDPANDNTKYDPKFQG
91	CDRH2	RIDPANGNTKYDPKFQG
92	CDRH2	RIDPANGNTHFDPKFQG
93	CDRH2	KIDPANGNTKYDPKFQG
94	CDRH3	YSYRYDVGGFDY
95	CDRH3	YYYRYDVGGFDY
96	CDRH3	YFYRYDVGGFDY
97	CDRH3	YFDKYDVGGCDY
98	CDRH3	YYYKYDVGGFDY
99	CDRH3	YYYRYEVGGFDY
100	CDRL1	SVSSSISSSNLH
101	CDRL1	SVSSSISSSTLH
102	CDRL1	SVSSSISSSNLY
103	CDRL1	SVSSGISSSNLH
104	CDRL2	GTSNLAG
105	CDRL3	QQWSSYPFT
106	CDRL3	QQWNSYPLT
107	CDRL3	QQWSSYPHT

SEQ ID NO:	Description	Amino acid sequence
108	CDRL3	QQWSSYPWT
109	CDRL3	QQWSSYPLT
110	CDRL3	QQWSTYPFT
111	CDRL3	QQWSRYPWT
112	CDRL3	QQWRSYPFT
113	VH FR1	QVQLVQSGAEVKKPGASVKVSCKASGFNIK
114	VH FR1	QVQMKQSGAEVKKPGASVKVSCTASGFNIK
115	VH FR1	QVQMKQSGAEVVKPGASVKVSCTASGFNIK
116	VH FR1	QVQLVQSGAEVKKPGASVKVSCTASGFNIK
117	VH FR1	QVQMVQSGAEVKKPGASVKVSCKASGFNIK
118	VH FR1	QVQMVQSGAEVKKPGASVKVSCTASGFNIK
119	VH FR1	QVQMKQSGAEVKKPGASVKVSCKASGFNIK
120	VH FR1	QVQLKQSGAEVKKPGASVKVSCKASGFNIK
121	VH FR1	QVQLKQSGAEVKKPGASVKVSCTASGFNIK
122	VH FR2	WVRQAPGQGLEWMG
123	VH FR2	WVRQAPGQGLEWIG
124	VH FR3	RVTMTTRDTSTSTVYMELSSLRSED TAVYYCAR
125	VH FR3	RVTITADTSTSTVYMELSSLRSED TAVYYCAT
126	VH FR3	RVTITADTSSNTVYMELSSLRSED TAVYYCAT
127	VH FR3	RVTITADTSSNTVYLQLSSLRSED TAVYYCAT
128	VH FR4	WGQGTLVTVSS
129	VL FR1	EIVLTQSPGTL SLSPGERATLSC
130	VL FR1	EILLTQSPGTL SLSPGERATLSC
131	VL FR1	EILLTQSPGTL SLSPGERATLTC
132	VL FR2	WYQQKPGQAPRLLIY
133	VL FR2	WFQQKPGQAPRLLIY
134	VL FR2	WFQQKPGQAPRLWIY
135	VL FR2	WFQQKPGQSPRLWIY
136	VL FR3	GIPDRFSGSGSGTDFTLTISRLEPEDFAVYYC
137	VL FR3	GIPDRFSGSGSGTSYTLTISRLEPEDFAVYYC
138	VL FR3	GVPDRFSGSGSGTSYTLTISRLEPEDFAVYYC
139	VL FR4	FGQGTKVEIK

SEQ ID NO:	Description	Amino acid sequence
140	CDRH1 consensus	DX ₁ YX ₂ X ₃ , wherein: X ₁ is T or N; X ₂ is I or M; and X ₃ is H, Y or D
141	CDRH1 consensus	DX ₁ YX ₂ X ₃ , wherein: X ₁ is T or N; X ₂ is I or M; and X ₃ is H or Y
142	CDRH2 consensus	X ₁ IDPANX ₂ X ₃ X ₄ X ₅ X ₆ X ₇ PX ₈ X ₉ QX ₁₀ , wherein: X ₁ is E, R, S, or K; X ₂ is D or G; X ₃ is N or H; X ₄ is T or S; X ₅ is K or H; X ₆ is Y or F; X ₇ is D or A; X ₈ is K or R; X ₉ is F or L; and X ₁₀ is G or D
143	CDRH2 consensus	X ₁ IDPANX ₂ X ₃ X ₄ KX ₅ X ₆ PX ₇ FQX ₈ , wherein: X ₁ is E, R, or S; X ₂ is D or G; X ₃ is N or H; X ₄ is T or S; X ₅ is Y or F; X ₆ is D or A; X ₇ is K or R; and X ₈ is G or D
144	CDRH3 consensus	YX ₁ X ₂ X ₃ YX ₄ VGGX ₅ DY, wherein: X ₁ is Y, F, or S; X ₂ is Y or D; X ₃ is K or R; X ₄ is D or E; and X ₅ is F or C
145	CDRH3 consensus	YX ₁ X ₂ X ₃ YDVGGX ₄ DY, wherein: X ₁ is Y, F, or S; X ₂ is Y or D; X ₃ is K or R; and X ₄ is F or C
146	CDRH3 consensus	YYYX ₁ YX ₂ VGGFDY, wherein: X ₁ is K or R; and X ₂ is D or E
147	CDRL1 consensus	SVSSX ₁ ISSSX ₂ LX ₃ , wherein: X ₁ is S or G; X ₂ is N or T; and X ₃ is H or Y
148	CDRL1 consensus	SVSSSISSSNLX ₁ , wherein: X ₁ is H or Y

SEQ ID NO:	Description	Amino acid sequence
149	CDRL3 consensus	QQWX ₁ X ₂ YPX ₃ T, wherein: X ₁ is S, N, or R; X ₂ is S, T or R; and X ₃ is F, L, H, or W
150	CDRL3 consensus	QQWX ₁ SYPX ₂ T, wherein: X ₁ is S, N, or R; and X ₂ is F, L, or H
151	Humanized VH consensus	QVQX ₁ X ₂ QSGAEVX ₃ KPGASVKVSCX ₄ ASGFNIKDTYIHWVRQAPGQGLEWX ₅ GEIDPANDNTKYDPKFQGRVTX ₆ TX ₇ DTSX ₈ X ₉ TVYX ₁₀ X ₁₁ LSSLRSEDYAVYYCAX ₁₂ YYYX ₁₃ YX ₁₄ VGGFDYWGQGTLLTVSS, wherein: X ₁ is M or L; X ₂ is V or K; X ₃ is K or V; X ₄ is K or T; X ₅ is M or I; X ₆ is I or M; X ₇ is A or R; X ₈ is T or S; X ₉ is S or N; X ₁₀ is M or L; X ₁₁ is E or Q; X ₁₂ is T or R; X ₁₃ is K or R; and X ₁₄ is D or E
218	Humanized VH consensus	QVQX ₁ X ₂ QSGAEVX ₃ KPGASVKVSCX ₄ ASGFNIKDTYIHWVRQAPGQGLEWX ₅ GEIDPANDNTKYDPKFQGRVTX ₆ TX ₇ DTSX ₈ X ₉ TVYX ₁₀ X ₁₁ LSSLRSEDYAVYYCAX ₁₂ YYYX ₁₃ YX ₁₄ VGGFDYWGQGTLLTVSS, wherein: X ₁ is M or L; X ₂ is V or K; X ₃ is K or V; X ₄ is K or T; X ₅ is M or I; X ₆ is I or M; X ₇ is A or R; X ₈ is T or S; X ₉ is S or N; X ₁₀ is M or L; X ₁₁ is E or Q; X ₁₂ is T or R; X ₁₃ is K or R; and X ₁₄ is D or E

SEQ ID NO:	Description	Amino acid sequence
222	Humanized VH consensus	<p>X₁VQX₂X₃QSGAEVX₄KPGASVKVSCASGFNIKDTYIHWVRQAPGQGLEWX₅GEIDPANDNTKYDPKFQGRVTX₆TX₇DTSX₈X₉TVYX₁₀X₁₁LSSLRSEDVAVYYCAX₁₂YYYX₁₃YX₁₄VGGFDYWGQGTLLTVSS, wherein:</p> <p>X₁ is Q or pE (pyroglutamate) X₂ is M or L; X₃ is V or K; X₄ is K or V; X₅ is M or I; X₆ is I or M; X₇ is A or R; X₈ is T or S; X₉ is S or N; X₁₀ is M or L; X₁₁ is E or Q; X₁₂ is T or R; X₁₃ is K or R; and X₁₄ is D or E</p>
223	Humanized VH consensus	<p>X₁VQX₂X₃QSGAEVX₄KPGASVKVSCX₅ASGFNIKDTYIHWVRQAPGQGLEWX₆GEIDPANDNTKYDPKFQGRVTX₇TX₈DTSX₉X₁₀TVYX₁₁X₁₂LSSLRSEDVAVYYCAX₁₃YYYX₁₄YX₁₅VGGFDYWGQGTLLTVSS, wherein:</p> <p>X₁ is Q or pE (pyroglutamate) X₂ is M or L; X₃ is V or K; X₄ is K or V; X₅ is K or T; X₆ is M or I; X₇ is I or M; X₈ is A or R; X₉ is T or S; X₁₀ is S or N; X₁₁ is M or L; X₁₂ is E or Q; X₁₃ is T or R; X₁₄ is K or R; and X₁₅ is D or E</p>
152	Humanized VL consensus	<p>EIX₁LTQSPGTLSPGERATLX₂CSVSSSISSSNLHWX₃QQKPGQX₄PRLX₅IYGTSNLSGX₆PDRFSGSGSGTX₇X₈TLTISRLEPEDFAVYYCQWSSYPFTFGQGTKVEIK, wherein:</p> <p>X₁ is V or L; X₂ is S or T; X₃ is Y or F; X₄ is A or S; X₅ is L or W; X₆ is I or V; X₇ is D or S; and X₈ is F or Y</p>

SEQ ID NO:	Description	Amino acid sequence
224	Humanized VL consensus	<p>X₁IX₂LTQSPGTL₃SLSPGERATLX₃CSVSSSISSSNLH WX₄QQKPGQX₅PRLX₆IYGTSN₇LASGX₇PDRFSGSGSGT X₈X₉TLTISRLEPEDFAVYYCQQWSSYPFTFGQGTKVE IK, wherein: X₁ is E or pE (pyroglutamate) X₂ is V or L; X₃ is S or T; X₄ is Y or F; X₅ is A or S; X₆ is L or W; X₇ is I or V; X₈ is D or S; and X₉ is F or Y</p>
168	H1_R98K_L4M full length IgG ₁ heavy chain	<p>QVQMVQSGAEVKKPGASVKVSCKASGFNIKDTYIHWV RQAPGQGLEWMGEIDPANDNTKYDPKFQGRVTITADT STSTVYMELSSLRSED₁TAVYYCATYYYKYDVGGFDYW GQGTLVTVSSASTKGPSVFPLAPSSKSTSGGTAALGC LVKDYFPEPVT₂SVWNSGALTSGVHTFPAVLQSSGLYS LSSVTVTPSSSLGTQTYICNVNHKPSNTKVDKRV₃EPK SCDKTHTCPPCPAPEL₄LGPSVFLFPPKPKDTLMISR TPEVTCVVVDVSHEDPEVKFNWYVDGVEVHNAKTKPR EEQYNSTYRVVSVLTVLHQDWLNGKEYKCKVSNKALP APIEKTISKAKGQPREPQVYTLPPSREEMTKNQVSLT CLVKGFYPSDIAVEWESNGQPENNYKTPPVLDSDGS FFLYSKLTVDKSRWQQGNVFSCSVMHEALHNHYTQKS LSLSPG</p>
225	H1_R98K_L4M full length IgG ₁ heavy chain	<p>XVQMVQSGAEVKKPGASVKVSCKASGFNIKDTYIHWV RQAPGQGLEWMGEIDPANDNTKYDPKFQGRVTITADT STSTVYMELSSLRSED₁TAVYYCATYYYKYDVGGFDYW GQGTLVTVSSASTKGPSVFPLAPSSKSTSGGTAALGC LVKDYFPEPVT₂SVWNSGALTSGVHTFPAVLQSSGLYS LSSVTVTPSSSLGTQTYICNVNHKPSNTKVDKRV₃EPK SCDKTHTCPPCPAPEL₄LGPSVFLFPPKPKDTLMISR TPEVTCVVVDVSHEDPEVKFNWYVDGVEVHNAKTKPR EEQYNSTYRVVSVLTVLHQDWLNGKEYKCKVSNKALP APIEKTISKAKGQPREPQVYTLPPSREEMTKNQVSLT CLVKGFYPSDIAVEWESNGQPENNYKTPPVLDSDGS FFLYSKLTVDKSRWQQGNVFSCSVMHEALHNHYTQKS LSLSPG, wherein X = glutamine (Q) or pyroglutamate (pE)</p>

SEQ ID NO:	Description	Amino acid sequence
169	H1_R98K_L4M full length IgG ₁ N297A heavy chain	QVQMVQSGAEVKKPGASVKVSCKASGFNIKDTYIHWV RQAPGQGLEWMGEIDPANDNTKYDPKFQGRVTITADT STSTVYMELSSLRSEDVAVYYCATYYYKYDVGGFDYW GQGTLVTVSSASTKGPSVFPLAPSSKSTSGGTAALGC LVKDYFPEPVTVSWNSGALTSGVHTFPAVLQSSGLYS LSSVTVTPSSSLGTQTYICNVNHKPSNTKVDKRVEPK SCDKTHTCPPCPAPELLGGPSVFLFPPKPKDTLMISR TPEVTCVVVDVSHEDPEVKFNWYVDGVEVHNAKT KPR EEQYASTYRVVSVLTVLHQDWLNGKEYKCKVSNKALP APIEKTISKAKGQPREPQVYTLPPSREEMTKNQVSLT CLVKGFYPSDIAVEWESNGQPENNYKTTPPVLDSDGS FFLYSKLTVDKSRWQQGNVFCFSVMHEALHNHYTQKS LSLSPG
226	H1_R98K_L4M full length IgG ₁ N297A heavy chain	XVQMVQSGAEVKKPGASVKVSCKASGFNIKDTYIHWV RQAPGQGLEWMGEIDPANDNTKYDPKFQGRVTITADT STSTVYMELSSLRSEDVAVYYCATYYYKYDVGGFDYW GQGTLVTVSSASTKGPSVFPLAPSSKSTSGGTAALGC LVKDYFPEPVTVSWNSGALTSGVHTFPAVLQSSGLYS LSSVTVTPSSSLGTQTYICNVNHKPSNTKVDKRVEPK SCDKTHTCPPCPAPELLGGPSVFLFPPKPKDTLMISR TPEVTCVVVDVSHEDPEVKFNWYVDGVEVHNAKT KPR EEQYASTYRVVSVLTVLHQDWLNGKEYKCKVSNKALP APIEKTISKAKGQPREPQVYTLPPSREEMTKNQVSLT CLVKGFYPSDIAVEWESNGQPENNYKTTPPVLDSDGS FFLYSKLTVDKSRWQQGNVFCFSVMHEALHNHYTQKS LSLSPG, wherein X = glutamine (Q) or pyroglutamate (pE)
170	H1_R98K_L4M full length IgG ₄ S228P heavy chain	QVQMVQSGAEVKKPGASVKVSCKASGFNIKDTYIHWV RQAPGQGLEWMGEIDPANDNTKYDPKFQGRVTITADT STSTVYMELSSLRSEDVAVYYCATYYYKYDVGGFDYW GQGTLVTVSSASTKGPSVFPLAPCSRSTSESTAALGC LVKDYFPEPVTVSWNSGALTSGVHTFPAVLQSSGLYS LSSVTVTPSSSLGTQTYTCNVNHHKPSNTKVDKRVESK YGPPCPPCPAPEFLGGPSVFLFPPKPKDTLMISRTPE VTCVVVDVSQEDPEVQFNWYVDGVEVHNAKT KPREEQ FNSTYRVVSVLTVLHQDWLNGKEYKCKVSNKGLPSSI EKTISKAKGQPREPQVYTLPPSQEEMTKNQVSLTCLV KGFYPSDIAVEWESNGQPENNYKTTPPVLDSDGSFFL YSRLTVDKSRWQEGNVFCFSVMHEALHNHYTQKSLSL SLG

SEQ ID NO:	Description	Amino acid sequence
227	H1_R98K_L4M full length IgG ₄ S228P heavy chain	XVQMVQSGAEVKKPGASVKVSCKASGFNIKDTYIHWV RQAPGQGLEWMGEIDPANDNTKYDPKFQGRVTITADT STSTVYMELSSLRSED TAVYYCATYYYKYDVGGFDYW GQGTLVTVSSASTKGPSVFPLAPCSRSTSESTAALGC LVKDYFPEPVTVSWNSGALTSGVHTFPAVLQSSGLYS LSSVTVTPSSSLGTQTYTCNV DHKPSNTKVDKRVESK YGPPCPPCPAPEFLGGPSVFLFPPKPKDTLMISRTPE VTCVVVDVSQEDPEVQFNWYVDGVEVHNAKTKPREEQ FNSTYRVVSVLTVLHQDWLNGKEYKCKVSNKGLPSSI EKTISKAKGQPREPQVYTLPPSQEEMTKNQVSLTCLV KGFYPSDIAVEWESNGQPENNYKTTTPVLDSGGSFFL YSRLTVDKSRWQEGNVFSCSVMHEALHNHYTQKSLSL SLG, wherein X = glutamine (Q) or pyroglutamate (pE)
171	H0 full length IgG ₁ N297A heavy chain	QVQLVQSGAEVKKPGASVKVSCKASGFNIKDTYIHWV RQAPGQGLEWMGEIDPANDNTKYDPKFQGRVTMTTRDT STSTVYMELSSLRSED TAVYYCARYYYRYDVGGFDYW GQGTLVTVSSASTKGPSVFPLAPSSKSTSGGTAALGC LVKDYFPEPVTVSWNSGALTSGVHTFPAVLQSSGLYS LSSVTVTPSSSLGTQTYICNVNHKPSNTKVDKRVEPK SCDKTHTCPPCPAPELLGGPSVFLFPPKPKDTLMISR TPEVTCVVVDVSHEDPEVKFNWYVDGVEVHNAKTKPR EEQYASTYRVVSVLTVLHQDWLNGKEYKCKVSNKALP APIEKTISKAKGQPREPQVYTLPPSREEMTKNQVSLT CLVKGFYPSDIAVEWESNGQPENNYKTTTPVLDSGGS FFLYSKLTVDKSRWQQGNVFSCSVMHEALHNHYTQKS LSLSPG
172	H1 full length IgG ₁ N297A heavy chain	QVQLVQSGAEVKKPGASVKVSCKASGFNIKDTYIHWV RQAPGQGLEWMGEIDPANDNTKYDPKFQGRVTITADT STSTVYMELSSLRSED TAVYYCATYYYRYDVGGFDYW GQGTLVTVSSASTKGPSVFPLAPSSKSTSGGTAALGC LVKDYFPEPVTVSWNSGALTSGVHTFPAVLQSSGLYS LSSVTVTPSSSLGTQTYICNVNHKPSNTKVDKRVEPK SCDKTHTCPPCPAPELLGGPSVFLFPPKPKDTLMISR TPEVTCVVVDVSHEDPEVKFNWYVDGVEVHNAKTKPR EEQYASTYRVVSVLTVLHQDWLNGKEYKCKVSNKALP APIEKTISKAKGQPREPQVYTLPPSREEMTKNQVSLT CLVKGFYPSDIAVEWESNGQPENNYKTTTPVLDSGGS FFLYSKLTVDKSRWQQGNVFSCSVMHEALHNHYTQKS LSLSPG

SEQ ID NO:	Description	Amino acid sequence
173	H2 full length IgG ₁ N297A heavy chain	QVQLVQSGAEVKKPGASVKVSCKASGFNIKDTYIHWV RQAPGQGLEWMGEIDPANDNTKYDPKFQGRVTITADT SSNTVMELSSLRSED TAVYYCATYYYRYDVGGFDYW GQGTLVTVSSASTKGPSVFPLAPSSKSTSGGTAALGC LVKDYFPEPVTVSWNSGALTSGVHTFPAVLQSSGLYS LSSVTVTPSSSLGTQTYICNVNHKPSNTKVDKRVEPK SCDKTHTCPPCPAPELLGGPSVFLFPPKPKDTLMISR TPEVTCVVVDVSHEDPEVKFNWYVDGVEVHNAKTKPR EEQYASTYRVVSVLTVLHQDWLNGKEYKCKVSNKALP APIEKTISKAKGQPREPQVYTLPPSREEMTKNQVSLT CLVKGFYPSDIAVEWESNGQPENNYKTTPPVLDSDGS FFLYSKLTVDKSRWQQGNVFSCSVMHEALHNHYTQKS LSLSPG
174	H3 full length IgG ₁ N297A heavy chain	QVQMKQSGAEVKKPGASVKVSCTASGFNIKDTYIHWV RQAPGQGLEWMGEIDPANDNTKYDPKFQGRVTITADT SSNTVMELSSLRSED TAVYYCATYYYRYDVGGFDYW GQGTLVTVSSASTKGPSVFPLAPSSKSTSGGTAALGC LVKDYFPEPVTVSWNSGALTSGVHTFPAVLQSSGLYS LSSVTVTPSSSLGTQTYICNVNHKPSNTKVDKRVEPK SCDKTHTCPPCPAPELLGGPSVFLFPPKPKDTLMISR TPEVTCVVVDVSHEDPEVKFNWYVDGVEVHNAKTKPR EEQYASTYRVVSVLTVLHQDWLNGKEYKCKVSNKALP APIEKTISKAKGQPREPQVYTLPPSREEMTKNQVSLT CLVKGFYPSDIAVEWESNGQPENNYKTTPPVLDSDGS FFLYSKLTVDKSRWQQGNVFSCSVMHEALHNHYTQKS LSLSPG
175	H4 full length IgG ₁ N297A heavy chain	QVQMKQSGAEVVKPGASVKVSCTASGFNIKDTYIHWV RQAPGQGLEWIGEIDPANDNTKYDPKFQGRVTITADT SSNTVYLQLSSLRSED TAVYYCATYYYRYDVGGFDYW GQGTLVTVSSASTKGPSVFPLAPSSKSTSGGTAALGC LVKDYFPEPVTVSWNSGALTSGVHTFPAVLQSSGLYS LSSVTVTPSSSLGTQTYICNVNHKPSNTKVDKRVEPK SCDKTHTCPPCPAPELLGGPSVFLFPPKPKDTLMISR TPEVTCVVVDVSHEDPEVKFNWYVDGVEVHNAKTKPR EEQYASTYRVVSVLTVLHQDWLNGKEYKCKVSNKALP APIEKTISKAKGQPREPQVYTLPPSREEMTKNQVSLT CLVKGFYPSDIAVEWESNGQPENNYKTTPPVLDSDGS FFLYSKLTVDKSRWQQGNVFSCSVMHEALHNHYTQKS LSLSPG

SEQ ID NO:	Description	Amino acid sequence
176	H4_R98K full length IgG ₁ N297A heavy chain	QVQMKQSGAEVVKPGASVKVSC TASGFNIKDTYIHWV RQAPGQGLEWIGEIDPANDNTKYDPKFQGRVTITADT SSNTVYLQLSSLRSED TAVYYCATYYYKYDVGGFDYW GQGTLVTVSSASTKGPSVFPLAPSSKSTSGGTAALGC LVKDYFPEPVT VSWNSGALTSGVHTFPAVLQSSGLYS LSSVTVTPSSSLGTQTYICNVNHKPSNTKVDKRVEPK SCDKTHTCPPCPAPELLGGPSVFLFPPKPKDTLMISR TPEVTCVVVDVSHEDPEVKFNWYVDGVEVHNAKTKPR EEQYASTYRVVSVLTVLHQDWLNGKEYKCKVSNKALP APIEKTISKAKGQPREPQVYTLPPSREEMTKNQVSLT CLVKGFYPSDIAVEWESNGQPENNYKTTPPVLDSDGS FFLYSKLTVDKSRWQQGNV FSCSV MHEALHNHYTQKS LSLSPG
177	H4_D100E full length IgG ₁ N297A heavy chain	QVQMKQSGAEVVKPGASVKVSC TASGFNIKDTYIHWV RQAPGQGLEWIGEIDPANDNTKYDPKFQGRVTITADT SSNTVYLQLSSLRSED TAVYYCATYYYRYEVGGFDYW GQGTLVTVSSASTKGPSVFPLAPSSKSTSGGTAALGC LVKDYFPEPVT VSWNSGALTSGVHTFPAVLQSSGLYS LSSVTVTPSSSLGTQTYICNVNHKPSNTKVDKRVEPK SCDKTHTCPPCPAPELLGGPSVFLFPPKPKDTLMISR TPEVTCVVVDVSHEDPEVKFNWYVDGVEVHNAKTKPR EEQYASTYRVVSVLTVLHQDWLNGKEYKCKVSNKALP APIEKTISKAKGQPREPQVYTLPPSREEMTKNQVSLT CLVKGFYPSDIAVEWESNGQPENNYKTTPPVLDSDGS FFLYSKLTVDKSRWQQGNV FSCSV MHEALHNHYTQKS LSLSPG
178	H1_R98K full length IgG ₁ N297A heavy chain	QVQLVQSGAEVKKPGASVKVSC KASGFNIKDTYIHWV RQAPGQGLEWMGEIDPANDNTKYDPKFQGRVTITADT STSTVYME LSSLRSED TAVYYCATYYYKYDVGGFDYW GQGTLVTVSSASTKGPSVFPLAPSSKSTSGGTAALGC LVKDYFPEPVT VSWNSGALTSGVHTFPAVLQSSGLYS LSSVTVTPSSSLGTQTYICNVNHKPSNTKVDKRVEPK SCDKTHTCPPCPAPELLGGPSVFLFPPKPKDTLMISR TPEVTCVVVDVSHEDPEVKFNWYVDGVEVHNAKTKPR EEQYASTYRVVSVLTVLHQDWLNGKEYKCKVSNKALP APIEKTISKAKGQPREPQVYTLPPSREEMTKNQVSLT CLVKGFYPSDIAVEWESNGQPENNYKTTPPVLDSDGS FFLYSKLTVDKSRWQQGNV FSCSV MHEALHNHYTQKS LSLSPG

SEQ ID NO:	Description	Amino acid sequence
179	H1_R98K_K23T full length IgG ₁ N297A heavy chain	QVQLVQSGAEVKKPGASVKVSCTASGFNIKDTYIHWV RQAPGQGLEWMGEIDPANDNTKYDPKFQGRVTITADT STSTVYMELSSLRSED TAVYYCATYYYKYDVGGFDYW GQGTLVTVSSASTKGPSVFPLAPSSKSTSGGTAALGC LVKDYFPEPVTVSWNSGALTSGVHTFPAVLQSSGLYS LSSVTVTPSSSLGTQTYICNVNHKPSNTKVDKRVEPK SCDKTHTCPPCPAPELLGGPSVFLFPPKPKDTLMISR TPEVTCVVVDVSHEDPEVKFNWYVDGVEVHNAKTKPR EEQYASTYRVVSVLTVLHQDWLNGKEYKCKVSNKALP APIEKTISKAKGQPREPQVYTLPPSREEMTKNQVSLT CLVKGFYPSDIAVEWESNGQPENNYKTTPPVLDSDGS FFLYSKLTVDKSRWQQGNVFSCSVMHEALHNHYTQKS LSLSPG
180	H1_R98K_L4M_K23T full length IgG ₁ N297A heavy chain	QVQMVQSGAEVKKPGASVKVSCTASGFNIKDTYIHWV RQAPGQGLEWMGEIDPANDNTKYDPKFQGRVTITADT STSTVYMELSSLRSED TAVYYCATYYYKYDVGGFDYW GQGTLVTVSSASTKGPSVFPLAPSSKSTSGGTAALGC LVKDYFPEPVTVSWNSGALTSGVHTFPAVLQSSGLYS LSSVTVTPSSSLGTQTYICNVNHKPSNTKVDKRVEPK SCDKTHTCPPCPAPELLGGPSVFLFPPKPKDTLMISR TPEVTCVVVDVSHEDPEVKFNWYVDGVEVHNAKTKPR EEQYASTYRVVSVLTVLHQDWLNGKEYKCKVSNKALP APIEKTISKAKGQPREPQVYTLPPSREEMTKNQVSLT CLVKGFYPSDIAVEWESNGQPENNYKTTPPVLDSDGS FFLYSKLTVDKSRWQQGNVFSCSVMHEALHNHYTQKS LSLSPG
181	H1_R98K_L4M_V5K full length IgG ₁ N297A heavy chain	QVQMKQSGAEVKKPGASVKVSCKASGFNIKDTYIHWV RQAPGQGLEWMGEIDPANDNTKYDPKFQGRVTITADT STSTVYMELSSLRSED TAVYYCATYYYKYDVGGFDYW GQGTLVTVSSASTKGPSVFPLAPSSKSTSGGTAALGC LVKDYFPEPVTVSWNSGALTSGVHTFPAVLQSSGLYS LSSVTVTPSSSLGTQTYICNVNHKPSNTKVDKRVEPK SCDKTHTCPPCPAPELLGGPSVFLFPPKPKDTLMISR TPEVTCVVVDVSHEDPEVKFNWYVDGVEVHNAKTKPR EEQYASTYRVVSVLTVLHQDWLNGKEYKCKVSNKALP APIEKTISKAKGQPREPQVYTLPPSREEMTKNQVSLT CLVKGFYPSDIAVEWESNGQPENNYKTTPPVLDSDGS FFLYSKLTVDKSRWQQGNVFSCSVMHEALHNHYTQKS LSLSPG

SEQ ID NO:	Description	Amino acid sequence
182	H1_R98K_L4M_V5K_K23T full length IgG ₁ N297A heavy chain	QVQMKQSGAEVKKPGASVKVSCTASGFNIKDTYIHWV RQAPGQGLEWMGEIDPANDNTKYDPKFQGRVTITADT STSTVYMELSSLRSED TAVYYCATYYYKYDVGGFDYW GQGTLVTVSSASTKGPSVFPLAPSSKSTSGGTAALGC LVKDYFPEPVTVSWNSGALTSGVHTFPAVLQSSGLYS LSSVTVTPSSSLGTQTYICNVNHKPSNTKVDKRVPEPK SCDKTHTCPPCPAPELLGGPSVFLFPPKPKDTLMISR TPEVTCVVVDVSHEDPEVKFNWYVDGVEVHNAKTKPR EEQYASTYRVVSVLTVLHQDWLNGKEYKCKVSNKALP APIEKTISKAKGQPREPQVYTLPPSREEMTKNQVSLT CLVKGFYPSDIAVEWESNGQPENNYKTTPPVLDSDGS FFLYSKLTVDKSRWQQGNVFSCSVMEALHNHYTQKS LSLSPG
183	H1_R98K_V5K full length IgG ₁ N297A heavy chain	QVQLKQSGAEVKKPGASVKVSCKASGFNIKDTYIHWV RQAPGQGLEWMGEIDPANDNTKYDPKFQGRVTITADT STSTVYMELSSLRSED TAVYYCATYYYKYDVGGFDYW GQGTLVTVSSASTKGPSVFPLAPSSKSTSGGTAALGC LVKDYFPEPVTVSWNSGALTSGVHTFPAVLQSSGLYS LSSVTVTPSSSLGTQTYICNVNHKPSNTKVDKRVPEPK SCDKTHTCPPCPAPELLGGPSVFLFPPKPKDTLMISR TPEVTCVVVDVSHEDPEVKFNWYVDGVEVHNAKTKPR EEQYASTYRVVSVLTVLHQDWLNGKEYKCKVSNKALP APIEKTISKAKGQPREPQVYTLPPSREEMTKNQVSLT CLVKGFYPSDIAVEWESNGQPENNYKTTPPVLDSDGS FFLYSKLTVDKSRWQQGNVFSCSVMEALHNHYTQKS LSLSPG
184	H1_R98K_V5K_K23T full length IgG ₁ N297A heavy chain	QVQLKQSGAEVKKPGASVKVSCTASGFNIKDTYIHWV RQAPGQGLEWMGEIDPANDNTKYDPKFQGRVTITADT STSTVYMELSSLRSED TAVYYCATYYYKYDVGGFDYW GQGTLVTVSSASTKGPSVFPLAPSSKSTSGGTAALGC LVKDYFPEPVTVSWNSGALTSGVHTFPAVLQSSGLYS LSSVTVTPSSSLGTQTYICNVNHKPSNTKVDKRVPEPK SCDKTHTCPPCPAPELLGGPSVFLFPPKPKDTLMISR TPEVTCVVVDVSHEDPEVKFNWYVDGVEVHNAKTKPR EEQYASTYRVVSVLTVLHQDWLNGKEYKCKVSNKALP APIEKTISKAKGQPREPQVYTLPPSREEMTKNQVSLT CLVKGFYPSDIAVEWESNGQPENNYKTTPPVLDSDGS FFLYSKLTVDKSRWQQGNVFSCSVMEALHNHYTQKS LSLSPG

SEQ ID NO:	Description	Amino acid sequence
185	H2_R98K full length IgG ₁ N297A heavy chain	QVQLVQSGAEVKKPGASVKVSCKASGFNIKDTYIHWV RQAPGQGLEWMGEIDPANDNTKYDPKFQGRVTITADT SSNTVYMELSSLRSEDVAVYYCATYYYKYDVGGFDYW GQGTLLTVSSASTKGPSVFPLAPSSKSTSGGTAALGC LVKDYFPEPVTVSWNSGALTSGVHTFPAVLQSSGLYS LSSVTVTPSSSLGTQTYICNVNHKPSNTKVDKRVKPK SCDKTHTCPPCPAPELLGGPSVFLFPPKPKDTLMISR TPEVTCVVVDVSHEDPEVKFNWYVDGVEVHNAKTKPR EEQYASTYRVVSVLTVLHQDWLNGKEYKCKVSNKALP APIEKTISKAKGQPREPQVYTLPPSREEMTKNQVSLT CLVKGFYPSDIAVEWESNGQPENNYKTPPVLDSDGS FFLYSKLTVDKSRWQQGNVFCFSVMHEALHNHYTQKS LSLSPG
186	H3_R98K full length IgG ₁ N297A heavy chain	QVQMKQSGAEVKKPGASVKVSCTASGFNIKDTYIHWV RQAPGQGLEWMGEIDPANDNTKYDPKFQGRVTITADT SSNTVYMELSSLRSEDVAVYYCATYYYKYDVGGFDYW GQGTLLTVSSASTKGPSVFPLAPSSKSTSGGTAALGC LVKDYFPEPVTVSWNSGALTSGVHTFPAVLQSSGLYS LSSVTVTPSSSLGTQTYICNVNHKPSNTKVDKRVKPK SCDKTHTCPPCPAPELLGGPSVFLFPPKPKDTLMISR TPEVTCVVVDVSHEDPEVKFNWYVDGVEVHNAKTKPR EEQYASTYRVVSVLTVLHQDWLNGKEYKCKVSNKALP APIEKTISKAKGQPREPQVYTLPPSREEMTKNQVSLT CLVKGFYPSDIAVEWESNGQPENNYKTPPVLDSDGS FFLYSKLTVDKSRWQQGNVFCFSVMHEALHNHYTQKS LSLSPG
187	L0 full length light chain	EIVLTQSPGTLSLSPGERATLSCSVSSSISSSNLHWY QQKPGQAPRLLIYGTSNLSAGIPDRFSGSGSGTDFTL TISRLEPEDFAVYYCQQWSSYPFTFGQGTKEIKRTV AAPSVFI FPPSDEQLKSGTASVVCLLNNFYPREAKVQ WKVDNALQSGNSQESVTEQDSKSTYSLSSTLTLSKA DYEKHKVYACEVTHQGLSSPVTKSFNREGC
228	L0 full length light chain	XIVLTQSPGTLSLSPGERATLSCSVSSSISSSNLHWY QQKPGQAPRLLIYGTSNLSAGIPDRFSGSGSGTDFTL TISRLEPEDFAVYYCQQWSSYPFTFGQGTKEIKRTV AAPSVFI FPPSDEQLKSGTASVVCLLNNFYPREAKVQ WKVDNALQSGNSQESVTEQDSKSTYSLSSTLTLSKA DYEKHKVYACEVTHQGLSSPVTKSFNREGC, wherein X = glutamate (E) or pyroglutamate (pE)
188	L1 full length light chain	EIVLTQSPGTLSLSPGERATLSCSVSSSISSSNLHWY QQKPGQAPRLLIYGTSNLSAGIPDRFSGSGSGTSYTL TISRLEPEDFAVYYCQQWSSYPFTFGQGTKEIKRTV AAPSVFI FPPSDEQLKSGTASVVCLLNNFYPREAKVQ WKVDNALQSGNSQESVTEQDSKSTYSLSSTLTLSKA DYEKHKVYACEVTHQGLSSPVTKSFNREGC

SEQ ID NO:	Description	Amino acid sequence
189	L2 full length light chain	EILLTQSPGTLSSLSPGERATLSCSVSSSISSSNLHWF QQKPGQAPRLLIYGTSNLASGIPDRFSGSGSGTSTYTL TISRLEPEDFAVYYCQQWSSYPFTFGQGTKEIKRTV AAPSVFIFPPSDEQLKSGTASVVCLLNNFYPREAKVQ WKVDNALQSGNSQESVTEQDSKDSTYLSSTLTLSKA DYEKHKVYACEVTHQGLSSPVTKSFNRGEC
190	L3 full length light chain	EILLTQSPGTLSSLSPGERATLSCSVSSSISSSNLHWF QQKPGQAPRLWIYGTSNLASGVPDRFSGSGSGTSTYTL TISRLEPEDFAVYYCQQWSSYPFTFGQGTKEIKRTV AAPSVFIFPPSDEQLKSGTASVVCLLNNFYPREAKVQ WKVDNALQSGNSQESVTEQDSKDSTYLSSTLTLSKA DYEKHKVYACEVTHQGLSSPVTKSFNRGEC
191	L4 full length light chain	EILLTQSPGTLSSLSPGERATLTCSVSSSISSSNLHWF QQKPGQSPRLWIYGTSNLASGVPDRFSGSGSGTSTYTL TISRLEPEDFAVYYCQQWSSYPFTFGQGTKEIKRTV AAPSVFIFPPSDEQLKSGTASVVCLLNNFYPREAKVQ WKVDNALQSGNSQESVTEQDSKDSTYLSSTLTLSKA DYEKHKVYACEVTHQGLSSPVTKSFNRGEC
192	Human IgG ₁ G1m3 allotype (without C-terminal lysine)	ASTKGPSVFPLAPSSKSTSGGTAALGCLVKDYFPEPV TVSWNSGALTSGVHTFPAVLQSSGLYSLSSVTVTPSS SLGTQTYICNVNHKPSNTKVDKRVKPKSCDKTHTCPP CPAPELLGGPSVFLFPPKPKDTLMI SRTPEVTCVVVD VSHEDPEVKFNWYVDGVEVHNAKTKPREEQYNSTYRV VSVLTVLHQDWLNGKEYKCKVSNKALPAPIEKTISKA KGQPREPQVYTLPPSREEMTKNQVSLTCLVKGFYPSD IAVEWESNGQPENNYKTTTPVLDSDGSFFLYSKLTVD KSRWQQGNVFSCSVMHEALHNHYTQKSLSLSPG
193	Human IgG ₁ G1m3 allotype	ASTKGPSVFPLAPSSKSTSGGTAALGCLVKDYFPEPV TVSWNSGALTSGVHTFPAVLQSSGLYSLSSVTVTPSS SLGTQTYICNVNHKPSNTKVDKRVKPKSCDKTHTCPP CPAPELLGGPSVFLFPPKPKDTLMI SRTPEVTCVVVD VSHEDPEVKFNWYVDGVEVHNAKTKPREEQYNSTYRV VSVLTVLHQDWLNGKEYKCKVSNKALPAPIEKTISKA KGQPREPQVYTLPPSREEMTKNQVSLTCLVKGFYPSD IAVEWESNGQPENNYKTTTPVLDSDGSFFLYSKLTVD KSRWQQGNVFSCSVMHEALHNHYTQKSLSLSPGK
208	Human IgG ₁ G1m17 allotype (without C-terminal lysine)	ASTKGPSVFPLAPSSKSTSGGTAALGCLVKDYFPEPV TVSWNSGALTSGVHTFPAVLQSSGLYSLSSVTVTPSS SLGTQTYICNVNHKPSNTKVDKKVEPKSCDKTHTCPP CPAPELLGGPSVFLFPPKPKDTLMI SRTPEVTCVVVD VSHEDPEVKFNWYVDGVEVHNAKTKPREEQYNSTYRV VSVLTVLHQDWLNGKEYKCKVSNKALPAPIEKTISKA KGQPREPQVYTLPPSRDELTKNQVSLTCLVKGFYPSD IAVEWESNGQPENNYKTTTPVLDSDGSFFLYSKLTVD KSRWQQGNVFSCSVMHEALHNHYTQKSLSLSPG

SEQ ID NO:	Description	Amino acid sequence
209	Human IgG ₁ G1m17 allotype	ASTKGPSVFPLAPSSKSTSGGTAALGCLVKDYFPEPV TVSWNSGALTSGVHTFPAVLQSSGLYSLSSVTVPS SLGTQTYICNVNHKPSNTKVDKKVEPKSCDKTHTCPP CPAPELLGGPSVFLFPPKPKDTLMI SRTPEVTCVVD VSHEDPEVKFNWYVDGVEVHNAKTKPREEQYNSTYRV VSVLTIVLHQDWLNGKEYKCKVSNKALPAPIEKTISKA KGQPREPQVYTLPPSRDELTKNQVSLTCLVKGFYPSD IAVEWESNGQPENNYKTTTPVLDSDGSFFLYSKLTVD KSRWQQGNVFSCSVMHEALHNHYTQKSLSLSPGK
194	IgG ₁ N297A (without C-terminal lysine)	ASTKGPSVFPLAPSSKSTSGGTAALGCLVKDYFPEPV TVSWNSGALTSGVHTFPAVLQSSGLYSLSSVTVPS SLGTQTYICNVNHKPSNTKVDKRVKVEPKSCDKTHTCPP CPAPELLGGPSVFLFPPKPKDTLMI SRTPEVTCVVD VSHEDPEVKFNWYVDGVEVHNAKTKPREEQYASTYRV VSVLTIVLHQDWLNGKEYKCKVSNKALPAPIEKTISKA KGQPREPQVYTLPPSREEMTKNQVSLTCLVKGFYPSD IAVEWESNGQPENNYKTTTPVLDSDGSFFLYSKLTVD KSRWQQGNVFSCSVMHEALHNHYTQKSLSLSPG
195	IgG ₁ N297A	ASTKGPSVFPLAPSSKSTSGGTAALGCLVKDYFPEPV TVSWNSGALTSGVHTFPAVLQSSGLYSLSSVTVPS SLGTQTYICNVNHKPSNTKVDKRVKVEPKSCDKTHTCPP CPAPELLGGPSVFLFPPKPKDTLMI SRTPEVTCVVD VSHEDPEVKFNWYVDGVEVHNAKTKPREEQYASTYRV VSVLTIVLHQDWLNGKEYKCKVSNKALPAPIEKTISKA KGQPREPQVYTLPPSREEMTKNQVSLTCLVKGFYPSD IAVEWESNGQPENNYKTTTPVLDSDGSFFLYSKLTVD KSRWQQGNVFSCSVMHEALHNHYTQKSLSLSPGK
196	IgG ₄ S228P (without C-terminal lysine)	ASTKGPSVFPLAPCSRSTSESTAALGCLVKDYFPEPV TVSWNSGALTSGVHTFPAVLQSSGLYSLSSVTVPS SLGKTYTCNVNHNKPSNTKVDKRVESKYGPCCPPCPA PEFLGGPSVFLFPPKPKDTLMI SRTPEVTCVVDVSQ EDPEVQFNWYVDGVEVHNAKTKPREEQFNSTYRVVSV LTVLHQDWLNGKEYKCKVSNKGLPSSIEKTISKAKGQ PREPQVYTLPPSQEEMTKNQVSLTCLVKGFYPSDIAV EWESNGQPENNYKTTTPVLDSDGSFFLYSRLTVDKSR WQEGNVFSCSVMHEALHNHYTQKSLSLSLG
197	IgG ₄ S228P	ASTKGPSVFPLAPCSRSTSESTAALGCLVKDYFPEPV TVSWNSGALTSGVHTFPAVLQSSGLYSLSSVTVPS SLGKTYTCNVNHNKPSNTKVDKRVESKYGPCCPPCPA PEFLGGPSVFLFPPKPKDTLMI SRTPEVTCVVDVSQ EDPEVQFNWYVDGVEVHNAKTKPREEQFNSTYRVVSV LTVLHQDWLNGKEYKCKVSNKGLPSSIEKTISKAKGQ PREPQVYTLPPSQEEMTKNQVSLTCLVKGFYPSDIAV EWESNGQPENNYKTTTPVLDSDGSFFLYSRLTVDKSR WQEGNVFSCSVMHEALHNHYTQKSLSLSLGK
198	Human kappa light chain constant region IGKC*01 Km3 allotype	RTVAAPSVFIFPPSDEQLKSGTASVVCLLNNFYPREA KVQWKVDNALQSGNSQESVTEQDSKDYSTLSSTLT SKADYEKHKVYACEVTHQGLSPVTKSFNRGEC

SEQ ID NO:	Description	Amino acid sequence
219	Human kappa light chain constant region IGKC*01 Km3 allotype	GTVAAPSVFIFPPSDEQLKSGTASVVCLLNNFYPREA KVQWKVDNALQSGNSQESVTEQDSKDSSTLSSTLT SKADYEKHKVYACEVTHQGLSSPVTKSFNRGEC

Table 2. Heavy chain CDR amino acid sequences of exemplary anti-LAG-3 antibodies. *

VH	CDRH1 (SEQ ID NO:)	CDRH2 (SEQ ID NO:)	CDRH3 (SEQ ID NO:)
P01C09 VH	DTYMY (78)	RIDPANGNTKYDPKLGQ (83)	YSYRYDVGGFDY (94)
P05E01 VH	DTYIH (79)	EIDPANGNTKYDPKFQG (84)	YYYRYDVGGFDY (95)
P01A12 VH	DTYMY (78)	RIDPANGNTKFDPKFQG (85)	YYYRYDVGGFDY (95)
P13B01 VH	DTYIY (80)	RIDPANGNTKFDPKFQG (85)	YFYRYDVGGFDY (96)
P13C10 VH	DTYMY (78)	RIDPANGNTKFDPKFQD (86)	YFYRYDVGGFDY (96)
P13C08 VH	DNYIH (81)	SIDPANGNTKYDPKFQG (87)	YFYRYDVGGFDY (96)
P13E02 VH	DTYIH (79)	EIDPANGNSKYAPRFQG (88)	YYYRYDVGGFDY (95)
P13F02 VH	DTYIH (79)	EIDPANGHTKYDPKFQG (89)	YYYRYDVGGFDY (95)
P13B02 VH	DTYIH (79)	EIDPANDNTKYDPKFQG (90)	YYYRYDVGGFDY (95)
P13A06 VH	DTYMY (78)	RIDPANGNTKYDPKFQG (91)	YSYRYDVGGFDY (94)
P14C04 VH	DTYMY (78)	RIDPANGNTHFDPKFQG (92)	YFYRYDVGGFDY (96)
P14A04 VH	DTYMY (78)	RIDPANGNTKFDPKFQD (86)	YFYRYDVGGFDY (96)
P15F06 VH	DTYMY (78)	RIDPANGNTKFDPKFQD (86)	YFYRYDVGGFDY (96)
P13B03 VH	DTYIH (79)	EIDPANGNTKYDPKFQG (84)	YYYRYDVGGFDY (95)
P15C02 VH	DTYMY (78)	RIDPANGNTKFDPKFQD (86)	YFYRYDVGGFDY (96)
P16D04 VH	DTYMY (78)	RIDPANGNTKFDPKFQD (86)	YFYRYDVGGFDY (96)
P13F01 VH	DTYMY (78)	RIDPANGNTKFDPKFQD (86)	YFYRYDVGGFDY (96)
P13A04 VH	DTYMY (78)	RIDPANGNTKFDPKFQD (86)	YFYRYDVGGFDY (96)
P16H05 VH	DNYIH (81)	SIDPANGNTKYDPKFQG (87)	YFYRYDVGGFDY (96)
P13F09 VH	DNYIH (81)	SIDPANGNTKYDPKFQG (87)	YFYRYDVGGFDY (96)
P13G01 VH	DTYMY (78)	RIDPANGNTKFDPKFQD (86)	YFYRYDVGGFDY (96)
P13H05 VH	DTYIH (79)	EIDPANDNTKYDPKFQG (90)	YYYRYDVGGFDY (95)
P13D04 VH	DNYMD (82)	KIDPANGNTKYDPKFQG (93)	YYYRYDVGGFDY (95)
P14G01 VH	DTYMY (78)	RIDPANGNTKFDPKFQD (86)	YFYRYDVGGFDY (96)
P14G03 VH	DTYIH (79)	EIDPANGNTKYDPKFQG (84)	YYYRYDVGGFDY (95)
P13G05 VH	DTYMY (78)	RIDPANGNTKFDPKFQD (86)	YFYRYDVGGFDY (96)
P13F06 VH	DTYMY (78)	RIDPANGNTKFDPKFQD (86)	YFYRYDVGGFDY (96)
P13B11 VH	DNYMD (82)	KIDPANGNTKYDPKFQG (93)	YYYRYDVGGFDY (95)
P14F01 VH	DTYMY (78)	RIDPANGNTKFDPKFQD (86)	YFYRYDVGGFDY (96)
P14F06 VH	DNYIH (81)	SIDPANGNTKYDPKFQG (87)	YFYRYDVGGFDY (96)
P13D05 VH	DTYMY (78)	RIDPANGNTKFDPKFQD (86)	YFYRYDVGGFDY (96)
P13G04 VH	DTYMY (78)	RIDPANGNTKFDPKFQD (86)	YFYRYDVGGFDY (96)
P15E06 VH	DTYMY (78)	RIDPANGNTKFDPKFQD (86)	YFYRYDVGGFDY (96)

VH	CDRH1 (SEQ ID NO:)	CDRH2 (SEQ ID NO:)	CDRH3 (SEQ ID NO:)
P15G05 VH	DTYMY (78)	RIDPANGNTKFDPKFQD (86)	YFYRYDVGGFY (96)
P15B06 VH	DTYMY (78)	RIDPANGNTHFDPKFQG (92)	YFYRYDVGGFY (96)
P14B07 VH	DNYIH (81)	SIDPANGNTKYDPKFQG (87)	YFYRYDVGGFY (96)
P05E03 VH	DTYMY (78)	RIDPANGNTKFDPKFQD (86)	YFYRYDVGGFY (96)
P13C06 VH	DTYMY (78)	RIDPANGNTKFDPKFQD (86)	YFDKYDVGCDY (97)
H0	DTYIH (79)	EIDPANDNTKYDPKFQG (90)	YYYRYDVGGFY (95)
H1	DTYIH (79)	EIDPANDNTKYDPKFQG (90)	YYYRYDVGGFY (95)
H2	DTYIH (79)	EIDPANDNTKYDPKFQG (90)	YYYRYDVGGFY (95)
H3	DTYIH (79)	EIDPANDNTKYDPKFQG (90)	YYYRYDVGGFY (95)
H4	DTYIH (79)	EIDPANDNTKYDPKFQG (90)	YYYRYDVGGFY (95)
H4_R98K	DTYIH (79)	EIDPANDNTKYDPKFQG (90)	YYYKYDVGGFY (98)
H4_D100E	DTYIH (79)	EIDPANDNTKYDPKFQG (90)	YYYRYEVGGFY (99)
H1_R98K	DTYIH (79)	EIDPANDNTKYDPKFQG (90)	YYYKYDVGGFY (98)
H1_R98K_K23T	DTYIH (79)	EIDPANDNTKYDPKFQG (90)	YYYKYDVGGFY (98)
H1_R98K_L4M	DTYIH (79)	EIDPANDNTKYDPKFQG (90)	YYYKYDVGGFY (98)
H1_R98K_L4M_K23T	DTYIH (79)	EIDPANDNTKYDPKFQG (90)	YYYKYDVGGFY (98)
H1_R98K_L4M_V5K	DTYIH (79)	EIDPANDNTKYDPKFQG (90)	YYYKYDVGGFY (98)
H1_R98K_L4M_V5K_K23T	DTYIH (79)	EIDPANDNTKYDPKFQG (90)	YYYKYDVGGFY (98)
H1_R98K_V5K	DTYIH (79)	EIDPANDNTKYDPKFQG (90)	YYYKYDVGGFY (98)
H1_R98K_V5K_K23T	DTYIH (79)	EIDPANDNTKYDPKFQG (90)	YYYKYDVGGFY (98)
H2_R98K	DTYIH (79)	EIDPANDNTKYDPKFQG (90)	YYYKYDVGGFY (98)
H3_R98K	DTYIH (79)	EIDPANDNTKYDPKFQG (90)	YYYKYDVGGFY (98)

*Defined according to the Kabat numbering system.

Table 3. Light chain CDR amino acid sequences of exemplary anti-LAG-3 antibodies. *

VL	CDRL1 (SEQ ID NO:)	CDRL2 (SEQ ID NO:)	CDRL3 (SEQ ID NO:)
P01C09 VL	SVSSSISSSNLH (100)	GTSNLAS (104)	QQWSSYPFT (105)
P05E01 VL	SVSSSISSSNLH (100)	GTSNLAS (104)	QQWNSYPLT (106)
P01A12 VL	SVSSSISSSNLH (100)	GTSNLAS (104)	QQWSSYPFT (105)
P13B01 VL	SVSSSISSSNLH (100)	GTSNLAS (104)	QQWSSYPFT (105)
P13C10 VL	SVSSSISSSNLH (100)	GTSNLAS (104)	QQWSSYPFT (105)
P13C08 VL	SVSSSISSSNLH (100)	GTSNLAS (104)	QQWSSYPFT (105)
P13E02 VL	SVSSSISSSNLH (100)	GTSNLAS (104)	QQWSSYPFT (105)
P13F02 VL	SVSSSISSSNLH (100)	GTSNLAS (104)	QQWSSYPFT (105)
P13B02 VL	SVSSSISSSNLH (100)	GTSNLAS (104)	QQWSSYPFT (105)
P13A06 VL	SVSSSISSSNLH (100)	GTSNLAS (104)	QQWSSYPHT (107)
P14C04 VL	SVSSSISSSNLH (100)	GTSNLAS (104)	QQWSSYPFT (105)

VL	CDRL1 (SEQ ID NO:)	CDRL2 (SEQ ID NO:)	CDRL3 (SEQ ID NO:)
P14A04 VL	SVSSSISSSNLH (100)	GTSNLAS (104)	QQWSSYPFT (105)
P15F06 VL	SVSSSISSSTLH (101)	GTSNLAS (104)	QQWSSYPWT (108)
P13B03 VL	SVSSSISSSNLH (100)	GTSNLAS (104)	QQWSSYPFT (105)
P15C02 VL	SVSSSISSSNLH (100)	GTSNLAS (104)	QQWSSYPFT (105)
P16D04 VL	SVSSSISSSNLH (100)	GTSNLAS (104)	QQWSSYPLT (109)
P13F01 VL	SVSSSISSSNLH (100)	GTSNLAS (104)	QQWSSYPFT (105)
P13A04 VL	SVSSSISSSNLH (100)	GTSNLAS (104)	QQWSSYPFT (105)
P16H05 VL	SVSSSISSSNLH (100)	GTSNLAS (104)	QQWSSYPHT (107)
P13F09 VL	SVSSSISSSNLH (100)	GTSNLAS (104)	QQWSSYPFT (105)
P13G01 VL	SVSSSISSSNLH (100)	GTSNLAS (104)	QQWSSYPFT (105)
P13H05 VL	SVSSSISSSNLH (100)	GTSNLAS (104)	QQWSSYPFT (105)
P13D04 VL	SVSSSISSSNLH (100)	GTSNLAS (104)	QQWSSYPFT (105)
P14G01 VL	SVSSSISSSNLH (100)	GTSNLAS (104)	QQWSSYPFT (105)
P14G03 VL	SVSSSISSSNLY (102)	GTSNLAS (104)	QQWSSYPFT (105)
P13G05 VL	SVSSSISSSNLH (100)	GTSNLAS (104)	QQWSSYPFT (105)
P13F06 VL	SVSSSISSSNLH (100)	GTSNLAS (104)	QQWSSYPFT (105)
P13B11 VL	SVSSSISSSNLH (100)	GTSNLAS (104)	QQWSSYPFT (105)
P14F01 VL	SVSSSISSSNLH (100)	GTSNLAS (104)	QQWSTYPFT (110)
P14F06 VL	SVSSSISSSNLH (100)	GTSNLAS (104)	QQWSSYPFT (105)
P13D05 VL	SVSSSISSSNLH (100)	GTSNLAS (104)	QQWSSYPFT (105)
P13G04 VL	SVSSSISSSNLH (100)	GTSNLAS (104)	QQWSSYPFT (105)
P15E06 VL	SVSSGISSSNLH (103)	GTSNLAS (104)	QQWSRYPWT (111)
P15G05 VL	SVSSSISSSNLH (100)	GTSNLAS (104)	QQWSSYPLT (109)
P15B06 VL	SVSSSISSSNLH (100)	GTSNLAS (104)	QQWSSYPLT (109)
P14B07 VL	SVSSSISSSNLH (100)	GTSNLAS (104)	QQWSSYPFT (105)
P05E03 VL	SVSSSISSSNLH (100)	GTSNLAS (104)	QQWSSYPFT (105)
P13C06 VL	SVSSSISSSNLY (102)	GTSNLAS (104)	QQWRSYPFT (112)
L0	SVSSSISSSNLH (100)	GTSNLAS (104)	QQWSSYPFT (105)
L1	SVSSSISSSNLH (100)	GTSNLAS (104)	QQWSSYPFT (105)
L2	SVSSSISSSNLH (100)	GTSNLAS (104)	QQWSSYPFT (105)
L3	SVSSSISSSNLH (100)	GTSNLAS (104)	QQWSSYPFT (105)
L4	SVSSSISSSNLH (100)	GTSNLAS (104)	QQWSSYPFT (105)

*Defined according to the Kabat numbering system.

Table 4. Heavy chain framework (FR) amino acid sequences of exemplary anti-LAG-3 antibodies. *

VH	VH FR1 (SEQ ID NO:)	VH FR2 (SEQ ID NO:)	VH FR3 (SEQ ID NO:)	VH FR4 (SEQ ID NO:)
H0	QVQLVQSGAEVKKPGASV KVSCKASGFNIK (113)	WVRQAPGQGL EWMG (122)	RVTMTRDTSTSTVYMELSSL RSEDVAVYYCAR (124)	WGQGTLLTVV SS (128)
H1	QVQLVQSGAEVKKPGASV KVSCKASGFNIK (113)	WVRQAPGQGL EWMG (122)	RVTITADTSTSTVYMELSSL RSEDVAVYYCAT (125)	WGQGTLLTVV SS (128)
H2	QVQLVQSGAEVKKPGASV KVSCKASGFNIK (113)	WVRQAPGQGL EWMG (122)	RVTITADTSSNTVYMELSSL RSEDVAVYYCAT (126)	WGQGTLLTVV SS (128)
H3	QVQMKQSGAEVKKPGASV KVSCASGFNIK (114)	WVRQAPGQGL EWMG (122)	RVTITADTSSNTVYMELSSL RSEDVAVYYCAT (126)	WGQGTLLTVV SS (128)
H4	QVQMKQSGAEVVKPGASV KVSCASGFNIK (115)	WVRQAPGQGL EWIG (123)	RVTITADTSSNTVYLQLSSL RSEDVAVYYCAT (127)	WGQGTLLTVV SS (128)
H4_R98K	QVQMKQSGAEVVKPGASV KVSCASGFNIK (115)	WVRQAPGQGL EWIG (123)	RVTITADTSSNTVYLQLSSL RSEDVAVYYCAT (127)	WGQGTLLTVV SS (128)
H4_D100E	QVQMKQSGAEVVKPGASV KVSCASGFNIK (115)	WVRQAPGQGL EWIG (123)	RVTITADTSSNTVYLQLSSL RSEDVAVYYCAT (127)	WGQGTLLTVV SS (128)
H1_R98K	QVQLVQSGAEVKKPGASV KVSCKASGFNIK (113)	WVRQAPGQGL EWMG (122)	RVTITADTSTSTVYMELSSL RSEDVAVYYCAT (125)	WGQGTLLTVV SS (128)
H1_R98K_K23T	QVQLVQSGAEVKKPGASV KVSCASGFNIK (116)	WVRQAPGQGL EWMG (122)	RVTITADTSTSTVYMELSSL RSEDVAVYYCAT (125)	WGQGTLLTVV SS (128)
H1_R98K_L4M	QVQMVQSGAEVKKPGASV KVSCKASGFNIK (117)	WVRQAPGQGL EWMG (122)	RVTITADTSTSTVYMELSSL RSEDVAVYYCAT (125)	WGQGTLLTVV SS (128)
H1_R98K_L4M_K23T	QVQMVQSGAEVKKPGASV KVSCASGFNIK (118)	WVRQAPGQGL EWMG (122)	RVTITADTSTSTVYMELSSL RSEDVAVYYCAT (125)	WGQGTLLTVV SS (128)
H1_R98K_L4M_V5K	QVQMKQSGAEVKKPGASV KVSCKASGFNIK (119)	WVRQAPGQGL EWMG (122)	RVTITADTSTSTVYMELSSL RSEDVAVYYCAT (125)	WGQGTLLTVV SS (128)
H1_R98K_L4M_V5K_K23T	QVQMKQSGAEVKKPGASV KVSCASGFNIK (114)	WVRQAPGQGL EWMG (122)	RVTITADTSTSTVYMELSSL RSEDVAVYYCAT (125)	WGQGTLLTVV SS (128)
H1_R98K_V5K	QVQLKQSGAEVKKPGASV KVSCKASGFNIK (120)	WVRQAPGQGL EWMG (122)	RVTITADTSTSTVYMELSSL RSEDVAVYYCAT (125)	WGQGTLLTVV SS (128)
H1_R98K_V5K_K23T	QVQLKQSGAEVKKPGASV KVSCASGFNIK (121)	WVRQAPGQGL EWMG (122)	RVTITADTSTSTVYMELSSL RSEDVAVYYCAT (125)	WGQGTLLTVV SS (128)
H2_R98K	QVQLVQSGAEVKKPGASV KVSCKASGFNIK (113)	WVRQAPGQGL EWMG (122)	RVTITADTSSNTVYMELSSL RSEDVAVYYCAT (126)	WGQGTLLTVV SS (128)
H3_R98K	QVQMKQSGAEVKKPGASV KVSCASGFNIK (114)	WVRQAPGQGL EWMG (122)	RVTITADTSSNTVYMELSSL RSEDVAVYYCAT (126)	WGQGTLLTVV SS (128)

* The heavy chain framework regions described in Table 4 are determined based upon the

boundaries of the Kabat numbering system for CDRs. In other words, the VH CDRs are

5 determined by Kabat and the framework regions are the amino acid residues surrounding the

CDRs in the variable region in the format FR1, CDR1, FR2, CDR2, FR3, CDR3, and FR4.

Table 5. Light chain framework (FR) amino acid sequences of exemplary anti-LAG-3 antibodies. *

VL	VL FR1 (SEQ ID NO:)	VL FR2 (SEQ ID NO:)	VL FR3 (SEQ ID NO:)	VL FR4 (SEQ ID NO:)
L0	EIVLTQSPGTLSSLSPGER ATLSC (129)	WYQQKPGQAPRLLIY (132)	GIPDRFSGSGSGTDFTLTISRL EPEDFAVYYC (136)	FGQGTKVEIK (139)
L1	EIVLTQSPGTLSSLSPGER ATLSC (129)	WYQQKPGQAPRLLIY (132)	GIPDRFSGSGSGTSYTLTISRL EPEDFAVYYC (137)	FGQGTKVEIK (139)
L2	EILLTQSPGTLSSLSPGER ATLSC (130)	WFQQKPGQAPRLLIY (133)	GIPDRFSGSGSGTSYTLTISRL EPEDFAVYYC (137)	FGQGTKVEIK (139)
L3	EILLTQSPGTLSSLSPGER ATLSC (130)	WFQQKPGQAPRLWIY (134)	GVPDRFSGSGSGTSYTLTISRL EPEDFAVYYC (138)	FGQGTKVEIK (139)
L4	EILLTQSPGTLSSLSPGER ATLTC (131)	WFQQKPGQSPRLWIY (135)	GVPDRFSGSGSGTSYTLTISRL EPEDFAVYYC (138)	FGQGTKVEIK (139)

* The light chain framework regions described in Table 5 are determined based upon the boundaries of the Kabat numbering system for CDRs. In other words, the VL CDRs are determined by Kabat and the framework regions are the amino acid residues surrounding the CDRs in the variable region in the format FR1, CDR1, FR2, CDR2, FR3, CDR3, and FR4.

Table 6. Exemplary murine anti-LAG-3 antibodies.

Antibody	Heavy chain variable region SEQ ID NO:	Light chain variable region SEQ ID NO:
P01C09	1	2
P05E01	3	4
P01A12	5	6
P13B01	7	8
P13C10	9	10
P13C08	11	12
P13E02	13	10
P13F02	14	10
P13B02	15	16
P13A06	17	18
P14C04	19	8
P14A04	20	21
P15F06	22	23

Antibody	Heavy chain variable region SEQ ID NO:	Light chain variable region SEQ ID NO:
P13B03	24	10
P15C02	9	25
P16D04	9	26
P13F01	20	2
P13A04	27	28
P16H05	29	30
P13F09	31	32
P13G01	33	34
P13H05	35	10
P13D04	36	37
P14G01	38	21
P14G03	39	40
P13G05	9	2
P13F06	9	41
P13B11	42	43
P14F01	9	44
P14F06	45	32
P13D05	46	47
P13G04	48	10
P15E06	20	49
P15G05	33	50
P15B06	51	52
P14B07	53	8
P05E03	38	2
P13C06	54	55

Table 7. Exemplary humanized anti-LAG-3 antibodies. *

Antibody	Heavy chain variable region (SEQ ID NO:)	Light chain variable region (SEQ ID NO:)
P13B02-01	H0 (56)	L0 (73)
P13B02-02	H0 (56)	L1 (74)
P13B02-03	H0 (56)	L2 (75)
P13B02-04	H0 (56)	L3 (76)
P13B02-05	H0 (56)	L4 (77)
P13B02-06	H1 (57)	L0 (73)
P13B02-07	H1 (57)	L1 (74)
P13B02-08	H1 (57)	L2 (75)
P13B02-09	H1 (57)	L3 (76)
P13B02-10	H1 (57)	L4 (77)
P13B02-11	H2 (58)	L0 (73)
P13B02-12	H2 (58)	L1 (74)
P13B02-13	H2 (58)	L2 (75)
P13B02-14	H2 (58)	L3 (76)
P13B02-15	H2 (58)	L4 (77)
P13B02-16	H3 (59)	L0 (73)
P13B02-17	H3 (59)	L1 (74)
P13B02-18	H3 (59)	L2 (75)
P13B02-19	H3 (59)	L3 (76)
P13B02-20	H3 (59)	L4 (77)
P13B02-21	H4 (60)	L0 (73)
P13B02-22	H4 (60)	L1 (74)
P13B02-23	H4 (60)	L2 (75)
P13B02-24	H4 (60)	L3 (76)
P13B02-25	H4 (60)	L4 (77)
P13B02-26	H4_R98K (61)	L4 (77)
P13B02-27	H4_D100E (62)	L4 (77)
P13B02-28	H1_R98K (63)	L0 (73)
P13B02-29	H1_R98K_K23T (64)	L0 (73)
P13B02-30	H1_R98K_L4M (65)	L0 (73)

Antibody	Heavy chain variable region (SEQ ID NO:)	Light chain variable region (SEQ ID NO:)
P13B02-31	H1_R98K_L4M_K23T (66)	L0 (73)
P13B02-32	H1_R98K_L4M_V5K (67)	L0 (73)
P13B02-33	H1_R98K_L4M_V5K_K23T (68)	L0 (73)
P13B02-34	H1_R98K_V5K (69)	L0 (73)
P13B02-35	H1_R98K_V5K_K23T (70)	L0 (73)
P13B02-36	H2_R98K (71)	L0 (73)
P13B02-37	H3_R98K (72)	L0 (73)

Table 8. Human germline sequences.

SEQ ID NO:	Description	Amino acid sequence
153	IGHV1-46*01	QVQLVQSGAEVKKPGASVKVSCKASGYTFTSYMHV RQAPGQGLEWMGIINPSGGSTSYAQKFQGRVTMT STSTVYMEISSLRSEDTAVYYCAR
154	IGHV1-69-2*01	EVQLVQSGAEVKKPGATVKISCKVSGYTFTDYMHV QQAPGKGLEWMGLVDPEDGETIYAEKFQGRVTIT STDTAYMEISSLRSEDTAVYYCAT
155	IGHV1-3*01	QVQLVQSGAEVKKPGASVKVSCKASGYTFTSYAMHV RQAPGQRLQWMGWINAGNGNTKYSQKFQGRVTIT SASTAYMEISSLRSEDTAVYYCAR
156	IGHV1-24*01	QVQLVQSGAEVKKPGASVKVSCKASGYTFTLSMHV RQAPGKGLEWMGGFDPEDGETIYAEKFQGRVTMT STDTAYMEISSLRSEDTAVYYCAT
157	IGHV1-2*01	QVQLVQSGAEVKKPGASVKVSCKASGYTFTGYMHV RQAPGQGLEWMGRINPNSGGTNYAQKFQGRVTIT SISTAYMEISRLRSDDTVYYCAR
158	IGHV1-45*01	QMQLVQSGAEVKKGTSSVKVSCKASGYTFTYRLHV RQAPGQALEWMGWITPFGNTNYAQKFQDRVTIT SMSTAYMEISSLRSEDTAMYYCAR
159	IGHV1-18*01	QVQLVQSGAEVKKPGASVKVSCKASGYTFTSYGISV RQAPGQGLEWMGWI SAYNGNTNYAQKLQGRVTMT STSTAYMEISRLRSDDTAVYYCAR
200	IGHJ1*01	AEYFQHWGQGTLVTVSS
160	IGKV3-20*01	EIVLTQSPGTLSPGERATLSCRASQSVSSSYLAWY QQKPGQAPRLLIYGASSRATGIPDRFSGSGSGTDF TISRLEPEDFAVYYCQQYGSSP
161	IGKV3D-15*01 or IGKV3-15*01	EIVMTQSPATLSVSPGERATLSCRASQSVSSNLAWYQ QKPGQAPRLLIYGASTRATGIPARFSGSGSGTEFTLT ISSLSQSEDFAVYYCQQYNNWP
162	IGKV3D-20*01	EIVLTQSPATLSLSPGERATLSCGASQSVSSSYLAWY QQKPGAPRLLIYDASSRATGIPDRFSGSGSGTDFTL TISRLEPEDFAVYYCQQYGSSP

SEQ ID NO:	Description	Amino acid sequence
163	IGKV3D-7*01	EIVMTQSPATLSLSPGERATLSCRASQSVSSSYLSWY QQKPGQAPRLLIYGASTRATGIPARFSGSGSGTDFTL TISSLQPEDFAVYYCQQDYNLPP
164	IGKV1-9*01	DIQLTQSPSFLSASVGDRTITCRASQGISSYLAWYQ QKPGKAPKLLIYAASLTQSGVPSRFSGSGSGTEFTLT ISSLQPEDFAVYYCQQLNLYP
165	IGKV3-11*01	EIVLTQSPATLSLSPGERATLSCRASQSVSSYLAWYQ QKPGQAPRLLIYDASNRATGIPARFSGSGSGTDFTLT ISSLQPEDFAVYYCQQRSNWP
201	IGKJ1*01	WTFGQGTKVEIK

Table 9. Exemplary sequences of LAG-3.

SEQ ID NO:	Description	Amino acid Sequence
166	Human LAG-3 immature protein (P18627-1)	MWEAQFLGLLFLQPLWVAPVKPLQPGAIEVPVW AQEGAPALPCSPITPLQDLSSLRRAGVTWQHQP DSGPPAAAPGHPLAPGHPAAPSWSGPRPRRYTV LSVGPGGLRSGRLPLQPRVQLDERGRQRGDFSLW LRPARRADAGEYRAAVHLRDRALSCRLRLRLGQ ASMTASPPGSLRASDWVILNCSFSRPDRPASVHW FRNRGQGRVPVRESPPHHLAESFLPLQVSPMDS GPWGCILTYRDGFNVSIMYNLTVLGLPPTPLTVY AGAGSRVGLPCRLPAGVGTRSFLLAKWTPPGGGP DLLVTGDNGDFTLRLEDVSQAQAGTYTCHHLQE QQLNATVTLAIITVTPKSFGSPGSLGKLLCEVTPVS GQERFVWSSLDTPSQRSFSGPWLEAQEAQLLSQP WQCQLYQGERLLGAAVYFTELSSPGAQRSGRAP GALPAGHLLLFLILGVLSLLLVTGAFGFHLWRRQ WRPRRFSALEQGIHPPQAQSKIEELEQEPEPEPEPE PEPEPEPEPEQL
167	Human LAG-3 mature protein	VPVWVAQEGAPALPCSPITPLQDLSSLRRAGVT WQHQPDSGPPAAAPGHPLAPGHPAAPSWSGPRP RRYTVLSVGPGGLRSGRLPLQPRVQLDERGRQRG DFSLWLRPARRADAGEYRAAVHLRDRALSCRLR LRLGQASMTASPPGSLRASDWVILNCSFSRPDRPA SVHWFNRNRGQGRVPVRESPPHHLAESFLPLQVS PMDSGPWGCILTYRDGFNVSIMYNLTVLGLPPTP LTVYAGAGSRVGLPCRLPAGVGTRSFLLAKWTPP GGGPDLLVTGDNGDFTLRLEDVSQAQAGTYTCHI HLQEQQNLNATVTLAIITVTPKSFGSPGSLGKLLCE VTPVSGQERFVWSSLDTPSQRSFSGPWLEAQEAQ LLSQPWQCQLYQGERLLGAAVYFTELSSPGAQRS GRAPGALPAGHLLLFLILGVLSLLLVTGAFGFHL WRRQWRPRRFSALEQGIHPPQAQSKIEELEQEPEP EPEPEPEPEPEPEQL

SEQ ID NO:	Description	Amino acid Sequence
210	Human LAG-3 mature protein	LQPGAIEVPVWVAQEGAPALPCSTIPLQDLSLLR RAGVTWQHQPDSGPPAAAPGHPLAPGPHPAAPSS WGPRPRRYTVLSVGPGLRSGRLPLQPRVQLDER GRQRGDFSLWLRPARRADAGEYRAAVHLRDRAL SCRLRLRLGQASMTASPPGSLRASDWVILNCSFSR PDRPASVHWFRNRGQGRVPVRESPHHHLAESFLF LPQVSPMDSGPWGCILTYRDGFNVSIMYNLTVLG LEPPTPLTVYAGAGSRVGLPCRLPAGVGTRSFLLTA KWTPPGGGPDLLVTGDNGDFTLRLEDVSQAQAG TYTCHIHLEQQQLNATVTLAIITVTPKSFGSPGSLG KLLCEVTPVSGQERFVWSSLDTPSQRSFSGPWLE AQEAQLLSQPWQCQLYQGERLLGAAVYFTELSSP GAQRSGRAPGALPAGHLLLFLILGVLSLLLLVTGA FGFHLWRRQWRPRRFSALEQGIHPPQAQSKIEELE QEPEPEPEPEPEPEPEPEPEQL
217	Human LAG-3 fragment	LQPGAIEVPVWVAQEGAPALPCSTIPLQDLSLLR RAGVTWQHQPDSGPPAAAPGHPLAPGPHPAAPSS WGPRPRRYTVLSVGPGLRSGRLPLQPRVQLDER GRQRGDFSLWLRPARRADAGEYRAAVHLRDRAL SCRLRLRLGQASMTASPPGSLRASDWVILNCSFSR PDRPASVHWFRNRGQGRVPVRESPHHHLAESFLF LPQVSPMDSGPWGCILTYRDGFNVSIMYNLTVLG LEPPTPLTVYAGAGSRVGLPCRLPAGVGTRSFLLTA KWTPPGGGPDLLVTGDNGDFTLRLEDVSQAQAG TYTCHIHLEQQQLNATVTLAIITVTPKSFGSPGSLG KLLCEVTPVSGQERFVWSSLDTPSQRSFSGPWLE AQEAQLLSQPWQCQLYQGERLLGAAVYFTELSSP GAQRSGRAPGALPAGHL
199	Human LAG-3 30 amino acid loop	GPPAAAPGHPLAPGPHPAAPSSWGPRPRRY
211	LAG-3 epitope	PTIPLQD
212	LAG-3 epitope	SPTIPLQD
213	LAG-3 epitope	SPTIPLQDL
214	LAG-3 epitope	SPTIPLQDLS
215	LAG-3 epitope	SPTIPLQDLSL
216	LAG-3 epitope	SPTIPLQDLSLL

[00142] In certain embodiments, the instant disclosure provides an isolated antibody that specifically binds to LAG-3 (*e.g.*, human LAG-3), the antibody comprising a VH domain comprising one, two, or all three of the CDRs of a VH domain set forth in Tables 1, 2, 6, and 7 herein. In certain embodiments, the antibody comprises the CDRH1 of one of the VH domains set forth in Tables 1, 2, 6, and 7. In certain embodiments, the antibody comprises the CDRH2 of one of the VH domains set forth in Tables 1, 2, 6, and 7. In certain embodiments, the antibody comprises the CDRH3 of one of the VH domains set forth in Tables 1, 2, 6, and 7.

[00143] In certain embodiments, the instant disclosure provides an isolated antibody that specifically binds to LAG-3 (*e.g.*, human LAG-3), the antibody comprising a VL domain comprising one, two, or all three of the CDRs of a VL domain disclosed in Tables 1, 3, 6, and 7 herein. In certain embodiments, the antibody comprises the CDRL1 of one of the VL domains set forth in Tables 1, 3, 6, and 7. In certain embodiments, the antibody comprises the CDRL2 of one of the VL domains set forth in Tables 1, 3, 6, and 7. In certain embodiments, the antibody comprises the CDRL3 of one of the VL domains set forth in Tables 1, 3, 6, and 7.

[00144] In certain embodiments, the CDRs of an antibody can be determined according to Kabat *et al.*, J. Biol. Chem. 252, 6609-6616 (1977) and Kabat *et al.*, Sequences of protein of immunological interest (1991), each of which is herein incorporated by reference in its entirety.

[00145] In certain embodiments, the instant disclosure provides an isolated antibody that specifically binds to LAG-3 (*e.g.*, human LAG-3), the antibody comprising the Kabat VH CDRs of a VH disclosed in Tables 1, 6, and 7 herein. In certain embodiments, the instant disclosure provides an isolated antibody that specifically binds to LAG-3 (*e.g.*, human LAG-3), the antibody comprising the Kabat VL CDRs of a VL disclosed in Tables 1, 6, and 7 herein. In certain embodiments, the instant disclosure provides an isolated antibody that specifically binds to LAG-3 (*e.g.*, human LAG-3), the antibody comprising the Kabat VH CDRs and Kabat VL CDRs of an antibody disclosed in Tables 1, 6, and 7 herein.

[00146] In certain embodiments, the CDRs of an antibody can be determined according to the Chothia numbering scheme, which refers to the location of immunoglobulin structural loops (*see, e.g.*, Chothia C & Lesk AM, (1987), J Mol Biol 196: 901-917; Al-Lazikani B *et al.*, (1997) J Mol Biol 273: 927-948; Chothia C *et al.*, (1992) J Mol Biol 227: 799-817; Tramontano A *et al.*, (1990) J Mol Biol 215(1): 175-82; and U.S. Patent No. 7,709,226, all of which are herein incorporated by reference in their entireties). Typically, when using the Kabat numbering convention, the Chothia CDRH1 loop is present at heavy chain amino acids 26 to 32, 33, or 34, the Chothia CDRH2 loop is present at heavy chain amino acids 52 to 56, and the Chothia CDRH3 loop is present at heavy chain amino acids 95 to 102, while the Chothia CDRL1 loop is present at light chain amino acids 24 to 34, the Chothia CDRL2 loop is present at light chain amino acids 50 to 56, and the Chothia CDRL3 loop is present at light chain amino acids 89 to 97. The end of the Chothia CDRH1 loop when numbered using the Kabat numbering convention varies between H32 and H34 depending on the length of the loop (this is because the Kabat numbering scheme places the insertions at H35A and H35B; if

neither 35A nor 35B is present, the loop ends at 32; if only 35A is present, the loop ends at 33; if both 35A and 35B are present, the loop ends at 34).

[00147] In certain embodiments, the instant disclosure provides an isolated antibody that specifically binds to LAG-3 (*e.g.*, human LAG-3), the antibody comprising the Chothia VH CDRs of a VH disclosed in Tables 1, 6, and 7 herein. In certain embodiments, the instant disclosure provides an isolated antibody that specifically binds to LAG-3 (*e.g.*, human LAG-3), the antibody comprising the Chothia VL CDRs of a VL disclosed in Tables 1, 6, and 7 herein. In certain embodiments, the instant disclosure provides an isolated antibody that specifically binds to LAG-3 (*e.g.*, human LAG-3), the antibody comprising the Chothia VH CDRs and Chothia VL CDRs of an antibody disclosed in Tables 1, 6, and 7 herein. In certain embodiments, antibodies that specifically bind to LAG-3 (*e.g.*, human LAG-3) comprise one or more CDRs, in which the Chothia and Kabat CDRs have the same amino acid sequence. In certain embodiments, the instant disclosure provides an isolated antibody that specifically binds to LAG-3 (*e.g.*, human LAG-3) and comprises combinations of Kabat CDRs and Chothia CDRs.

[00148] In certain embodiments, the CDRs of an antibody can be determined according to the IMGT numbering system as described in Lefranc M-P, (1999) *The Immunologist* 7: 132-136 and Lefranc M-P *et al.*, (1999) *Nucleic Acids Res* 27: 209-212, each of which is herein incorporated by reference in its entirety. According to the IMGT numbering scheme, CDRH1 is at positions 26 to 35, CDRH2 is at positions 51 to 57, CDRH3 is at positions 93 to 102, CDRL1 is at positions 27 to 32, CDRL2 is at positions 50 to 52, and CDRL3 is at positions 89 to 97.

[00149] In certain embodiments, the instant disclosure provides antibodies that specifically bind to LAG-3 (*e.g.*, human LAG-3) and comprise CDRs of an antibody disclosed in Tables 1, 6, and 7 herein, as determined by the IMGT numbering system, for example, as described in Lefranc M-P (1999) *supra* and Lefranc M-P *et al.*, (1999) *supra*.

[00150] In certain embodiments, the CDRs of an antibody can be determined according to the AbM numbering scheme, which refers to AbM hypervariable regions, which represent a compromise between the Kabat CDRs and Chothia structural loops, and are used by Oxford Molecular's AbM antibody modeling software (Oxford Molecular Group, Inc.). In a particular embodiment, the instant disclosure provides antibodies that specifically bind to LAG-3 (*e.g.*, human LAG-3) and comprise CDRs of an antibody disclosed in Tables 1, 6, and 7 herein as determined by the AbM numbering scheme.

[00151] In certain embodiments, the CDRs of an antibody can be determined according to

MacCallum RM *et al.*, (1996) J Mol Biol 262: 732-745, which is herein incorporated by reference in its entirety. *See also, e.g.*, Martin A. "Protein Sequence and Structure Analysis of Antibody Variable Domains," in *Antibody Engineering*, Kontermann and Dübel, eds., Chapter 31, pp. 422-439, Springer-Verlag, Berlin (2001), which is herein incorporated by reference in its entirety. In a particular embodiment, the instant disclosure provides antibodies that specifically bind to LAG-3 (*e.g.*, human LAG-3) and comprise CDRs of an antibody disclosed in Tables 1, 6, and 7 herein as described in MacCallum RM *et al.*, (1996) *supra*.

[00152] In certain embodiments, the instant disclosure provides an isolated antibody that specifically binds to LAG-3 (*e.g.*, human LAG-3), wherein the antibody comprises a heavy chain variable region comprising the CDRH1, CDRH2, and CDRH3 region amino acid sequences of a VH domain set forth in SEQ ID NO: 1, 3, 5, 7, 9, 11, 13, 14, 15, 17, 19, 20, 22, 24, 27, 29, 31, 33, 35, 36, 38, 39, 42, 45, 46, 48, 51, 53, 54, 56, 57, 58, 59, 60, 61, 62, 63, 64, 65, 66, 67, 68, 69, 70, 71, or 72, and a light chain variable region comprising the CDRL1, CDRL2, and CDRL3 region amino acid sequences of a VL domain set forth in SEQ ID NO: 2, 4, 6, 8, 10, 12, 16, 18, 21, 23, 25, 26, 28, 30, 32, 34, 37, 40, 41, 43, 44, 47, 49, 50, 52, 55, 73, 74, 75, 76, or 77, wherein each CDR is defined in accordance with the Kabat definition, the Chothia definition, the combination of the Kabat definition and the Chothia definition, the IMGT numbering system, the AbM definition, or the MacCallum definition of CDR.

[00153] In certain embodiments, the instant disclosure provides an isolated antibody that specifically binds to LAG-3 (*e.g.*, human LAG-3), the antibody comprising:

(a) a CDRH1 comprises the amino acid sequence of DX₁YX₂X₃ (SEQ ID NO: 140), wherein

X₁ is T or N,

X₂ is I or M, and

X₃ is H, Y or D; and/or

(b) a CDRH2 comprises the amino acid sequence of X₁IDPANX₂X₃X₄X₅X₆X₇PX₈X₉QX₁₀ (SEQ ID NO: 142), wherein

X₁ is E, R, S, or K,

X₂ is D or G,

X₃ is N or H,

X₄ is T or S,

X₅ is K or H,

X₆ is Y or F,

X₇ is D or A,

X₈ is K or R,

X₉ is F or L, and

X₁₀ is G or D; and/or

- 5 (c) a CDRH3 comprises the amino acid sequence of YX₁X₂X₃YX₄VGGX₅DY (SEQ ID NO: 144), wherein

X₁ is Y, F, or S,

X₂ is Y or D,

X₃ is K or R,

10 X₄ is D or E, and

X₅ is F or C; and/or

- (d) a CDRL1 comprises the amino acid sequence of SVSSX₁ISSSX₂LX₃ (SEQ ID NO: 147), wherein

X₁ is S or G,

15 X₂ is N or T, and

X₃ is H or Y; and/or

- (e) a CDRL2 comprises the amino acid sequence of GTSNLAS (SEQ ID NO: 104); and/or

- (f) a CDRL3 comprises the amino acid sequence of QQWX₁X₂YPX₃T (SEQ ID NO: 20 149), wherein

X₁ is S, N, or R,

X₂ is S, T or R, and

X₃ is F, L, H, or W.

[00154] In certain embodiments, CDRH1 comprises the amino acid sequence of DX₁YX₂X₃ (SEQ ID NO: 141), wherein: X₁ is T or N; X₂ is I or M; and X₃ is H or Y. In certain embodiments, CDRH2 comprises the amino acid sequence of X₁IDPANX₂X₃X₄KX₅X₆PX₇FQX₈ (SEQ ID NO: 143), wherein: X₁ is E, R, or S; X₂ is D or G; X₃ is N or H; X₄ is T or S; X₅ is Y or F; X₆ is D or A; X₇ is K or R; and X₈ is G or D. In certain embodiments, CDRH3 comprises the amino acid sequence of YX₁X₂X₃YDVGGX₄DY (SEQ ID NO: 145), wherein: X₁ is Y, F, or S; X₂ is Y or D; X₃ is K or R; and X₄ is F or C. In certain embodiments, CDRH3 comprises the amino acid sequence of YYYYX₁YX₂VGGFDY (SEQ ID NO: 146), wherein: X₁ is K or R; and X₂ is D or E. In certain embodiments, CDRL1 comprises the amino acid sequence of SVSSSISSSNLX₁ (SEQ ID NO: 148), wherein: X₁ is H or Y. In certain embodiments, CDRL3 comprises the amino

acid sequence of QQWX₁SYPX₂T (SEQ ID NO: 150), wherein: X₁ is S, N, or R; and X₂ is F, L, or H.

[00155] In certain embodiments, the instant disclosure provides an isolated antibody that specifically binds to LAG-3 (*e.g.*, human LAG-3), the antibody comprising:

- 5 (a) a CDRH1 comprises the amino acid sequence of DTYIH (SEQ ID NO: 79); and/or
- (b) a CDRH2 comprises the amino acid sequence of EIDPANDNTKYDPKFQG (SEQ ID NO: 90); and/or
- (c) a CDRH3 comprises the amino acid sequence of YYYX₁YX₂VGGFDY (SEQ ID NO: 146), wherein: X₁ is K or R; and X₂ is D or E; and/or
- 10 (d) a CDRL1 comprises the amino acid sequence of SVSSSISSSNLH (SEQ ID NO: 100); and/or
- (e) a CDRL2 comprises the amino acid sequence of GTSNLAS (SEQ ID NO: 104); and/or
- (f) a CDRL3 comprises the amino acid sequence of QQWSSYPFT (SEQ ID NO: 105).

- 15 [00156] In certain embodiments, CDRH1 comprises an amino acid sequence selected from the group consisting of SEQ ID NOs: 78-82. In certain embodiments, CDRH2 comprises an amino acid sequence selected from the group consisting of SEQ ID NOs: 83-93. In certain embodiments, CDRH3 comprises an amino acid sequence selected from the group consisting of SEQ ID NOs: 94-99. In certain embodiments, CDRL1 comprises an amino acid sequence
- 20 selected from the group consisting of SEQ ID NOs: 100-103. In certain embodiments, CDRL3 comprises an amino acid sequence selected from the group consisting of SEQ ID NOs: 105-112.

- [00157] In certain embodiments, the instant disclosure provides an isolated antibody that specifically binds to LAG-3 (*e.g.*, human LAG-3), wherein the antibody comprises a VH
- 25 domain comprising the CDRH1, CDRH2 and CDRH3 amino acid sequences set forth in SEQ ID NOs: 78, 83, and 94; 78, 85, and 95; 78, 86, and 96; 78, 86, and 97; 78, 91, and 94; 78, 92, and 96; 79, 84, and 95; 79, 88, and 95; 79, 89, and 95; 79, 90, and 95; 79, 90, and 98; 79, 90, and 99; 80, 85, and 96; 81, 87, and 96; or, 82, 93, and 95, respectively. In certain embodiments, the instant disclosure provides an isolated antibody that specifically binds to
- 30 LAG-3 (*e.g.*, human LAG-3), wherein the antibody comprises a VH domain comprising the CDRH1, CDRH2 and CDRH3 amino acid sequences set forth in SEQ ID NOs: 79, 90, and 95, respectively. In certain embodiments, the instant disclosure provides an isolated antibody that specifically binds to LAG-3 (*e.g.*, human LAG-3), wherein the antibody comprises a VH domain comprising the CDRH1, CDRH2 and CDRH3 amino acid sequences set forth in SEQ

ID NOs: 79, 90, and 98, respectively.

[00158] In certain embodiments, the instant disclosure provides an isolated antibody that specifically binds to LAG-3 (*e.g.*, human LAG-3), wherein the antibody comprises a VL domain comprising the CDRL1, CDRL2 and CDRL3 amino acid sequences set forth in SEQ ID NOs: 100, 104, and 105; 100, 104, and 106; 100, 104, and 107; 100, 104, and 109; 100, 104, and 110; 101, 104, and 108; 102, 104, and 105; 102, 104, and 112; or, 103, 104, and 111, respectively. In certain embodiments, the instant disclosure provides an isolated antibody that specifically binds to LAG-3 (*e.g.*, human LAG-3), wherein the antibody comprises a VL domain comprising the CDRL1, CDRL2 and CDRL3 amino acid sequences set forth in SEQ ID NOs: 100, 104, and 105, respectively.

[00159] In certain embodiments, the instant disclosure provides an isolated antibody that specifically binds to LAG-3 (*e.g.*, human LAG-3), wherein the antibody comprises a heavy chain variable region comprising CDRH1, CDRH2, and CDRH3 regions, and a light chain variable region comprising CDRL1, CDRL2, and CDRL3 regions, wherein the CDRH1, CDRH2, CDRH3, CDRL1, CDRL2, and CDRL3 regions comprise the amino acid sequences set forth in SEQ ID NOs: 78, 83, 94, 100, 104, and 105; 78, 85, 95, 100, 104, and 105; 78, 86, 96, 100, 104, and 105; 78, 86, 96, 100, 104, and 109; 78, 86, 96, 100, 104, and 110; 78, 86, 96, 101, 104, and 108; 78, 86, 96, 103, 104, and 111; 78, 86, 97, 102, 104, and 112; 78, 91, 94, 100, 104, and 107; 78, 92, 96, 100, 104, and 105; 78, 92, 96, 100, 104, and 109; 79, 84, 95, 100, 104, and 105; 79, 84, 95, 100, 104, and 106; 79, 84, 95, 102, 104, and 105; 79, 88, 95, 100, 104, and 105; 79, 89, 95, 100, 104, and 105; 79, 90, 95, 100, 104, and 105; 79, 90, 98, 100, 104, and 105; 79, 90, 99, 100, 104, and 105; 80, 85, 96, 100, 104, and 105; 81, 87, 96, 100, 104, and 105; 81, 87, 96, 100, 104, and 107; or, 82, 93, 95, 100, 104, and 105, respectively. In certain embodiments, the instant disclosure provides an isolated antibody that specifically binds to LAG-3 (*e.g.*, human LAG-3), wherein the antibody comprises a heavy chain variable region comprising CDRH1, CDRH2, and CDRH3 regions, and a light chain variable region comprising CDRL1, CDRL2, and CDRL3 regions, wherein the CDRH1, CDRH2, CDRH3, CDRL1, CDRL2, and CDRL3 regions comprise the amino acid sequences set forth in SEQ ID NOs: 79, 90, 95, 100, 104, and 105, respectively. In certain embodiments, the instant disclosure provides an isolated antibody that specifically binds to LAG-3 (*e.g.*, human LAG-3), wherein the antibody comprises a heavy chain variable region comprising CDRH1, CDRH2, and CDRH3 regions, and a light chain variable region comprising CDRL1, CDRL2, and CDRL3 regions, wherein the CDRH1, CDRH2, CDRH3, CDRL1, CDRL2, and CDRL3 regions comprise the amino acid sequences set forth in SEQ

ID NOs: 79, 90, 98, 100, 104, and 105, respectively.

[00160] In certain embodiments, the instant disclosure provides an isolated antibody that specifically binds to LAG-3 (*e.g.*, human LAG-3), wherein the antibody comprises a heavy chain variable region (VH) comprising one, two or all three of the VH CDRs of an antibody in Tables 1, 2, 6, and 7. In some embodiments, the antibody comprises one, two, three or all four of the VH framework regions described herein. In specific embodiments, the antibody comprises one, two, three or all four of the VH framework regions (FRs) set forth in Table 4 (*e.g.*, one, two, three, or four of the framework regions in one row in Table 4). In certain embodiments, the antibody comprises one, two, three or all four of the framework regions of the heavy chain variable region sequence of SEQ ID NO: 151 or 222. In certain embodiments, the antibody comprises one, two, three or all four of the framework regions of the heavy chain variable region sequence of SEQ ID NO: 218 or 223. In certain embodiments, the antibody comprises one, two, three or four of the framework regions of a heavy chain variable region sequence which is at least 75%, 80%, 85%, 90%, 95%, or 100% (*e.g.*, at least 86, 87, 88, 89, 90, 91, 92, 93, 94, 95, 96, 97, 98 or 99%) identical to one, two, three or four of the framework regions of a heavy chain variable region sequence selected from the group consisting of SEQ ID NOs: 56-72 and 220. In certain embodiments, the antibody comprises a heavy chain variable framework region that is or is derived from an amino acid sequence encoded by a human gene, wherein the amino acid sequence is selected from the group consisting of IGHV1-46 (*e.g.*, IGHV1-46*01, *e.g.*, having the amino acid sequence of SEQ ID NO: 153), IGHV1-69-2 (*e.g.*, IGHV1-69-2*01, *e.g.*, having the amino acid sequence of SEQ ID NO: 154), IGHV1-3 (*e.g.*, IGHV1-3*01, *e.g.*, having the amino acid sequence of SEQ ID NO: 155), IGHV1-24 (*e.g.*, IGHV1-24*01, *e.g.*, having the amino acid sequence of SEQ ID NO: 156), IGHV1-2 (*e.g.*, IGHV1-2*01, *e.g.*, having the amino acid sequence of SEQ ID NO: 157), IGHV1-45 (*e.g.*, IGHV1-45*01, *e.g.*, having the amino acid sequence of SEQ ID NO: 158), and IGHV1-18 (*e.g.*, IGHV1-18*01, *e.g.*, having the amino acid sequence of SEQ ID NO: 159). In specific embodiments, the heavy chain variable framework region that is derived from said amino acid sequence consists of said amino acid sequence but for the presence of up to 20 amino acid substitutions, deletions, and/or insertions, preferably up to 20 amino acid substitutions. In a particular embodiment, the heavy chain variable framework region that is derived from said amino acid sequence consists of said amino acid sequence with 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, or 20 amino acid residues being substituted for an amino acid found in an analogous position in a corresponding non-human heavy chain variable framework region. In

some embodiments, the antibody comprises a heavy chain variable framework region that is derived from the amino acid sequence of SEQ ID NO: 153), wherein at least one amino acid in the amino acid sequence of SEQ ID NO: 153 is substituted with an amino acid in an analogous position in a corresponding non-human heavy chain variable framework region. In a specific embodiment, the amino acid substitution is at an amino acid position selected from the group consisting of 4, 5, 12, 23, 27, 28, 29, 30, 48, 69, 71, 75, 76, 80, 81, and 94, wherein the amino acid position is indicated according to the Kabat numbering system. In particular embodiments, the amino acid substitution is selected from the group consisting of 4M, 5K, 12V, 23T, 27F, 28N, 29I, 30K, 48I, 69I, 71A, 75S, 76N, 80L, 81Q, and 94T, wherein the position of the amino acid substitution is indicated according to the Kabat numbering system. In another specific embodiment, the amino acid substitution is at an amino acid position selected from the group consisting of 4, 27, 28, 29, 30, 69, 71, and 94, wherein the amino acid position is indicated according to the Kabat numbering system. In particular embodiments, the amino acid substitution is selected from the group consisting of 4M, 27F, 28N, 29I, 30K, 69I, 71A, and 94T, wherein the position of the amino acid substitution is indicated according to the Kabat numbering system.

[00161] In certain embodiments, the instant disclosure provides an isolated antibody that specifically binds to LAG-3 (*e.g.*, human LAG-3), wherein the antibody comprises a light chain variable region (VH) comprising one, two or all three of the VL CDRs of an antibody in Tables 1, 3, 6, and 7. In some embodiments, the antibody comprises one, two, three or all four of the VL framework regions described herein. In specific embodiments, the antibody comprises one, two, three or all four of the VL framework regions (FRs) set forth in Table 5 (*e.g.*, one, two, three, or four of the framework regions in one row in Table 5). In certain embodiments, the antibody comprises one, two, three or all four of the framework regions of the light chain variable region sequence of SEQ ID NO: 152 or 224. In certain embodiments, the antibody comprises one, two, three or four of the framework regions of a light chain variable region sequence which is at least 75%, 80%, 85%, 90%, 95%, or 100% (*e.g.*, at least 86, 87, 88, 89, 90, 91, 92, 93, 94, 95, 96, 97, 98 or 99%) identical to one, two, three or four of the framework regions of a light chain variable region sequence selected from the group consisting of SEQ ID NOs: 73-77. In certain embodiments, the antibody comprises a light chain variable framework region that is or is derived from an amino acid sequence encoded by a human gene, wherein the amino acid sequence is selected from the group consisting of IGKV3-20 (*e.g.*, IGKV3-20*01, *e.g.*, having the amino acid sequence of SEQ ID NO: 160), IGKV3D-15 (*e.g.*, IGKV3D-15*01, *e.g.*, having the amino acid sequence of SEQ ID NO:

161), IGKV3-15 (*e.g.*, IGKV3-15*01, *e.g.*, having the amino acid sequence of SEQ ID NO: 161), IGKV3D-20 (*e.g.*, IGKV3D-20*01, *e.g.*, having the amino acid sequence of SEQ ID NO: 162), IGKV3D-7 (*e.g.*, IGKV3D-7*01, *e.g.*, having the amino acid sequence of SEQ ID NO: 163), IGKV1-9 (*e.g.*, IGKV1-9*01, *e.g.*, having the amino acid sequence of SEQ ID NO: 164), and IGKV3-11 (*e.g.*, IGKV3-11*01, *e.g.*, having the amino acid sequence of SEQ ID NO: 165). In specific embodiments, the light chain variable framework region that is derived from said amino acid sequence consists of said amino acid sequence but for the presence of up to 20 amino acid substitutions, deletions, and/or insertions, preferably up to 20 amino acid substitutions. In a particular embodiment, the light chain variable framework region that is derived from said amino acid sequence consists of said amino acid sequence with 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, or 20 amino acid residues being substituted for an amino acid found in an analogous position in a corresponding non-human light chain variable framework region. In some embodiments, the antibody comprises a light chain variable framework region that is derived from the amino acid sequence of SEQ ID NO: 160, wherein at least one amino acid in the amino acid sequence of SEQ ID NO: 160 is substituted with an amino acid in an analogous position in a corresponding non-human light chain variable framework region. In a specific embodiment, the amino acid substitution is at an amino acid position selected from the group consisting of 3, 22, 36, 43, 47, 58, 70, and 71, wherein the amino acid position is indicated according to the Kabat numbering system. In particular embodiments, the amino acid substitution is selected from the group consisting of 3L, 22T, 36F, 43S, 47W, 58V, 70S, and 71Y, wherein the position of the amino acid substitution is indicated according to the Kabat numbering system.

[00162] In certain embodiments, the instant disclosure provides an isolated antibody that specifically binds to LAG-3 (*e.g.*, human LAG-3), wherein the antibody comprises a heavy chain variable region (VH) comprising one, two or all three of the VH CDRs of an antibody in Tables 1, 2, 6, and 7 (*e.g.*, the VH CDRs in one row of Table 2) and a light chain variable region (VL) comprising one, two or all three of the VL CDRs of an antibody in Tables 1, 3, 6, and 7 (*e.g.*, the VL CDRs in one row of Table 3). In some embodiments, the antibody comprises the VH framework regions and the VL framework regions described herein. In specific embodiments, the antibody comprises the VH framework regions (FRs) set forth in Table 4 (*e.g.*, one, two, three, or four of the framework regions in one row in Table 4) and the VL framework regions (FRs) set forth in Table 5 (*e.g.*, one, two, three, or four of the framework regions in one row in Table 5).

[00163] In certain embodiments, the instant disclosure provides an isolated antibody that

specifically binds to LAG-3 (*e.g.*, human LAG-3), wherein the antibody comprises a VH domain comprising the CDRH1, CDRH2 and CDRH3 amino acid sequences set forth in SEQ ID NOs: 79, 90, and 95; or 79, 90, and 98, respectively. In certain embodiments, the antibody comprises one, two, three or all four VH framework regions derived from the VH of a human or primate antibody. In some embodiments, the antibody comprises VH framework regions of an antibody set forth in Table 4.

[00164] In certain embodiments, the instant disclosure provides an isolated antibody that specifically binds to LAG-3 (*e.g.*, human LAG-3), wherein the antibody comprises a VL domain comprising the CDRL1, CDRL2 and CDRL3 amino acid sequences set forth in SEQ ID NOs: 100, 104, and 105, respectively. In certain embodiments, the antibody comprises one, two, three or all four VL framework regions derived from the VL of a human or primate antibody. In some embodiments, the antibody comprises VL framework regions of an antibody set forth in Table 5.

[00165] In certain embodiments, the instant disclosure provides an isolated antibody that specifically binds to LAG-3 (*e.g.*, human LAG-3), wherein the antibody comprises a heavy chain variable region comprising CDRH1, CDRH2, and CDRH3 regions, and a light chain variable region comprising CDRL1, CDRL2, and CDRL3 regions, wherein the CDRH1, CDRH2, CDRH3, CDRL1, CDRL2, and CDRL3 regions comprise the amino acid sequences set forth in SEQ ID NOs: 79, 90, 95, 100, 104, and 105; or 79, 90, 98, 100, 104, and 105, respectively. In certain embodiments, the antibody comprises one, two, three or all four VH framework regions derived from the VH of a human or primate antibody and one, two, three or all four VL framework regions derived from the VL of a human or primate antibody. In some embodiments, the antibody comprises VH framework regions and VL framework regions of an antibody set forth in Tables 4 and 5, respectively.

[00166] In certain embodiments, the instant disclosure provides an isolated antibody that specifically binds to LAG-3 (*e.g.*, human LAG-3), comprising a heavy chain variable region comprising an amino acid sequence of SEQ ID NO: 151 or 222. In certain embodiments, the instant disclosure provides an isolated antibody that specifically binds to LAG-3 (*e.g.*, human LAG-3), comprising a heavy chain variable region comprising an amino acid sequence of SEQ ID NO: 218 or 223. In certain embodiments, the instant disclosure provides an isolated antibody that specifically binds to LAG-3 (*e.g.*, human LAG-3), comprising a heavy chain variable region comprising an amino acid sequence that is at least 75%, 80%, 85%, 90%, 95%, or 100% (*e.g.*, at least 86, 87, 88, 89, 90, 91, 92, 93, 94, 95, 96, 97, 98 or 99%) identical to the amino acid sequence set forth in SEQ ID NO: 56, 57, 58, 59, 60, 61, 62, 63, 64, 65,

220, 66, 67, 68, 69, 70, 71, or 72. In certain embodiments, the antibody comprises a heavy chain variable region having the amino acid sequence set forth in SEQ ID NO: 56, 57, 58, 59, 60, 61, 62, 63, 64, 65, 220, 66, 67, 68, 69, 70, 71, or 72, optionally wherein the amino acid residue at position 1 of the heavy chain variable region has been converted to pyroglutamate.

5 In certain embodiments, the antibody comprises a heavy chain variable region having the amino acid sequence set forth in SEQ ID NO: 65. In certain embodiments, the antibody comprises a heavy chain variable region having the amino acid sequence set forth in SEQ ID NO: 220. In certain embodiments, the X in SEQ ID NO: 220 is Q. In certain embodiments, the X in SEQ ID NO: 220 is pyroglutamate. In certain embodiments, the antibody comprises

10 a heavy chain having the amino acid sequence set forth in SEQ ID NO: 168, 225, 169, 226, 170, 227, 171, 172, 173, 174, 175, 176, 177, 178, 179, 180, 181, 182, 183, 184, 185, or 186, optionally wherein the amino acid residue at position 1 of the heavy chain has been converted to pyroglutamate. In certain embodiments, the antibody comprises a heavy chain having the amino acid sequence set forth in SEQ ID NO: 168. In certain embodiments, the antibody

15 comprises a heavy chain having the amino acid sequence set forth in SEQ ID NO: 225. In certain embodiments, the X in SEQ ID NO: 225 is Q. In certain embodiments, the X in SEQ ID NO: 225 is pyroglutamate. In certain embodiments, the antibody comprises a heavy chain having the amino acid sequence set forth in SEQ ID NO: 169. In certain embodiments, the antibody comprises a heavy chain having the amino acid sequence set forth in SEQ ID NO:

20 226. In certain embodiments, the X in SEQ ID NO: 226 is Q. In certain embodiments, the X in SEQ ID NO: 226 is pyroglutamate. In certain embodiments, the antibody comprises a heavy chain having the amino acid sequence set forth in SEQ ID NO: 170. In certain embodiments, the antibody comprises a heavy chain having the amino acid sequence set forth in SEQ ID NO: 227. In certain embodiments, the X in SEQ ID NO: 227 is Q. In certain

25 embodiments, the X in SEQ ID NO: 227 is pyroglutamate.

[00167] In certain embodiments, the instant disclosure provides an isolated antibody that specifically binds to LAG-3 (*e.g.*, human LAG-3), comprising a light chain variable region comprising an amino acid sequence of SEQ ID NO: 152 or 224. In certain embodiments, the instant disclosure provides an isolated antibody that specifically binds to LAG-3 (*e.g.*, human

30 LAG-3), comprising a light chain variable region comprising an amino acid sequence that is at least 75%, 80%, 85%, 90%, 95%, or 100% (*e.g.*, at least 86, 87, 88, 89, 90, 91, 92, 93, 94, 95, 96, 97, 98 or 99%) identical to the amino acid sequence set forth in SEQ ID NO: 73, 74, 75, 76, or 77. In certain embodiments, the antibody comprises a light chain variable region having the amino acid sequence set forth in SEQ ID NO: 73, 74, 75, 76, or 77, optionally

wherein the amino acid residue at position 1 of the light chain variable region has been converted to pyroglutamate. In certain embodiments, the antibody comprises a light chain variable region having the amino acid sequence set forth in SEQ ID NO: 73. In certain embodiments, the antibody comprises a light chain variable region having the amino acid sequence set forth in SEQ ID NO: 221. In certain embodiments, the X in SEQ ID NO: 221 is E. In certain embodiments, the X in SEQ ID NO: 221 is pyroglutamate. In certain embodiments, the antibody comprises a light chain variable region having the amino acid sequence set forth in SEQ ID NO: 187, 188, 189, 190, or 191, optionally wherein the amino acid residue at position 1 of the light chain has been converted to pyroglutamate. In certain embodiments, the antibody comprises a light chain variable region having the amino acid sequence set forth in SEQ ID NO: 187. In certain embodiments, the antibody comprises a light chain variable region having the amino acid sequence set forth in SEQ ID NO: 228. In certain embodiments, the X in SEQ ID NO: 228 is E. In certain embodiments, the X in SEQ ID NO: 228 is pyroglutamate.

[00168] In certain embodiments, the instant disclosure provides an isolated antibody that specifically binds to LAG-3 (*e.g.*, human LAG-3), comprising a heavy chain variable region comprising an amino acid sequence of SEQ ID NO: 151 or 222, and a light chain variable region comprising an amino acid sequence of SEQ ID NO: 152 or 224. In certain embodiments, the instant disclosure provides an isolated antibody that specifically binds to LAG-3 (*e.g.*, human LAG-3), comprising a heavy chain variable region comprising an amino acid sequence of SEQ ID NO: 218 or 223, and a light chain variable region comprising an amino acid sequence of SEQ ID NO: 152 or 224. In certain embodiments, the instant disclosure provides an isolated antibody that specifically binds to LAG-3 (*e.g.*, human LAG-3), comprising a heavy chain variable region comprising an amino acid sequence that is at least 75%, 80%, 85%, 90%, 95%, or 100% (*e.g.*, at least 86, 87, 88, 89, 90, 91, 92, 93, 94, 95, 96, 97, 98 or 99%) identical to the amino acid sequence set forth in SEQ ID NO: 56, 57, 58, 59, 60, 61, 62, 63, 64, 65, 220, 66, 67, 68, 69, 70, 71, or 72, and a light chain variable region comprising an amino acid sequence that is at least 75%, 80%, 85%, 90%, 95%, or 100% (*e.g.*, at least 86, 87, 88, 89, 90, 91, 92, 93, 94, 95, 96, 97, 98 or 99%) identical to the amino acid sequence set forth in SEQ ID NO: 73, 221, 74, 75, 76, or 77. In certain embodiments, the antibody comprises a heavy chain variable region having the amino acid sequence set forth in SEQ ID NO: 56, 57, 58, 59, 60, 61, 62, 63, 64, 65, 220, 66, 67, 68, 69, 70, 71, or 72, and a light chain variable region having the amino acid sequence set forth in SEQ ID NO: 73, 221, 74, 75, 76, or 77. In certain embodiments, the antibody comprises a heavy chain variable

region and light chain variable region having the amino acid sequences set forth in SEQ ID NO: 56 and 73; 56 and 74; 56 and 75; 56 and 76; 56 and 77; 57 and 73; 57 and 74; 57 and 75; 57 and 76; 57 and 77; 58 and 73; 58 and 74; 58 and 75; 58 and 76; 58 and 77; 59 and 73; 59 and 74; 59 and 75; 59 and 76; 59 and 77; 60 and 73; 60 and 74; 60 and 75; 60 and 76; 60 and 77; 61 and 77; 62 and 77; 63 and 73; 64 and 73; 65 and 73; 220 and 73; 65 and 221; 220 and 221; 66 and 73; 67 and 73; 68 and 73; 69 and 73; 70 and 73; 71 and 73; or 72 and 73, respectively. In certain embodiments, the antibody comprises a heavy chain variable region and light chain variable region having the amino acid sequences set forth in SEQ ID NO: 56 and 73, respectively. In certain embodiments, the antibody comprises a heavy chain variable region and light chain variable region having the amino acid sequences set forth in SEQ ID NO: 220 and 221, respectively. In certain embodiments, the X in SEQ ID NO: 220 is Q. In certain embodiments, the X in SEQ ID NO: 220 is pyroglutamate. In certain embodiments, the X in SEQ ID NO: 221 is E. In certain embodiments, the X in SEQ ID NO: 221 is pyroglutamate. In certain embodiments, the X in SEQ ID NO: 220 is Q, and the X in SEQ ID NO: 221 is E. In certain embodiments, the X in SEQ ID NO: 220 is Q, and the X in SEQ ID NO: 221 is pyroglutamate. In certain embodiments, the X in SEQ ID NO: 220 is pyroglutamate, and the X in SEQ ID NO: 221 is E. In certain embodiments, the X in SEQ ID NO: 220 is pyroglutamate, and the X in SEQ ID NO: 221 is pyroglutamate. In certain embodiments, the antibody comprises a heavy chain variable region and light chain variable region having the amino acid sequences set forth in SEQ ID NO: 56 and 74, respectively. In certain embodiments, the antibody comprises a heavy chain variable region and light chain variable region having the amino acid sequences set forth in SEQ ID NO: 56 and 75, respectively. In certain embodiments, the antibody comprises a heavy chain variable region and light chain variable region having the amino acid sequences set forth in SEQ ID NO: 56 and 76, respectively. In certain embodiments, the antibody comprises a heavy chain variable region and light chain variable region having the amino acid sequences set forth in SEQ ID NO: 56 and 77, respectively. In certain embodiments, the antibody comprises a heavy chain variable region and light chain variable region having the amino acid sequences set forth in SEQ ID NO: 57 and 73, respectively. In certain embodiments, the antibody comprises a heavy chain variable region and light chain variable region having the amino acid sequences set forth in SEQ ID NO: 57 and 74, respectively. In certain embodiments, the antibody comprises a heavy chain variable region and light chain variable region having the amino acid sequences set forth in SEQ ID NO: 57 and 75, respectively. In certain embodiments, the antibody comprises a heavy chain variable region and light chain variable region having the

[illegible]

amino acid sequences set forth in SEQ ID NO: 60 and 77, respectively. In certain embodiments, the antibody comprises a heavy chain variable region and light chain variable region having the amino acid sequences set forth in SEQ ID NO: 61 and 77, respectively. In certain embodiments, the antibody comprises a heavy chain variable region and light chain variable region having the amino acid sequences set forth in SEQ ID NO: 62 and 77, respectively. In certain embodiments, the antibody comprises a heavy chain variable region and light chain variable region having the amino acid sequences set forth in SEQ ID NO: 63 and 73, respectively. In certain embodiments, the antibody comprises a heavy chain variable region and light chain variable region having the amino acid sequences set forth in SEQ ID NO: 64 and 73, respectively. In certain embodiments, the antibody comprises a heavy chain variable region and light chain variable region having the amino acid sequences set forth in SEQ ID NO: 65 and 73, respectively. In certain embodiments, the antibody comprises a heavy chain variable region and light chain variable region having the amino acid sequences set forth in SEQ ID NO: 66 and 73, respectively. In certain embodiments, the antibody comprises a heavy chain variable region and light chain variable region having the amino acid sequences set forth in SEQ ID NO: 67 and 73, respectively. In certain embodiments, the antibody comprises a heavy chain variable region and light chain variable region having the amino acid sequences set forth in SEQ ID NO: 68 and 73, respectively. In certain embodiments, the antibody comprises a heavy chain variable region and light chain variable region having the amino acid sequences set forth in SEQ ID NO: 69 and 73, respectively. In certain embodiments, the antibody comprises a heavy chain variable region and light chain variable region having the amino acid sequences set forth in SEQ ID NO: 70 and 73, respectively. In certain embodiments, the antibody comprises a heavy chain variable region and light chain variable region having the amino acid sequences set forth in SEQ ID NO: 71 and 73, respectively. In certain embodiments, the antibody comprises a heavy chain variable region and light chain variable region having the amino acid sequences set forth in SEQ ID NO: 72 and 73, respectively. In certain embodiments, the amino acid residue at position 1 of the heavy chain variable region has been converted to pyroglutamate. In certain embodiments, the amino acid residue at position 1 of the light chain variable region has been converted to pyroglutamate. In certain embodiments, the amino acid residue at position 1 of the heavy chain variable region has been converted to pyroglutamate, and the amino acid residue at position 1 of the light chain variable region has been converted to pyroglutamate.

[00169] In certain embodiments, the instant disclosure provides an isolated antibody that cross-competes for binding to LAG-3 (*e.g.*, human LAG-3) with an antibody comprising the

heavy and light chain variable region amino acid sequences set forth in SEQ ID NOs: 1 and 2; 3 and 4; 5 and 6; 7 and 8; 9 and 10; 11 and 12; 13 and 10; 14 and 10; 15 and 16; 17 and 18; 19 and 8; 20 and 21; 22 and 23; 24 and 10; 9 and 25; 9 and 26; 20 and 2; 27 and 28; 29 and 30; 31 and 32; 33 and 34; 35 and 10; 36 and 37; 38 and 21; 39 and 40; 9 and 2; 9 and 41; 42 and 43; 9 and 44; 45 and 32; 46 and 47; 48 and 10; 20 and 49; 33 and 50; 51 and 52; 53 and 8; 38 and 2; or 54 and 55, respectively. In certain embodiments, the instant disclosure provides an isolated antibody that cross-competes for binding to LAG-3 (*e.g.*, human LAG-3) with an antibody comprising the heavy and light chain variable region amino acid sequences set forth in SEQ ID NOs: 56 and 73; 56 and 74; 56 and 75; 56 and 76; 56 and 77; 57 and 73; 57 and 74; 57 and 75; 57 and 76; 57 and 77; 58 and 73; 58 and 74; 58 and 75; 58 and 76; 58 and 77; 59 and 73; 59 and 74; 59 and 75; 59 and 76; 59 and 77; 60 and 73; 60 and 74; 60 and 75; 60 and 76; 60 and 77; 61 and 77; 62 and 77; 63 and 73; 64 and 73; 65 and 73; 220 and 73; 65 and 221; 220 and 221; 66 and 73; 67 and 73; 68 and 73; 69 and 73; 70 and 73; 71 and 73; or 72 and 73, respectively.

[00170] In certain embodiments, the instant disclosure provides an isolated antibody that binds to the same or an overlapping epitope of LAG-3 (*e.g.*, an epitope of human LAG-3) as an antibody described herein, *e.g.*, an antibody comprising the heavy and light chain variable region amino acid sequences set forth in SEQ ID NOs: 1 and 2; 3 and 4; 5 and 6; 7 and 8; 9 and 10; 11 and 12; 13 and 10; 14 and 10; 15 and 16; 17 and 18; 19 and 8; 20 and 21; 22 and 23; 24 and 10; 9 and 25; 9 and 26; 20 and 2; 27 and 28; 29 and 30; 31 and 32; 33 and 34; 35 and 10; 36 and 37; 38 and 21; 39 and 40; 9 and 2; 9 and 41; 42 and 43; 9 and 44; 45 and 32; 46 and 47; 48 and 10; 20 and 49; 33 and 50; 51 and 52; 53 and 8; 38 and 2; or 54 and 55, respectively. In certain embodiments, the instant disclosure provides an isolated antibody that binds to the same or an overlapping epitope of LAG-3 (*e.g.*, an epitope of human LAG-3) as an antibody described herein, *e.g.*, an antibody comprising the heavy and light chain variable region amino acid sequences set forth in SEQ ID NOs: 56 and 73; 56 and 74; 56 and 75; 56 and 76; 56 and 77; 57 and 73; 57 and 74; 57 and 75; 57 and 76; 57 and 77; 58 and 73; 58 and 74; 58 and 75; 58 and 76; 58 and 77; 59 and 73; 59 and 74; 59 and 75; 59 and 76; 59 and 77; 60 and 73; 60 and 74; 60 and 75; 60 and 76; 60 and 77; 61 and 77; 62 and 77; 63 and 73; 64 and 73; 65 and 73; 220 and 73; 65 and 221; 220 and 221; 66 and 73; 67 and 73; 68 and 73; 69 and 73; 70 and 73; 71 and 73; or 72 and 73, respectively. In certain embodiments, the epitope of an antibody can be determined by, *e.g.*, NMR spectroscopy, surface plasmon resonance (BIAcore[®]), X-ray diffraction crystallography studies, ELISA assays, hydrogen/deuterium exchange coupled with mass spectrometry (*e.g.*, liquid chromatography

electrospray mass spectrometry), array-based oligo-peptide scanning assays, and/or mutagenesis mapping (*e.g.*, site-directed mutagenesis mapping). For X-ray crystallography, crystallization may be accomplished using any of the known methods in the art (*e.g.*, Giegé R *et al.*, (1994) *Acta Crystallogr D Biol Crystallogr* 50(Pt 4): 339-350; McPherson A (1990) *Eur J Biochem* 189: 1-23; Chayen NE (1997) *Structure* 5: 1269-1274; McPherson A (1976) *J Biol Chem* 251: 6300-6303, all of which are herein incorporated by reference in their entirety). Antibody:antigen crystals may be studied using well known X-ray diffraction techniques and may be refined using computer software such as X-PLOR (Yale University, 1992, distributed by Molecular Simulations, Inc.; *see, e.g.*, *Meth Enzymol* (1985) volumes 114 & 115, eds Wyckoff HW *et al.*; U.S. Patent Application No. 2004/0014194), and BUSTER (Bricogne G (1993) *Acta Crystallogr D Biol Crystallogr* 49(Pt 1): 37-60; Bricogne G (1997) *Meth Enzymol* 276A: 361-423, ed Carter CW; Roversi P *et al.*, (2000) *Acta Crystallogr D Biol Crystallogr* 56(Pt 10): 1316-1323, all of which are herein incorporated by reference in their entirety). Mutagenesis mapping studies may be accomplished using any method known to one of skill in the art. *See, e.g.*, Champe M *et al.*, (1995) *supra* and Cunningham BC & Wells JA (1989) *supra* for a description of mutagenesis techniques, including alanine scanning mutagenesis techniques. In a specific embodiment, the epitope of an antibody is determined using alanine scanning mutagenesis studies. In addition, antibodies that recognize and bind to the same or overlapping epitopes of LAG-3 (*e.g.*, human LAG-3) can be identified using routine techniques such as an immunoassay, for example, by showing the ability of one antibody to block the binding of another antibody to a target antigen, *i.e.*, a competitive binding assay. Competitive binding assays also can be used to determine whether two antibodies have similar binding specificity for an epitope. Competitive binding can be determined in an assay in which the immunoglobulin under test inhibits specific binding of a reference antibody to a common antigen, such as LAG-3 (*e.g.*, human LAG-3). Numerous types of competitive binding assays are known, for example: solid phase direct or indirect radioimmunoassay (RIA), solid phase direct or indirect enzyme immunoassay (EIA), sandwich competition assay (*see* Stahli C *et al.*, (1983) *Methods Enzymol* 9: 242-253); solid phase direct biotin-avidin EIA (*see* Kirkland TN *et al.*, (1986) *J Immunol* 137: 3614-9); solid phase direct labeled assay, solid phase direct labeled sandwich assay (*see* Harlow E & Lane D, (1988) *Antibodies: A Laboratory Manual*, Cold Spring Harbor Press); solid phase direct label RIA using I-125 label (*see* Morel GA *et al.*, (1988) *Mol Immunol* 25(1): 7-15); solid phase direct biotin-avidin EIA (*see* Cheung RC *et al.*, (1990) *Virology* 176: 546-52); and direct labeled RIA (*see* Moldenhauer G *et al.*, (1990)

Scand J Immunol 32: 77-82), all of which are herein incorporated by reference in their entireties. Typically, such an assay involves the use of purified antigen (*e.g.*, LAG-3 such as human LAG-3) bound to a solid surface or cells bearing either of these, an unlabeled test immunoglobulin and a labeled reference immunoglobulin. Competitive inhibition can be measured by determining the amount of label bound to the solid surface or cells in the presence of the test immunoglobulin. Usually the test immunoglobulin is present in excess. Usually, when a competing antibody is present in excess, it will inhibit specific binding of a reference antibody to a common antigen by at least 50-55%, 55-60%, 60-65%, 65-70%, 70-75% or more. A competition binding assay can be configured in a large number of different formats using either labeled antigen or labeled antibody. In a common version of this assay, the antigen is immobilized on a 96-well plate. The ability of unlabeled antibodies to block the binding of labeled antibodies to the antigen is then measured using radioactive or enzyme labels. For further details *see*, for example, Wagener C *et al.*, (1983) J Immunol 130: 2308-2315; Wagener C *et al.*, (1984) J Immunol Methods 68: 269-274; Kuroki M *et al.*, (1990) Cancer Res 50: 4872-4879; Kuroki M *et al.*, (1992) Immunol Invest 21: 523-538; Kuroki M *et al.*, (1992) Hybridoma 11: 391-407 and Antibodies: A Laboratory Manual, Ed Harlow E & Lane D editors *supra*, pp. 386-389, all of which are herein incorporated by reference in their entireties.

[00171] In certain embodiments, the instant disclosure provides an isolated antibody that specifically binds to LAG-3 (*e.g.*, human LAG-3), the antibody comprising a heavy chain comprising the amino acid sequence set forth in SEQ ID NO: 168, 225, 169, 226, 170, 227, 171, 172, 173, 174, 175, 176, 177, 178, 179, 180, 181, 182, 183, 184, 185, or 186, optionally wherein the amino acid residue at position 1 of the heavy chain has been converted to pyroglutamate. In certain embodiments, the antibody comprises a heavy chain comprising the amino acid sequence set forth in SEQ ID NO: 168. In certain embodiments, the antibody comprises a heavy chain comprising the amino acid sequence set forth in SEQ ID NO: 225. In certain embodiments, the X in SEQ ID NO: 225 is Q. In certain embodiments, the X in SEQ ID NO: 225 is pyroglutamate. In certain embodiments, the antibody comprises a heavy chain comprising the amino acid sequence set forth in SEQ ID NO: 169. In certain embodiments, the antibody comprises a heavy chain comprising the amino acid sequence set forth in SEQ ID NO: 226. In certain embodiments, the X in SEQ ID NO: 226 is Q. In certain embodiments, the X in SEQ ID NO: 226 is pyroglutamate. In certain embodiments, the antibody comprises a heavy chain comprising the amino acid sequence set forth in SEQ ID NO: 170. In certain embodiments, the antibody comprises a heavy chain comprising the

amino acid sequence set forth in SEQ ID NO: 227. In certain embodiments, the X in SEQ ID NO: 227 is Q. In certain embodiments, the X in SEQ ID NO: 227 is pyroglutamate. In certain embodiments, the antibody comprises a heavy chain comprising the amino acid sequence set forth in SEQ ID NO: 171. In certain embodiments, the antibody comprises a heavy chain comprising the amino acid sequence set forth in SEQ ID NO: 172. In certain embodiments, the antibody comprises a heavy chain comprising the amino acid sequence set forth in SEQ ID NO: 173. In certain embodiments, the antibody comprises a heavy chain comprising the amino acid sequence set forth in SEQ ID NO: 174. In certain embodiments, the antibody comprises a heavy chain comprising the amino acid sequence set forth in SEQ ID NO: 175. In certain embodiments, the antibody comprises a heavy chain comprising the amino acid sequence set forth in SEQ ID NO: 176. In certain embodiments, the antibody comprises a heavy chain comprising the amino acid sequence set forth in SEQ ID NO: 177. In certain embodiments, the antibody comprises a heavy chain comprising the amino acid sequence set forth in SEQ ID NO: 178. In certain embodiments, the antibody comprises a heavy chain comprising the amino acid sequence set forth in SEQ ID NO: 179. In certain embodiments, the antibody comprises a heavy chain comprising the amino acid sequence set forth in SEQ ID NO: 180. In certain embodiments, the antibody comprises a heavy chain comprising the amino acid sequence set forth in SEQ ID NO: 181. In certain embodiments, the antibody comprises a heavy chain comprising the amino acid sequence set forth in SEQ ID NO: 182. In certain embodiments, the antibody comprises a heavy chain comprising the amino acid sequence set forth in SEQ ID NO: 183. In certain embodiments, the antibody comprises a heavy chain comprising the amino acid sequence set forth in SEQ ID NO: 184. In certain embodiments, the antibody comprises a heavy chain comprising the amino acid sequence set forth in SEQ ID NO: 185. In certain embodiments, the antibody comprises a heavy chain comprising the amino acid sequence set forth in SEQ ID NO: 186. In certain embodiments, the amino acid residue at position 1 of the heavy chain has been converted to pyroglutamate.

[00172] In certain embodiments, the instant disclosure provides an isolated antibody that specifically binds to LAG-3 (*e.g.*, human LAG-3), the antibody comprising a light chain comprising the amino acid sequence set forth in SEQ ID NO: 187, 228, 188, 189, 190, or 191, optionally wherein the amino acid residue at position 1 of the light chain has been converted to pyroglutamate. In certain embodiments, the antibody comprises a light chain comprising the amino acid sequence set forth in SEQ ID NO: 187. In certain embodiments, the antibody comprises a light chain comprising the amino acid sequence set forth in SEQ ID

NO: 228. In certain embodiments, the X in SEQ ID NO: 228 is E. In certain embodiments, the X in SEQ ID NO: 228 is pyroglutamate. In certain embodiments, the antibody comprises a light chain comprising the amino acid sequence set forth in SEQ ID NO: 188. In certain embodiments, the antibody comprises a light chain comprising the amino acid sequence set forth in SEQ ID NO: 189. In certain embodiments, the antibody comprises a light chain comprising the amino acid sequence set forth in SEQ ID NO: 190. In certain embodiments, the antibody comprises a light chain comprising the amino acid sequence set forth in SEQ ID NO: 191. In certain embodiments, the amino acid residue at position 1 of the light chain has been converted to pyroglutamate.

10 [00173] In certain embodiments, the instant disclosure provides an isolated antibody that specifically binds to LAG-3 (*e.g.*, human LAG-3), the antibody comprising a heavy chain comprising the amino acid sequence of SEQ ID NO: 168; and a light chain comprising the amino acid sequence of SEQ ID NO: 187. In certain embodiments, the instant disclosure provides an isolated antibody that specifically binds to LAG-3 (*e.g.*, human LAG-3), the antibody comprising a heavy chain comprising the amino acid sequence of SEQ ID NO: 225; and a light chain comprising the amino acid sequence of SEQ ID NO: 228. In certain embodiments, the X in SEQ ID NO: 225 is Q, and the X in SEQ ID NO: 228 is pyroglutamate. In certain embodiments, the X in SEQ ID NO: 225 is pyroglutamate, and the X in SEQ ID NO: 228 is E. In certain embodiments, the X in SEQ ID NO: 225 is pyroglutamate, and the X in SEQ ID NO: 228 is pyroglutamate.

20 [00174] In certain embodiments, the instant disclosure provides an isolated antibody that specifically binds to LAG-3 (*e.g.*, human LAG-3), the antibody comprising a heavy chain comprising the amino acid sequence of SEQ ID NO: 169; and a light chain comprising the amino acid sequence of SEQ ID NO: 187. In certain embodiments, the instant disclosure provides an isolated antibody that specifically binds to LAG-3 (*e.g.*, human LAG-3), the antibody comprising a heavy chain comprising the amino acid sequence of SEQ ID NO: 226; and a light chain comprising the amino acid sequence of SEQ ID NO: 228. In certain embodiments, the X in SEQ ID NO: 226 is Q, and the X in SEQ ID NO: 228 is pyroglutamate. In certain embodiments, the X in SEQ ID NO: 226 is pyroglutamate, and the X in SEQ ID NO: 228 is E. In certain embodiments, the X in SEQ ID NO: 226 is pyroglutamate, and the X in SEQ ID NO: 228 is pyroglutamate.

30 [00175] In certain embodiments, the instant disclosure provides an isolated antibody that specifically binds to LAG-3 (*e.g.*, human LAG-3), the antibody comprising a heavy chain comprising the amino acid sequence of SEQ ID NO: 170; and a light chain comprising the

amino acid sequence of SEQ ID NO: 187. In certain embodiments, the instant disclosure provides an isolated antibody that specifically binds to LAG-3 (*e.g.*, human LAG-3), the antibody comprising a heavy chain comprising the amino acid sequence of SEQ ID NO: 227; and a light chain comprising the amino acid sequence of SEQ ID NO: 228. In certain
5 embodiments, the X in SEQ ID NO: 227 is Q, and the X in SEQ ID NO: 228 is pyroglutamate. In certain embodiments, the X in SEQ ID NO: 227 is pyroglutamate, and the X in SEQ ID NO: 228 is E. In certain embodiments, the X in SEQ ID NO: 227 is pyroglutamate, and the X in SEQ ID NO: 228 is pyroglutamate.

[00176] In certain embodiments, the instant disclosure provides an isolated antibody that
10 specifically binds to LAG-3 (*e.g.*, human LAG-3), the antibody comprising a heavy chain comprising the amino acid sequence of SEQ ID NO: 171; and a light chain comprising the amino acid sequence of SEQ ID NO: 187. In certain embodiments, the instant disclosure provides an isolated antibody that specifically binds to LAG-3 (*e.g.*, human LAG-3), the antibody comprising a heavy chain comprising the amino acid sequence of SEQ ID NO: 172;
15 and a light chain comprising the amino acid sequence of SEQ ID NO: 187. In certain embodiments, the instant disclosure provides an isolated antibody that specifically binds to LAG-3 (*e.g.*, human LAG-3), the antibody comprising a heavy chain comprising the amino acid sequence of SEQ ID NO: 173; and a light chain comprising the amino acid sequence of SEQ ID NO: 187. In certain embodiments, the instant disclosure provides an isolated
20 antibody that specifically binds to LAG-3 (*e.g.*, human LAG-3), the antibody comprising a heavy chain comprising the amino acid sequence of SEQ ID NO: 174; and a light chain comprising the amino acid sequence of SEQ ID NO: 187. In certain embodiments, the instant disclosure provides an isolated antibody that specifically binds to LAG-3 (*e.g.*, human LAG-3), the antibody comprising a heavy chain comprising the amino acid sequence of SEQ
25 ID NO: 175; and a light chain comprising the amino acid sequence of SEQ ID NO: 187. In certain embodiments, the instant disclosure provides an isolated antibody that specifically binds to LAG-3 (*e.g.*, human LAG-3), the antibody comprising a heavy chain comprising the amino acid sequence of SEQ ID NO: 176; and a light chain comprising the amino acid sequence of SEQ ID NO: 187. In certain embodiments, the instant disclosure provides an
30 isolated antibody that specifically binds to LAG-3 (*e.g.*, human LAG-3), the antibody comprising a heavy chain comprising the amino acid sequence of SEQ ID NO: 177; and a light chain comprising the amino acid sequence of SEQ ID NO: 187. In certain embodiments, the instant disclosure provides an isolated antibody that specifically binds to LAG-3 (*e.g.*, human LAG-3), the antibody comprising a heavy chain comprising the amino

acid sequence of SEQ ID NO: 178; and a light chain comprising the amino acid sequence of SEQ ID NO: 187. In certain embodiments, the instant disclosure provides an isolated antibody that specifically binds to LAG-3 (*e.g.*, human LAG-3), the antibody comprising a heavy chain comprising the amino acid sequence of SEQ ID NO: 179; and a light chain comprising the amino acid sequence of SEQ ID NO: 187. In certain embodiments, the instant disclosure provides an isolated antibody that specifically binds to LAG-3 (*e.g.*, human LAG-3), the antibody comprising a heavy chain comprising the amino acid sequence of SEQ ID NO: 180; and a light chain comprising the amino acid sequence of SEQ ID NO: 187. In certain embodiments, the instant disclosure provides an isolated antibody that specifically binds to LAG-3 (*e.g.*, human LAG-3), the antibody comprising a heavy chain comprising the amino acid sequence of SEQ ID NO: 181; and a light chain comprising the amino acid sequence of SEQ ID NO: 187. In certain embodiments, the instant disclosure provides an isolated antibody that specifically binds to LAG-3 (*e.g.*, human LAG-3), the antibody comprising a heavy chain comprising the amino acid sequence of SEQ ID NO: 182; and a light chain comprising the amino acid sequence of SEQ ID NO: 187. In certain embodiments, the instant disclosure provides an isolated antibody that specifically binds to LAG-3 (*e.g.*, human LAG-3), the antibody comprising a heavy chain comprising the amino acid sequence of SEQ ID NO: 183; and a light chain comprising the amino acid sequence of SEQ ID NO: 187. In certain embodiments, the instant disclosure provides an isolated antibody that specifically binds to LAG-3 (*e.g.*, human LAG-3), the antibody comprising a heavy chain comprising the amino acid sequence of SEQ ID NO: 184; and a light chain comprising the amino acid sequence of SEQ ID NO: 187. In certain embodiments, the instant disclosure provides an isolated antibody that specifically binds to LAG-3 (*e.g.*, human LAG-3), the antibody comprising a heavy chain comprising the amino acid sequence of SEQ ID NO: 185; and a light chain comprising the amino acid sequence of SEQ ID NO: 187. In certain embodiments, the instant disclosure provides an isolated antibody that specifically binds to LAG-3 (*e.g.*, human LAG-3), the antibody comprising a heavy chain comprising the amino acid sequence of SEQ ID NO: 186; and a light chain comprising the amino acid sequence of SEQ ID NO: 187. In certain embodiments, the amino acid residue at position 1 of the heavy chain has been converted to pyroglutamate. In certain embodiments, the amino acid residue at position 1 of the light chain has been converted to pyroglutamate. In certain embodiments, the amino acid residue at position 1 of the heavy chain has been converted to pyroglutamate, and the amino acid residue at position 1 of the light chain has been converted to pyroglutamate.

[00177] Any Ig constant region can be used in the antibodies disclosed herein. In certain embodiments, the Ig region is a human IgG, IgE, IgM, IgD, IgA, or IgY immunoglobulin molecule, any class (*e.g.*, IgG₁, IgG₂, IgG₃, IgG₄, IgA₁, and IgA₂), or any subclass (*e.g.*, IgG_{2a} and IgG_{2b}) of immunoglobulin molecule.

5 [00178] In certain embodiments, the instant disclosure provides an isolated antibody that specifically binds to LAG-3 (*e.g.*, human LAG-3), the antibody comprising a heavy chain constant region comprising the amino acid sequence of SEQ ID NO: 192, 193, 194, 195, 196, 197, 208, or 209. In certain embodiments, the instant disclosure provides an isolated antibody that specifically binds to LAG-3 (*e.g.*, human LAG-3), the antibody comprising a heavy chain constant region comprising the amino acid sequence of SEQ ID NO: 194. In certain embodiments, the instant disclosure provides an isolated antibody that specifically binds to LAG-3 (*e.g.*, human LAG-3), the antibody comprising a heavy chain constant region comprising the amino acid sequence of SEQ ID NO: 195. In certain embodiments, the instant disclosure provides an isolated antibody that specifically binds to LAG-3 (*e.g.*, human LAG-3), the antibody comprising a light chain constant region comprising the amino acid sequence of SEQ ID NO: 198. In certain embodiments, the instant disclosure provides an isolated antibody that specifically binds to LAG-3 (*e.g.*, human LAG-3), the antibody comprising a light chain constant region comprising the amino acid sequence of SEQ ID NO: 219.

20 [00179] In certain embodiments, one, two, or more mutations (*e.g.*, amino acid substitutions) are introduced into the Fc region of an antibody described herein (*e.g.*, CH2 domain (residues 231-340 of human IgG₁) and/or CH3 domain (residues 341-447 of human IgG₁) and/or the hinge region, numbered according to the EU numbering system to alter one or more functional properties of the antibody, such as serum half-life, complement fixation, Fc receptor binding and/or antigen-dependent cellular cytotoxicity.

25 [00180] In certain embodiments, one, two, or more mutations (*e.g.*, amino acid substitutions) are introduced into the hinge region of the Fc region (CH1 domain) such that the number of cysteine residues in the hinge region are altered (*e.g.*, increased or decreased) as described in, *e.g.*, U.S. Patent No. 5,677,425. The number of cysteine residues in the hinge region of the CH1 domain may be altered to, *e.g.*, facilitate assembly of the light and heavy chains, or to alter (*e.g.*, increase or decrease) the stability of the antibody.

30 [00181] In a specific embodiment, one, two, or more amino acid mutations (*e.g.*, substitutions, insertions or deletions) are introduced into an IgG constant domain, or FcRn-binding fragment thereof (preferably an Fc or hinge-Fc domain fragment) to alter (*e.g.*,

decrease or increase) half-life of the antibody *in vivo*. See, e.g., International Publication Nos. WO 02/060919; WO 98/23289; and WO 97/34631; and U.S. Patent Nos. 5,869,046, 6,121,022, 6,277,375 and 6,165,745, all of which are herein incorporated by reference in their entireties, for examples of mutations that will alter (e.g., decrease or increase) the half-life of an antibody *in vivo*. In some embodiments, one, two or more amino acid mutations (e.g., substitutions, insertions, or deletions) are introduced into an IgG constant domain, or FcRn-binding fragment thereof (preferably an Fc or hinge-Fc domain fragment) to decrease the half-life of the antibody *in vivo*. In other embodiments, one, two or more amino acid mutations (e.g., substitutions, insertions or deletions) are introduced into an IgG constant domain, or FcRn-binding fragment thereof (preferably an Fc or hinge-Fc domain fragment) to increase the half-life of the antibody *in vivo*. In a specific embodiment, the antibodies may have one or more amino acid mutations (e.g., substitutions) in the second constant (CH2) domain (residues 231-340 of human IgG₁) and/or the third constant (CH3) domain (residues 341-447 of human IgG₁), numbered according to the EU numbering system. In a specific embodiment, the constant region of the IgG₁ of an antibody described herein comprises a methionine (M) to tyrosine (Y) substitution in position 252, a serine (S) to threonine (T) substitution in position 254, and a threonine (T) to glutamic acid (E) substitution in position 256, numbered according to the EU numbering system. See U.S. Patent No. 7,658,921, which is herein incorporated by reference in its entirety. This type of mutant IgG, referred to as "YTE mutant" has been shown to display fourfold increased half-life as compared to wild-type versions of the same antibody (see Dall'Acqua WF *et al.*, (2006) J Biol Chem 281: 23514-24, which is herein incorporated by reference in its entirety). In certain embodiments, an antibody comprises an IgG constant domain comprising one, two, three or more amino acid substitutions of amino acid residues at positions 251-257, 285-290, 308-314, 385-389, and 428-436, numbered according to the EU numbering system.

[00182] In some embodiments, one, two, or more mutations (e.g., amino acid substitutions) are introduced into the Fc region of an antibody described herein (e.g., CH2 domain (residues 231-340 of human IgG₁) and/or CH3 domain (residues 341-447 of human IgG₁) and/or the hinge region, numbered according to the EU numbering system to increase or decrease the affinity of the antibody for an Fc receptor (e.g., an activated Fc receptor) on the surface of an effector cell. Mutations in the Fc region of an antibody that decrease or increase the affinity of an antibody for an Fc receptor and techniques for introducing such mutations into the Fc receptor or fragment thereof are known to one of skill in the art. Examples of mutations in the Fc receptor of an antibody that can be made to alter the affinity

of the antibody for an Fc receptor are described in, *e.g.*, Smith P *et al.*, (2012) PNAS 109: 6181-6186, U.S. Patent No. 6,737,056, and International Publication Nos. WO 02/060919; WO 98/23289; and WO 97/34631, all of which are herein incorporated by reference in their entireties.

5 [00183] In a further embodiment, one, two, or more amino acid substitutions are introduced into an IgG constant domain Fc region to alter the effector function(s) of the antibody. For example, one or more amino acids selected from amino acid residues 234, 235, 236, 237, 297, 318, 320 and 322, numbered according to the EU numbering system, can be replaced with a different amino acid residue such that the antibody has an altered affinity for
10 an effector ligand but retains the antigen-binding ability of the parent antibody. The effector ligand to which affinity is altered can be, for example, an Fc receptor or the C1 component of complement. This approach is described in further detail in U.S. Patent Nos. 5,624,821 and 5,648,260, each of which is herein incorporated by reference in its entirety. In some embodiments, the deletion or inactivation (through point mutations or other means) of a
15 constant region domain may reduce Fc receptor binding of the circulating antibody thereby increasing tumor localization. *See, e.g.*, U.S. Patent Nos. 5,585,097 and 8,591,886, each of which is herein incorporated by reference in its entirety, for a description of mutations that delete or inactivate the constant domain and thereby increase tumor localization. In certain embodiments, one or more amino acid substitutions may be introduced into the Fc region of
20 an antibody described herein to remove potential glycosylation sites on Fc region, which may reduce Fc receptor binding (*see, e.g.*, Shields RL *et al.*, (2001) J Biol Chem 276: 6591-604, which is herein incorporated by reference in its entirety). In various embodiments, one or more of the following mutations in the constant region of an antibody described herein may be made: an N297A substitution; an N297Q substitution; a L235A substitution and a L237A
25 substitution; a L234A substitution and a L235A substitution; a E233P substitution; a L234V substitution; a L235A substitution; a C236 deletion; a P238A substitution; a D265A substitution; a A327Q substitution; or a P329A substitution, numbered according to the EU numbering system. In certain embodiments, a mutation selected from the group consisting of D265A, P329A, and a combination thereof may be made in the constant region of an
30 antibody described herein.

[00184] In a specific embodiment, an antibody described herein comprises the constant domain of an IgG₁ with an N297Q or N297A amino acid substitution, numbered according to the EU numbering system. In one embodiment, an antibody described herein comprises the constant domain of an IgG₁ with a mutation selected from the group consisting of D265A,

P329A, and a combination thereof, numbered according to the EU numbering system. In another embodiment, an antibody described herein comprises the constant domain of an IgG₁ with a mutation selected from the group consisting of L234A, L235A, and a combination thereof, numbered according to the EU numbering system. In certain embodiments, amino acid residues in the constant region of an antibody described herein in the positions corresponding to positions L234, L235, and D265 in a human IgG₁ heavy chain, numbered according to the EU index of numbering, are not L, L, and D, respectively. This approach is described in detail in International Publication No. WO 14/108483, which is herein incorporated by reference in its entirety. In a particular embodiment, the amino acids corresponding to positions L234, L235, and D265 in a human IgG₁ heavy chain are F, E, and A; or A, A, and A, respectively.

[00185] In certain embodiments, one or more amino acids selected from amino acid residues 329, 331, and 322 in the constant region of an antibody described herein, numbered according to the EU numbering system, can be replaced with a different amino acid residue such that the antibody has altered C1q binding and/or reduced or abolished complement dependent cytotoxicity (CDC). This approach is described in further detail in U.S. Patent No. 6,194,551 (Idusogie *et al*), which is herein incorporated by reference in its entirety. In some embodiments, one or more amino acid residues within amino acid positions 231 to 238 in the N-terminal region of the CH2 domain of an antibody described herein are altered to thereby alter the ability of the antibody to fix complement. This approach is described further in International Publication No. WO 94/29351, which is herein incorporated by reference in its entirety. In certain embodiments, the Fc region of an antibody described herein is modified to increase the ability of the antibody to mediate antibody dependent cellular cytotoxicity (ADCC) and/or to increase the affinity of the antibody for an Fcγ receptor by mutating one or more amino acids (*e.g.*, introducing amino acid substitutions) at the following positions: 238, 239, 248, 249, 252, 254, 255, 256, 258, 265, 267, 268, 269, 270, 272, 276, 278, 280, 283, 285, 286, 289, 290, 292, 293, 294, 295, 296, 298, 301, 303, 305, 307, 309, 312, 315, 320, 322, 324, 326, 327, 328, 329, 330, 331, 333, 334, 335, 337, 338, 340, 360, 373, 376, 378, 382, 388, 389, 398, 414, 416, 419, 430, 434, 435, 437, 438, or 439, numbered according to the EU numbering system. This approach is described further in International Publication No. WO 00/42072, which is herein incorporated by reference in its entirety.

[00186] In certain embodiments, an antibody described herein comprises the constant region of an IgG₄ antibody and the serine at amino acid residue 228 of the heavy chain, numbered according to the EU numbering system, is substituted for proline. In certain

embodiments, the instant disclosure provides an isolated antibody that specifically binds to LAG-3 (*e.g.*, human LAG-3), the antibody comprising a heavy chain constant region comprising the amino acid sequence of SEQ ID NO: 196. In certain embodiments, the instant disclosure provides an isolated antibody that specifically binds to LAG-3 (*e.g.*, human LAG-3), the antibody comprising a heavy chain constant region comprising the amino acid sequence of SEQ ID NO: 197.

[00187] In certain embodiments, any of the constant region mutations or modifications described herein can be introduced into one or both heavy chain constant regions of an antibody described herein having two heavy chain constant regions.

[00188] In certain embodiments, the instant disclosure provides an isolated antibody that specifically binds to LAG-3 (*e.g.*, human LAG-3) and functions as an antagonist.

[00189] In certain embodiments, the instant disclosure provides an isolated antibody that specifically binds to LAG-3 (*e.g.*, human LAG-3) and decreases LAG-3 (*e.g.*, human LAG-3) activity by at least 5%, 10%, 15%, 20%, 25%, 30%, 35%, 40%, 45%, 50%, 55%, 60%, 65%, 70%, 75%, 80%, 85%, 90%, 95%, 98%, or 99% as assessed by methods described herein and/or known to one of skill in the art, relative to LAG-3 (*e.g.*, human LAG-3) activity without any antibody or with an unrelated antibody (*e.g.*, an antibody that does not specifically bind to LAG-3 (*e.g.*, human LAG-3)). In certain embodiments, the instant disclosure provides an isolated antibody that specifically binds to LAG-3 (*e.g.*, human LAG-3) and decreases LAG-3 (*e.g.*, human LAG-3) activity by at least about 1.2 fold, 1.3 fold, 1.4 fold, 1.5 fold, 2 fold, 2.5 fold, 3 fold, 3.5 fold, 4 fold, 4.5 fold, 5 fold, 6 fold, 7 fold, 8 fold, 9 fold, 10 fold, 15 fold, 20 fold, 30 fold, 40 fold, 50 fold, 60 fold, 70 fold, 80 fold, 90 fold, or 100 fold as assessed by methods described herein and/or known to one of skill in the art, relative to LAG-3 (*e.g.*, human LAG-3) activity without any antibody or with an unrelated antibody (*e.g.*, an antibody that does not specifically bind to LAG-3 (*e.g.*, human LAG-3)). Non-limiting examples of LAG-3 (*e.g.*, human LAG-3) activity can include LAG-3 (*e.g.*, human LAG-3) signaling, LAG-3 (*e.g.*, human LAG-3) binding to LAG-3 (*e.g.*, human LAG-3) ligand (*e.g.*, MHC class II), and inhibition of cytokine production (*e.g.*, IL-2 and/or TNF- α). In certain embodiments, the instant disclosure provides an isolated antibody that specifically binds to LAG-3 (*e.g.*, human LAG-3) and deactivates, reduces, or inhibits a LAG-3 (*e.g.*, human LAG-3) activity. In specific embodiments, a decrease in a LAG-3 (*e.g.*, human LAG-3) activity is assessed as described in the Examples, *infra*.

[00190] In specific embodiments, the instant disclosure provides an isolated antibody that specifically binds to LAG-3 (*e.g.*, human LAG-3) and reduces LAG-3 (*e.g.*, human LAG-3)

- binding to its ligand (e.g., MHC class II) by at least about 5%, 10%, 15%, 20%, 25%, 30%, 35%, 40%, 45%, 50%, 55%, 60%, 65%, 70%, 75%, 80%, 85%, 90%, 95%, 98%, or 99%, as assessed by methods described herein (*see* the Examples, *infra*) or known to one of skill in the art, relative to LAG-3 (e.g., human LAG-3) binding to its ligand (e.g., MHC class II) without any antibody or with an unrelated antibody (e.g., an antibody that does not specifically bind to LAG-3 (e.g., human LAG-3)). In specific embodiments, the instant disclosure provides an isolated antibody that specifically binds to LAG-3 (e.g., human LAG-3) and reduces LAG-3 (e.g., human LAG-3) binding to its ligand (e.g., MHC class II) by at least about 1.2 fold, 1.3 fold, 1.4 fold, 1.5 fold, 2 fold, 2.5 fold, 3 fold, 3.5 fold, 4 fold, 4.5 fold, 5 fold, 6 fold, 7 fold, 8 fold, 9 fold, 10 fold, 15 fold, 20 fold, 30 fold, 40 fold, 50 fold, 60 fold, 70 fold, 80 fold, 90 fold, or 100 fold, as assessed by methods described herein (*see* the Examples, *infra*) or known to one of skill in the art, relative to LAG-3 (e.g., human LAG-3) binding to its ligand (e.g., MHC class II) without any antibody or with an unrelated antibody (e.g., an antibody that does not specifically bind to LAG-3 (e.g., human LAG-3)).
- 15 [00191] In specific embodiments, the instant disclosure provides an isolated antibody that specifically binds to LAG-3 (e.g., human LAG-3) and increases cytokine production (e.g., IL-2 and/or TNF- α) by at least about 5%, 10%, 15%, 20%, 25%, 30%, 35%, 40%, 45%, 50%, 55%, 60%, 65%, 70%, 75%, 80%, 85%, 90%, 95%, 98%, or 99%, as assessed by methods described herein (*see* the Examples, *infra*) or known to one of skill in the art, relative to cytokine production without any antibody or with an unrelated antibody (e.g., an antibody that does not specifically bind to LAG-3 (e.g., human LAG-3)). In specific embodiments, the instant disclosure provides an isolated antibody that specifically binds to LAG-3 (e.g., human LAG-3) and increases cytokine production (e.g., IL-2 and/or TNF- α) by at least about 1.2 fold, 1.3 fold, 1.4 fold, 1.5 fold, 2 fold, 2.5 fold, 3 fold, 3.5 fold, 4 fold, 4.5 fold, 5 fold, 6 fold, 7 fold, 8 fold, 9 fold, 10 fold, 15 fold, 20 fold, 30 fold, 40 fold, 50 fold, 60 fold, 70 fold, 80 fold, 90 fold, or 100 fold, as assessed by methods described herein (*see* the Examples, *infra*) or known to one of skill in the art, relative to cytokine production without any antibody or with an unrelated antibody (e.g., an antibody that does not specifically bind to LAG-3 (e.g., human LAG-3)).
- 25 [00192] In specific embodiments, the instant disclosure provides an isolated antibody that specifically binds to LAG-3 (e.g., human LAG-3) and either alone or in combination with an anti-PD-1 antibody (e.g., pembrolizumab or nivolumab), an anti-PD-L1 antibody (e.g., avelumab, durvalumab, or atezolizumab), or an anti-CTLA-4 antibody (e.g., ipilimumab) increases IL-2 production in human peripheral blood mononuclear cells (PBMCs) in response

to Staphylococcus Enterotoxin A (SEA) stimulation by at least about 1.2 fold, 1.3 fold, 1.4 fold, 1.5 fold, 2 fold, 2.5 fold, 3 fold, 3.5 fold, 4 fold, 4.5 fold, 5 fold, 6 fold, 7 fold, 8 fold, 9 fold, 10 fold, 15 fold, 20 fold, 30 fold, 40 fold, 50 fold, 60 fold, 70 fold, 80 fold, 90 fold, or 100 fold, as assessed by methods described herein (*see* the Examples, *infra*) or known to one of skill in the art, relative to IL-2 production without any antibody or with an unrelated antibody (*e.g.*, an antibody that does not specifically bind to LAG-3 (*e.g.*, human LAG-3)).

[00193] In certain embodiments, human peripheral blood mononuclear cells (PBMCs) stimulated with Staphylococcus Enterotoxin A (SEA) in the presence of an antibody described herein, which specifically binds to LAG-3 (*e.g.*, human LAG-3), have increased IL-2 production by at least about 1.2 fold, 1.3 fold, 1.4 fold, 1.5 fold, 2 fold, 2.5 fold, 3 fold, 3.5 fold, 4 fold, 4.5 fold, 5 fold, 6 fold, 7 fold, 8 fold, 9 fold, 10 fold, 15 fold, 20 fold, 30 fold, 40 fold, 50 fold, 60 fold, 70 fold, 80 fold, 90 fold, or 100 fold relative to PBMCs only stimulated with SEA without any antibody or with an unrelated antibody (*e.g.*, an antibody that does not specifically bind to LAG-3 (*e.g.*, human LAG-3)), as assessed by methods described herein (*see* the Examples, *infra*) or known to one of skill in the art.

[00194] In specific embodiments, the instant disclosure provides an isolated antibody that specifically binds to LAG-3 (*e.g.*, human LAG-3) and either alone or in combination with an anti-PD-1 antibody (*e.g.*, pembrolizumab or nivolumab) increases TNF α production in tumor infiltrating lymphocytes (TILs) in response to anti-CD3 antibody and anti-CD28 antibody stimulation by at least about 1.2 fold, 1.3 fold, 1.4 fold, 1.5 fold, 2 fold, 2.5 fold, 3 fold, 3.5 fold, 4 fold, 4.5 fold, 5 fold, 6 fold, 7 fold, 8 fold, 9 fold, 10 fold, 15 fold, 20 fold, 30 fold, 40 fold, 50 fold, 60 fold, 70 fold, 80 fold, 90 fold, or 100 fold, as assessed by methods described herein (*see* the Examples, *infra*) or known to one of skill in the art, relative to TNF α production without an antibody that specifically binds to LAG-3 (*e.g.*, human LAG-3). In one embodiment, the TILs are from renal cell carcinoma tumor. In another embodiment, the TILs are from colorectal cancer tumor.

[00195] In certain embodiments, tumor infiltrating lymphocytes (TILs) stimulated with anti-CD3 and anti-CD28 antibodies in the presence of an antibody described herein, which specifically binds to LAG-3 (*e.g.*, human LAG-3), have increased TNF α production by at least about 1.2 fold, 1.3 fold, 1.4 fold, 1.5 fold, 2 fold, 2.5 fold, 3 fold, 3.5 fold, 4 fold, 4.5 fold, 5 fold, 6 fold, 7 fold, 8 fold, 9 fold, 10 fold, 15 fold, 20 fold, 30 fold, 40 fold, 50 fold, 60 fold, 70 fold, 80 fold, 90 fold, or 100 fold relative to TILs only stimulated with anti-CD3 and anti-CD28 antibodies without an antibody that specifically binds to LAG-3 (*e.g.*, human LAG-3), as assessed by methods described herein (*see* the Examples, *infra*) or known to one

of skill in the art. In one embodiment, the TILs are from renal cell carcinoma tumor. In another embodiment, the TILs are from colorectal cancer tumor.

6.3 Pharmaceutical Compositions

[00196] Provided herein are compositions (e.g., pharmaceutical compositions) comprising an anti-LAG-3 (e.g., human LAG-3) antibody described herein having the desired degree of purity in a physiologically acceptable carrier, excipient or stabilizer (Remington's Pharmaceutical Sciences (1990) Mack Publishing Co., Easton, PA). Acceptable carriers, excipients, or stabilizers are nontoxic to recipients at the dosages and concentrations employed, and include buffers such as phosphate, citrate, and other organic acids; antioxidants including ascorbic acid and methionine; preservatives (such as octadecyldimethylbenzyl ammonium chloride; hexamethonium chloride; benzalkonium chloride, benzethonium chloride; phenol, butyl or benzyl alcohol; alkyl parabens such as methyl or propyl paraben; catechol; resorcinol; cyclohexanol; 3-pentanol; and m-cresol); low molecular weight (less than about 10 residues) polypeptides; proteins, such as serum albumin, gelatin, or immunoglobulins; hydrophilic polymers such as polyvinylpyrrolidone; amino acids such as glycine, glutamine, asparagine, histidine, arginine, or lysine; monosaccharides, disaccharides, and other carbohydrates including glucose, mannose, or dextrans; chelating agents such as EDTA; sugars such as sucrose, mannitol, trehalose or sorbitol; salt-forming counter-ions such as sodium; metal complexes (e.g., Zn-protein complexes); and/or non-ionic surfactants such as TWEEN™, PLURONICS™ or polyethylene glycol (PEG).

[00197] In certain embodiments, the composition comprises one or more anti-LAG-3 (e.g., human LAG-3) antibodies as disclosed herein, wherein in a portion of the antibodies, the N-terminal amino acid residue(s) of the heavy chain and/or the light chain have been converted to pyroglutamate (e.g., as a result of post-translational cyclization of the free amino group of the N-terminal E or Q residue). In certain embodiments, the N-terminal amino acid residue of at least 50% (e.g., at least 60%, 70%, 75%, 80%, 85%, 90%, 95%, 96%, 97%, 98%, or 99%) of the heavy chains in the composition has been converted to pyroglutamate. In certain embodiments, the N-terminal amino acid residue of no more than 50% (e.g., no more than 1%, 2%, 3%, 4%, 5%, 10%, 15%, 20%, 25%, 30%, or 40%) of the light chains in the composition has been converted to pyroglutamate. In certain embodiments, the N-terminal amino acid residue of at least 50% (e.g., at least 60%, 70%, 75%, 80%, 85%, 90%, 95%, 96%, 97%, 98%, or 99%) of the heavy chains in the composition has been converted to pyroglutamate, and the N-terminal amino acid residue of no more than 50% (e.g., no more than 1%, 2%, 3%, 4%, 5%, 10%, 15%, 20%, 25%, 30%, or 40%) of the light chains in the

composition has been converted to pyroglutamate.

[00198] In a specific embodiment, pharmaceutical compositions comprise an anti-LAG-3 (e.g., human LAG-3) antibody described herein, and optionally one or more additional prophylactic or therapeutic agents, in a pharmaceutically acceptable carrier. In a specific
5 embodiment, pharmaceutical compositions comprise an effective amount of an antibody described herein, and optionally one or more additional prophylactic or therapeutic agents, in a pharmaceutically acceptable carrier. In some embodiments, the antibody is the only active ingredient included in the pharmaceutical composition. Pharmaceutical compositions described herein can be useful in inhibiting LAG-3 (e.g., human LAG-3) activity and treating
10 a condition, such as cancer or an infectious disease.

[00199] Pharmaceutically acceptable carriers used in parenteral preparations include aqueous vehicles, nonaqueous vehicles, antimicrobial agents, isotonic agents, buffers, antioxidants, local anesthetics, suspending and dispersing agents, emulsifying agents, sequestering or chelating agents and other pharmaceutically acceptable substances.
15 Examples of aqueous vehicles include Sodium Chloride Injection, Ringers Injection, Isotonic Dextrose Injection, Sterile Water Injection, Dextrose and Lactated Ringers Injection. Nonaqueous parenteral vehicles include fixed oils of vegetable origin, cottonseed oil, corn oil, sesame oil and peanut oil. Antimicrobial agents in bacteriostatic or fungistatic concentrations can be added to parenteral preparations packaged in multiple-dose containers
20 which include phenols or cresols, mercurials, benzyl alcohol, chlorobutanol, methyl and propyl p-hydroxybenzoic acid esters, thimerosal, benzalkonium chloride and benzethonium chloride. Isotonic agents include sodium chloride and dextrose. Buffers include phosphate and citrate. Antioxidants include sodium bisulfate. Local anesthetics include procaine hydrochloride. Suspending and dispersing agents include sodium carboxymethylcellulose,
25 hydroxypropyl methylcellulose and polyvinylpyrrolidone. Emulsifying agents include Polysorbate 80 (TWEEN[®] 80). A sequestering or chelating agent of metal ions includes EDTA. Pharmaceutical carriers also include ethyl alcohol, polyethylene glycol and propylene glycol for water miscible vehicles; and sodium hydroxide, hydrochloric acid, citric acid or lactic acid for pH adjustment.

[00200] A pharmaceutical composition may be formulated for any route of administration to a subject. Specific examples of routes of administration include intranasal, oral, pulmonary, transdermal, intradermal, and parenteral. Parenteral administration, characterized by either subcutaneous, intramuscular or intravenous injection, is also contemplated herein. Injectables can be prepared in conventional forms, either as liquid solutions or suspensions,

solid forms suitable for solution or suspension in liquid prior to injection, or as emulsions. The injectables, solutions and emulsions also contain one or more excipients. Suitable excipients are, for example, water, saline, dextrose, glycerol or ethanol. In addition, if desired, the pharmaceutical compositions to be administered can also contain minor amounts of non-toxic auxiliary substances such as wetting or emulsifying agents, pH buffering agents, stabilizers, solubility enhancers, and other such agents, such as for example, sodium acetate, sorbitan monolaurate, triethanolamine oleate and cyclodextrins.

[00201] Preparations for parenteral administration of an antibody include sterile solutions ready for injection, sterile dry soluble products, such as lyophilized powders, ready to be combined with a solvent just prior to use, including hypodermic tablets, sterile suspensions ready for injection, sterile dry insoluble products ready to be combined with a vehicle just prior to use and sterile emulsions. The solutions may be either aqueous or nonaqueous.

[00202] If administered intravenously, suitable carriers include physiological saline or phosphate buffered saline (PBS), and solutions containing thickening and solubilizing agents, such as glucose, polyethylene glycol, and polypropylene glycol and mixtures thereof.

[00203] Topical mixtures comprising an antibody are prepared as described for the local and systemic administration. The resulting mixture can be a solution, suspension, emulsions or the like and can be formulated as creams, gels, ointments, emulsions, solutions, elixirs, lotions, suspensions, tinctures, pastes, foams, aerosols, irrigations, sprays, suppositories, bandages, dermal patches or any other formulations suitable for topical administration.

[00204] An anti-LAG-3 (*e.g.*, human LAG-3) antibody described herein can be formulated as an aerosol for topical application, such as by inhalation (see, *e.g.*, U.S. Patent Nos. 4,044,126, 4,414,209 and 4,364,923, which describe aerosols for delivery of a steroid useful for treatment of inflammatory diseases, particularly asthma and are incorporated by reference in their entireties). These formulations for administration to the respiratory tract can be in the form of an aerosol or solution for a nebulizer, or as a microfine powder for insufflations, alone or in combination with an inert carrier such as lactose. In such a case, the particles of the formulation will, in one embodiment, have diameters of less than 50 microns, in one embodiment less than 10 microns.

[00205] An anti-LAG-3 (*e.g.*, human LAG-3) antibody described herein can be formulated for local or topical application, such as for topical application to the skin and mucous membranes, such as in the eye, in the form of gels, creams, and lotions and for application to the eye or for intracisternal or intraspinal application. Topical administration is contemplated for transdermal delivery and also for administration to the eyes or mucosa, or for inhalation

therapies. Nasal solutions of the antibody alone or in combination with other pharmaceutically acceptable excipients can also be administered.

[00206] Transdermal patches, including iontophoretic and electrophoretic devices, are well known to those of skill in the art, and can be used to administer an antibody. For example, such patches are disclosed in U.S. Patent Nos. 6,267,983, 6,261,595, 6,256,533, 6,167,301, 6,024,975, 6,010,715, 5,985,317, 5,983,134, 5,948,433, and 5,860,957, all of which are herein incorporated by reference in their entireties.

[00207] In certain embodiments, a pharmaceutical composition comprising an antibody described herein is a lyophilized powder, which can be reconstituted for administration as solutions, emulsions and other mixtures. It may also be reconstituted and formulated as solids or gels. The lyophilized powder is prepared by dissolving an antibody described herein, or a pharmaceutically acceptable derivative thereof, in a suitable solvent. In some embodiments, the lyophilized powder is sterile. The solvent may contain an excipient which improves the stability or other pharmacological component of the powder or reconstituted solution, prepared from the powder. Excipients that may be used include, but are not limited to, dextrose, sorbitol, fructose, corn syrup, xylitol, glycerin, glucose, sucrose or other suitable agent. The solvent may also contain a buffer, such as citrate, sodium or potassium phosphate or other such buffer known to those of skill in the art at, in one embodiment, about neutral pH. Subsequent sterile filtration of the solution followed by lyophilization under standard conditions known to those of skill in the art provides the desired formulation. In one embodiment, the resulting solution will be apportioned into vials for lyophilization. Each vial will contain a single dosage or multiple dosages of the compound. The lyophilized powder can be stored under appropriate conditions, such as at about 4°C to room temperature. Reconstitution of this lyophilized powder with water for injection provides a formulation for use in parenteral administration. For reconstitution, the lyophilized powder is added to sterile water or other suitable carrier. The precise amount depends upon the selected compound. Such amount can be empirically determined.

[00208] The anti-LAG-3 (*e.g.*, human LAG-3) antibodies described herein and other compositions provided herein can also be formulated to be targeted to a particular tissue, receptor, or other area of the body of the subject to be treated. Many such targeting methods are well known to those of skill in the art. All such targeting methods are contemplated herein for use in the instant compositions. For non-limiting examples of targeting methods, see, *e.g.*, U.S. Patent Nos. 6,316,652, 6,274,552, 6,271,359, 6,253,872, 6,139,865, 6,131,570, 6,120,751, 6,071,495, 6,060,082, 6,048,736, 6,039,975, 6,004,534, 5,985,307, 5,972,366,

5,900,252, 5,840,674, 5,759,542 and 5,709,874, all of which are herein incorporated by reference in their entireties. In a specific embodiment, an antibody described herein is targeted to a tumor.

[00209] The compositions to be used for *in vivo* administration can be sterile. This is readily accomplished by filtration through, *e.g.*, sterile filtration membranes.

6.4 Methods of Use and Uses

[00210] In another aspect, the instant disclosure provides a method of treating a subject using the anti-LAG-3 (*e.g.*, human LAG-3) antibodies disclosed herein. Any disease or disorder in a subject that would benefit from inhibition of LAG-3 (*e.g.*, human LAG-3) function can be treated using the anti-LAG-3 (*e.g.*, human LAG-3) antibodies disclosed herein. The anti-LAG-3 (*e.g.*, human LAG-3) antibodies disclosed herein are particularly useful for inhibiting immune system tolerance to tumors, and accordingly can be used as an immunotherapy for subjects with cancer. For example, in certain embodiments, the instant disclosure provides a method of increasing T cell activation in response to an antigen in a subject, the method comprising administering to the subject an effective amount of an anti-LAG-3 (*e.g.*, human LAG-3) antibody or pharmaceutical composition thereof, as disclosed herein. In certain embodiments, the instant disclosure provides a method of treating cancer in a subject, the method comprising administering to the subject an effective amount of the antibody or pharmaceutical composition, as disclosed herein.

[00211] Cancers that can be treated with the anti-LAG-3 (*e.g.*, human LAG-3) antibodies or pharmaceutical compositions disclosed herein include, without limitation, a solid tumor, a hematologic cancer, leukemia, lymphoma, osteosarcoma, rhabdomyosarcoma, neuroblastoma, kidney cancer, renal transitional cell cancer, bladder cancer, Wilm's cancer, ovarian cancer, pancreatic cancer, breast cancer (*e.g.*, characterized by a mutation in BRCA1 and/or BRCA2), prostate cancer, bone cancer, lung cancer (*e.g.*, non-small cell lung cancer or small cell lung cancer), gastric cancer, colorectal cancer, cervical cancer, synovial sarcoma, head and neck cancer, squamous cell carcinoma, multiple myeloma, renal cell cancer, retinoblastoma, hepatoblastoma, hepatocellular carcinoma, melanoma, rhabdoid tumor of the kidney, Ewing's sarcoma, chondrosarcoma, brain cancer, glioblastoma, meningioma, pituitary adenoma, vestibular schwannoma, a primitive neuroectodermal tumor, medulloblastoma, astrocytoma, anaplastic astrocytoma, oligodendroglioma, ependymoma, choroid plexus papilloma, polycythemia vera, thrombocythemia, idiopathic myelofibrosis, soft tissue sarcoma, thyroid cancer, endometrial cancer, carcinoid cancer, liver cancer, epithelial cancer, and peritoneal cancer. In certain embodiments, the cancer is metastatic cancer, *e.g.*, of the

varieties described above.

[00212] In certain embodiments, the cancer is a solid tumor, a hematological cancer (*e.g.*, leukemia, lymphoma, myeloma), and a metastatic lesion thereof. In one embodiment, the cancer is a solid tumor. Examples of solid tumors include malignancies, *e.g.*, sarcomas and carcinomas (*e.g.*, adenocarcinomas) of the various organ systems, such as those affecting lung, breast, lymphoid, gastrointestinal or colorectal, genitals and genitourinary tract (*e.g.*, renal, urothelial, bladder cells), pharynx, CNS (*e.g.*, brain, neural or glial cells), skin (*e.g.*, melanoma), head and neck (*e.g.*, head and neck squamous cell carcinoma (HNSC)), and pancreas. For example, melanoma, colon cancers, gastric cancer, rectal cancer, renal-cell carcinoma, breast cancer (*e.g.*, a breast cancer that does not express one, two or all of estrogen receptor, progesterone receptor, or Her2/neu, *e.g.*, a triple negative breast cancer), liver cancer, a lung cancer (*e.g.*, a non-small cell lung cancer (NSCLC) (*e.g.*, a NSCLC with squamous and/or non-squamous histology) or small cell lung cancer), prostate cancer, cancer of head or neck (*e.g.*, HPV+ squamous cell carcinoma), cancer of the small intestine and cancer of the esophagus.

[00213] In one embodiment, the cancer is a hematological cancer, for example, a leukemia, a lymphoma, or a myeloma. In one embodiment, the cancer is a leukemia, for example, acute lymphoblastic leukemia (ALL), acute myelogenous leukemia (AML), acute myeloblastic leukemia (AML), chronic lymphocytic leukemia (CLL), chronic myelogenous leukemia (CML), chronic myeloid leukemia (CML), chronic myelomonocytic leukemia (CMML), chronic lymphocytic leukemia (CLL), or hairy cell leukemia. In one embodiment, the cancer is a lymphoma, for example, B cell lymphoma, diffuse large B-cell lymphoma (DLBCL), activated B-cell like (ABC) diffuse large B cell lymphoma, germinal center B cell (GCB) diffuse large B cell lymphoma, mantle cell lymphoma, Hodgkin lymphoma, non-Hodgkin lymphoma, relapsed non-Hodgkin lymphoma, refractory non-Hodgkin lymphoma, recurrent follicular non-Hodgkin lymphoma, Burkitt lymphoma, small lymphocytic lymphoma, follicular lymphoma, lymphoplasmacytic lymphoma, or extranodal marginal zone lymphoma. In one embodiment the cancer is a myeloma, for example, multiple myeloma.

[00214] In certain embodiments, the instant disclosure provides a method of preventing or treating an infectious disease in a subject, the method comprising administering to the subject an effective amount of an anti-LAG-3 (*e.g.*, human LAG-3) antibody or pharmaceutical composition thereof, as disclosed herein. In one embodiment, provided herein are methods for preventing and/or treating an infection (*e.g.*, a viral infection, a bacterial infection, a fungal infection, a protozoal infection, or a parasitic infection). The infection prevented

and/or treated in accordance with the methods can be caused by an infectious agent identified herein. In a specific embodiment, an anti-LAG-3 (*e.g.*, human LAG-3) antibody described herein or a composition thereof is the only active agent administered to a subject. In some embodiments, an anti-LAG-3 (*e.g.*, human LAG-3) antibody described herein or a composition thereof is used in combination with anti-infective interventions (*e.g.*, antivirals, antibacterials, antifungals, or anti-helminthics) for the treatment of infectious diseases.

[00215] Infectious diseases that can be treated and/or prevented by anti-LAG-3 (*e.g.*, human LAG-3) antibodies or pharmaceutical compositions disclosed herein are caused by infectious agents including but not limited to bacteria, parasites, fungi, protozoa, and viruses.

In a specific embodiment, the infectious disease treated and/or prevented by anti-LAG-3 (*e.g.*, human LAG-3) antibodies or pharmaceutical compositions disclosed herein is caused by a virus. Viral diseases or viral infections that can be prevented and/or treated in accordance with the methods described herein include, but are not limited to, those caused by hepatitis type A, hepatitis type B, hepatitis type C, influenza (*e.g.*, influenza A or influenza B), varicella, adenovirus, herpes simplex type I (HSV-I), herpes simplex type II (HSV-II), rinderpest, rhinovirus, echovirus, rotavirus, respiratory syncytial virus, papilloma virus, papova virus, cytomegalovirus, echinovirus, arbovirus, huntavirus, coxsackie virus, mumps virus, measles virus, rubella virus, polio virus, small pox, Epstein Barr virus, human immunodeficiency virus type I (HIV-I), human immunodeficiency virus type II (HIV-II), and agents of viral diseases such as viral meningitis, encephalitis, dengue or small pox.

[00216] Bacterial infections that can be prevented and/or treated include infections caused by *Escherichia coli*, *Klebsiella pneumoniae*, *Staphylococcus aureus*, *Enterococcus faecalis*, *Proteus vulgaris*, *Staphylococcus viridans*, and *Pseudomonas aeruginosa*. Bacterial diseases caused by bacteria (*e.g.*, *Escherichia coli*, *Klebsiella pneumoniae*, *Staphylococcus aureus*, *Enterococcus faecalis*, *Proteus vulgaris*, *Staphylococcus viridans*, and *Pseudomonas aeruginosa*) that can be prevented and/or treated in accordance with the methods described herein include, but are not limited to, *Mycobacteria rickettsia*, *Mycoplasma*, *Neisseria*, *S. pneumonia*, *Borrelia burgdorferi* (Lyme disease), *Bacillus anthracis* (anthrax), tetanus, *Streptococcus*, *Staphylococcus*, *mycobacterium*, pertissus, cholera, plague, diphtheria, chlamydia, *S. aureus* and legionella.

[00217] Protozoal diseases or protozoal infections caused by protozoa that can be prevented and/or treated in accordance with the methods described herein include, but are not limited to, leishmania, coccidiosis, *trypanosoma schistosoma* or malaria. Parasitic diseases or parasitic infections caused by parasites that can be prevented and/or treated in accordance

with the methods described herein include, but are not limited to, chlamydia and rickettsia.

[00218] Fungal diseases or fungal infections that can be prevented and/or treated in accordance with the methods described herein include, but are not limited to, those caused by *Candida* infections, zygomycosis, *Candida* mastitis, progressive disseminated
5 trichosporonosis with latent trichosporonemia, disseminated candidiasis, pulmonary paracoccidioidomycosis, pulmonary aspergillosis, *Pneumocystis carinii* pneumonia, cryptococcal meningitis, *coccidioidal* meningoencephalitis and cerebrospinal vasculitis, *Aspergillus niger* infection, *Fusarium keratitis*, paranasal sinus mycoses, *Aspergillus fumigatus* endocarditis, tibial dyschondroplasia, *Candida glabrata* vaginitis, oropharyngeal
10 candidiasis, X-linked chronic granulomatous disease, tinea pedis, cutaneous candidiasis, mycotic placentitis, disseminated trichosporonosis, allergic bronchopulmonary aspergillosis, mycotic keratitis, *Cryptococcus neoformans* infection, fungal peritonitis, *Curvularia geniculata* infection, staphylococcal endophthalmitis, sporotrichosis, and dermatophytosis.

[00219] In certain embodiments, the instant disclosure provides a method of preventing or
15 treating a disease or disorder of the nervous system in a subject, the method comprising administering to the subject an effective amount of an anti-LAG-3 (*e.g.*, human LAG-3) antibody or pharmaceutical composition thereof, as disclosed herein. In some embodiments, the disease or disorder of the nervous system is a synucleinopathy. In some embodiments, the disease or disorder of the nervous system is Parkinson's disease.

[00220] In certain embodiments, these methods further comprise administering an
20 additional therapeutic agent to the subject. In certain embodiments, the additional therapeutic agent is a chemotherapeutic, radiotherapeutic, or a checkpoint targeting agent. In certain embodiments, the chemotherapeutic agent is a hypomethylating agent (*e.g.*, azacitidine). In certain embodiments, the checkpoint targeting agent is selected from the group consisting of
25 an antagonist anti-CTLA-4 antibody, an antagonist anti-PD-L1 antibody, an antagonist anti-PD-L2 antibody, an antagonist anti-PD-1 antibody, an antagonist anti-TIM-3 antibody, an antagonist anti-LAG-3 antibody, an antagonist anti-CEACAM1 antibody, an agonist anti-GITR antibody, an agonist anti-OX40 antibody, an antagonist anti-TIGIT antibody, an agonist anti-CD137 antibody, an antagonist anti-VISTA antibody, an antagonist anti-CD73
30 antibody, and an antagonist anti-CD96 antibody.

[00221] In one embodiment, the present invention relates to an antibody and/or pharmaceutical composition of the present invention for use in a method of the present invention, wherein the method further comprises administering an additional therapeutic agent to the subject. In one embodiment, the present invention relates to (a) an antibody

and/or pharmaceutical composition of the present invention and (b) an additional therapeutic agent for use as a medicament. In one embodiment, the present invention relates to (a) an antibody and/or pharmaceutical composition of the present invention, and (b) an additional therapeutic agent for use in a method for the treatment of cancer. In a further embodiment, the present invention relates to a pharmaceutical composition, kit or kit-of-parts comprising (a) an antibody and/or pharmaceutical composition of the present invention and (b) an additional therapeutic agent. In one embodiment, the additional therapeutic agent is a chemotherapeutic, a radiotherapeutic, or a checkpoint targeting agent.

[00222] In certain embodiments, an anti-PD-1 antibody is used in methods disclosed herein. In certain embodiments, the anti-PD-1 antibody is nivolumab, also known as BMS-936558 or MDX1106, developed by Bristol-Myers Squibb. In certain embodiments, the anti-PD-1 antibody is pembrolizumab, also known as lambrolizumab or MK-3475, developed by Merck & Co. In certain embodiments, the anti-PD-1 antibody is pidilizumab, also known as CT-011, developed by CureTech. In certain embodiments, the anti-PD-1 antibody is MEDI0680, also known as AMP-514, developed by Medimmune. In certain embodiments, the anti-PD-1 antibody is PDR001 developed by Novartis Pharmaceuticals. In certain embodiments, the anti-PD-1 antibody is REGN2810 developed by Regeneron Pharmaceuticals. In certain embodiments, the anti-PD-1 antibody is PF-06801591 developed by Pfizer. In certain embodiments, the anti-PD-1 antibody is BGB-A317 developed by BeiGene. In certain embodiments, the anti-PD-1 antibody is TSR-042 developed by AnaptysBio and Tesaro. In certain embodiments, the anti-PD-1 antibody is SHR-1210 developed by Hengrui.

[00223] Further non-limiting examples of anti-PD-1 antibodies that may be used in treatment methods disclosed herein are disclosed in the following patents and patent applications, all of which are herein incorporated by reference in their entireties: U.S. Patent No. 6,808,710; U.S. Patent No. 7,332,582; U.S. Patent No. 7,488,802; U.S. Patent No. 8,008,449; U.S. Patent No. 8,114,845; U.S. Patent No. 8,168,757; U.S. Patent No. 8,354,509; U.S. Patent No. 8,686,119; U.S. Patent No. 8,735,553; U.S. Patent No. 8,747,847; U.S. Patent No. 8,779,105; U.S. Patent No. 8,927,697; U.S. Patent No. 8,993,731; U.S. Patent No. 9,102,727; U.S. Patent No. 9,205,148; U.S. Publication No. US 2013/0202623 A1; U.S. Publication No. US 2013/0291136 A1; U.S. Publication No. US 2014/0044738 A1; U.S. Publication No. US 2014/0356363 A1; U.S. Publication No. US 2016/0075783 A1; and PCT Publication No. WO 2013/033091 A1; PCT Publication No. WO 2015/036394 A1; PCT Publication No. WO 2014/179664 A2; PCT Publication No. WO 2014/209804 A1; PCT

Publication No. WO 2014/206107 A1; PCT Publication No. WO 2015/058573 A1; PCT Publication No. WO 2015/085847 A1; PCT Publication No. WO 2015/200119 A1; PCT Publication No. WO 2016/015685 A1; and PCT Publication No. WO 2016/020856 A1.

[00224] In certain embodiments, an anti-PD-L1 antibody is used in methods disclosed
5 herein. In certain embodiments, the anti-PD-L1 antibody is atezolizumab developed by Genentech. In certain embodiments, the anti-PD-L1 antibody is durvalumab developed by AstraZeneca, Celgene and Medimmune. In certain embodiments, the anti-PD-L1 antibody is avelumab, also known as MSB0010718C, developed by Merck Serono and Pfizer. In certain
10 embodiments, the anti-PD-L1 antibody is MDX-1105 developed by Bristol-Myers Squibb. In certain embodiments, the anti-PD-L1 antibody is AMP-224 developed by Amplimmune and GSK.

[00225] Non-limiting examples of anti-PD-L1 antibodies that may be used in treatment methods disclosed herein are disclosed in the following patents and patent applications, all of which are herein incorporated by reference in their entireties: US Patent No. 7,943,743; US
15 Patent No. 8,168,179; US Patent No. 8,217,149; U.S. Patent No. 8,552,154; U.S. Patent No. 8,779,108; U.S. Patent No. 8,981,063; U.S. Patent No. 9,175,082; U.S. Publication No. US 2010/0203056 A1; U.S. Publication No. US 2003/0232323 A1; U.S. Publication No. US 2013/0323249 A1; U.S. Publication No. US 2014/0341917 A1; U.S. Publication No. US 2014/0044738 A1; U.S. Publication No. US 2015/0203580 A1; U.S. Publication No. US
20 2015/0225483 A1; U.S. Publication No. US 2015/0346208 A1; U.S. Publication No. US 2015/0355184 A1; and PCT Publication No. WO 2014/100079 A1; PCT Publication No. WO 2014/022758 A1; PCT Publication No. WO 2014/055897 A2; PCT Publication No. WO 2015/061668 A1; PCT Publication No. WO 2015/109124 A1; PCT Publication No. WO 2015/195163 A1; PCT Publication No. WO 2016/000619 A1; and PCT Publication No. WO
25 2016/030350 A1.

[00226] In certain embodiments, an anti-CTLA-4 antibody is used in methods disclosed herein. In certain embodiments, the anti-CTLA-4 antibody is ipilimumab developed by Bristol-Myers Squibb. In certain embodiments, the anti-CTLA-4 antibody is ipilimumab developed by Bristol-Myers Squibb. In certain embodiments, the anti-CTLA-4 antibody is
30 tremelimumab developed by Pfizer and Medimmune.

[00227] Non-limiting examples of anti-CTLA-4 antibodies that may be used in treatment methods disclosed herein are disclosed in the following patents and patent applications, all of which are herein incorporated by reference in their entireties: US Patent No. 6,984,720; US Patent No. 7,411,057; US Patent No. 7,034,121; US Patent No. 8,697,845; U.S. Publication

No. US 2009/0123477 A1; U.S. Publication No. US 2014/0105914 A1; U.S. Publication No. US 2013/0267688 A1; U.S. Publication No. US 2016/0145355 A1; PCT Publication No. WO 2014/207064 A1; and PCT Publication No. WO 2016/015675 A1.

[00228] In certain embodiments, an anti-LAG-3 (*e.g.*, human LAG-3) antibody disclosed
5 herein is administered to a subject in combination with a compound that targets an immunomodulatory enzyme(s) such as IDO (indoleamine-(2,3)-dioxygenase) and/or TDO (tryptophan 2,3-dioxygenase). In certain embodiments, such compound is selected from the group consisting of epacadostat (Incyte Corp; *see, e.g.*, WO 2010/005958 which is herein incorporated by reference in its entirety), BMS-986205 (Flexus Biosciences/Bristol-Myers
10 Squibb), indoximod (NewLink Genetics), and NLG919 (NewLink Genetics). In one embodiment, the compound is epacadostat. In another embodiment, the compound is BMS-986205. In another embodiment, the compound is indoximod. In another embodiment, the compound is NLG919. In a specific embodiment, an anti-LAG-3 (*e.g.*, human LAG-3) antibody disclosed herein is administered to a subject in combination with an IDO inhibitor
15 for treating cancer. The IDO inhibitor as described herein for use in treating cancer is present in a solid dosage form of a pharmaceutical composition such as a tablet, a pill or a capsule, wherein the pharmaceutical composition includes an IDO inhibitor and a pharmaceutically acceptable excipient. As such, the antibody as described herein and the IDO inhibitor as described herein can be administered separately, sequentially or concurrently as separate
20 dosage forms. In one embodiment, the antibody is administered parenterally, and the IDO inhibitor is administered orally. In particular embodiments, the inhibitor is selected from the group consisting of epacadostat (Incyte Corporation), BMS-986205 (Flexus Biosciences/Bristol-Myers Squibb), indoximod (NewLink Genetics), and NLG919 (NewLink Genetics). Epacadostat has been described in PCT Publication No. WO 2010/005958, which
25 is herein incorporated by reference in its entirety. In one embodiment, the inhibitor is epacadostat. In another embodiment, the inhibitor is BMS-986205. In another embodiment, the inhibitor is indoximod. In another embodiment, the inhibitor is NLG919.

[00229] In certain embodiments, the instant disclosure provides an isolated antibody that specifically binds to LAG-3 (*e.g.*, human LAG-3) in combination with an anti-PD-1 antibody
30 (*e.g.*, pembrolizumab or nivolumab) and an inhibitor of indoleamine-2,3-dioxygenase (IDO). In certain embodiments, the anti-PD-1 antibody is pembrolizumab. In certain embodiments, the anti-PD-1 antibody is nivolumab. In certain embodiments, the IDO inhibitor is selected from the group consisting of epacadostat, BMS-986205, indoximod, and NLG919. In certain embodiments, the IDO inhibitor is epacadostat. In certain embodiments, the IDO inhibitor is

BMS-986205. In certain embodiments, the IDO inhibitor is indoximod.

[00230] In certain embodiments, an anti-LAG-3 (*e.g.*, human LAG-3) antibody disclosed herein is administered to a subject in combination with another anti-cancer agent. Exemplary anti-cancer agents include antibody therapeutics such as trastuzumab (Herceptin), antibodies to co-stimulatory or co-inhibitory molecules such as CTLA-4, CD137, and PD-1, and antibodies to cytokines such as IL-10 and TGF- β .

[00231] In certain embodiments, the additional therapeutic agent is an inhibitor of JAK, PI3Kdelta, BRD, PI3Kgamma, or Axl/Mer. In certain embodiments, the additional therapeutic agent is an inhibitor of JAK, including JAK1 and/or JAK2. In certain embodiments, the additional therapeutic agent is an inhibitor of PI3Kdelta. In certain embodiments, the additional therapeutic agent is an inhibitor of BRD. In certain embodiments, the additional therapeutic agent is an inhibitor of PI3Kgamma. In certain embodiments, the additional therapeutic agent is an inhibitor of Axl/Mer.

[00232] Additional examples of anti-cancer agents include those that block immune cell migration such as antagonists to chemokine receptors, including CCR2 and CCR4, and those that augment the immune system such as adjuvants or adoptive T cell transfer.

[00233] One or more additional immune checkpoint modulators can be used in combination with an anti-LAG-3 (*e.g.*, human LAG-3) antibody disclosed herein for treatment of any diseases, disorders, or conditions described herein, *e.g.*, TAM-associated diseases, disorders, or conditions. Exemplary immune checkpoint modulators include modulators against immune checkpoint molecules such as CD27, CD28, CD40, CD122, CD96, CD73, CD47, CD96, CD137, OX40, GITR, CSF1R, JAK, PI3K delta, PI3K gamma, TAM, arginase, CD137 (also known as 4-1BB), ICOS, A2AR, B7-H3, B7-H4, BTLA, CTLA-4, LAG-3, TIM-3, TIGIT, VISTA, PD-1, PD-L1, and PD-L2. In some embodiments, the immune checkpoint molecule is a co-stimulatory checkpoint molecule selected from CD27, CD28, CD40, ICOS, OX40, GITR, and CD137. In some embodiments, the immune checkpoint molecule is a co-inhibitory checkpoint molecule selected from A2AR, B7-H3, B7-H4, BTLA, CTLA-4, IDO, KIR, PD-1, TIM-3, and VISTA. In some embodiments, an anti-LAG-3 (*e.g.*, human LAG-3) antibody disclosed herein can be used in combination with one or more agents selected from KIR inhibitors, TIGIT inhibitors, LAIR1 inhibitors, CD160 inhibitors, 2B4 inhibitors, and TGFR beta inhibitors.

[00234] In some embodiments, the modulator of an immune checkpoint molecule is an antagonistic anti-PD1 antibody, an antagonistic anti-PD-L1 antibody, or an antagonistic anti-CTLA-4 antibody.

[00235] In some embodiments, the modulator of an immune checkpoint molecule is an agonist of GITR, *e.g.*, an agonistic anti-GITR antibody. In some embodiments, the agonistic anti-GITR antibody is TRX518 or MK-4166.

5 [00236] In some embodiments, the modulator of an immune checkpoint molecule is an agonist of OX40, *e.g.*, an agonistic anti-OX40 antibody or OX40L fusion protein. In some embodiments, the agonistic anti-OX40 antibody is MEDI0562. In some embodiments, the OX40L fusion protein is MEDI6383.

[00237] An anti-LAG-3 (*e.g.*, human LAG-3) antibody disclosed herein can be used in combination with one or more agents for the treatment of diseases such as cancer. In some
10 embodiments, the agent is an alkylating agent, a proteasome inhibitor, a corticosteroid, or an immunomodulatory agent. Examples of an alkylating agent include cyclophosphamide (CY), melphalan (MEL), and bendamustine. In some embodiments, the proteasome inhibitor is carfilzomib. In some embodiments, the corticosteroid is dexamethasone (DEX). In some embodiments, the immunomodulatory agent is lenalidomide (LEN) or pomalidomide (POM).

15 [00238] In certain embodiments, an anti-LAG-3 (*e.g.*, human LAG-3) antibody disclosed herein is administered to a subject in combination with a vaccine. The vaccine can be, *e.g.*, a peptide vaccine, a DNA vaccine, or an RNA vaccine. In certain embodiments, the vaccine is a heat shock protein based tumor vaccine or a heat shock protein based pathogen vaccine. In a specific embodiment, an anti-LAG-3 (*e.g.*, human LAG-3) antibody disclosed herein is
20 administered to a subject in combination with a heat shock protein based tumor-vaccine. Heat shock proteins (HSPs) are a family of highly conserved proteins found ubiquitously across all species. Their expression can be powerfully induced to much higher levels as a result of heat shock or other forms of stress, including exposure to toxins, oxidative stress or glucose deprivation. Five families have been classified according to molecular weight: HSP-
25 110, -90, -70, -60 and -28. HSPs deliver immunogenic peptides through the cross-presentation pathway in antigen presenting cells (APCs) such as macrophages and dendritic cells (DCs), leading to T cell activation. HSPs function as chaperone carriers of tumor-associated antigenic peptides forming complexes able to induce tumor-specific immunity. Upon release from dying tumor cells, the HSP-antigen complexes are taken up by antigen-
30 presenting cells (APCs) wherein the antigens are processed into peptides that bind MHC class I and class II molecules leading to the activation of anti-tumor CD8⁺ and CD4⁺ T cells. The immunity elicited by HSP complexes derived from tumor preparations is specifically directed against the unique antigenic peptide repertoire expressed by the cancer of each subject. Therefore, in one embodiment, the present invention relates to (a) an antibody and/or

pharmaceutical composition of the present invention and (b) a vaccine for use as a medicament, for example for use in a method for the treatment of cancer. In one embodiment, the present invention relates to a pharmaceutical composition, kit or kit-of-parts comprising (a) an antibody and/or pharmaceutical composition of the present invention and (b) a vaccine.

5 In one embodiment, the vaccine is a heat shock protein based tumor vaccine. In one embodiment, the vaccine is a heat shock protein based pathogen vaccine.

[00239] A heat shock protein peptide complex (HSPPC) is a protein peptide complex consisting of a heat shock protein non-covalently complexed with antigenic peptides. HSPPCs elicit both innate and adaptive immune responses. In a specific embodiment, the antigenic peptide(s) displays antigenicity for the cancer being treated. HSPPCs are efficiently seized by APCs via membrane receptors (mainly CD91) or by binding to Toll-like receptors. HSPPC internalization results in functional maturation of the APCs with chemokine and cytokine production leading to activation of natural killer cells (NK), monocytes and Th1 and Th-2-mediated immune responses. In certain embodiments, HSPPCs used in methods disclosed herein comprise one or more heat shock proteins from the hsp60, hsp70, or hsp90 family of stress proteins complexed with antigenic peptides. In certain embodiments, HSPPCs comprise hsc70, hsp70, hsp90, hsp110, grp170, gp96, calreticulin, or combinations of two or more thereof.

[00240] In a specific embodiment, the heat shock protein peptide complex (HSPPC) comprises recombinant heat shock proteins (*e.g.*, hsp70 or hsc70) or a peptide-binding domain thereof complexed with recombinant antigenic peptides. Recombinant heat shock proteins can be produced by recombinant DNA technology, for example, using human hsc70 sequence as described in Dworniczak and Mirault, *Nucleic Acids Res.* 15:5181-5197 (1987) and GenBank accession no. P11142 and/or Y00371, each of which is incorporated herein by reference in its entirety. In certain embodiments, Hsp70 sequences are as described in Hunt and Morimoto *Proc. Natl. Acad. Sci. U.S.A.* 82 (19), 6455-6459 (1985) and GenBank accession no. P0DMV8 and/or M11717, each of which is incorporated herein by reference in its entirety. Antigenic peptides can also be prepared by recombinant DNA methods known in the art.

30 [00241] In certain embodiments, the antigenic peptides comprise a modified amino acid. In certain embodiments, the modified amino acid comprises a post-translational modification. In certain embodiments, the modified amino acid comprises a mimetic of a post-translational modification. In certain embodiments, the modified amino acid is a Tyr, Ser, Thr, Arg, Lys, or His that has been phosphorylated on a side chain hydroxyl or amine. In certain

embodiments, the modified amino acid is a mimetic of a Tyr, Ser, Thr, Arg, Lys, or His amino acid that has been phosphorylated on a side chain hydroxyl or amine.

[00242] In a specific embodiment, an anti-LAG-3 (*e.g.*, human LAG-3) antibody disclosed herein is administered to a subject in combination with a heat shock protein peptide complex (HSPPC), *e.g.*, heat shock protein peptide complex-96 (HSPPC-96), to treat cancer. HSPPC-96 comprises a 96 kDa heat shock protein (Hsp), gp96, complexed to antigenic peptides. HSPPC-96 is a cancer immunotherapy manufactured from a subject's tumor and contains the cancer's antigenic "fingerprint." In certain embodiments, this fingerprint contains unique antigens that are present only in that particular subject's specific cancer cells and injection of the vaccine is intended to stimulate the subject's immune system to recognize and attack any cells with the specific cancer fingerprint. Therefore, in one embodiment, the present invention relates to an antibody and/or pharmaceutical composition of the present invention in combination with a heat shock protein peptide complex (HSPPC) for use as a medicament and/or for use in a method for the treatment of cancer.

[00243] In certain embodiments, the HSPPC, *e.g.*, HSPPC-96, is produced from the tumor tissue of a subject. In a specific embodiment, the HSPPC (*e.g.*, HSPPC-96) is produced from a tumor of the type of cancer or metastasis thereof being treated. In another specific embodiment, the HSPPC (*e.g.*, HSPPC-96) is autologous to the subject being treated. In certain embodiments, the tumor tissue is non-necrotic tumor tissue. In certain embodiments, at least 1 gram (*e.g.*, at least 1, at least 2, at least 3, at least 4, at least 5, at least 6, at least 7, at least 8, at least 9, or at least 10 grams) of non-necrotic tumor tissue is used to produce a vaccine regimen. In certain embodiments, after surgical resection, non-necrotic tumor tissue is frozen prior to use in vaccine preparation. In some embodiments, the HSPPC, *e.g.*, HSPPC-96, is isolated from the tumor tissue by purification techniques, filtered and prepared for an injectable vaccine. In certain embodiments, a subject is administered 6-12 doses of the HSPPC, *e.g.*, HSPPC-96. In such embodiments, the HSPPC, *e.g.*, HSPPC-96, doses may be administered weekly for the first 4 doses and then biweekly for the 2-8 additional doses.

[00244] Further examples of HSPPCs that may be used in accordance with the methods described herein are disclosed in the following patents and patent applications, all of which are herein incorporated by reference in their entireties: U.S. Patent Nos. 6,391,306, 6,383,492, 6,403,095, 6,410,026, 6,436,404, 6,447,780, 6,447,781 and 6,610,659, all of which are herein incorporated by reference in their entireties.

[00245] In certain embodiments, an anti-LAG-3 antibody disclosed herein is administered to a subject in combination with an adjuvant. Various adjuvants can be used depending on

the treatment context. Non-limiting examples of appropriate adjuvants include, but not limited to, Complete Freund's Adjuvant (CFA), Incomplete Freund's Adjuvant (IFA), montanide ISA (incomplete Seppic adjuvant), the Ribi adjuvant system (RAS), Titer Max, muramyl peptides, Syntex Adjuvant Formulation (SAF), alum (aluminum hydroxide and/or aluminum phosphate), aluminum salt adjuvants, Gerbu[®] adjuvants, nitrocellulose absorbed antigen, encapsulated or entrapped antigen, 3 De-O-acylated monophosphoryl lipid A (3 D-MPL), immunostimulatory oligonucleotides, toll-like receptor (TLR) ligands, mannan-binding lectin (MBL) ligands, STING agonists, immuno-stimulating complexes such as saponins, Quil A, QS-21, QS-7, ISCOMATRIX, and others. Other adjuvants include CpG oligonucleotides and double stranded RNA molecules, such as poly(A) and poly(U). Combinations of the above adjuvants may also be used. See, *e.g.*, U.S. Patent Nos. 6,645,495; 7,029,678; and 7,858,589, all of which are incorporated herein by reference in their entireties. In one embodiment, the adjuvant used herein is QS-21 STIMULON.

[00246] In certain embodiments, an anti-LAG-3 antibody disclosed herein is administered to a subject in combination with an additional therapeutic agent comprising a TCR. In certain embodiments, the additional therapeutic agent is a soluble TCR. In certain embodiments, the additional therapeutic agent is a cell expressing a TCR. Therefore, in one embodiment, the present invention relates to an antibody and/or pharmaceutical composition of the present invention in combination with an additional therapeutic agent comprising a TCR for use as a medicament and/or for use in a method for the treatment of cancer.

[00247] In certain embodiments, an anti-LAG-3 antibody disclosed herein is administered to a subject in combination with a cell expressing a chimeric antigen receptor (CAR). In certain embodiments, the cell is a T cell.

[00248] In certain embodiments, an anti-LAG-3 antibody disclosed herein is administered to a subject in combination with a TCR mimic antibody. In certain embodiments, the TCR mimic antibody is an antibody that specifically binds to a peptide-MHC complex. For non-limiting examples of TCR mimic antibodies, see, *e.g.*, U.S. Patent No. 9,074,000 and U.S. Publication Nos. US 2009/0304679 A1 and US 2014/0134191 A1, all of which are incorporated herein by reference in their entireties.

[00249] The anti-LAG-3 (*e.g.*, human LAG-3) antibody and the additional therapeutic agent (*e.g.*, chemotherapeutic, radiotherapeutic, checkpoint targeting agent, IDO inhibitor, vaccine, adjuvant, a soluble TCR, a cell expressing a TCR, a cell expressing a chimeric antigen receptor, and/or a TCR mimic antibody) can be administered separately, sequentially or concurrently as separate dosage forms. In one embodiment, an anti-LAG-3 (*e.g.*, human

LAG-3) antibody is administered parenterally, and an IDO inhibitor is administered orally.

[00250] An antibody or pharmaceutical composition described herein may be delivered to a subject by a variety of routes. These include, but are not limited to, parenteral, intranasal, intratracheal, oral, intradermal, topical, intramuscular, intraperitoneal, transdermal, 5 intravenous, intratumoral, conjunctival, intra-arterial, and subcutaneous routes. Pulmonary administration can also be employed, *e.g.*, by use of an inhaler or nebulizer, and formulation with an aerosolizing agent for use as a spray. In certain embodiments, the antibody or pharmaceutical composition described herein is delivered subcutaneously or intravenously. In certain embodiments, the antibody or pharmaceutical composition described herein is 10 delivered intratumorally. In certain embodiments, the antibody or pharmaceutical composition described herein is delivered intra-arterially. In certain embodiments, the antibody or pharmaceutical composition described herein is delivered into a tumor draining lymph node. In certain embodiments, the antibody or pharmaceutical composition described herein is delivered intranasally.

15 [00251] The amount of an antibody or composition which will be effective in the treatment and/or prevention of a condition will depend on the nature of the disease, and can be determined by standard clinical techniques.

[00252] The precise dose to be employed in a composition will also depend on the route of administration, and the seriousness of the infection or disease caused by it, and should be 20 decided according to the judgment of the practitioner and each subject's circumstances. For example, effective doses may also vary depending upon means of administration, target site, physiological state of the patient (including age, body weight and health), whether the patient is human or an animal, other medications administered, or whether treatment is prophylactic or therapeutic. Usually, the patient is a human but non-human mammals including transgenic 25 mammals can also be treated. Treatment dosages are optimally titrated to optimize safety and efficacy.

[00253] An anti-LAG-3 (*e.g.*, human LAG-3) antibody described herein can also be used to assay LAG-3 (*e.g.*, human LAG-3) protein levels in a biological sample using classical immunohistological methods known to those of skill in the art, including immunoassays, 30 such as the enzyme linked immunosorbent assay (ELISA), immunoprecipitation, or Western blotting. Suitable antibody assay labels are known in the art and include enzyme labels, such as, glucose oxidase; radioisotopes, such as iodine (^{125}I , ^{121}I), carbon (^{14}C), sulfur (^{35}S), tritium (^3H), indium (^{121}In), and technetium (^{99}Tc); luminescent labels, such as luminol; and fluorescent labels, such as fluorescein and rhodamine, and biotin. Such labels can be used to

label an antibody described herein. Alternatively, a second antibody that recognizes an anti-LAG-3 (*e.g.*, human LAG-3) antibody described herein can be labeled and used in combination with an anti-LAG-3 (*e.g.*, human LAG-3) antibody to detect LAG-3 (*e.g.*, human LAG-3) protein levels. Therefore, in one embodiment, the present invention relates to the use of an antibody of the present invention for *in vitro* detection of LAG-3 (*e.g.*, human LAG-3) protein in a biological sample. In a further embodiment, the present invention relates to the use of an anti-LAG-3 antibody of the invention, for assaying and/or detecting LAG-3 (*e.g.*, human LAG-3) protein levels in a biological sample *in vitro*, optionally wherein the anti-LAG-3 antibody is conjugated to a radionuclide or detectable label, and/or carries a label described herein, and/or wherein an immunohistological method is used.

[00254] Assaying for the expression level of LAG-3 (*e.g.*, human LAG-3) protein is intended to include qualitatively or quantitatively measuring or estimating the level of LAG-3 (*e.g.*, human LAG-3) protein in a first biological sample either directly (*e.g.*, by determining or estimating absolute protein level) or relatively (*e.g.*, by comparing to the disease associated protein level in a second biological sample). LAG-3 (*e.g.*, human LAG-3) polypeptide expression level in the first biological sample can be measured or estimated and compared to a standard LAG-3 (*e.g.*, human LAG-3) protein level, the standard being taken from a second biological sample obtained from an individual not having the disorder or being determined by averaging levels from a population of individuals not having the disorder. As will be appreciated in the art, once the “standard” LAG-3 (*e.g.*, human LAG-3) polypeptide level is known, it can be used repeatedly as a standard for comparison. Therefore, in a further embodiment, the present invention relates to an *in vitro* method for assaying and/or detecting LAG-3 protein levels, for example human LAG-3 protein levels, in a biological sample, comprising qualitatively or quantitatively measuring or estimating the level of LAG-3 protein, for example of human LAG-3 protein, in a biological sample, by an immunohistological method.

[00255] As used herein, the term “biological sample” refers to any biological sample obtained from a subject, cell line, tissue, or other source of cells potentially expressing LAG-3 (*e.g.*, human LAG-3). Methods for obtaining tissue biopsies and body fluids from animals (*e.g.*, humans) are well known in the art. Biological samples include peripheral mononuclear blood cells.

[00256] An anti-LAG-3 (*e.g.*, human LAG-3) antibody described herein can be used for prognostic, diagnostic, monitoring and screening applications, including *in vitro* and *in vivo* applications well known and standard to the skilled artisan and based on the present

description. Prognostic, diagnostic, monitoring and screening assays and kits for *in vitro* assessment and evaluation of immune system status and/or immune response may be utilized to predict, diagnose and monitor to evaluate patient samples including those known to have or suspected of having an immune system-dysfunction or with regard to an anticipated or desired immune system response, antigen response or vaccine response. The assessment and evaluation of immune system status and/or immune response is also useful in determining the suitability of a patient for a clinical trial of a drug or for the administration of a particular chemotherapeutic agent, a radiotherapeutic agent, or an antibody, including combinations thereof, versus a different agent or antibody. This type of prognostic and diagnostic monitoring and assessment is already in practice utilizing antibodies against the HER2 protein in breast cancer (HercepTest™, Dako) where the assay is also used to evaluate patients for antibody therapy using Herceptin®. *In vivo* applications include directed cell therapy and immune system modulation and radio imaging of immune responses. Therefore, in one embodiment, the present invention relates to an anti-LAG-3 antibody and/or pharmaceutical composition of the present invention for use as a diagnostic. In one embodiment, the present invention relates to an anti-LAG-3 antibody and/or pharmaceutical composition of the present invention for use in a method for the prediction, diagnosis and/or monitoring of a subject having or suspected to have an immune system-dysfunction and/or with regard to an anticipated or desired immune system response, antigen response or vaccine response. In another embodiment, the present invention relates to the use of anti-LAG-3 antibody of the invention, for predicting, diagnosing and/or monitoring of a subject having or suspected to have an immune system-dysfunction and/or with regard to an anticipated or desired immune system response, antigen response or vaccine response by assaying and/or detecting human LAG-3 protein levels in a biological sample of the subject *in vitro*.

[00257] In one embodiment, an anti-LAG-3 (*e.g.*, human LAG-3) antibody can be used in immunohistochemistry of biopsy samples. In another embodiment, an anti-LAG-3 (*e.g.*, human LAG-3) antibody can be used to detect levels of LAG-3 (*e.g.*, human LAG-3), or levels of cells which contain LAG-3 (*e.g.*, human LAG-3) on their membrane surface, which levels can then be linked to certain disease symptoms. Anti- LAG-3 (*e.g.*, human LAG-3) antibodies described herein may carry a detectable or functional label. When fluorescence labels are used, currently available microscopy and fluorescence-activated cell sorter analysis (FACS) or combination of both methods procedures known in the art may be utilized to identify and to quantitate the specific binding members. Anti-LAG-3 (*e.g.*, human LAG-3) antibodies described herein may carry a fluorescence label. Exemplary fluorescence labels

include, for example, reactive and conjugated probes *e.g.*, Aminocoumarin, Fluorescein and Texas red, Alexa Fluor dyes, Cy dyes and DyLight dyes. An anti-LAG-3 (*e.g.*, human LAG-3) antibody may carry a radioactive label, such as the isotopes ^3H , ^{14}C , ^{32}P , ^{35}S , ^{36}Cl , ^{51}Cr , ^{57}Co , ^{58}Co , ^{59}Fe , ^{67}Cu , ^{90}Y , ^{99}Tc , ^{111}In , ^{117}Lu , ^{121}I , ^{124}I , ^{125}I , ^{131}I , ^{198}Au , ^{211}At , ^{213}Bi , ^{225}Ac and ^{186}Re . When radioactive labels are used, currently available counting procedures known in the art may be utilized to identify and quantitate the specific binding of anti-LAG-3 (*e.g.*, human LAG-3) antibody to LAG-3 (*e.g.*, human LAG-3). In the instance where the label is an enzyme, detection may be accomplished by any of the presently utilized colorimetric, spectrophotometric, fluorospectrophotometric, amperometric or gasometric techniques as known in the art. This can be achieved by contacting a sample or a control sample with an anti-LAG-3 (*e.g.*, human LAG-3) antibody under conditions that allow for the formation of a complex between the antibody and LAG-3 (*e.g.*, human LAG-3). Any complexes formed between the antibody and LAG-3 (*e.g.*, human LAG-3) are detected and compared in the sample and the control. In light of the specific binding of the antibodies described herein for LAG-3 (*e.g.*, human LAG-3), the antibodies can be used to specifically detect LAG-3 (*e.g.*, human LAG-3) expression on the surface of cells. The antibodies described herein can also be used to purify LAG-3 (*e.g.*, human LAG-3) via immunoaffinity purification. Also included herein is an assay system which may be prepared in the form of a test kit for the quantitative analysis of the extent of the presence of, for instance, LAG-3 (*e.g.*, human LAG-3) or LAG-3 (*e.g.*, human LAG-3)/ LAG-3 (*e.g.*, human LAG-3) ligand complexes. The system or test kit, kit, or kit-of-parts may comprise a labeled component, *e.g.*, a labeled antibody, and one or more additional immunochemical reagents.

6.5 Polynucleotides, Vectors and Methods of Producing Anti-LAG-3 Antibodies

[00258] In another aspect, provided herein are polynucleotides comprising a nucleotide sequence encoding an antibody described herein or a fragment thereof (*e.g.*, a light chain variable region and/or heavy chain variable region) that specifically binds to a LAG-3 (*e.g.*, human LAG-3) antigen, and vectors, *e.g.*, vectors comprising such polynucleotides for recombinant expression in host cells (*e.g.*, *E. coli* and mammalian cells). Provided herein are polynucleotides comprising nucleotide sequences encoding a heavy and/or light chain of any of the antibodies provided herein, as well as vectors comprising such polynucleotide sequences, *e.g.*, expression vectors for their efficient expression in host cells, *e.g.*, mammalian cells.

[00259] As used herein, an “isolated” polynucleotide or nucleic acid molecule is one

which is separated from other nucleic acid molecules which are present in the natural source (*e.g.*, in a mouse or a human) of the nucleic acid molecule. Moreover, an “isolated” nucleic acid molecule, such as a cDNA molecule, can be substantially free of other cellular material, or culture medium when produced by recombinant techniques, or substantially free of chemical precursors or other chemicals when chemically synthesized. For example, the language “substantially free” includes preparations of polynucleotide or nucleic acid molecule having less than about 15%, 10%, 5%, 2%, 1%, 0.5%, or 0.1% (in particular less than about 10%) of other material, *e.g.*, cellular material, culture medium, other nucleic acid molecules, chemical precursors and/or other chemicals. In a specific embodiment, a nucleic acid molecule(s) encoding an antibody described herein is isolated or purified.

[00260] In particular aspects, provided herein are polynucleotides comprising nucleotide sequences encoding antibodies, which specifically bind to a LAG-3 (*e.g.*, human LAG-3) polypeptide and comprises an amino acid sequence as described herein, as well as antibodies which compete with such antibodies for binding to a LAG-3 (*e.g.*, human LAG-3) polypeptide (*e.g.*, in a dose-dependent manner), or which binds to the same epitope as that of such antibodies.

[00261] In certain aspects, provided herein are polynucleotides comprising a nucleotide sequence encoding the light chain or heavy chain of an antibody described herein. The polynucleotides can comprise nucleotide sequences encoding a light chain comprising the VL FRs and CDRs of antibodies described herein (see, *e.g.*, Tables 1, 3, 5, 6, and 7) or nucleotide sequences encoding a heavy chain comprising the VH FRs and CDRs of antibodies described herein (see, *e.g.*, Tables 1, 2, 4, 6, and 7).

[00262] Also provided herein are polynucleotides encoding an anti-LAG-3 (*e.g.*, human LAG-3) antibody that are optimized, *e.g.*, by codon/RNA optimization, replacement with heterologous signal sequences, and elimination of mRNA instability elements. Methods to generate optimized nucleic acids encoding an anti-LAG-3 (*e.g.*, human LAG-3) antibody or a fragment thereof (*e.g.*, light chain, heavy chain, VH domain, or VL domain) for recombinant expression by introducing codon changes and/or eliminating inhibitory regions in the mRNA can be carried out by adapting the optimization methods described in, *e.g.*, U.S. Patent Nos. 5,965,726; 6,174,666; 6,291,664; 6,414,132; and 6,794,498, accordingly, all of which are herein incorporated by reference in their entireties. For example, potential splice sites and instability elements (*e.g.*, A/T or A/U rich elements) within the RNA can be mutated without altering the amino acids encoded by the nucleic acid sequences to increase stability of the RNA for recombinant expression. The alterations utilize the degeneracy of the genetic code,

e.g., using an alternative codon for an identical amino acid. In some embodiments, it can be desirable to alter one or more codons to encode a conservative mutation, *e.g.*, a similar amino acid with similar chemical structure and properties and/or function as the original amino acid. Such methods can increase expression of an anti-LAG-3 (*e.g.*, human LAG-3) antibody or
5 fragment thereof by at least 1 fold, 2 fold, 3 fold, 4 fold, 5 fold, 10 fold, 20 fold, 30 fold, 40 fold, 50 fold, 60 fold, 70 fold, 80 fold, 90 fold, or 100 fold or more relative to the expression of an anti-LAG-3 (*e.g.*, human LAG-3) antibody encoded by polynucleotides that have not been optimized.

[00263] In certain embodiments, an optimized polynucleotide sequence encoding an anti-
10 LAG-3 (*e.g.*, human LAG-3) antibody described herein or a fragment thereof (*e.g.*, VL domain and/or VH domain) can hybridize to an antisense (*e.g.*, complementary) polynucleotide of an unoptimized polynucleotide sequence encoding an anti-LAG-3 (*e.g.*, human LAG-3) antibody described herein or a fragment thereof (*e.g.*, VL domain and/or VH domain). In specific embodiments, an optimized nucleotide sequence encoding an anti-LAG-
15 3 (*e.g.*, human LAG-3) antibody described herein or a fragment hybridizes under high stringency conditions to antisense polynucleotide of an unoptimized polynucleotide sequence encoding an anti-LAG-3 (*e.g.*, human LAG-3) antibody described herein or a fragment thereof. In a specific embodiment, an optimized nucleotide sequence encoding an anti-LAG-
20 3 (*e.g.*, human LAG-3) antibody described herein or a fragment thereof hybridizes under high stringency, intermediate or lower stringency hybridization conditions to an antisense polynucleotide of an unoptimized nucleotide sequence encoding an anti-LAG-3 (*e.g.*, human LAG-3) antibody described herein or a fragment thereof. Information regarding hybridization conditions has been described, see, *e.g.*, U.S. Patent Application Publication No. US 2005/0048549 (*e.g.*, paragraphs 72-73), which is herein incorporated by reference in
25 its entirety.

[00264] The polynucleotides can be obtained, and the nucleotide sequence of the polynucleotides determined, by any method known in the art. Nucleotide sequences encoding antibodies described herein, *e.g.*, antibodies described in Tables 1, 6, and 7, and modified versions of these antibodies can be determined using methods well known in the art,
30 *i.e.*, nucleotide codons known to encode particular amino acids are assembled in such a way to generate a nucleic acid that encodes the antibody. Such a polynucleotide encoding the antibody can be assembled from chemically synthesized oligonucleotides (*e.g.*, as described in Kutmeier G *et al.*, (1994), *BioTechniques* 17: 242-6, which is herein incorporated by reference in its entirety), which, briefly, involves the synthesis of overlapping

oligonucleotides containing portions of the sequence encoding the antibody, annealing and ligating of those oligonucleotides, and then amplification of the ligated oligonucleotides by PCR.

5 [00265] Alternatively, a polynucleotide encoding an antibody described herein can be generated from nucleic acid from a suitable source (*e.g.*, a hybridoma) using methods well known in the art (*e.g.*, PCR and other molecular cloning methods). For example, PCR amplification using synthetic primers hybridizable to the 3' and 5' ends of a known sequence can be performed using genomic DNA obtained from hybridoma cells producing the antibody of interest. Such PCR amplification methods can be used to obtain nucleic acids comprising
10 the sequence encoding the light chain and/or heavy chain of an antibody. Such PCR amplification methods can be used to obtain nucleic acids comprising the sequence encoding the variable light chain region and/or the variable heavy chain region of an antibody. The amplified nucleic acids can be cloned into vectors for expression in host cells and for further cloning, for example, to generate chimeric and humanized antibodies.

15 [00266] If a clone containing a nucleic acid encoding a particular antibody is not available, but the sequence of the antibody molecule is known, a nucleic acid encoding the immunoglobulin can be chemically synthesized or obtained from a suitable source (*e.g.*, an antibody cDNA library or a cDNA library generated from, or nucleic acid, preferably poly A+ RNA, isolated from, any tissue or cells expressing the antibody, such as hybridoma cells
20 selected to express an antibody described herein) by PCR amplification using synthetic primers hybridizable to the 3' and 5' ends of the sequence or by cloning using an oligonucleotide probe specific for the particular gene sequence to identify, *e.g.*, a cDNA clone from a cDNA library that encodes the antibody. Amplified nucleic acids generated by PCR can then be cloned into replicable cloning vectors using any method well known in the
25 art.

[00267] DNA encoding anti-LAG-3 (*e.g.*, human LAG-3) antibodies described herein can be readily isolated and sequenced using conventional procedures (*e.g.*, by using oligonucleotide probes that are capable of binding specifically to genes encoding the heavy and light chains of the anti-LAG-3 (*e.g.*, human LAG-3) antibodies). Hybridoma cells can
30 serve as a source of such DNA. Once isolated, the DNA can be placed into expression vectors, which are then transfected into host cells such as *E. coli* cells, simian COS cells, Chinese hamster ovary (CHO) cells (*e.g.*, CHO cells from the CHO GS System™ (Lonza)), or myeloma cells that do not otherwise produce immunoglobulin protein, to obtain the synthesis of anti-LAG-3 (*e.g.*, human LAG-3) antibodies in the recombinant host cells.

[00268] To generate whole antibodies, PCR primers including VH or VL nucleotide sequences, a restriction site, and a flanking sequence to protect the restriction site can be used to amplify the VH or VL sequences in scFv clones. Utilizing cloning techniques known to those of skill in the art, the PCR amplified VH domains can be cloned into vectors expressing a heavy chain constant region, *e.g.*, the human gamma 4 constant region, and the PCR amplified VL domains can be cloned into vectors expressing a light chain constant region, *e.g.*, human kappa or lambda constant regions. In certain embodiments, the vectors for expressing the VH or VL domains comprise an EF-1 α promoter, a secretion signal, a cloning site for the variable region, constant domains, and a selection marker such as neomycin. The VH and VL domains can also be cloned into one vector expressing the necessary constant regions. The heavy chain conversion vectors and light chain conversion vectors are then co-transfected into cell lines to generate stable or transient cell lines that express full-length antibodies, *e.g.*, IgG, using techniques known to those of skill in the art.

[00269] The DNA also can be modified, for example, by substituting the coding sequence for human heavy and light chain constant domains in place of the murine sequences, or by covalently joining to the immunoglobulin coding sequence all or part of the coding sequence for a non-immunoglobulin polypeptide.

[00270] Also provided are polynucleotides that hybridize under high stringency, intermediate or lower stringency hybridization conditions to polynucleotides that encode an antibody described herein. In specific embodiments, polynucleotides described herein hybridize under high stringency, intermediate or lower stringency hybridization conditions to polynucleotides encoding a VH domain and/or VL domain provided herein.

[00271] Hybridization conditions have been described in the art and are known to one of skill in the art. For example, hybridization under stringent conditions can involve hybridization to filter-bound DNA in 6x sodium chloride/sodium citrate (SSC) at about 45° C followed by one or more washes in 0.2xSSC/0.1% SDS at about 50-65° C; hybridization under highly stringent conditions can involve hybridization to filter-bound nucleic acid in 6xSSC at about 45° C followed by one or more washes in 0.1xSSC/0.2% SDS at about 68° C. Hybridization under other stringent hybridization conditions are known to those of skill in the art and have been described, see, for example, Ausubel FM *et al.*, eds., (1989) Current Protocols in Molecular Biology, Vol. I, Green Publishing Associates, Inc. and John Wiley & Sons, Inc., New York at pages 6.3.1-6.3.6 and 2.10.3, which is herein incorporated by reference in its entirety.

[00272] In certain aspects, provided herein are cells (*e.g.*, host cells) expressing (*e.g.*,

recombinantly) antibodies described herein which specifically bind to LAG-3 (*e.g.*, human LAG-3) and related polynucleotides and expression vectors. Provided herein are vectors (*e.g.*, expression vectors) comprising polynucleotides comprising nucleotide sequences encoding anti-LAG-3 (*e.g.*, human LAG-3) antibodies or a fragment for recombinant
5 expression in host cells, preferably in mammalian cells. Also provided herein are host cells comprising such vectors for recombinantly expressing anti-LAG-3 (*e.g.*, human LAG-3) antibodies described herein (*e.g.*, human or humanized antibody). In a particular aspect, provided herein are methods for producing an antibody described herein, comprising expressing such antibody from a host cell.

10 [00273] Recombinant expression of an antibody described herein (*e.g.*, a full-length antibody, heavy and/or light chain of an antibody, or a single chain antibody described herein) that specifically binds to LAG-3 (*e.g.*, human LAG-3) involves construction of an expression vector containing a polynucleotide that encodes the antibody. Once a polynucleotide encoding an antibody molecule, heavy and/or light chain of an antibody, or a
15 fragment thereof (*e.g.*, heavy and/or light chain variable regions) described herein has been obtained, the vector for the production of the antibody molecule can be produced by recombinant DNA technology using techniques well known in the art. Thus, methods for preparing a protein by expressing a polynucleotide containing an antibody or antibody fragment (*e.g.*, light chain or heavy chain) encoding nucleotide sequence are described
20 herein. Methods which are well known to those skilled in the art can be used to construct expression vectors containing antibody or antibody fragment (*e.g.*, light chain or heavy chain) coding sequences and appropriate transcriptional and translational control signals. These methods include, for example, *in vitro* recombinant DNA techniques, synthetic techniques, and *in vivo* genetic recombination. Also provided are replicable vectors comprising a
25 nucleotide sequence encoding an antibody molecule described herein, a heavy or light chain of an antibody, a heavy or light chain variable region of an antibody or a fragment thereof, or a heavy or light chain CDR, operably linked to a promoter. Such vectors can, for example, include the nucleotide sequence encoding the constant region of the antibody molecule (see, *e.g.*, International Publication Nos. WO 86/05807 and WO 89/01036; and U.S. Patent No.
30 5,122,464, which are herein incorporated by reference in their entireties) and variable regions of the antibody can be cloned into such a vector for expression of the entire heavy, the entire light chain, or both the entire heavy and light chains.

[00274] An expression vector can be transferred to a cell (*e.g.*, host cell) by conventional techniques and the resulting cells can then be cultured by conventional techniques to produce

an antibody described herein or a fragment thereof. Thus, provided herein are host cells containing a polynucleotide encoding an antibody described herein or fragments thereof, or a heavy or light chain thereof, or fragment thereof, or a single chain antibody described herein, operably linked to a promoter for expression of such sequences in the host cell. In certain
5 embodiments, for the expression of double-chained antibodies, vectors encoding both the heavy and light chains, individually, can be co-expressed in the host cell for expression of the entire immunoglobulin molecule, as detailed below. In certain embodiments, a host cell contains a vector comprising a polynucleotide encoding both the heavy chain and light chain of an antibody described herein, or a fragment thereof. In specific embodiments, a host cell
10 contains two different vectors, a first vector comprising a polynucleotide encoding a heavy chain or a heavy chain variable region of an antibody described herein, or a fragment thereof, and a second vector comprising a polynucleotide encoding a light chain or a light chain variable region of an antibody described herein, or a fragment thereof. In other embodiments, a first host cell comprises a first vector comprising a polynucleotide encoding
15 a heavy chain or a heavy chain variable region of an antibody described herein, or a fragment thereof, and a second host cell comprises a second vector comprising a polynucleotide encoding a light chain or a light chain variable region of an antibody described herein. In specific embodiments, a heavy chain/heavy chain variable region expressed by a first cell associated with a light chain/light chain variable region of a second cell to form an anti-LAG-
20 3 (*e.g.*, human LAG-3) antibody described herein. In certain embodiments, provided herein is a population of host cells comprising such first host cell and such second host cell.

[00275] In a particular embodiment, provided herein is a population of vectors comprising a first vector comprising a polynucleotide encoding a light chain/light chain variable region of an anti-LAG-3 (*e.g.*, human LAG-3) antibody described herein, and a second vector
25 comprising a polynucleotide encoding a heavy chain/heavy chain variable region of an anti-LAG-3 (*e.g.*, human LAG-3) antibody described herein.

[00276] A variety of host-expression vector systems can be utilized to express antibody molecules described herein (see, *e.g.*, U.S. Patent No. 5,807,715, which is herein incorporated by reference in its entirety). Such host-expression systems represent vehicles by which the
30 coding sequences of interest can be produced and subsequently purified, but also represent cells which can, when transformed or transfected with the appropriate nucleotide coding sequences, express an antibody molecule described herein *in situ*. These include but are not limited to microorganisms such as bacteria (*e.g.*, *E. coli* and *B. subtilis*) transformed with recombinant bacteriophage DNA, plasmid DNA or cosmid DNA expression vectors

containing antibody coding sequences; yeast (*e.g.*, *Saccharomyces Pichia*) transformed with recombinant yeast expression vectors containing antibody coding sequences; insect cell systems infected with recombinant virus expression vectors (*e.g.*, baculovirus) containing antibody coding sequences; plant cell systems (*e.g.*, green algae such as *Chlamydomonas reinhardtii*) infected with recombinant virus expression vectors (*e.g.*, cauliflower mosaic virus, CaMV; tobacco mosaic virus, TMV) or transformed with recombinant plasmid expression vectors (*e.g.*, Ti plasmid) containing antibody coding sequences; or mammalian cell systems (*e.g.*, COS (*e.g.*, COS1 or COS), CHO, BHK, MDCK, HEK 293, NS0, PER.C6, VERO, CRL7030, HsS78Bst, HeLa, and NIH 3T3, HEK-293T, HepG2, SP210, R1.1, B-W, L-M, BSC1, BSC40, YB/20 and BMT10 cells) harboring recombinant expression constructs containing promoters derived from the genome of mammalian cells (*e.g.*, metallothionein promoter) or from mammalian viruses (*e.g.*, the adenovirus late promoter; the vaccinia virus 7.5K promoter). In a specific embodiment, cells for expressing antibodies described herein are CHO cells, for example CHO cells from the CHO GS System™ (Lonza). In certain embodiments, the heavy chain and/or light chain of an antibody produced by a CHO cell may have an N-terminal glutamine or glutamate residue replaced by pyroglutamate. In a particular embodiment, cells for expressing antibodies described herein are human cells, *e.g.*, human cell lines. In a specific embodiment, a mammalian expression vector is pOptiVEC™ or pcDNA3.3. In a particular embodiment, bacterial cells such as *Escherichia coli*, or eukaryotic cells (*e.g.*, mammalian cells), especially for the expression of whole recombinant antibody molecule, are used for the expression of a recombinant antibody molecule. For example, mammalian cells such as Chinese hamster ovary (CHO) cells, in conjunction with a vector such as the major intermediate early gene promoter element from human cytomegalovirus is an effective expression system for antibodies (Foecking MK & Hofstetter H (1986) Gene 45: 101-5; and Cockett MI *et al.*, (1990) Biotechnology 8(7): 662-7, each of which is herein incorporated by reference in its entirety). In certain embodiments, antibodies described herein are produced by CHO cells or NS0 cells. In a specific embodiment, the expression of nucleotide sequences encoding antibodies described herein which specifically bind LAG-3 (*e.g.*, human LAG-3) is regulated by a constitutive promoter, inducible promoter or tissue specific promoter.

[00277] In bacterial systems, a number of expression vectors can be advantageously selected depending upon the use intended for the antibody molecule being expressed. For example, when a large quantity of such an antibody is to be produced, for the generation of pharmaceutical compositions of an antibody molecule, vectors which direct the expression of

high levels of fusion protein products that are readily purified can be desirable. Such vectors include, but are not limited to, the *E. coli* expression vector pUR278 (Ruether U & Mueller-Hill B (1983) EMBO J 2: 1791-1794), in which the antibody coding sequence can be ligated individually into the vector in frame with the lac Z coding region so that a fusion protein is produced; pIN vectors (Inouye S & Inouye M (1985) Nuc Acids Res 13: 3101-3109; Van Heeke G & Schuster SM (1989) J Biol Chem 24: 5503-5509); and the like, all of which are herein incorporated by reference in their entireties. For example, pGEX vectors can also be used to express foreign polypeptides as fusion proteins with glutathione S-transferase (GST). In general, such fusion proteins are soluble and can easily be purified from lysed cells by adsorption and binding to matrix glutathione agarose beads followed by elution in the presence of free glutathione. The pGEX vectors are designed to include thrombin or factor Xa protease cleavage sites so that the cloned target gene product can be released from the GST moiety.

[00278] In an insect system, *Autographa californica* nuclear polyhedrosis virus (AcNPV), for example, can be used as a vector to express foreign genes. The virus grows in *Spodoptera frugiperda* cells. The antibody coding sequence can be cloned individually into non-essential regions (for example the polyhedrin gene) of the virus and placed under control of an AcNPV promoter (for example the polyhedrin promoter).

[00279] In mammalian host cells, a number of viral-based expression systems can be utilized. In cases where an adenovirus is used as an expression vector, the antibody coding sequence of interest can be ligated to an adenovirus transcription/translation control complex, e.g., the late promoter and tripartite leader sequence. This chimeric gene can then be inserted in the adenovirus genome by *in vitro* or *in vivo* recombination. Insertion in a non-essential region of the viral genome (e.g., region EI or E3) will result in a recombinant virus that is viable and capable of expressing the antibody molecule in infected hosts (e.g., see Logan J & Shenk T (1984) PNAS 81(12): 3655-9, which is herein incorporated by reference in its entirety). Specific initiation signals can also be required for efficient translation of inserted antibody coding sequences. These signals include the ATG initiation codon and adjacent sequences. Furthermore, the initiation codon must be in phase with the reading frame of the desired coding sequence to ensure translation of the entire insert. These exogenous translational control signals and initiation codons can be of a variety of origins, both natural and synthetic. The efficiency of expression can be enhanced by the inclusion of appropriate transcription enhancer elements, transcription terminators, etc. (see, e.g., Bitter G *et al.*, (1987) Methods Enzymol. 153: 516-544, which is herein incorporated by reference in its

entirety).

[00280] In addition, a host cell strain can be chosen which modulates the expression of the inserted sequences, or modifies and processes the gene product in the specific fashion desired. Such modifications (*e.g.*, glycosylation) and processing (*e.g.*, cleavage) of protein products can be important for the function of the protein. Different host cells have characteristic and specific mechanisms for the post-translational processing and modification of proteins and gene products. Appropriate cell lines or host systems can be chosen to ensure the correct modification and processing of the foreign protein expressed. To this end, eukaryotic host cells which possess the cellular machinery for proper processing of the primary transcript, glycosylation, and phosphorylation of the gene product can be used. Such mammalian host cells include but are not limited to CHO, VERO, BHK, Hela, MDCK, HEK 293, NIH 3T3, W138, BT483, Hs578T, HTB2, BT20 and T47D, NS0 (a murine myeloma cell line that does not endogenously produce any immunoglobulin chains), CRL7030, COS (*e.g.*, COS1 or COS), PER.C6, VERO, HsS78Bst, HEK-293T, HepG2, SP210, R1.1, B-W, L-M, BSC1, BSC40, YB/20, BMT10 and HsS78Bst cells. In certain embodiments, anti-LAG-3 (*e.g.*, human LAG-3) antibodies described herein are produced in mammalian cells, such as CHO cells.

[00281] In a specific embodiment, the antibodies described herein have reduced fucose content or no fucose content. Such antibodies can be produced using techniques known one skilled in the art. For example, the antibodies can be expressed in cells deficient or lacking the ability of to fucosylate. In a specific example, cell lines with a knockout of both alleles of α 1,6-fucosyltransferase can be used to produce antibodies with reduced fucose content. The Potelligent[®] system (Lonza) is an example of such a system that can be used to produce antibodies with reduced fucose content.

[00282] For long-term, high-yield production of recombinant proteins, stable expression cells can be generated. For example, cell lines which stably express an anti-LAG-3 (*e.g.*, human LAG-3) antibody described herein can be engineered. In specific embodiments, a cell provided herein stably expresses a light chain/light chain variable region and a heavy chain/heavy chain variable region which associate to form an antibody described herein.

[00283] In certain aspects, rather than using expression vectors which contain viral origins of replication, host cells can be transformed with DNA controlled by appropriate expression control elements (*e.g.*, promoter, enhancer, sequences, transcription terminators, polyadenylation sites, *etc.*), and a selectable marker. Following the introduction of the foreign DNA/polynucleotide, engineered cells can be allowed to grow for 1-2 days in an

enriched media, and then are switched to a selective media. The selectable marker in the recombinant plasmid confers resistance to the selection and allows cells to stably integrate the plasmid into their chromosomes and grow to form foci which in turn can be cloned and expanded into cell lines. This method can advantageously be used to engineer cell lines which express an anti-LAG-3 (*e.g.*, human LAG-3) antibody described herein or a fragment thereof. Such engineered cell lines can be particularly useful in screening and evaluation of compositions that interact directly or indirectly with the antibody molecule.

[00284] A number of selection systems can be used, including but not limited to the herpes simplex virus thymidine kinase (Wigler M *et al.*, (1977) Cell 11(1): 223-32), hypoxanthineguanine phosphoribosyltransferase (Szybalska EH & Szybalski W (1962) PNAS 48(12): 2026-2034) and adenine phosphoribosyltransferase (Lowy I *et al.*, (1980) Cell 22(3): 817-23) genes in tk-, hgp^{rt}- or ap^{rt}-cells, respectively, all of which are herein incorporated by reference in their entireties. Also, antimetabolite resistance can be used as the basis of selection for the following genes: *dhfr*, which confers resistance to methotrexate (Wigler M *et al.*, (1980) PNAS 77(6): 3567-70; O'Hare K *et al.*, (1981) PNAS 78: 1527-31); *gpt*, which confers resistance to mycophenolic acid (Mulligan RC & Berg P (1981) PNAS 78(4): 2072-6); neo, which confers resistance to the aminoglycoside G-418 (Wu GY & Wu CH (1991) Biotherapy 3: 87-95; Tolstoshev P (1993) Ann Rev Pharmacol Toxicol 32: 573-596; Mulligan RC (1993) Science 260: 926-932; and Morgan RA & Anderson WF (1993) Ann Rev Biochem 62: 191-217; Nabel GJ & Felgner PL (1993) Trends Biotechnol 11(5): 211-5); and *hygro*, which confers resistance to hygromycin (Santerre RF *et al.*, (1984) Gene 30(1-3): 147-56), all of which are herein incorporated by reference in their entireties. Methods commonly known in the art of recombinant DNA technology can be routinely applied to select the desired recombinant clone and such methods are described, for example, in Ausubel FM *et al.*, (eds.), Current Protocols in Molecular Biology, John Wiley & Sons, NY (1993); Kriegler M, Gene Transfer and Expression, A Laboratory Manual, Stockton Press, NY (1990); and in Chapters 12 and 13, Dracopoli NC *et al.*, (eds.), Current Protocols in Human Genetics, John Wiley & Sons, NY (1994); Colbère-Garapin F *et al.*, (1981) J Mol Biol 150: 1-14, which are incorporated by reference herein in their entireties.

[00285] The expression levels of an antibody molecule can be increased by vector amplification (for a review, see Bebbington CR & Hentschel CCG, The use of vectors based on gene amplification for the expression of cloned genes in mammalian cells in DNA cloning, Vol. 3 (Academic Press, New York, 1987), which is herein incorporated by reference in its entirety). When a marker in the vector system expressing antibody is

amplifiable, increase in the level of inhibitor present in culture of host cell will increase the number of copies of the marker gene. Since the amplified region is associated with the antibody gene, production of the antibody will also increase (Crouse GF *et al.*, (1983) Mol Cell Biol 3: 257-66, which is herein incorporated by reference in its entirety).

5 [00286] The host cell can be co-transfected with two or more expression vectors described herein, the first vector encoding a heavy chain derived polypeptide and the second vector encoding a light chain derived polypeptide. The two vectors can contain identical selectable markers which enable equal expression of heavy and light chain polypeptides. The host cells can be co-transfected with different amounts of the two or more expression vectors. For
10 example, host cells can be transfected with any one of the following ratios of a first expression vector and a second expression vector: 1:1, 1:2, 1:3, 1:4, 1:5, 1:6, 1:7, 1:8, 1:9, 1:10, 1:12, 1:15, 1:20, 1:25, 1:30, 1:35, 1:40, 1:45, or 1:50.

[00287] Alternatively, a single vector can be used which encodes, and is capable of expressing, both heavy and light chain polypeptides. In such situations, the light chain
15 should be placed before the heavy chain to avoid an excess of toxic free heavy chain (Proudfoot NJ (1986) Nature 322: 562-565; and Köhler G (1980) PNAS 77: 2197-2199, each of which is herein incorporated by reference in its entirety). The coding sequences for the heavy and light chains can comprise cDNA or genomic DNA. The expression vector can be monocistronic or multicistronic. A multicistronic nucleic acid construct can encode 2, 3, 4, 5,
20 6, 7, 8, 9, 10 or more, or in the range of 2-5, 5-10 or 10-20 genes/nucleotide sequences. For example, a bicistronic nucleic acid construct can comprise in the following order a promoter, a first gene (*e.g.*, heavy chain of an antibody described herein), and a second gene and (*e.g.*, light chain of an antibody described herein). In such an expression vector, the transcription of both genes can be driven by the promoter, whereas the translation of the mRNA from the
25 first gene can be by a cap-dependent scanning mechanism and the translation of the mRNA from the second gene can be by a cap-independent mechanism, *e.g.*, by an IRES.

[00288] Once an antibody molecule described herein has been produced by recombinant expression, it can be purified by any method known in the art for purification of an immunoglobulin molecule, for example, by chromatography (*e.g.*, ion exchange, affinity,
30 particularly by affinity for the specific antigen after Protein A, and sizing column chromatography), centrifugation, differential solubility, or by any other standard technique for the purification of proteins. Further, the antibodies described herein can be fused to heterologous polypeptide sequences described herein or otherwise known in the art to facilitate purification.

[00289] In specific embodiments, an antibody described herein is isolated or purified. Generally, an isolated antibody is one that is substantially free of other antibodies with different antigenic specificities than the isolated antibody. For example, in a particular embodiment, a preparation of an antibody described herein is substantially free of cellular material and/or chemical precursors. The language “substantially free of cellular material” includes preparations of an antibody in which the antibody is separated from cellular components of the cells from which it is isolated or recombinantly produced. Thus, an antibody that is substantially free of cellular material includes preparations of antibody having less than about 30%, 20%, 10%, 5%, 2%, 1%, 0.5%, or 0.1% (by dry weight) of heterologous protein (also referred to herein as a “contaminating protein”) and/or variants of an antibody, for example, different post-translational modified forms of an antibody or other different versions of an antibody (*e.g.*, antibody fragments). When the antibody is recombinantly produced, it is also generally substantially free of culture medium, *i.e.*, culture medium represents less than about 20%, 10%, 2%, 1%, 0.5%, or 0.1% of the volume of the protein preparation. When the antibody is produced by chemical synthesis, it is generally substantially free of chemical precursors or other chemicals, *i.e.*, it is separated from chemical precursors or other chemicals which are involved in the synthesis of the protein. Accordingly, such preparations of the antibody have less than about 30%, 20%, 10%, or 5% (by dry weight) of chemical precursors or compounds other than the antibody of interest. In a specific embodiment, antibodies described herein are isolated or purified.

[00290] Antibodies or fragments thereof that specifically bind to LAG-3 (*e.g.*, human LAG-3) can be produced by any method known in the art for the synthesis of antibodies, for example, by chemical synthesis or by recombinant expression techniques. The methods described herein employs, unless otherwise indicated, conventional techniques in molecular biology, microbiology, genetic analysis, recombinant DNA, organic chemistry, biochemistry, PCR, oligonucleotide synthesis and modification, nucleic acid hybridization, and related fields within the skill of the art. These techniques are described, for example, in the references cited herein and are fully explained in the literature. See, *e.g.*, Maniatis T *et al.*, (1982) *Molecular Cloning: A Laboratory Manual*, Cold Spring Harbor Laboratory Press; Sambrook J *et al.*, (1989), *Molecular Cloning: A Laboratory Manual*, Second Edition, Cold Spring Harbor Laboratory Press; Sambrook J *et al.*, (2001) *Molecular Cloning: A Laboratory Manual*, Cold Spring Harbor Laboratory Press, Cold Spring Harbor, NY; Ausubel FM *et al.*, *Current Protocols in Molecular Biology*, John Wiley & Sons (1987 and annual updates); *Current Protocols in Immunology*, John Wiley & Sons (1987 and annual updates)

Gait (ed.) (1984) *Oligonucleotide Synthesis: A Practical Approach*, IRL Press; Eckstein (ed.) (1991) *Oligonucleotides and Analogues: A Practical Approach*, IRL Press; Birren B *et al.*, (eds.) (1999) *Genome Analysis: A Laboratory Manual*, Cold Spring Harbor Laboratory Press, all of which are herein incorporated by reference in their entirety.

5 [00291] In a specific embodiment, an antibody described herein is an antibody (*e.g.*, recombinant antibody) prepared, expressed, created or isolated by any means that involves creation, *e.g.*, via synthesis, genetic engineering of DNA sequences. In certain embodiments, such antibody comprises sequences (*e.g.*, DNA sequences or amino acid sequences) that do not naturally exist within the antibody germline repertoire of an animal or mammal (*e.g.*,
10 human) *in vivo*.

[00292] In one aspect, provided herein is a method of making an antibody which specifically binds to LAG-3 (*e.g.*, human LAG-3) comprising culturing a cell or host cell described herein. In one embodiment, the method is performed *in vitro*. In a certain aspect, provided herein is a method of making an antibody which specifically binds to LAG-3 (*e.g.*,
15 human LAG-3) comprising expressing (*e.g.*, recombinantly expressing) the antibody using a cell or host cell described herein (*e.g.*, a cell or a host cell comprising polynucleotides encoding an antibody described herein). In a particular embodiment, the cell is an isolated cell. In a particular embodiment, the exogenous polynucleotides have been introduced into the cell. In a particular embodiment, the method further comprises the step of purifying the
20 antibody obtained from the cell or host cell.

[00293] Methods for producing polyclonal antibodies are known in the art (see, for example, Chapter 11 in: *Short Protocols in Molecular Biology*, (2002) 5th Ed., Ausubel FM *et al.*, eds., John Wiley and Sons, New York, which is herein incorporated by reference in its entirety).

25 [00294] Monoclonal antibodies can be prepared using a wide variety of techniques known in the art including the use of hybridoma, recombinant, and phage display technologies, or a combination thereof. For example, monoclonal antibodies can be produced using hybridoma techniques including those known in the art and taught, for example, in Harlow E & Lane D, *Antibodies: A Laboratory Manual*, (Cold Spring Harbor Laboratory Press, 2nd ed. 1988);
30 Hammerling GJ *et al.*, in: *Monoclonal Antibodies and T-Cell Hybridomas* 563 681 (Elsevier, N.Y., 1981), each of which is herein incorporated by reference in its entirety. The term “monoclonal antibody” as used herein is not limited to antibodies produced through hybridoma technology. For example, monoclonal antibodies can be produced recombinantly from host cells exogenously expressing an antibody described herein or a fragment thereof,

for example, light chain and/or heavy chain of such antibody.

[00295] In specific embodiments, a “monoclonal antibody,” as used herein, is an antibody produced by a single cell (*e.g.*, hybridoma or host cell producing a recombinant antibody), wherein the antibody specifically binds to LAG-3 (*e.g.*, human LAG-3) as determined, *e.g.*,
5 by ELISA or other antigen-binding or competitive binding assay known in the art or in the examples provided herein. In particular embodiments, a monoclonal antibody can be a chimeric antibody or a humanized antibody. In certain embodiments, a monoclonal antibody is a monovalent antibody or multivalent (*e.g.*, bivalent) antibody. In particular embodiments, a monoclonal antibody is a monospecific or multispecific antibody (*e.g.*, bispecific antibody).
10 Monoclonal antibodies described herein can, for example, be made by the hybridoma method as described in Kohler G & Milstein C (1975) *Nature* 256: 495, which is herein incorporated by reference in its entirety, or can, *e.g.*, be isolated from phage libraries using the techniques as described herein, for example. Other methods for the preparation of clonal cell lines and of monoclonal antibodies expressed thereby are well known in the art (see, for example,
15 Chapter 11 in: *Short Protocols in Molecular Biology*, (2002) 5th Ed., Ausubel FM *et al.*, *supra*).

[00296] Methods for producing and screening for specific antibodies using hybridoma technology are routine and well known in the art. For example, in the hybridoma method, a mouse or other appropriate host animal, such as a sheep, goat, rabbit, rat, hamster or macaque
20 monkey, is immunized to elicit lymphocytes that produce or are capable of producing antibodies that will specifically bind to the protein (*e.g.*, LAG-3 (*e.g.*, human LAG-3)) used for immunization. Alternatively, lymphocytes may be immunized *in vitro*. Lymphocytes then are fused with myeloma cells using a suitable fusing agent, such as polyethylene glycol, to form a hybridoma cell (Goding JW (Ed), *Monoclonal Antibodies: Principles and Practice*,
25 pp. 59-103 (Academic Press, 1986), herein incorporated by reference in its entirety). Additionally, a RIMMS (repetitive immunization multiple sites) technique can be used to immunize an animal (Kilpatrick KE *et al.*, (1997) *Hybridoma* 16:381-9, herein incorporated by reference in its entirety).

[00297] In some embodiments, mice (or other animals, such as rats, monkeys, donkeys,
30 pigs, sheep, hamster, or dogs) can be immunized with an antigen (*e.g.*, LAG-3 (*e.g.*, human LAG-3)) and once an immune response is detected, *e.g.*, antibodies specific for the antigen are detected in the mouse serum, the mouse spleen is harvested and splenocytes isolated. The splenocytes are then fused by well-known techniques to any suitable myeloma cells, for example cells from cell line SP20 available from the American Type Culture Collection

(ATCC[®]) (Manassas, VA), to form hybridomas. Hybridomas are selected and cloned by limited dilution. In certain embodiments, lymph nodes of the immunized mice are harvested and fused with NS0 myeloma cells.

[00298] The hybridoma cells thus prepared are seeded and grown in a suitable culture medium that preferably contains one or more substances that inhibit the growth or survival of the unfused, parental myeloma cells. For example, if the parental myeloma cells lack the enzyme hypoxanthine guanine phosphoribosyl transferase (HGPRT or HPRT), the culture medium for the hybridomas typically will include hypoxanthine, aminopterin, and thymidine (HAT medium), which substances prevent the growth of HGPRT-deficient cells.

[00299] Specific embodiments employ myeloma cells that fuse efficiently, support stable high-level production of antibody by the selected antibody-producing cells, and are sensitive to a medium such as HAT medium. Among these myeloma cell lines are murine myeloma lines, such as NS0 cell line or those derived from MOPC-21 and MPC-11 mouse tumors available from the Salk Institute Cell Distribution Center, San Diego, CA, USA, and SP-2 or X63-Ag8.653 cells available from the American Type Culture Collection, Rockville, MD, USA. Human myeloma and mouse-human heteromyeloma cell lines also have been described for the production of human monoclonal antibodies (Kozbor D (1984) J Immunol 133: 3001-5; Brodeur *et al.*, Monoclonal Antibody Production Techniques and Applications, pp. 51-63 (Marcel Dekker, Inc., New York, 1987), each of which is herein incorporated by reference in its entirety).

[00300] Culture medium in which hybridoma cells are growing is assayed for production of monoclonal antibodies directed against LAG-3 (*e.g.*, human LAG-3). The binding specificity of monoclonal antibodies produced by hybridoma cells is determined by methods known in the art, for example, immunoprecipitation or by an *in vitro* binding assay, such as radioimmunoassay (RIA) or enzyme-linked immunoabsorbent assay (ELISA).

[00301] After hybridoma cells are identified that produce antibodies of the desired specificity, affinity, and/or activity, the clones may be subcloned by limiting dilution procedures and grown by standard methods (Goding JW (Ed), Monoclonal Antibodies: Principles and Practice, *supra*). Suitable culture media for this purpose include, for example, D-MEM or RPMI 1640 medium. In addition, the hybridoma cells may be grown *in vivo* as ascites tumors in an animal.

[00302] The monoclonal antibodies secreted by the subclones are suitably separated from the culture medium, ascites fluid, or serum by conventional immunoglobulin purification procedures such as, for example, protein A-Sepharose, hydroxylapatite chromatography, gel

electrophoresis, dialysis, or affinity chromatography.

[00303] Antibodies described herein include antibody fragments which recognize specific LAG-3 (*e.g.*, human LAG-3) and can be generated by any technique known to those of skill in the art. For example, Fab and F(ab')₂ fragments described herein can be produced by
5 proteolytic cleavage of immunoglobulin molecules, using enzymes such as papain (to produce Fab fragments) or pepsin (to produce F(ab')₂ fragments). A Fab fragment corresponds to one of the two identical arms of an antibody molecule and contains the complete light chain paired with the VH and CH1 domains of the heavy chain. A F(ab')₂ fragment contains the two antigen-binding arms of an antibody molecule linked by disulfide
10 bonds in the hinge region.

[00304] Further, the antibodies described herein can also be generated using various phage display methods known in the art. In phage display methods, functional antibody domains are displayed on the surface of phage particles which carry the polynucleotide sequences encoding them. In particular, DNA sequences encoding VH and VL domains are amplified
15 from animal cDNA libraries (*e.g.*, human or murine cDNA libraries of affected tissues). The DNA encoding the VH and VL domains are recombined together with a scFv linker by PCR and cloned into a phagemid vector. The vector is electroporated in *E. coli* and the *E. coli* is infected with helper phage. Phage used in these methods are typically filamentous phage including fd and M13, and the VH and VL domains are usually recombinantly fused to either
20 the phage gene III or gene VIII. Phage expressing an antigen binding domain that binds to a particular antigen can be selected or identified with antigen, *e.g.*, using labeled antigen or antigen bound or captured to a solid surface or bead. Examples of phage display methods that can be used to make the antibodies described herein include those disclosed in Brinkman
U *et al.*, (1995) J Immunol Methods 182: 41-50; Ames RS *et al.*, (1995) J Immunol Methods
25 184: 177-186; Kettleborough CA *et al.*, (1994) Eur J Immunol 24: 952-958; Persic L *et al.*, (1997) Gene 187: 9-18; Burton DR & Barbas CF (1994) Advan Immunol 57: 191-280; PCT Application No. PCT/GB91/001134; International Publication Nos. WO 90/02809, WO 91/10737, WO 92/01047, WO 92/18619, WO 93/1 1236, WO 95/15982, WO 95/20401, and WO 97/13844; and U.S. Patent Nos. 5,698,426, 5,223,409, 5,403,484, 5,580,717, 5,427,908,
30 5,750,753, 5,821,047, 5,571,698, 5,427,908, 5,516,637, 5,780,225, 5,658,727, 5,733,743 and 5,969,108, all of which are herein incorporated by reference in their entireties.

[00305] As described in the above references, after phage selection, the antibody coding regions from the phage can be isolated and used to generate whole antibodies, including human antibodies, or any other desired antigen binding fragment, and expressed in any

desired host, including mammalian cells, insect cells, plant cells, yeast, and bacteria, *e.g.*, as described below. Techniques to recombinantly produce antibody fragments such as Fab, Fab' and F(ab')₂ fragments can also be employed using methods known in the art such as those disclosed in PCT publication No. WO 92/22324; Mullinax RL *et al.*, (1992) BioTechniques 12(6): 864-9; Sawai H *et al.*, (1995) Am J Reprod Immunol 34: 26-34; and Better M *et al.*, (1988) Science 240: 1041-1043, all of which are herein incorporated by reference in their entirety.

[00306] In certain embodiments, to generate whole antibodies, PCR primers including VH or VL nucleotide sequences, a restriction site, and a flanking sequence to protect the restriction site can be used to amplify the VH or VL sequences from a template, *e.g.*, scFv clones. Utilizing cloning techniques known to those of skill in the art, the PCR amplified VH domains can be cloned into vectors expressing a VH constant region, and the PCR amplified VL domains can be cloned into vectors expressing a VL constant region, *e.g.*, human kappa or lambda constant regions. The VH and VL domains can also be cloned into one vector expressing the necessary constant regions. The heavy chain conversion vectors and light chain conversion vectors are then co-transfected into cell lines to generate stable or transient cell lines that express full-length antibodies, *e.g.*, IgG, using techniques known to those of skill in the art.

[00307] A chimeric antibody is a molecule in which different portions of the antibody are derived from different immunoglobulin molecules. For example, a chimeric antibody can contain a variable region of a mouse or rat monoclonal antibody fused to a constant region of a human antibody. Methods for producing chimeric antibodies are known in the art. See, *e.g.*, Morrison SL (1985) Science 229: 1202-7; Oi VT & Morrison SL (1986) BioTechniques 4: 214-221; Gillies SD *et al.*, (1989) J Immunol Methods 125: 191-202; and U.S. Patent Nos. 5,807,715, 4,816,567, 4,816,397, and 6,331,415, all of which are herein incorporated by reference in their entirety.

[00308] A humanized antibody is capable of binding to a predetermined antigen and which comprises a framework region having substantially the amino acid sequence of a human immunoglobulin and CDRs having substantially the amino acid sequence of a non-human immunoglobulin (*e.g.*, a murine immunoglobulin). In particular embodiments, a humanized antibody also comprises at least a portion of an immunoglobulin constant region (Fc), typically that of a human immunoglobulin. The antibody also can include the CH1, hinge, CH2, CH3, and CH4 regions of the heavy chain. A humanized antibody can be selected from any class of immunoglobulins, including IgM, IgG, IgD, IgA and IgE, and any isotype,

including IgG₁, IgG₂, IgG₃ and IgG₄. Humanized antibodies can be produced using a variety of techniques known in the art, including but not limited to, CDR-grafting (European Patent No. EP 239400; International Publication No. WO 91/09967; and U.S. Patent Nos. 5,225,539, 5,530,101, and 5,585,089), veneering or resurfacing (European Patent Nos. EP 592106 and 5
EP 519596; Padlan EA (1991) *Mol Immunol* 28(4/5): 489-498; Studnicka GM *et al.*, (1994) *Prot Engineering* 7(6): 805-814; and Roguska MA *et al.*, (1994) *PNAS* 91: 969-973), chain shuffling (U.S. Patent No. 5,565,332), and techniques disclosed in, *e.g.*, U.S. Pat. No. 6,407,213, U.S. Pat. No. 5,766,886, International Publication No. WO 93/17105; Tan P *et al.*, (2002) *J Immunol* 169: 1119-25; Caldas C *et al.*, (2000) *Protein Eng.* 13(5): 353-60; Morea V
10 *et al.*, (2000) *Methods* 20(3): 267-79; Baca M *et al.*, (1997) *J Biol Chem* 272(16): 10678-84; Roguska MA *et al.*, (1996) *Protein Eng* 9(10): 895-904; Couto JR *et al.*, (1995) *Cancer Res.* 55 (23 Supp): 5973s-5977s; Couto JR *et al.*, (1995) *Cancer Res* 55(8): 1717-22; Sandhu JS (1994) *Gene* 150(2): 409-10 and Pedersen JT *et al.*, (1994) *J Mol Biol* 235(3): 959-73, all of which are herein incorporated by reference in their entireties. See also U.S. Application
15 Publication No. US 2005/0042664 A1 (Feb. 24, 2005), which is incorporated by reference herein in its entirety.

[00309] Methods for making multispecific (*e.g.*, bispecific antibodies) have been described, see, for example, U.S. Patent Nos. 7,951,917; 7,183,076; 8,227,577; 5,837,242; 5,989,830; 5,869,620; 6,132,992 and 8,586,713, all of which are herein incorporated by
20 reference in their entireties.

[00310] Single domain antibodies, for example, antibodies lacking the light chains, can be produced by methods well known in the art. See Riechmann L & Muyldermans S (1999) *J Immunol* 231: 25-38; Nuttall SD *et al.*, (2000) *Curr Pharm Biotechnol* 1(3): 253-263; Muyldermans S, (2001) *J Biotechnol* 74(4): 277-302; U.S. Patent No. 6,005,079; and
25 International Publication Nos. WO 94/04678, WO 94/25591 and WO 01/44301, all of which are herein incorporated by reference in their entireties.

[00311] Further, antibodies that specifically bind to a LAG-3 (*e.g.*, human LAG-3) antigen can, in turn, be utilized to generate anti-idiotypic antibodies that “mimic” an antigen using techniques well known to those skilled in the art. See, *e.g.*, Greenspan NS & Bona CA
30 (1989) *FASEB J* 7(5): 437-444; and Nissinoff A (1991) *J Immunol* 147(8): 2429-2438, each of which is herein incorporated by reference in its entirety.

[00312] In particular embodiments, an antibody described herein, which binds to the same epitope of LAG-3 (*e.g.*, human LAG-3) as an anti-LAG-3 (*e.g.*, human LAG-3) antibody described herein, is a human antibody. In particular embodiments, an antibody described

herein, which competitively blocks (*e.g.*, in a dose-dependent manner) any one of the antibodies described herein, from binding to LAG-3 (*e.g.*, human LAG-3), is a human antibody. Human antibodies can be produced using any method known in the art. For example, transgenic mice which are incapable of expressing functional endogenous immunoglobulins, but which can express human immunoglobulin genes, can be used. In particular, the human heavy and light chain immunoglobulin gene complexes can be introduced randomly or by homologous recombination into mouse embryonic stem cells. Alternatively, the human variable region, constant region, and diversity region can be introduced into mouse embryonic stem cells in addition to the human heavy and light chain genes. The mouse heavy and light chain immunoglobulin genes can be rendered non-functional separately or simultaneously with the introduction of human immunoglobulin loci by homologous recombination. In particular, homozygous deletion of the J_H region prevents endogenous antibody production. The modified embryonic stem cells are expanded and microinjected into blastocysts to produce chimeric mice. The chimeric mice are then bred to produce homozygous offspring which express human antibodies. The transgenic mice are immunized in the normal fashion with a selected antigen, *e.g.*, all or a portion of an antigen (*e.g.*, LAG-3 (*e.g.*, human LAG-3)). Monoclonal antibodies directed against the antigen can be obtained from the immunized, transgenic mice using conventional hybridoma technology. The human immunoglobulin transgenes harbored by the transgenic mice rearrange during B cell differentiation, and subsequently undergo class switching and somatic mutation. Thus, using such a technique, it is possible to produce therapeutically useful IgG, IgA, IgM and IgE antibodies. For an overview of this technology for producing human antibodies, see Lonberg N & Huszar D (1995) *Int Rev Immunol* 13:65-93, herein incorporated by reference in its entirety. For a detailed discussion of this technology for producing human antibodies and human monoclonal antibodies and protocols for producing such antibodies, see, *e.g.*, International Publication Nos. WO 98/24893, WO 96/34096 and WO 96/33735; and U.S. Patent Nos. 5,413,923, 5,625,126, 5,633,425, 5,569,825, 5,661,016, 5,545,806, 5,814,318 and 5,939,598, all of which are herein incorporated by reference in their entireties. Examples of mice capable of producing human antibodies include the XenomouseTM (Abgenix, Inc.; U.S. Patent Nos. 6,075,181 and 6,150,184), the HuAb-MouseTM (Medarex, Inc./Gen Pharm; U.S. Patent Nos. 5,545,806 and 5,569, 825), the Trans Chromo MouseTM (Kirin) and the KM MouseTM (Medarex/Kirin), all of which are herein incorporated by reference in their entireties.

[00313] Human antibodies which specifically bind to LAG-3 (*e.g.*, human LAG-3) can be

made by a variety of methods known in the art including phage display methods described above using antibody libraries derived from human immunoglobulin sequences. *See also* U.S. Patent Nos. 4,444,887, 4,716,111, and 5,885,793; and International Publication Nos. WO 98/46645, WO 98/50433, WO 98/24893, WO 98/16654, WO 96/34096, WO 96/33735, and WO 91/10741, all of which are herein incorporated by reference in their entireties.

[00314] In some embodiments, human antibodies can be produced using mouse-human hybridomas. For example, human peripheral blood lymphocytes transformed with Epstein-Barr virus (EBV) can be fused with mouse myeloma cells to produce mouse-human hybridomas secreting human monoclonal antibodies, and these mouse-human hybridomas can be screened to determine ones which secrete human monoclonal antibodies that specifically bind to a target antigen (*e.g.*, LAG-3 (*e.g.*, human LAG-3)). Such methods are known and are described in the art, *see, e.g.*, Shinmoto H *et al.*, (2004) *Cytotechnology* 46: 19-23; Naganawa Y *et al.*, (2005) *Human Antibodies* 14: 27-31, each of which is herein incorporated by reference in its entirety.

6.6 Kits

[00315] Also provided, are kits comprising one or more antibodies described herein, or pharmaceutical composition or conjugates thereof. In a specific embodiment, provided herein is a pharmaceutical pack or kit comprising one or more containers filled with one or more of the ingredients of the pharmaceutical compositions described herein, such as one or more antibodies provided herein. In some embodiments, the kits contain a pharmaceutical composition described herein and any prophylactic or therapeutic agent, such as those described herein. In certain embodiments, the kits may contain a T cell mitogen, such as, *e.g.*, phytohaemagglutinin (PHA) and/or phorbol myristate acetate (PMA), or a TCR complex stimulating antibody, such as an anti-CD3 antibody and anti-CD28 antibody. Optionally associated with such container(s) can be a notice in the form prescribed by a governmental agency regulating the manufacture, use or sale of pharmaceuticals or biological products, which notice reflects approval by the agency of manufacture, use or sale for human administration.

[00316] Also provided, are kits that can be used in the above methods. In one embodiment, a kit comprises an antibody described herein, preferably a purified antibody, in one or more containers. In a specific embodiment, kits described herein contain a substantially isolated LAG-3 (*e.g.*, human LAG-3) antigen as a control. In another specific embodiment, the kits described herein further comprise a control antibody which does not react with a LAG-3 (*e.g.*, human LAG-3) antigen. In another specific embodiment, kits

described herein contain one or more elements for detecting the binding of an antibody to a LAG-3 (*e.g.*, human LAG-3) antigen (*e.g.*, the antibody can be conjugated to a detectable substrate such as a fluorescent compound, an enzymatic substrate, a radioactive compound or a luminescent compound, or a second antibody which recognizes the first antibody can be conjugated to a detectable substrate). In specific embodiments, a kit provided herein can include a recombinantly produced or chemically synthesized LAG-3 (*e.g.*, human LAG-3) antigen. The LAG-3 (*e.g.*, human LAG-3) antigen provided in the kit can also be attached to a solid support. In a more specific embodiment, the detecting means of the above described kit includes a solid support to which a LAG-3 (*e.g.*, human LAG-3) antigen is attached. Such a kit can also include a non-attached reporter-labeled anti-human antibody or anti-mouse/rat antibody. In this embodiment, binding of the antibody to the LAG-3 (*e.g.*, human LAG-3) antigen can be detected by binding of the said reporter-labeled antibody. In one embodiment, the present invention relates to the use of a kit of the present invention for *in vitro* assaying and/or detecting LAG-3 antigen (*e.g.*, human LAG-3) in a biological sample.

7. EXAMPLES

[00317] The examples in this Section (*i.e.*, Section 6) are offered by way of illustration, and not by way of limitation.

7.1 Example 1: Generation and characterization of novel antibodies against human LAG-3

[00318] This example describes the generation and characterization of antibodies that bind to human Lymphocyte activation gene 3 (LAG-3), also known as CD223. In particular, this example describes the generation of murine antibodies that specifically bind to human LAG-3 and inhibit the function of human LAG-3.

7.1.1 Generation of anti-LAG-3 antibodies

[00319] Anti-LAG-3 antibodies were identified by generation and selection of an immunized Fab phage display library. First, total RNA was purified from single-cell suspension of splenocytes from three individual mice previously immunized with recombinant human LAG-3-Fc protein (R&D Systems, Cat# 2319-L3-050) and recombinant cynomolgus monkey LAG-3-Fc protein (Evitria, Custom order). Per mouse, a Fab library was then generated by random-primed cDNA using total RNA as a template to amplify variable regions from mouse antibody genes. Heavy and kappa chain amplicons were combined and cloned into phagemid vectors. Three rounds of selection were performed against recombinant LAG-3 proteins (human LAG-3-Fc, human LAG-3-6His, and/or a 30 amino acid human LAG-3 peptide) and/or cells expressing cynomolgus LAG-3 to identify

LAG-3-specific Fab phage clones. The 30 amino acid human LAG-3 peptide is a biotinylated peptide comprising the amino acid sequence of GPPAAAPGHPLAPGPHPAAPSSWGPRPRRY (SEQ ID NO: 199). Periplasmic extracts of selected Fab clones were then screened by ELISA or flow cytometry. Antibody sequencing and off-rate analysis were then performed on anti-LAG-3 Fabs.

[00320] A set of murine antibodies that bind to human LAG-3 were identified and designated as P01A12, P01C09, P05E01, P05E03, P13A04, P13A06, P13B01, P13B02, P13B03, P13B11, P13C06, P13C08, P13C10, P13D04, P13D05, P13E02, P13F01, P13F02, P13F06, P13F09, P13G01, P13G04, P13G05, P13H05, P14A04, P14B07, P14C04, P14F01, P14F06, P14G01, P14G03, P15B06, P15C02, P15E06, P15F06, P15G05, P16D04, and P16H05. The sequence information of the variable regions of these antibodies is summarized in Table 6.

7.1.2 Binding of anti-LAG-3 Fabs to LAG-3-expressing cells

[00321] The anti-LAG-3 Fabs from periplasmic extracts were tested for binding to LAG-3-expressing cells using flow cytometry. Briefly, wild type and human LAG-3-expressing Jurkat cells were plated at 2×10^5 cells/well in sample buffer (PBS (Gibco, Cat# 10010-015) + 0.5% FBS (Gibco, Cat# 10270-106)) in 96-well U-bottom plates (Sarstedt). 22 μ l of anti-LAG-3 Fab periplasmic extract and 83 μ l of diluted anti-c-myc antibody (Gentaur, Clone# 9E10, Cat# 04-cmyc-9E10) were incubated for 30 minutes at room temperature. 100 μ l of the periplasmic extract/anti-c-myc mixture was added to cells and then incubated for one hour on ice. Cells were washed three times and then incubated with a goat anti-mouse APC (BD Biosciences, Cat# 550826) for 30 minutes on ice. Cells were washed three times and then analyzed with a FACS machine (BD Accuri6).

[00322] As shown in Figures 1A-1C, all the anti-LAG-3 Fabs tested exhibited binding to Jurkat cells expressing human LAG-3. The apparent low level of binding of P05E01 was possibly due to low Fab concentration in the periplasmic extracts. The binding of P05E01 were confirmed in later experiments (data not shown).

7.1.3 Ligand blocking activity of anti-LAG-3 Fabs or antibodies

[00323] Anti-LAG-3 Fabs were tested for their ability to block the binding of recombinant human LAG-3 to MHC class II expressing cells. LAG-3-6His (Acro Biosystems, Cat# LA3-H5222) was pre-incubated at 10 μ g/ml with anti-His biotin (Genscript, Clone# A00186, Cat# A00613) at 6 μ g/ml for 10 minutes at 4°C. This mixture was then incubated with either 10000 ng/ml (Figure 2A) or a serial dilution (21170, 7056.6, 2352.2, 784.0, 261.3, 87.1, 29.0, or 9.7 ng/ml) (Figure 2B) of anti-LAG-3 Fab or a negative control Fab not specific for LAG-

3 for 60 minutes at 4°C and then incubated with 50,000 Raji cells for another 60 minutes at 4°C. Cultures were washed twice with sample buffer (PBS + 2% FBS + 0.09% sodium azide) and then incubated with streptavidin-PE (Biolegend, Cat# 405204) in sample buffer for 30 minutes at 4°C. Cells were washed twice and then analyzed with a FACS Fortessa
5 cytometer (Becton Dickinson).

[00324] As shown in Figures 2A and 2B, all the anti-LAG-3 Fabs tested blocked the binding of cross-linked recombinant LAG-3-6His to MHC class II positive Raji cells.

[00325] The variable regions of selected Fabs were cloned into human heavy chain (Ch1, Ch2, and Ch3) and light chain (CL) constant regions and expressed as full-length IgG₁
10 chimeric antibodies and tested in a similar ligand blocking assay as described above at various concentrations (96360, 48180, 24090, 12045, 6022, 3011, 1505, 753, 376, 188, or 94 ng/ml).

[00326] The full length chimeric anti-LAG-3 antibodies tested all blocked the interaction between recombinant LAG-3 and MHC class II expressing Raji cells (Figure 3).

15 **7.1.4 Effect of anti-LAG-3 antibody on human PBMCs upon Staphylococcus Enterotoxin A (SEA) stimulation**

[00327] The functional activity of the chimeric anti-LAG-3 antibody P13B02 was tested using primary human peripheral blood mononuclear cells (PBMCs) stimulated with Staphylococcus Enterotoxin A (SEA). Briefly, cryopreserved human PBMCs (Research
20 Blood Components) were plated at 10⁵ cells/well in RPMI1640 supplemented with Normocin™ (Invivogen, Cat# ant-nr-1) and 10% heat-inactivated FBS (Thermo Fisher Scientific, Cat# 26140079) in 96-well NUNC LON delta surface plates (NUNC™). Cells were cultured with 100 ng/ml SEA (Toxin Technologies, Cat# at101red) and 10 µg/ml P13B02 or an isotype control antibody for 5 days at 37°C, 5% CO₂, and 97% humidity.
25 Clarified supernatant was collected and stored at -80°C until analysis. IL-2 levels were determined using AlphaLISA (Perkin Elmer, Cat# AL221C).

[00328] As shown in Figure 4, the anti-LAG-3 antibody P13B02 increased IL-2 production in human PBMCs stimulated with the SEA superantigen.

30 **7.2 Example 2: Generation and characterization of humanized antibodies against human LAG-3**

[00329] This example describes the humanization of the murine antibody P13B02 and the characterization of the humanized antibodies.

7.2.1 Humanization of murine antibody P13B02

[00330] Homology matching was used to choose human acceptor framework regions to

graft the CDRs of the murine antibody P13B02. Databases, *e.g.*, a database of germline variable genes from the immunoglobulin loci of human and mouse (the IMGT database (the international ImMunoGeneTics information system[®]; Lefranc MP *et al.*, (1999) *Nucleic Acids Res* 27(1): 209-12; Ruiz M *et al.*, (2000) *Nucleic Acids Res* 28(1): 219-21; Lefranc MP (2001) *Nucleic Acids Res* 29(1): 207-9; Lefranc MP (2003) *Nucleic Acids Res* 31(1): 307-10; Lefranc MP *et al.*, (2005) *Dev Comp Immunol* 29(3): 185-203; Kaas Q *et al.*, (2007) *Briefings in Functional Genomics & Proteomics* 6(4): 253-64) or the VBASE2 (Retter I *et al.*, (2005) *Nucleic Acids Res* 33, Database issue D671-D674) or the Kabat database (Johnson G *et al.*, (2000) *Nucleic Acids Res* 28: 214-218)) or publications (*e.g.*, Kabat EA *et al.*, (1991) *Sequences of Proteins of Immunological Interest*, Fifth Edition, U.S. Department of Health and Human Services, NIH Publication No. 91-3242), all of which are herein incorporated by reference in their entirety, may be used to identify the human subfamilies to which the murine heavy and light chain variable regions belong and determine the best-fit human germline framework to use as the acceptor molecule. Selection of heavy and light chain variable region sequences within these subfamilies to be used as acceptor may be based upon sequence homology and/or a match of structure of the CDR1 and CDR2 regions to help preserve the appropriate relative presentation of the six CDRs after grafting.

[00331] Searching of the IMGT database, downloaded from IMGT.org, using BioEdit Sequence Alignment Editor (Hall, T.A. 1999. BioEdit: a user-friendly biological sequence alignment editor and analysis program for Windows 95/98/NT. *Nucl. Acids. Symp. Ser.* 41:95-98, herein incorporated by reference in its entirety) indicated good homology between the P13B02 heavy chain variable region framework and members of the human heavy chain variable region subgroup IGHV1. Highest homologies and identities of both CDR and framework sequences were observed for germline sequences: IGHV1-46*01 (SEQ ID NO: 153) (62% identity; 61 amino acid residues out of 98); IGHV1-69-2*01 (SEQ ID NO: 154) (63% identity; 62/98); IGHV1-3*01 (SEQ ID NO: 155) (64% identity; 63/98); IGHV1-24*01 (SEQ ID NO: 156) (61% identity; 60/98); IGHV1-2*01 (SEQ ID NO: 157) (60% identity; 59/98); IGHV1-45*01 (SEQ ID NO: 158) (59% identity; 58/98); and IGHV1-18*01 (SEQ ID NO: 159) (60% identity; 59/98).

[00332] Using the same approach, P13B02 light chain variable region sequence showed good homology to members of the human light chain variable region kappa subgroups IGKV3 and IGKV1. Highest homologies and identities of both CDRs and framework sequences were observed for germline sequences: IGKV3-20*01 (SEQ ID NO: 160) (59% identity; 57 amino acid residues out of 96); IGKV3D-15*01 and IGKV3-15*01 (SEQ ID NO:

161) (58% identity; 56/96); IGKV3D-20*01 (SEQ ID NO: 162) (59% identity; 57/96); IGKV3D-7*01 (SEQ ID NO: 163) (58% identity; 56/96); IGKV1-9*01 (SEQ ID NO: 164) (63% identity; 61/96); and IGKV3-11*01 (SEQ ID NO: 165) (60% identity; 58/96).

[00333] As a starting point to the humanization process, a CDR grafted version of mouse
 5 P13B02 VH was generated using framework regions 1,2 and 3 of human IGHV1-46*01 (SEQ ID NO: 153) and framework region 4 of human IGHJ1*01 (SEQ ID NO: 200) as human framework acceptor. A number of back mutations were made at positions that may affect the conformation of CDRs or inter-variable region packing and therefore may be structurally important for maintaining the full activity of the antibody (H0-H4; SEQ ID NOs:
 10 56-60 and 220, respectively) (Figure 5A). Similarly, a CDR grafted version of mouse P13B02 VL was generated using framework regions 1, 2, and 3 of human IGKV3-20*01 (SEQ ID NO: 160) and framework 4 of human IGKJ1*01 (SEQ ID NO: 201) as human framework acceptor (L0; SEQ ID NO: 73) (Figure 5B). Back mutations were generated at various positions (L1-L4; SEQ ID NOs: 74-77, respectively) (Figure 5B). The sequences of
 15 the humanized VHs H0-H4 and the humanized VLs L0-L4 are provided in Table 1. Table 10 shows positions that are different between mouse and human antibody framework regions and are subjected to back mutation in at least one of the humanized VHs or VLs described above. Table 11 shows positions that are different between mouse and human antibody framework regions and are subjected to back mutation in H1_R98K_L4M.
 20 **Table 10.** Summary of framework back mutations in at least one of the humanized VHs or VLs.

Heavy chain variable region		
Kabat position	P13B02 VH	IGHV1-46*01 (SEQ ID NO: 153)
H4	M	L
H5	K	V
H12	V	K
H23	T	K
H27	F	Y
H28	N	T
H29	I	F
H30	K	T
H48	I	M
H69	I	M
H71	A	R

H75	S	T
H76	N	S
H80	L	M
H81	Q	E
H94	T	R
Light chain variable region		
Kabat position	P13B02 VL	IGKV3-20*01 (SEQ ID NO: 160)
L3	L	V
L22	T	S
L36	F	Y
L43	S	A
L47	W	L
L58	V	I
L70	S	D
L71	Y	F

Table 11. Summary of framework back mutations in H1_R98K_L4M.

Kabat position	P13B02 VH	IGHV1-46*01 (SEQ ID NO: 153)
H4	M	L
H27	F	Y
H28	N	T
H29	I	F
H30	K	T
H69	I	M
H71	A	R
H94	T	R

[00334] In addition, the HCDR3 of P13B02 contains a “RYD” motif, which has been demonstrated previously to mimic the integrin binding motif “RGD.” In order to test whether the “RYD” motif could be removed without impacting binding to LAG-3, an amino acid substitution R98K or D100E, numbered according to Kabat definition, was introduced in the heavy chain.

[00335] A panel of 37 humanized antibodies, designated as P13B02-01 to P13B02-37, were designed based on the description above and generated as full length IgG₁ antibodies.

P13B02-30 were generated in three versions: a full length antibody containing IgG₁ G1m17 allotype, referred to as P13B02-30 (IgG₁); a full length antibody containing IgG₁ G1m17 allotype with a N297A mutation, referred to as P13B02-30 (IgG₁ G1m17 N297A); and a full length antibody containing IgG₁ G1m3 allotype with a N297A mutation, referred to as P13B02-30 (IgG₁ G1m3 N297A). The sequence information of the variable regions of P13B02-01 to P13B02-37 is summarized in Table 7. Two batches of P13B02-30 (IgG₁ G1m3 N297A) were examined for post-translational processing in the producer cells. All these 37 humanized antibodies retained binding to recombinant human LAG-3 in surface plasmon resonance analysis (data not shown).

7.2.2 Binding of humanized anti-LAG-3 antibodies to human LAG-3

[00336] The anti-LAG-3 antibodies were tested for binding to primary human T cells using flow cytometry. Cryopreserved human PBMCs (Research Blood Components) were plated at 10^6 cells/ml in RPMI1640 supplemented with Normocin™ (Invivogen, Cat# ant-nr-1) and 10% heat-inactivated FBS (Thermo Fisher Scientific, Cat# 26140079) in a T-75 flask (Corning) in the presence of 100 ng/ml SEA (Toxin Technologies, Cat# at101red) for 5 days at 37°C, 5% CO₂, and 97% humidity. Cultured PBMCs were then plated at 10^5 cells/well in 96-well U-bottom plates (Nunc). Cells were incubated with 10 µg/ml of anti-LAG-3 antibody for 30 minutes on ice. Cells were washed three times and then incubated with anti-CD4-PE/Cy7 (Biolegend, Clone# OKT4, Cat# 317414), anti-CD8-FITC (Biolegend, Clone# RPA-T8, Cat# 301060), anti-CD3-APC (BD Biosciences, Clone# SP34-2, Cat# 557597), LIVE/DEAD® Fixable Near-IR Dead Cell Stain (Life Technologies, Cat# L10119), Fc block (BD Biosciences, Cat# 422302), and goat anti-human IgG-PE (ThermoFisher, Cat# PA1-74408). Cells were incubated for 30 minutes on ice, washed, and analyzed with a FACS machine (BD Canto). As shown in Figure 6A, the chimeric antibody P13B02 and all the humanized antibodies tested exhibited binding to primary CD4⁺ human T cells stimulated with the SEA superantigen.

[00337] Next, human PBMCs were activated using the SEA superantigen similarly as described above and incubated with serially diluted (50000, 15000, 4500, 1350, 407, 122, 37, or 11 ng/ml) P13B02-16 or an isotype control antibody. Binding was analyzed with a FACS Fortessa cytometer (Becton Dickinson). The humanized antibody P13B02-16 bound to activated primary human CD4⁺ T cells in a dose-dependent manner (Figure 6B).

7.2.3 Ligand blocking activity of humanized anti-LAG-3 antibodies

[00338] Next, the ability of the humanized anti-LAG-3 antibodies to block the interaction between cross-linked recombinant LAG-3-6His and MHC class II expressing Raji cells was

examined as described above. The humanized antibodies P13B02-06, P13B02-07, P13B02-16, P13B02-26, and P13B02-27, all of which include a human IgG₁ constant region, were tested at 57820, 28910, 14455, 7228, 3613, 1807, 903, 452, 226, 113, and 56 ng/ml (Figure 7A). The humanized antibody P13B02-30 (IgG₁ G1m17 N297A) was tested at 96360, 48180, 24090, 12045, 6022, 3011, 1505, 753, 376, 188, and 94 ng/ml (Figure 7B).

[00339] As shown in Figures 7A and 7B, all the humanized antibodies tested effectively blocked LAG-3 binding to MHC class II positive Raji cells.

7.2.4 Effect of humanized anti-LAG-3 antibody on human PBMCs upon Staphylococcus Enterotoxin A (SEA) stimulation

[00340] The functional activity of the humanized antibody P13B02-30 (IgG₁) was assessed using primary human PBMCs stimulated by Staphylococcus Enterotoxin A (SEA). Cryopreserved human PBMCs (Research Blood Components) were plated at 10⁵ cells/well in RPMI1640 supplemented with Normocin™ (Invivogen, Cat# ant-nr-1) and 10% heat-inactivated FBS (Thermo Fisher Scientific, Cat# 26140079) in 96-well NUNC LON delta surface plates (NUNC™). Cells were cultured with 100 ng/ml SEA (Toxin Technologies, Cat# at101red) and 10 µg/ml P13B02-30 (IgG₁) or an isotype control antibody for 5 days at 37°C, 5% CO₂, and 97% humidity. Clarified supernatant was collected and stored at -80°C until analysis. IL-2 levels were determined using AlphaLISA (Perkin Elmer, Cat# AL221C).

[00341] As shown in Figure 8A, the anti-LAG-3 antibody P13B02-30 (IgG₁) enhanced IL-2 production in human PBMCs stimulated with the SEA superantigen.

[00342] In a similar experiment, cryopreserved human PBMCs were cultured with 100 ng/ml SEA (Toxin Technologies, Cat# at101red) and 10 µg/ml P13B02-16 (IgG₁) or an isotype control antibody in the presence or absence of 5 µg/ml of anti-PD-1 antibody pembrolizumab (Pembro) (Myoderm), anti-PD-1 antibody nivolumab (Nivo) (Myoderm), three different anti-PD-L1 antibodies, or anti-CTLA-4 antibody ipilimumab (Ipi) (Myoderm) for 5 days at 37°C, 5% CO₂, and 97% humidity. Clarified supernatant was collected and stored at -80°C until analysis. IL-2 levels were determined using AlphaLISA (Perkin Elmer, Cat# AL221C). Anti-PD-L1 antibody #1 was generated based on the variable region sequences of antibody A09-246-2 provided in U.S. Application Publication No. US2014/0341917 (herein incorporated by reference in its entirety). Anti-PD-L1 antibody #2 was generated based on the variable region sequences of antibody 2.14H9OPT provided in U.S. Patent No. 8,779,108 (herein incorporated by reference in its entirety). Anti-PD-L1 antibody #3 was generated based on sequences provided in U.S. Patent No. 8,217,149 (herein incorporated by reference in its entirety). The sequences of these three anti-PD-L1 antibodies

are listed in Table 12.

Table 12. Sequences of anti-PD-L1 antibodies

SEQ ID NO:	Description	Amino acid sequence
202	anti-PD-L1 antibody #1 heavy chain	EVQLLES GGGLVQPGGSLRLSCAASGFTFSSYIMMWVRQAPGKGLE WVSSIYPSGGITFYADTVKGRFTISRDN SKNTLYLQMNSLRAEDTA VYYCARIKLGTVT TVDYWGQGLTVTVSSASTKGPSVFPLAPSSKST SGGTAALGCLVKDYFPEPVT VSWNSGALTSGVHTFPAVLQSSGLYS LSSVVTVPSSSLGTQTYICNVNHKPSNTKVDKRVEPKSCDKTHTCP PCPAPELLGGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSHEDPEV KFNWYVDGVEVHNAKTKPREEQYNSTYRVVSVLTVLHQDWLNGKEY KCKVSNKALPAPIEKTISKAKGQPREPQVYTLPPSREEMTKNQVSL TCLVKGFYPSDIAVEWESNGQPENNYKTTTPVLDSDGSFFLYSKLT VDKSRWQQGNVFSCSVMHEALHNHYTQKSLSLSPG
203	anti-PD-L1 antibody #1 light chain	QSALTQPASVSGSPGQSITISCTGTSSDVGGYNYVSWYQQHPGKAP KLMIYDVSNRPSGVSNRFGSGSKSGNTASLTISGLQAED EADYYCSS YTSSSTRVFGTGTKVTVLQPKANPTVTLFPPSSEELQANKATLVCL ISDFYPGAVTVAWKADGSPVKAGVETTKPSKQSN NKYAASSYLSLT PEQWKSHRSYSCQVTHEGSTVEKTVAPTECS
204	anti-PD-L1 antibody #2 heavy chain	EVQLVES GGGLVQPGGSLRLSCAASGFTFSRYWMSWVRQAPGKGLE WVANIKQDGSEKYYVDSVKGRFTISRDN AKNSLYLQMNSLRAEDTA VYYCAREGGWFGELAFDYWGQGLTVTVSSASTKGPSVFPLAPSSKS TSGGTAALGCLVKDYFPEPVT VSWNSGALTSGVHTFPAVLQSSGLY SLSSVVTVPSSSLGTQTYICNVNHKPSNTKVDKRVEPKSCDKTHTC PPCPAPELLGGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSHEDPE VKFNWYVDGVEVHNAKTKPREEQYNSTYRVVSVLTVLHQDWLNGKE YKCKVSNKALPAPIEKTISKAKGQPREPQVYTLPPSREEMTKNQVS LTCLVKGFYPSDIAVEWESNGQPENNYKTTTPVLDSDGSFFLYSKL TVDKSRWQQGNVFSCSVMHEALHNHYTQKSLSLSPG
205	anti-PD-L1 antibody #2 light chain	EIVLTQSPGTL SLSPGERATLSCRASQRVSSSYLAWYQQKPGQAPR LLIYDASSRATGIPDRFSGSGSGTDFTLTISRLEPEDFAVYYCQQY GSLPWTFGQGTKVEIKRTVAAPSVFI FPPSDEQLKSGTASVCLLN NFYPREAKVQWKVDNALQSGNSQESVTEQDSKDS TYSLSSTLTLSK

		ADYEKHKVYACEVTHQGLSSPVTKSFNRGEC
206	anti-PD-L1 antibody #3 heavy chain	EVQLVESGGGLVQPGGSLRLSCAASGFTFSDSWIHWVRQAPGKGLE WVAWISPYGGSTYYADSVKGRFTISADTSKNTAYLQMNSLRAEDTA VYYCARRHWPGGFDYWGQGLTVTVSSASTKGPSVFPLAPSSKSTSG GTAALGCLVKDYFPEPVTVSWNSGALTSGVHTFPAVLQSSGLYSLS SVVTVPSSSLGTQTYICNVNHKPSNTKVDKRVEPKSCDKTHTCPPC PAPELLGGPSVFLFPPKPKDTLMISRTPEVTCVVDVSHEDPEVKF NWIYVDGVEVHNAKTKPREEQYNSTYRVVSVLTVLHQDWLNGKEYKC KVSNAKALPAPIEKTISKAKGQPREPQVYTLPPSREEMTKNQVSLTC LVKGFYPSDIAVEWESNGQPENNYKTTTPVLDSDGSFFLYSKLTVD KSRWQQGNVFCSCVMHEALHNNHYTQKSLSLSPG
207	anti-PD-L1 antibody #3 light chain	DIQMTQSPSSLSASVGDRVTITCRASQDVSTAVAWYQQKPGKAPKL LIYSASFYSGVPSRFSGSGSGTDFTLTISLQPEDFATYYCQYYL YHPATFGQGTKVEIKRTVAAPSVFIFPPSDEQLKSGTASVVCCLNN FYPREAKVQWKVDNALQSGNSQESVTEQDSKDYSLSTLTLSKA ADYEKHKVYACEVTHQGLSSPVTKSFNRGEC

[00343] As shown in Figure 8B, P13B02-16 (IgG₁), either alone or in combination with the anti-PD-1 antibody pembrolizumab or nivolumab, the anti-PD-L1 antibody #1, #2, or #3, or the anti-CTLA-4 antibody ipilimumab, enhanced IL-2 production in human PBMCs in the presence of the SEA superantigen.

7.2.5 Effect of humanized anti-LAG-3 antibody on cytokine production of tumor infiltrating lymphocytes

[00344] The anti-LAG-3 antibody P13B02-30 (IgG₁ G1m3 N297A) was further assessed for its ability to stimulate cytokine production of activated primary tumor infiltrating lymphocytes (TILs), alone or in combination with an anti-PD-1 antibody. Single-cell suspensions from fresh renal cell carcinoma (RCC) (stage I) or colorectal cancer (CRC) (stage II) tumors (UMass Medical School, Worcester, MA) were isolated *via* mechanical microdissection. In some cases, depending on the level of fibrosis, enzymatic digestion was necessary (Liberase and DNaseI, Roche). Cells were rested at 5×10^4 cells/well in RPMI1640 supplemented with NormocinTM (Invivogen, Cat# ant-nr-1), recombinant human IL-2 (20 U/ml, R&D Systems, Cat# 202-IL-010), and 10% heat-inactivated FBS (Thermo Fisher Scientific, Cat# 26140079) in 96-well NUNCTM delta surface plates (NUNCTM, Cat# 143761) for 1 day. On the following day, the samples were centrifuged and fresh

culture media containing the antibodies of interest, P13B02-30 (IgG₁ G1m3 N297A) at 20 µg/ml and the anti-PD-1 antibody pembrolizumab (Pembro) (Myoderm) at 5 µg/ml, and anti-CD3/CD28 microbeads (1:1 bead:cell ratio), was added at a final volume of 100 µl and allowed to incubate for 3 days at 37°C and 5% CO₂. Cell-free supernatant was collected and stored at -80°C until analysis. TNFα levels were determined using AlphaLISA (Perkin Elmer, Cat# AL208C).

[00345] As shown in Figures 9A and 9B, the anti-LAG-3 antibody P13B02-30 (IgG₁ G1m3 N297A) enhanced TNFα production of activated primary TILs from RCC or CRC tumors.

10 7.2.6 Anti-LAG-3 antibody enhances T cell activation in a LAG-3-mediated cell suppression assay

[00346] In this example, an NFAT-luciferase reporter line was used to assess the inhibitory effect of the anti-LAG-3 antibody P13B02-30 (IgG₁ G1m3 N297A) against LAG-3 in a cell suppression assay. Two experiments were performed, as described below.

15 [00347] In each of the two experiments, Jurkat-NFAT-LAG-3 cells were suspended to a 2.5× working concentration of 1×10⁶ cells/mL in assay medium (RPMI + 10% heat-inactivated FBS + 1% Pen/Strep). Raji cells were suspended to a 3.33× working concentration of 1×10⁶ cells/mL in assay medium. Staphylococcal Enterotoxin E (Toxin Technology) was prepared at a 10× working concentration of 0.04 ng/mL in assay medium.

20 A serial dilution of anti-LAG-3 antibody or isotype antibody was prepared in assay medium. In the first experiment, the antibody concentrations ranged from 0.2-50 µg/mL. In the second experiment, the antibody concentrations ranged from 0.1-100 µg/mL). After the serial dilutions of the antibodies were prepared, 40 µL of Jurkat-NFAT-LAG-3 cells and 20 µL of antibody solution were pre-incubated for 30 minutes at 37°C and 5% CO₂ in U-bottom 96-well plates. 30 µL of Raji cells and 10 µL of Staphylococcal Enterotoxin E were added to the

25 96-well plates and incubated for 5-6 hours. 100 µL of Bio-Glo Luciferase (Promega) was then added to each well and luminescence was recorded using EnVision Plate Reader (Perkin Elmer) after 10-15 minutes.

[00348] As shown in Figures 10A and 10B, the anti-LAG-3 antibody significantly increased NFAT-luciferase reporter signal, relative to isotype control antibody, in a dose-dependent manner.

7.3 Example 3: Epitope mapping of anti-LAG-3 antibody

[00349] In this example, the epitope of the anti-LAG-3 antibody P13B02-30 was characterized as described below.

7.3.1 Epitope mapping of anti-LAG-3 antibody using hydrogen-deuterium exchange (HDX) mass spectrometry

[00350] Anti-LAG-3 F(ab')₂ was generated from P13B02-30 (IgG₄ S228P) using the FragIT Kit (Genovis, Cat# A2-FR2-100). The interaction of anti-LAG-3 F(ab')₂ with human LAG-3 was studied using hydrogen-deuterium exchange (HDX) mass spectrometry.

[00351] For pepsin/protease XIII digestion, 7.9 µg of recombinant his-tagged human LAG-3 (Sino Biological, Cat# 16498-H08H) in 125 µl control buffer (50 mM phosphate, 100 mM sodium chloride, pH 7.4) was denatured by adding 125 µl of 4 M guanidine hydrochloride, 0.85 M TCEP buffer (final pH 2.5) and incubating the mixture for 5 minutes at 20°C. The mixture was then subjected to on-column pepsin/protease XIII digestion using an in-house packed pepsin/protease XIII (w/w, 1:1) column and the resultant peptides were analyzed using a UPLC-MS system comprised of a Waters Acquity UPLC coupled to a Q ExactiveTM Hybrid Quadrupole-Orbitrap Mass Spectrometer (Thermo). The peptides were separated on a 50 mm x 1 mm C8 column with a 19-minute gradient from 2-30% solvent B (0.2% formic acid in acetonitrile). Peptide identification was conducted through searching MS/MS data against the human LAG-3 sequence with Mascot. The mass tolerance for the precursor and productions was 10 ppm and 0.05 Da, respectively.

[00352] 20 µl human LAG-3 (7.9 µg) or 20 µl human LAG-3/F(ab')₂ mixture (7.9 µg: 15.8 µg) was incubated with 105 µl deuterium oxide labeling buffer (50 mM sodium phosphate, 100 mM sodium chloride, pH 7.4) for 0 second, 60 seconds, 300 seconds, 1800 seconds, 7200 seconds, and 14400 seconds at 20°C. Hydrogen/deuterium exchange was quenched by adding 125 µl of 4 M guanidine hydrochloride, 0.85 M TCEP buffer (final pH 2.5). Subsequently, the quenched samples were subjected to on column pepsin/protease XIII digestion and LC-MS analysis as described above. The mass spectra were recorded in MS only mode. Raw MS data were processed using HDX WorkBench, software for the analysis of H/D exchange MS data (J. Am. Soc. Mass Spectrom. 2012, 23 (9), 1512-1521, herein incorporated by reference in its entirety). The deuterium levels were calculated using the average mass difference between the deuterated peptide and its native form (t₀).

[00353] The sequence coverage achieved for human LAG-3 was 96.6%. While most of the human LAG-3 peptides displayed identical or similar deuterium levels with and without the anti-LAG-3 F(ab')₂, several peptide segments were found to have significantly decreased deuterium incorporation upon F(ab')₂ binding. A strong decrease in deuterium uptake was observed at a region consisting of the amino acid sequence of SEQ ID NO: 215 (SPTIPLQDLSL). The residues are numbered according to SEQ ID NO: 166.

* * *

[00354] The invention is not to be limited in scope by the specific embodiments described herein. Indeed, various modifications of the invention in addition to those described will become apparent to those skilled in the art from the foregoing description and accompanying
5 figures. Such modifications are intended to fall within the scope of the appended claims.

[00355] All references (*e.g.*, publications or patents or patent applications) cited herein are incorporated herein by reference in their entirety and for all purposes to the same extent as if each individual reference (*e.g.*, publication or patent or patent application) was specifically and individually indicated to be incorporated by reference in its entirety for all purposes.

10 [00356] Other embodiments are within the following claims.

CLAIMS

1. An isolated antibody that specifically binds to human LAG-3, the antibody comprising a heavy chain variable region comprising complementarity determining regions CDRH1, CDRH2 and CDRH3 and a light chain variable region comprising complementarity determining regions CDRL1, CDRL2 and CDRL3, wherein CDRH1, CDRH2, CDRH3, CDRL1, CDRL2, and CDRL3 comprise the amino acid sequences set forth in SEQ ID NOs: 78, 83, 94, 100, 104, and 105; 78, 85, 95, 100, 104, and 105; 78, 86, 96, 100, 104, and 105; 78, 86, 96, 100, 104, and 109; 78, 86, 96, 100, 104, and 110; 78, 86, 96, 101, 104, and 108; 78, 86, 96, 103, 104, and 111; 78, 86, 97, 102, 104, and 112; 78, 91, 94, 100, 104, and 107; 78, 92, 96, 100, 104, and 105; 78, 92, 96, 100, 104, and 109; 79, 84, 95, 100, 104, and 105; 79, 84, 95, 100, 104, and 106; 79, 84, 95, 102, 104, and 105; 79, 88, 95, 100, 104, and 105; 79, 89, 95, 100, 104, and 105; 79, 90, 95, 100, 104, and 105; 79, 90, 98, 100, 104, and 105; 79, 90, 99, 100, 104, and 105; 80, 85, 96, 100, 104, and 105; 81, 87, 96, 100, 104, and 105; 81, 87, 96, 100, 104, and 107; or, 82, 93, 95, 100, 104, and 105, respectively.
2. An isolated antibody that specifically binds to human LAG-3, the antibody comprising a heavy chain variable region comprising complementarity determining regions CDRH1, CDRH2 and CDRH3 and a light chain variable region comprising complementarity determining regions CDRL1, CDRL2 and CDRL3, wherein CDRH1, CDRH2, CDRH3, CDRL1, CDRL2, and CDRL3 comprise the amino acid sequences set forth in SEQ ID NOs: 79, 90, 98, 100, 104, and 105, respectively.
3. The isolated antibody of claim 1 or claim 2, wherein the heavy chain variable region comprises an amino acid sequence selected from the group consisting of SEQ ID NOs: 61, 63-72, and 220; and/or the light chain variable region comprises an amino acid sequence selected from the group consisting of SEQ ID NOs: 73-77, 152, 221, or 224.
4. The isolated antibody of any one of claims 1-3, wherein the heavy chain variable region and the light chain variable region, respectively, comprise the amino acid sequences set forth in SEQ ID NOs: 61 and 73; 61 and 74; 61 and 75; 61 and 76; 61 and 77; 61 and 221; 63 and 73; 63 and 74; 63 and 75; 63 and 76; 63 and 77; 63 and 221; 64 and 73; 64 and 74; 64 and 75; 64 and 76; 64 and 77; 64 and 221; 65 and 73;

65 and 74; 65 and 75; 65 and 76; 65 and 77; 65 and 221; 66 and 73; 66 and 74; 66 and 75; 66 and 76; 66 and 77; 66 and 221; 67 and 73; 67 and 74; 67 and 75; 67 and 76; 67 and 77; 67 and 221; 68 and 73; 68 and 74; 68 and 75; 68 and 76; 68 and 77; 68 and 221; 69 and 73; 69 and 74; 69 and 75; 69 and 76; 69 and 77; 69 and 221; 70 and 73; 70 and 74; 70 and 75; 70 and 76; 70 and 77; 70 and 221; 71 and 73; 71 and 74; 71 and 75; 71 and 76; 71 and 77; 71 and 221; 72 and 73; 72 and 74; 72 and 75; 72 and 76; 72 and 77; 72 and 221; 220 and 73; 220 and 74; 220 and 75; 220 and 76; 220 and 77; or 220 and 221.

5. The isolated antibody of claim 4, wherein the heavy chain variable region and the light chain variable region, respectively, comprise SEQ ID NO: 65 and 73.
6. The isolated antibody of any one of claims 1-5, wherein the antibody comprises a heavy chain comprising an amino acid sequence selected from the group consisting of SEQ ID NOs: 168, 169, 170, 176, 178-186, and 225-227.
7. The isolated antibody of any one of claims 1-6, wherein the antibody comprises a light chain comprising an amino acid sequence selected from the group consisting of SEQ ID NOs: 187-191 and 228.
8. The isolated antibody of any one of claims 1-7, wherein the antibody comprises a heavy chain constant region, wherein the heavy chain constant region:
 - (a) is selected from the group consisting of human IgG₁, IgG₂, IgG₃, IgG₄, IgA₁, and IgA₂;
 - (b) is an IgG₁ heavy chain constant region;
 - (c) is an IgG₁ heavy chain constant region comprising a N297A mutation, numbered according to the EU numbering system;
 - (d) comprises the amino acid sequence of SEQ ID NO: 194;
 - (e) is an IgG₁ heavy chain constant region comprising a N297Q mutation, numbered according to the EU numbering system;
 - (f) is a non-fucosylated IgG₁;
 - (g) is an IgG₄ heavy chain constant region;
 - (h) is an IgG₄ heavy chain constant region comprising a S228P mutation, numbered according to the EU numbering system; or
 - (i) comprises the amino acid sequence of SEQ ID NO: 196.

9. The isolated antibody of any one of claims 1-8, wherein the antibody comprises a human kappa or human lambda light chain constant region.
10. The isolated antibody of claim 9, wherein the light chain constant region is a human kappa light chain constant region.
11. The isolated antibody of claim 10, wherein the antibody comprises a light chain constant region comprising the amino acid sequence of SEQ ID NO: 198 or 219.
12. The isolated antibody of any one of claims 1-11, comprising a heavy chain and a light chain, wherein the heavy chain and the light chain, respectively, comprise the amino acid sequences set forth in SEQ ID NOs: 168 and 187; 168 and 188; 168 and 189; 168 and 190; 168 and 191; 168 and 228; 169 and 187; 169 and 188; 169 and 189; 169 and 190; 169 and 191; 169 and 228; 170 and 187; 170 and 188; 170 and 189; 170 and 190; 170 and 191; 170 and 228; 176 and 187; 176 and 188; 176 and 189; 176 and 190; 176 and 191; 176 and 228; 178 and 187; 178 and 188; 178 and 189; 178 and 190; 178 and 191; 178 and 228; 179 and 187; 179 and 188; 179 and 189; 179 and 190; 179 and 191; 179 and 228; 180 and 187; 180 and 188; 180 and 189; 180 and 190; 180 and 191; 180 and 228; 181 and 187; 181 and 188; 181 and 189; 181 and 190; 181 and 191; 181 and 228; 182 and 187; 182 and 188; 182 and 189; 182 and 190; 182 and 191; 182 and 228; 183 and 187; 183 and 188; 183 and 189; 183 and 190; 183 and 191; 183 and 228; 184 and 187; 184 and 188; 184 and 189; 184 and 190; 184 and 191; 184 and 228; 185 and 187; 185 and 188; 185 and 189; 185 and 190; 185 and 191; 185 and 228; 186 and 187; 186 and 188; 186 and 189; 186 and 190; 186 and 191; 186 and 228; 225 and 187; 225 and 188; 225 and 189; 225 and 190; 225 and 191; 225 and 228; 226 and 187; 226 and 188; 226 and 189; 226 and 190; 226 and 191; 226 and 228; 227 and 187; 227 and 188; 227 and 189; 227 and 190; 227 and 191; or 227 and 228.
13. The isolated antibody of claim 12, wherein the heavy chain and the light chain, respectively comprise the amino acid sequences set forth in SEQ ID NOs: 169 and 187.
14. The isolated antibody of claim 13, wherein the amino acid sequences of the heavy chain and the light chain, respectively, consist of the amino acid sequences set forth

in SEQ ID NOs: 169 and 187.

15. The isolated antibody of any one of claims 1-14, wherein the antibody:
 - (a) is a human antibody,
 - (b) is a humanized antibody,
 - (c) is antagonistic to human LAG-3,
 - (d) deactivates, reduces, or inhibits an activity of human LAG-3,
 - (e) inhibits binding of human LAG-3 to MHC class II,
 - (f) induces IL-2 production by peripheral blood mononuclear cells (PBMCs) stimulated with staphylococcal enterotoxin A (SEA), and/or
 - (g) induces TNF α production by tumor infiltrating lymphocytes (TILs) stimulated with anti-CD3 and anti-CD28 antibodies.
16. The isolated antibody of any one of claims 1-15, conjugated to a cytotoxic agent, cytostatic agent, toxin, radionuclide, or detectable label.
17. A pharmaceutical composition comprising the antibody of any one of claims 1-16 and a pharmaceutically acceptable carrier or excipient.
18. Use of the antibody or pharmaceutical composition of any one of claims 1-17 in the manufacture of a medicament for:
 - (a) the treatment of cancer in a subject in need thereof;
 - (b) increasing T cell activation in response to an antigen in a subject;
 - (c) the treatment of a bacterial infection caused by staphylococcus enterotoxin A in a subject in need thereof.
19. The antibody or pharmaceutical composition of any one of claims 1-17 for use in the treatment of cancer in a subject in need thereof.
20. An isolated polynucleotide encoding
 - a) a heavy chain variable region and/or a light chain variable region of the antibody of any one of claims 1-16; or
 - b) a heavy chain and/or a light chain of the antibody of any one of claims 1-16; or
 - c) the amino acid sequence of any one of SEQ ID NOs: 61, 63-77, 152, 168-170,

176, 178-191, 220, 221, or 224-228.

21. A vector comprising the polynucleotide of claim 20.
22. A recombinant host cell comprising:
 - a) an isolated polynucleotide encoding a heavy chain variable region of the antibody of any one of claims 1-16, and an isolated polynucleotide encoding a light chain variable region of the antibody of any one of claims 1-16; or
 - b) an isolated polynucleotide encoding a heavy chain of the antibody of any one of claims 1-16, and an isolated polynucleotide encoding a light chain of the antibody of any one of claims 1-16.
23. A method of producing an antibody that binds to human LAG-3, the method comprising culturing the host cell of claim 22 so that the polynucleotide is expressed and the antibody is produced.