

SINGLE-DOMAIN ANTIBODIES AGAINST LAG-3 AND USES THEREOF**CROSS-REFERENCE TO RELATED APPLICATIONS**

[0001] This application claims the priority benefit of International Patent Application No. PCT/CN2018/081356, filed March 30, 2018, the disclosure of which is incorporated by reference in its entirety.

SUBMISSION OF SEQUENCE LISTING ON ASCII TEXT FILE

[0002] The content of the following submission on ASCII text file is incorporated herein by reference in its entirety: a computer readable form (CRF) of the Sequence Listing (file name: 761422001442SEQLIST.txt, date recorded: March 19, 2019, size: 514 KB).

FIELD OF THE INVENTION

[0003] The present invention relates to constructs comprising a single-domain antibody (sdAb) that specifically recognize LAG-3, and methods of making and using thereof.

BACKGROUND OF THE INVENTION

[0004] Lymphocyte-activation protein 3 (LAG-3), comprised of 503 amino acids, belongs to the Ig superfamily and contains 4 extracellular Ig-like domains, designated D1 to D4. LAG3 is closely related to CD4. LAG-3 is a cell surface protein expressed on activated T cells, NK cells, B cells, and plasmacytoid dendritic cells, and plays a role in the function of these lymphocyte subsets that is important but not completely understood. The LAG-3 protein negatively regulates cellular proliferation, activation, and homeostasis of T cells. LAG-3 also helps maintain CD8⁺ T cells in a tolerogenic state. The interaction between LAG-3 and its major ligand, Class II MHC, is thought to play a role in modulating dendritic cell functions. Recent preclinical studies have documented a role for LAG-3 in CD8 T cell exhaustion, and blockade of the LAG-3/Class II MHC interaction using LAG-3 blocking antibodies or LAG-3-Ig fusion proteins is being evaluated in a number of clinical trials in cancer patients.

[0005] Programmed Cell Death Receptor 1 (PD-1) is another inhibitory immune checkpoint molecule with important negative regulation on T cell functions. T-cell responses can be attenuated by PD-1 signaling when PD-1 binds to Programmed Cell Death Ligand 1 (PD-L1) and/or Programmed Cell Death Ligand 2 (PD-L2), which regulate T-cell receptor (TCR) signaling. Blockade of the PD-1/PD-L1 axis using antibodies targeting either PD-1 or PD-L1 has been shown to promote tumor-specific T cell immunity with significant clinical benefits to cancer patients. However, there are still huge unmet clinical needs due to resistance or relapse upon PD-1/PD-L1 blockade.

[0006] The disclosures of all publications, patents, patent applications and published patent applications referred to herein are hereby incorporated herein by reference in their entirety.

BRIEF SUMMARY OF THE INVENTION

[0007] The present invention relates to anti-LAG-3 constructs comprising an sdAb that specifically recognizes LAG-3 (hereinafter referred to as “anti-LAG-3 sdAb”), such as anti-LAG-3 sdAb, anti-LAG-3 HCAb (*e.g.*, anti-LAG-3 sdAb-Fc fusion protein comprising an anti-LAG-3 sdAb fused to a crystalline fragment (Fc) of human immunoglobulin G (IgG), and multispecific (such as bispecific) antigen binding proteins comprising an anti-LAG-3 sdAb fused to, for example, other sdAbs, a full-length four-chain antibody or antigen binding fragments thereof (*e.g.*, Fab or scFv), and methods of making and using thereof.

[0008] One aspect of the present application provides an isolated anti-LAG-3 construct comprising a single-domain antibody (sdAb) specifically recognizing LAG-3, wherein the sdAb comprises a CDR1 comprising the amino acid sequence of any one of SEQ ID NOs: 39-76, or a variant thereof comprising up to about 3 amino acid substitutions; a CDR2 comprising the amino acid sequence of any one of SEQ ID NOs: 115-152, or a variant thereof comprising up to about 3 amino acid substitutions; and a CDR3 comprising the amino acid sequence of any one of SEQ ID NOs: 191-228, or a variant thereof comprising up to about 3 amino acid substitutions. In some embodiments, the isolated anti-LAG-3 construct comprises an sdAb specifically recognizing LAG-3, wherein the sdAb comprises a CDR1 comprising the amino acid sequence of any one of SEQ ID NOs: 39-76; a CDR2 comprising the amino acid sequence of any one of SEQ ID NOs: 115-152; and a CDR3 comprising the amino acid sequence of any one of SEQ ID NOs: 191-228; or a variant thereof comprising up to about 3 amino acid substitutions in the CDR regions.

[0009] In some embodiments according to any one of the isolated anti-LAG-3 constructs described above, the sdAb specifically recognizing LAG-3 comprises any one of the following:

- (1) a CDR1 comprising the amino acid sequence of SEQ ID NO: 39, or a variant thereof comprising up to about 3 amino acid substitutions; a CDR2 comprising the amino acid sequence of SEQ ID NO: 115, or a variant thereof comprising up to about 3 amino acid substitutions; and a CDR3 comprising the amino acid sequence of SEQ ID NO: 191, or a variant thereof comprising up to about 3 amino acid substitutions;
- (2) a CDR1 comprising the amino acid sequence of SEQ ID NO: 40, or a variant thereof comprising up to about 3 amino acid substitutions; a CDR2 comprising the amino acid sequence of SEQ ID NO: 116, or a variant thereof comprising up to about 3 amino acid substitutions; and a CDR3 comprising the

amino acid sequence of SEQ ID NO: 192, or a variant thereof comprising up to about 3 amino acid substitutions;

- (3) a CDR1 comprising the amino acid sequence of SEQ ID NO: 41, or a variant thereof comprising up to about 3 amino acid substitutions; a CDR2 comprising the amino acid sequence of SEQ ID NO: 117, or a variant thereof comprising up to about 3 amino acid substitutions; and a CDR3 comprising the amino acid sequence of SEQ ID NO: 193, or a variant thereof comprising up to about 3 amino acid substitutions;
- (4) a CDR1 comprising the amino acid sequence of SEQ ID NO: 42, or a variant thereof comprising up to about 3 amino acid substitutions; a CDR2 comprising the amino acid sequence of SEQ ID NO: 118, or a variant thereof comprising up to about 3 amino acid substitutions; and a CDR3 comprising the amino acid sequence of SEQ ID NO: 194, or a variant thereof comprising up to about 3 amino acid substitutions;
- (5) a CDR1 comprising the amino acid sequence of SEQ ID NO: 43, or a variant thereof comprising up to about 3 amino acid substitutions; a CDR2 comprising the amino acid sequence of SEQ ID NO: 119, or a variant thereof comprising up to about 3 amino acid substitutions; and a CDR3 comprising the amino acid sequence of SEQ ID NO: 195, or a variant thereof comprising up to about 3 amino acid substitutions;
- (6) a CDR1 comprising the amino acid sequence of SEQ ID NO: 44, or a variant thereof comprising up to about 3 amino acid substitutions; a CDR2 comprising the amino acid sequence of SEQ ID NO: 120, or a variant thereof comprising up to about 3 amino acid substitutions; and a CDR3 comprising the amino acid sequence of SEQ ID NO: 196, or a variant thereof comprising up to about 3 amino acid substitutions;
- (7) a CDR1 comprising the amino acid sequence of SEQ ID NO: 45, or a variant thereof comprising up to about 3 amino acid substitutions; a CDR2 comprising the amino acid sequence of SEQ ID NO: 121, or a variant thereof comprising up to about 3 amino acid substitutions; and a CDR3 comprising the amino acid sequence of SEQ ID NO: 197, or a variant thereof comprising up to about 3 amino acid substitutions;
- (8) a CDR1 comprising the amino acid sequence of SEQ ID NO: 46, or a variant thereof comprising up to about 3 amino acid substitutions; a CDR2 comprising the amino acid sequence of SEQ ID NO: 122, or a variant thereof comprising up to about 3 amino acid substitutions; and a CDR3 comprising the amino acid sequence of SEQ ID NO: 198, or a variant thereof comprising up to about 3 amino acid substitutions;
- (9) a CDR1 comprising the amino acid sequence of SEQ ID NO: 54, or a variant thereof comprising up to about 3 amino acid substitutions; a CDR2 comprising the amino acid sequence of SEQ ID NO: 130,

or a variant thereof comprising up to about 3 amino acid substitutions; and a CDR3 comprising the amino acid sequence of SEQ ID NO: 206, or a variant thereof comprising up to about 3 amino acid substitutions;

- (10) a CDR1 comprising the amino acid sequence of SEQ ID NO: 61, or a variant thereof comprising up to about 3 amino acid substitutions; a CDR2 comprising the amino acid sequence of SEQ ID NO: 137, or a variant thereof comprising up to about 3 amino acid substitutions; and a CDR3 comprising the amino acid sequence of SEQ ID NO: 213, or a variant thereof comprising up to about 3 amino acid substitutions;
- (11) a CDR1 comprising the amino acid sequence of SEQ ID NO: 70, or a variant thereof comprising up to about 3 amino acid substitutions; a CDR2 comprising the amino acid sequence of SEQ ID NO: 146, or a variant thereof comprising up to about 3 amino acid substitutions; and a CDR3 comprising the amino acid sequence of SEQ ID NO: 222, or a variant thereof comprising up to about 3 amino acid substitutions;
- (12) a CDR1 comprising the amino acid sequence of SEQ ID NO: 72, or a variant thereof comprising up to about 3 amino acid substitutions; a CDR2 comprising the amino acid sequence of SEQ ID NO: 148, or a variant thereof comprising up to about 3 amino acid substitutions; and a CDR3 comprising the amino acid sequence of SEQ ID NO: 224, or a variant thereof comprising up to about 3 amino acid substitutions; or
- (13) a CDR1 comprising the amino acid sequence of SEQ ID NO: 73, or a variant thereof comprising up to about 3 amino acid substitutions; a CDR2 comprising the amino acid sequence of SEQ ID NO: 149, or a variant thereof comprising up to about 3 amino acid substitutions; and a CDR3 comprising the amino acid sequence of SEQ ID NO: 225, or a variant thereof comprising up to about 3 amino acid substitutions.

[0010] In some embodiments according to any one of the isolated anti-LAG-3 constructs described above, the sdAb specifically recognizing LAG-3 comprises a CDR1 comprising the amino acid sequence of SEQ ID NO: 39; a CDR2 comprising the amino acid sequence of SEQ ID NO: 115; and a CDR3 comprising the amino acid sequence of SEQ ID NO: 191. In some embodiments, the sdAb specifically recognizing LAG-3 comprises a CDR1 comprising the amino acid sequence of SEQ ID NO: 40; a CDR2 comprising the amino acid sequence of SEQ ID NO: 116; and a CDR3 comprising the amino acid sequence of SEQ ID NO: 192. In some embodiments, the sdAb specifically recognizing LAG-3 comprises a CDR1 comprising the amino acid sequence of SEQ ID NO: 41; a CDR2 comprising the amino acid sequence of SEQ ID NO: 117; and a CDR3 comprising the amino acid sequence of SEQ ID NO: 193. In some embodiments, the sdAb specifically recognizing LAG-3 comprises a CDR1 comprising

the amino acid sequence of SEQ ID NO: 42; a CDR2 comprising the amino acid sequence of SEQ ID NO: 118; and a CDR3 comprising the amino acid sequence of SEQ ID NO: 194. In some embodiments, the sdAb specifically recognizing LAG-3 comprises a CDR1 comprising the amino acid sequence of SEQ ID NO: 43; a CDR2 comprising the amino acid sequence of SEQ ID NO: 119; and a CDR3 comprising the amino acid sequence of SEQ ID NO: 195. In some embodiments, the sdAb specifically recognizing LAG-3 comprises a CDR1 comprising the amino acid sequence of SEQ ID NO: 44; a CDR2 comprising the amino acid sequence of SEQ ID NO: 120; and a CDR3 comprising the amino acid sequence of SEQ ID NO: 196. In some embodiments, the sdAb specifically recognizing LAG-3 comprises a CDR1 comprising the amino acid sequence of SEQ ID NO: 45; a CDR2 comprising the amino acid sequence of SEQ ID NO: 121; and a CDR3 comprising the amino acid sequence of SEQ ID NO: 197. In some embodiments, the sdAb specifically recognizing LAG-3 comprises a CDR1 comprising the amino acid sequence of SEQ ID NO: 46; a CDR2 comprising the amino acid sequence of SEQ ID NO: 122; and a CDR3 comprising the amino acid sequence of SEQ ID NO: 198. In some embodiments, the sdAb specifically recognizing LAG-3 comprises a CDR1 comprising the amino acid sequence of SEQ ID NO: 54; a CDR2 comprising the amino acid sequence of SEQ ID NO: 130; and a CDR3 comprising the amino acid sequence of SEQ ID NO: 206. In some embodiments, the sdAb specifically recognizing LAG-3 comprises a CDR1 comprising the amino acid sequence of SEQ ID NO: 61; a CDR2 comprising the amino acid sequence of SEQ ID NO: 137; and a CDR3 comprising the amino acid sequence of SEQ ID NO: 213. In some embodiments, the sdAb specifically recognizing LAG-3 comprises a CDR1 comprising the amino acid sequence of SEQ ID NO: 70; a CDR2 comprising the amino acid sequence of SEQ ID NO: 146; and a CDR3 comprising the amino acid sequence of SEQ ID NO: 222. In some embodiments, the sdAb specifically recognizing LAG-3 comprises a CDR1 comprising the amino acid sequence of SEQ ID NO: 72; a CDR2 comprising the amino acid sequence of SEQ ID NO: 148; and a CDR3 comprising the amino acid sequence of SEQ ID NO: 224. In some embodiments, the sdAb specifically recognizing LAG-3 comprises a CDR1 comprising the amino acid sequence of SEQ ID NO: 73; a CDR2 comprising the amino acid sequence of SEQ ID NO: 149; and a CDR3 comprising the amino acid sequence of SEQ ID NO: 225.

[0011] In some embodiments, there is provided an isolated anti-LAG-3 construct comprising an sdAb specifically recognizing LAG-3, wherein the sdAb comprises CDR1, CDR2, and CDR3 of any one of SEQ ID NOs: 274-311.

[0012] In some embodiments according to any one of the isolated anti-LAG-3 constructs described above, the sdAb comprises V_HH domain comprising: (1) an FR1 comprising the amino acid sequence of any one of SEQ ID NOs: 1-38, or a variant thereof comprising up to about 3 amino acid substitutions; (2) an FR2 comprising the amino acid sequence of any one of SEQ ID NOs: 77-114, or a variant thereof

comprising up to about 3 amino acid substitutions; (3) an FR3 comprising the amino acid sequence of any one of SEQ ID NOs: 153-190, or a variant thereof comprising up to about 3 amino acid substitutions; and/or (4) an FR4 comprising the amino acid sequence of any one of SEQ ID NOs: 229-266, or a variant thereof comprising up to about 3 amino acid substitutions.

[0013] In some embodiments according to any one of the isolated anti-LAG-3 constructs described above, the sdAb comprises a V_HH domain comprising the amino acid sequence having at least about 80% sequence identity to any one of SEQ ID NOs: 274-311. In some embodiments, the sdAb comprises a V_HH domain comprising the amino acid sequence of any one of SEQ ID NOs: 274-311, or a variant thereof comprising up to about 3 amino acid substitutions in the V_HH domain. In some embodiments, the sdAb comprises a V_HH domain comprising the amino acid sequence of any one of SEQ ID NOs: 274-311.

[0014] In some embodiments according to any one of the isolated anti-LAG-3 constructs described above, the K_D of the binding between the sdAb and LAG-3 is about 10⁻⁷ M to about 10⁻¹² M. In some embodiments, the K_D of the binding between the sdAb and LAG-3 is about 10⁻⁹ M to about 10⁻¹¹ M.

[0015] In some embodiments according to any one of the isolated anti-LAG-3 constructs described above, the sdAb cross-reacts with a LAG-3 from a non-human mammal. In some embodiments, the sdAb specifically recognizing LAG-3 is camelid, chimeric, human, partially humanized, or fully humanized.

[0016] In some embodiments according to any one of the isolated anti-LAG-3 constructs described above, the isolated anti-LAG-3 construct is a heavy chain-only antibody (HCAb) comprising the sdAb specifically recognizing LAG-3 fused to an Fc fragment. In some embodiments, the HCAb is monomeric or dimeric. In some embodiments, the Fc fragment is a human IgG1 (hIgG1) Fc, effectorless (inert) hIgG1 Fc, hIgG4 Fc, or hIgG4 Fc (S228P). In some embodiments, the sdAb is fused to the Fc fragment via a peptide linker. In some embodiments, the peptide linker comprises the amino acid sequence of any one of SEQ ID NOs: 352-353 and 366-372. In some embodiments, the HCAb comprises the amino acid sequence of any one of SEQ ID NOs: 312-349.

[0017] In some embodiments according to any one of the isolated anti-LAG-3 constructs described above, the anti-LAG-3 construct comprises: (a) a first antigen binding portion comprising the sdAb specifically recognizing LAG-3; and (b) a second antigen binding portion that specifically recognizes a second epitope. In some embodiments, the second antigen binding portion comprises a full-length antibody, a Fab, a Fab', a (Fab')₂, an Fv, a single chain Fv (scFv), an scFv-scFv, a minibody, a diabody, or a second sdAb. In some embodiments, the anti-LAG-3 construct is multispecific (such as bispecific). In some embodiments, the first antigen binding portion and the second antigen binding portion are fused to each other via a peptide linker. In some embodiments, the peptide linker comprises the amino acid

sequence of any one of SEQ ID NOs: 352-353 and 366-372. In some embodiments, the N-terminus or the C-terminus of the anti-LAG-3 sdAb is fused to a peptide sequence. In some embodiments, the peptide sequence comprises the amino acid sequence of any one of SEQ ID NOs: 352-353 and 366-372. In some embodiments, the second antigen binding portion comprises a full-length antibody consisting of two heavy chains and two light chains. In some embodiments, the Fc fragment of the full-length antibody is human IgG1 (hIgG1) Fc, effectorless hIgG1 Fc, hIgG4 Fc, or hIgG4 Fc (S228P). In some embodiments, the N-terminus of the sdAb specifically recognizing LAG-3 is fused to the C-terminus of a heavy chain of the full-length antibody. In some embodiments, the C-terminus of the sdAb specifically recognizing LAG-3 is fused to the N-terminus of a heavy chain of the full-length antibody. In some embodiments, the N-terminus of the sdAb specifically recognizing LAG-3 is fused to the C-terminus of a light chain of the full-length antibody. In some embodiments, the C-terminus of the sdAb specifically recognizing LAG-3 is fused to the N-terminus of a light chain of the full-length antibody. In some embodiments, the second antigen binding portion specifically recognizes an immune checkpoint molecule selected from the group consisting of PD-1, 4-1BB, PD-L1, TIM-3, TIGIT, CTLA-4, VISTA, B7-1, B7-H3, CD47, OX40 and GITR. In some embodiments, the second antigen binding portion specifically recognizes PD-1. In some embodiments, the second antigen binding portion comprises HC-CDR1, HC-CDR2, and HC-CDR3 of a heavy chain comprising the amino acid sequence of SEQ ID NO: 356, and LC-CDR1, LC-CDR2, and LC-CDR3 of a light chain comprising the amino acid sequence of SEQ ID NO: 357. In some embodiments, the second antigen binding portion comprises HC-CDR1, HC-CDR2, and HC-CDR3 of a heavy chain comprising the amino acid sequence of SEQ ID NO: 373 and LC-CDR1, LC-CDR2, and LC-CDR3 of a light chain comprising the amino acid sequence of SEQ ID NO: 374. In some embodiments, the second antigen binding portion is a full-length antibody comprising a heavy chain comprising the amino acid sequence of SEQ ID NO: 356, and a light chain comprising the amino acid sequence of SEQ ID NO: 357. In some embodiments, the second antigen binding portion is a full-length antibody comprising a heavy chain comprising the amino acid sequence of SEQ ID NO: 373, and a light chain comprising the amino acid sequence of SEQ ID NO: 374.

[0018] Further provided is an isolated anti-LAG-3 construct that specifically binds to LAG-3 competitively with any one of the isolated anti-LAG-3 constructs described above.

[0019] Further provided is a pharmaceutical composition comprising any one of the isolated anti-LAG-3 constructs described above, and a pharmaceutically acceptable carrier.

[0020] Another aspect of the present application provides a method of treating an individual having a LAG-3-related disease, comprising administering to the individual an effective amount of any one of the pharmaceutical compositions described above. In some embodiments, the LAG-3-related disease is

cancer. In some embodiments, the cancer is colon cancer. In some embodiments, the individual is a human.

[0021] Further provided is an isolated nucleic acid encoding any one of the isolated anti-LAG-3 constructs described above, a vector comprising the isolated nucleic acid, or an isolated host cell comprising the isolated nucleic acid or the vector. In some embodiments, there is provided a method of producing any one of isolated anti-LAG-3 constructs described above, comprising culturing a host cell comprising any one of the isolated nucleic acids or vectors described above, or culturing any one of the isolated host cells described above, under conditions effective to express the encoded anti-LAG-3 construct; and obtaining the expressed anti-LAG-3 construct from the host cell.

[0022] Also provided are compositions, kits and articles of manufacture comprising the any one of the anti-LAG-3 constructs described herein.

BRIEF DESCRIPTION OF THE DRAWINGS

[0023] FIG. 1 depicts the evaluation of immune response against LAG3-Fc or Fc alone for pre-immune serum, and post-immune serum after final boost.

[0024] FIG. 2 depicts the evaluation of immune response against LAG3-Fc or Fc alone for regular antibodies (IgG1) and heavy chain antibodies (IgG2 and IgG3) in post-immune serum after final boost. Corresponding immunoglobulin fragments isolated from pre-immune serum were used as controls.

[0025] FIG. 3 depicts epitope binning test for non-humanized anti-LAG-3 sdAb-Fc fusion proteins and the benchmark antibody, BMS-986016.

[0026] FIG. 4 depicts the binding of non-humanized anti-LAG-3 sdAb-Fc fusion proteins to CHO-K1 cells expressing human LAG-3, tested with FACS. BMS-986016 was used as a positive control.

[0027] FIG. 5 depicts the blocking of interaction between MHC II and human LAG-3 by non-humanized anti-LAG-3 sdAb-Fc fusion proteins, as measured by flow cytometry. BMS-986016 was used as a positive control.

[0028] FIG. 6 depicts the *in vitro* functional blockade of LAG-3 by humanized anti-LAG-3 sdAb-Fc fusion proteins, measured with a reporter assay. BMS-986016 was used as a positive control and human IgG4 isotype control as a negative control.

[0029] FIG. 7 depicts a schematic structure of an exemplary BABP comprising a monospecific full-length antibody having two identical heavy chains and two identical light chains, and two identical anti-LAG-3 sdAbs, wherein the C-terminus of each anti-LAG-3 sdAb is fused to the N-terminus of one heavy

chain via an optional peptide linker. An optional peptide sequence can be fused to the N-terminus of each anti-LAG-3 sdAb. Each of the two anti-LAG-3 sdAbs specifically binds a first epitope (LAG-3). The full-length antibody has two antigen binding sites, each specifically binding a second epitope. For example, the BABP can consist of four polypeptide chains with structures from the N-terminus to the C-terminus as follows: (1) V_L-C_L ; (2) $V_{HH}-V_H-C_{H1}-C_{H2}-C_{H3}$; (3) $V_{HH}-V_H-C_{H1}-C_{H2}-C_{H3}$; and (4) V_L-C_L , wherein V_H and V_L of polypeptide chains (1) and (2) form an antigen binding site that specifically binds a first copy of the second epitope, V_H and V_L of polypeptide chains (3) and (4) form an antigen binding site that specifically binds a second copy of the second epitope, and each V_{HH} specifically binds a copy of the first epitope (LAG-3). In alternative formats, each anti-LAG-3 sdAb may be omitted, or replaced with two identical or different anti-LAG-3 sdAbs fused to each other. The monospecific full-length antibody may be replaced with a bispecific full-length antibody to further expand binding specificity.

[0030] FIG. 8 depicts a schematic structure of an exemplary BABP comprising a monospecific full-length antibody having two identical heavy chains and two identical light chains, and two identical anti-LAG-3 sdAbs, wherein the N-terminus of each anti-LAG-3 sdAb is fused to the C-terminus of one heavy chain via an optional peptide linker. An optional peptide sequence can be fused to the C-terminus of each anti-LAG-3 sdAb. Each of the two anti-LAG-3 sdAbs specifically binds a first epitope (LAG-3). The full-length antibody has two antigen binding sites, each specifically binding a second epitope. For example, the BABP can consist of four polypeptide chains with structures from the N-terminus to the C-terminus as follows: (1) V_L-C_L ; (2) $V_H-C_{H1}-C_{H2}-C_{H3}-V_{HH}$; (3) $V_H-C_{H1}-C_{H2}-C_{H3}-V_{HH}$; and (4) V_L-C_L , wherein V_H and V_L of polypeptide chains (1) and (2) form an antigen binding site that specifically binds a first copy of the second epitope, V_H and V_L of polypeptide chains (3) and (4) form an antigen binding site that specifically binds a second copy of the second epitope, and each V_{HH} specifically binds a copy of the first epitope (LAG-3). In alternative formats, each anti-LAG-3 sdAb may be omitted, or replaced with two identical or different anti-LAG-3 sdAbs fused to each other. The monospecific full-length antibody may be replaced with a bispecific full-length antibody to further expand binding specificity.

[0031] FIG. 9 depicts a schematic structure of an exemplary BABP comprising a monospecific full-length antibody having two identical heavy chains and two identical light chains, and two identical anti-LAG-3 sdAbs, wherein the C-terminus of each anti-LAG-3 sdAb is fused to the N-terminus of one light chain via an optional peptide linker. An optional peptide sequence can be fused to the N-terminus of each anti-LAG-3 sdAb. Each of the two anti-LAG-3 sdAbs specifically binds a first epitope (LAG-3). The full-length antibody has two antigen binding sites, each specifically binding a second epitope. For example, the BABP can consist of four polypeptide chains with structures from the N-terminus to the C-terminus as follows: (1) $V_{HH}-V_L-C_L$; (2) $V_H-C_{H1}-C_{H2}-C_{H3}$; (3) $V_H-C_{H1}-C_{H2}-C_{H3}$; and (4) $V_{HH}-V_L-C_L$, wherein V_H

and V_L of polypeptide chains (1) and (2) form an antigen binding site that specifically binds a first copy of the second epitope, V_H and V_L of polypeptide chains (3) and (4) form an antigen binding site that specifically binds a second copy of the second epitope, and each V_{HH} specifically binds a copy of the first epitope (LAG-3). In alternative formats, each anti-LAG-3 sdAb may be omitted, or replaced with two identical or different anti-LAG-3 sdAbs fused to each other. The monospecific full-length antibody may be replaced with a bispecific full-length antibody to further expand binding specificity.

[0032] FIG. 10 depicts a schematic structure of an exemplary BABP comprising a monospecific full-length antibody having two identical heavy chains and two identical light chains, and two identical anti-LAG-3 sdAbs, wherein the N-terminus of each anti-LAG-3 sdAb is fused to the C-terminus of one light chain via an optional peptide linker. An optional peptide sequence can be fused to the C-terminus of each anti-LAG-3 sdAb. Each of the two anti-LAG-3 sdAbs specifically binds a first epitope. The full-length antibody has two antigen binding sites, each specifically binding a second epitope. For example, the BABP can consist of four polypeptide chains with structures from the N-terminus to the C-terminus as follows: (1) V_L - C_L - V_{HH} ; (2) V_H - C_H1 - C_H2 - C_H3 ; (3) V_H - C_H1 - C_H2 - C_H3 ; and (4) V_L - C_L - V_{HH} , wherein V_H and V_L of polypeptide chains (1) and (2) form an antigen binding site that specifically binds a first copy of the second epitope, V_H and V_L of polypeptide chains (3) and (4) form an antigen binding site that specifically binds a second copy of the second epitope, and each V_{HH} specifically binds a copy of the first epitope (LAG-3). In alternative formats, each anti-LAG-3 sdAb may be omitted, or replaced with two identical or different anti-LAG-3 sdAbs fused to each other. The monospecific full-length antibody may be replaced with a bispecific full-length antibody to further expand binding specificity.

[0033] FIG. 11 depicts a schematic structure of an exemplary BABP comprising a monospecific full-length antibody having two identical heavy chains and two identical light chains, and four identical anti-LAG-3 sdAbs, wherein the C-terminus of each anti-LAG-3 sdAb is fused to the N-terminus of a heavy chain or a light chain of the monospecific full-length antibody via an optional peptide linker. An optional peptide sequence can be fused to the N-terminus of each anti-LAG-3 sdAb. Each anti-LAG-3 sdAb specifically binds to a first epitope (LAG-3). The full-length antibody has two antigen binding sites that each specifically binds a second epitope. For example, the BABP can consist of four polypeptide chains with structures from the N-terminus to the C-terminus as follows: (1) V_{HH} - V_L - C_L ; (2) V_{HH} - V_H - C_H1 - C_H2 - C_H3 ; (3) V_{HH} - V_H - C_H1 - C_H2 - C_H3 ; and (4) V_{HH} - V_L - C_L , wherein V_H and V_L of polypeptide chains (1) and (2) form an antigen binding site that specifically binds a first copy of the second epitope, V_H and V_L of polypeptide chains (3) and (4) form an antigen binding site that specifically binds a second copy of the second epitope, and each V_{HH} specifically binds a copy of the first epitope (LAG-3). In alternative formats, each anti-LAG-3 sdAb may be omitted, or replaced with two identical or different anti-LAG-3

sdAbs fused to each other. The monospecific full-length antibody may be replaced with a bispecific full-length antibody to further expand binding specificity.

[0034] FIG. 12 depicts a schematic structure of an exemplary BABP comprising a monospecific full-length antibody having two identical heavy chains and two identical light chains, and four identical anti-LAG-3 sdAbs, wherein fused to the N-terminus of each heavy chain are two identical anti-LAG-3 sdAbs, the two anti-LAG-3 sdAbs being fused to each other via an optional peptide linker, and the two anti-LAG-3 sdAbs are fused to the N-terminus of each heavy chain via an optional peptide linker. An optional peptide sequence can be fused to the N-terminus of the chimeric heavy chain. Each anti-LAG-3 sdAb specifically binds a first epitope (LAG-3). The full-length antibody has two antigen binding sites that each specifically binds a second epitope. For example, the BABP can consist of four polypeptide chains with structures from the N-terminus to the C-terminus as follows: (1) V_L-C_L ; (2) $V_{HH}-V_{HH}-V_{HH}-C_{H1}-C_{H2}-C_{H3}$; (3) $V_{HH}-V_{HH}-V_{HH}-C_{H1}-C_{H2}-C_{H3}$; and (4) V_L-C_L , wherein V_H and V_L of polypeptide chains (1) and (2) form an antigen binding site that specifically binds a first copy of the second epitope, V_H and V_L of polypeptide chains (3) and (4) form an antigen binding site that specifically binds a second copy of the second epitope, and each V_{HH} specifically binds a copy of the first epitope (LAG-3). In alternative formats, each anti-LAG-3 sdAb may be omitted, or replaced with two identical or different anti-LAG-3 sdAbs fused to each other. The monospecific full-length antibody may be replaced with a bispecific full-length antibody to further expand binding specificity.

[0035] FIG. 13 depicts a schematic structure of an exemplary BABP comprising two identical antigen-binding (Fab) fragments, two identical anti-LAG-3 sdAbs, and an Fc region, wherein the N-terminus of each anti-LAG-3 sdAb is fused to the C-terminus of the C_{H1} region of the Fab fragment via an optional peptide linker and the C-terminus of each anti-LAG-3 sdAb is fused to the N-terminus of the C_{H2} region of the Fc region. Each anti-LAG-3 sdAb specifically binds a first epitope (LAG-3). Each Fab fragment specifically binds a second epitope. For example, the BABP can consist of four polypeptide chains with structures from the N-terminus to the C-terminus as follows: (1) V_L-C_L ; (2) $V_H-C_{H1}-V_{HH}-C_{H2}-C_{H3}$; (3) $V_H-C_{H1}-V_{HH}-C_{H2}-C_{H3}$; and (4) V_L-C_L , wherein V_H and V_L of polypeptide chains (1) and (2) form an antigen binding site that specifically binds a first copy of the second epitope, V_H and V_L of polypeptide chains (3) and (4) form an antigen binding site that specifically binds a second copy of the second epitope, and each V_{HH} specifically binds a copy of the first epitope (LAG-3). In alternative formats, each anti-LAG-3 sdAb may be omitted, or replaced with two identical or different anti-LAG-3 sdAbs fused to each other. In alternative formats, to expand specificity, the two Fab fragments can specifically bind different epitopes, and/or the V_{HH} fragments can specifically bind different epitopes.

[0036] FIG. 14 depicts a schematic structure of an exemplary BABP comprising two identical single chain variable fragments (scFvs), two identical anti-LAG-3 sdAbs, and an Fc region, wherein the N-terminus of each anti-LAG-3 sdAb is fused to the C-terminus of an scFv via an optional peptide linker and the C-terminus of each anti-LAG-3 sdAb is fused to the N-terminus of the Fc region. Each anti-LAG-3 sdAb specifically binds a first epitope (LAG-3). Each scFv specifically binds a second epitope. For example, the BABP can consist of two polypeptide chains each with a structure from the N-terminus to the C-terminus as follows: $V_L-V_H-V_{HH}-C_{H2}-C_{H3}$, wherein V_H and V_L of each polypeptide chain form a scFv domain that specifically binds a copy of the second epitope, and each V_{HH} specifically binds a copy of the first epitope (LAG-3). In alternative formats, the scFv domain can comprise from the N-terminus to the C-terminus: V_H-V_L . In alternative formats, each anti-LAG-3 sdAb may be omitted, or replaced with two identical or different anti-LAG-3 sdAbs fused to each other. Additionally, to expand specificity, the two scFvs can specifically bind different epitopes, and/or the V_{HH} fragments can specifically bind different epitopes.

[0037] FIG. 15 depicts a schematic structure of an exemplary BABP comprising two identical Fab fragments, two identical Fab-like fragments each comprising two V_{HH} fragments, and an Fc region. In each Fab-like fragment, the V_H and V_L regions are each replaced by an anti-LAG-3 sdAb. Each Fab-like fragment specifically binds a first epitope (LAG-3). Each Fab fragment specifically binds a second epitope. For example, the BABP can consist of four polypeptide chains with structures from the N-terminus to the C-terminus as follows: (1) $V_L-C_L-V_{HH}-C_L$; (2) $V_H-C_{H1}-V_{HH}-C_{H1}-C_{H2}-C_{H3}$; (3) $V_H-C_{H1}-V_{HH}-C_{H1}-C_{H2}-C_{H3}$; and (4) $V_L-C_L-V_{HH}-C_L$, wherein V_H and V_L of polypeptide chains (1) and (2) forms an antigen binding site that specifically binds a first copy of the second epitope, V_H and V_L of polypeptide chains (3) and (4) forms an antigen binding site that specifically binds a second copy of the second epitope, and each V_{HH} specifically binds a copy of the first epitope (LAG-3). In alternative formats, to expand specificity, the two Fab fragments can specifically bind different epitopes, and/or the Fab-like fragments can specifically bind different epitopes (*e.g.*, different epitopes from LAG-3).

[0038] FIG. 16 depicts a schematic structure of an exemplary BABP comprising two identical scFvs, two identical Fab-like fragments each comprising two V_{HH} fragments, and an Fc region. In each Fab-like fragment, the V_H and V_L regions are each replaced by an anti-LAG-3 sdAb. Each Fab-like fragment specifically binds a first epitope (LAG-3). Each scFv specifically binds a second epitope. For example, the BABP can consist of four polypeptide chains with structures from the N-terminus to the C-terminus as follows: (1) $V_{HH}-C_L$; (2) $V_L-V_H-V_{HH}-C_{H1}-C_{H2}-C_{H3}$; (3) $V_L-V_H-V_{HH}-C_{H1}-C_{H2}-C_{H3}$; and (4) $V_{HH}-C_L$, wherein V_H and V_L of polypeptide chains (2) and (3) each forms an scFv that specifically binds a copy of the second epitope, and each V_{HH} specifically binds a copy of the first epitope (LAG-3). In alternative

formats, the C-terminus of the scFv may be fused to the N-terminus of the chain in the Fab-like fragment comprising V_{HH} - C_L ; and/or the scFv domain can comprise from the N-terminus to the C-terminus: V_{HH} - V_L . Additionally, to expand specificity, the two scFvs can specifically bind different epitopes, and/or the V_{HH} fragments can specifically bind different epitopes (*e.g.*, different epitopes from LAG-3).

[0039] FIGs. 17A-17B depict *in vitro* activities of PD-1 $\times$ LAG-3 BABPs in a PD-1 cell based reporter assay (FIG. 17A) and a LAG-3 blockade reporter assay (FIG. 17B). Pembrolizumab and PD1-BM-min were used as controls for PD-1 cell based reporter assay and BMS-986016 was used as a control for LAG-3 blockade reporter assay.

[0040] FIGs. 18A-18B depict *in vivo* efficacy of PD-1 $\times$ LAG-3 BLP-4 in C57BL/6 human PD-1/LAG-3 double knock-in mice bearing MC38 tumor, as compared to parental elements of BLP-4 (*i.e.*, PD1-BM-min and AS20846-Fc) and combination thereof. FIG. 18A shows average tumor volume in each treatment group. FIG. 18B shows spider plots of tumor volume of each animal. IgG4 was used as a negative control.

DETAILED DESCRIPTION OF THE INVENTION

[0041] The present invention provides novel single-domain antibodies (sdAbs) specifically recognizing LAG-3 (*i.e.*, anti-LAG-3 sdAb), and constructs comprising the anti-LAG-3 sdAbs. The anti-LAG3 sdAbs described herein have strong binding affinity and are capable of cross-reacting with LAG-3 of a non-human mammal, such as a cynomolgus monkey. The anti-LAG3 constructs described herein are useful for treating LAG-3-related diseases such as cancer.

[0042] Accordingly, one aspect of the present application provides an isolated anti-LAG-3 construct comprising an sdAb specifically recognizing LAG-3. The isolated anti-LAG-3 construct can be, for example, an anti-LAG-3 sdAb (*e.g.*, camelid or humanized), a polypeptide comprising a plurality of anti-LAG-3 sdAbs fused together, an anti-LAG-3 sdAb-Fc fusion protein comprising an anti-LAG-3 sdAb fused to an Fc fragment (*e.g.*, a human IgG1 Fc, effectorless IgG1 Fc, IgG2 Fc, or IgG4 Fc), or a multispecific antigen binding protein ("MABP") comprising an anti-LAG-3 sdAb fused to a full-length antibody (such as anti-PD-1 antibody) or an antigen binding fragment thereof. The anti-LAG-3 construct can be monospecific or multispecific (such as bispecific), monovalent or multivalent (such as bivalent).

[0043] Also provided are compositions (such as pharmaceutical compositions), kits and articles of manufacture comprising the anti-LAG-3 constructs described herein, methods of making thereof, and methods of treating LAG-3-related disease (such as cancer) using the anti-LAG-3 constructs described herein.

I. Definitions

[0044] The term “epitope” means a protein determinant capable of specific binding to an antibody. Epitopes usually consist of chemically active surface groupings of molecules such as amino acids or sugar side chains and usually have specific three dimensional structural characteristics, as well as specific charge characteristics. Conformational and non-conformational epitopes are distinguished in that the binding to the former but not the latter is lost in the presence of denaturing solvents.

[0045] As used herein, “treatment” or “treating” is an approach for obtaining beneficial or desired results including clinical results. For purposes of this invention, beneficial or desired clinical results include, but are not limited to, one or more of the following: alleviating one or more symptoms resulting from the disease, diminishing the extent of the disease, stabilizing the disease (*e.g.*, preventing or delaying the worsening of the disease), preventing or delaying the spread (*e.g.*, metastasis) of the disease, preventing or delaying the recurrence of the disease, delay or slowing the progression of the disease, ameliorating the disease state, providing a remission (partial or total) of the disease, decreasing the dose of one or more other medications required to treat the disease, delaying the progression of the disease, increasing the quality of life, and/or prolonging survival. Also encompassed by “treatment” is a reduction of pathological consequence of cancer. The methods of the invention contemplate any one or more of these aspects of treatment.

[0046] The term “prevent,” and similar words such as “prevented,” “preventing” *etc.*, indicate an approach for preventing, inhibiting, or reducing the likelihood of the recurrence of, a disease or condition, *e.g.*, cancer. It also refers to delaying the recurrence of a disease or condition or delaying the recurrence of the symptoms of a disease or condition. As used herein, “prevention” and similar words also includes reducing the intensity, effect, symptoms and/or burden of a disease or condition prior to recurrence of the disease or condition.

[0047] As used herein, “delaying” the development of cancer means to defer, hinder, slow, retard, stabilize, and/or postpone development of the disease. This delay can be of varying lengths of time, depending on the history of the disease and/or individual being treated. A method that “delays” development of cancer is a method that reduces probability of disease development in a given time frame and/or reduces the extent of the disease in a given time frame, when compared to not using the method. Such comparisons are typically based on clinical studies, using a statistically significant number of individuals. Cancer development can be detectable using standard methods, including, but not limited to, computerized axial tomography (CAT Scan), Magnetic Resonance Imaging (MRI), abdominal ultrasound,

clotting tests, arteriography, or biopsy. Development may also refer to cancer progression that may be initially undetectable and includes occurrence, recurrence, and onset.

[0048] The term “effective amount” used herein refers to an amount of an agent or a combination of agents, sufficient to treat a specified disorder, condition or disease such as ameliorate, palliate, lessen, and/or delay one or more of its symptoms. In reference to cancer, an effective amount comprises an amount sufficient to cause a tumor to shrink and/or to decrease the growth rate of the tumor (such as to suppress tumor growth) or to prevent or delay other unwanted cell proliferation. In some embodiments, an effective amount is an amount sufficient to delay development. In some embodiments, an effective amount is an amount sufficient to prevent or delay recurrence. An effective amount can be administered in one or more administrations. The effective amount of the drug or composition may: (i) reduce the number of cancer cells; (ii) reduce tumor size; (iii) inhibit, retard, slow to some extent and preferably stop cancer cell infiltration into peripheral organs; (iv) inhibit (*i.e.*, slow to some extent and preferably stop) tumor metastasis; (v) inhibit tumor growth; (vi) prevent or delay occurrence and/or recurrence of tumor; and/or (vii) relieve to some extent one or more of the symptoms associated with the cancer.

[0049] As used herein, an “individual” or a “subject” refers to a mammal, including, but not limited to, human, bovine, horse, feline, canine, rodent, or primate. In some embodiments, the individual is a human.

[0050] The term “antibody” is used in its broadest sense and encompasses various antibody structures, including but not limited to monoclonal antibodies, polyclonal antibodies, multispecific antibodies (*e.g.*, bispecific antibodies), full-length antibodies and antigen-binding fragments thereof, so long as they exhibit the desired antigen-binding activity. The term “antibody” includes conventional 4-chain antibodies, single-domain antibodies, and antigen-binding fragments thereof.

[0051] The basic 4-chain antibody unit is a heterotetrameric glycoprotein composed of two identical light (L) chains and two identical heavy (H) chains. An IgM antibody consists of 5 of the basic heterotetramer units along with an additional polypeptide called a J chain, and contains 10 antigen-binding sites, while IgA antibodies comprise from 2-5 of the basic 4-chain units which can polymerize to form polyvalent assemblages in combination with the J chain. In the case of IgGs, the 4-chain unit is generally about 150,000 Daltons. Each L chain is linked to an H chain by one covalent disulfide bond, while the two H chains are linked to each other by one or more disulfide bonds depending on the H chain isotype. Each H and L chain also has regularly spaced intrachain disulfide bridges. Each H chain has at the N-terminus, a variable domain (V_H) followed by three constant domains (C_H) for each of the α and γ chains and four C_H domains for μ and ϵ isotypes. Each L chain has at the N-terminus, a variable domain (V_L) followed by a constant domain at its other end. The V_L is aligned with the V_H and the C_L is aligned

with the first constant domain of the heavy chain (C_{H1}). Particular amino acid residues are believed to form an interface between the light chain and heavy chain variable domains. The pairing of a V_H and V_L together forms a single antigen-binding site. For the structure and properties of the different classes of antibodies, see *e.g.*, *Basic and Clinical Immunology*, 8th Edition, Daniel P. Sties, Abba I. Terr and Tristram G. Parslow (eds), Appleton & Lange, Norwalk, Conn., 1994, page 71 and Chapter 6. The L chain from any vertebrate species can be assigned to one of two clearly distinct types, called kappa and lambda, based on the amino acid sequences of their constant domains. Depending on the amino acid sequence of the constant domain of their heavy chains (C_H), immunoglobulins can be assigned to different classes or isotypes. There are five classes of immunoglobulins: IgA, IgD, IgE, IgG and IgM, having heavy chains designated α , δ , ϵ , γ and μ , respectively. The γ and α classes are further divided into subclasses on the basis of relatively minor differences in the C_H sequence and function, *e.g.*, humans express the following subclasses: IgG1, IgG2A, IgG2B, IgG3, IgG4, IgA1 and IgA2.

[0052] The term “heavy chain-only antibody” or “HCAb” refers to a functional antibody, which comprises heavy chains, but lacks the light chains usually found in 4-chain antibodies. Camelid animals (such as camels, llamas, or alpacas) are known to produce HCABs.

[0053] The term “single-domain antibody” or “sdAb” refers to a single antigen-binding polypeptide having three complementary determining regions (CDRs). The sdAb alone is capable of binding to the antigen without pairing with a corresponding CDR-containing polypeptide. In some cases, single-domain antibodies are engineered from camelid HCABs, and their heavy chain variable domains are referred herein as “ V_{HH} ” (Variable domain of the heavy chain of the Heavy chain antibody). Camelid sdAb is one of the smallest known antigen-binding antibody fragments (see, *e.g.*, Hamers-Casterman *et al.*, Nature 363:446-8 (1993); Greenberg *et al.*, Nature 374:168-73 (1995); Hassanzadeh-Ghassabeh *et al.*, Nanomedicine (Lond), 8:1013-26 (2013)). A basic V_{HH} has the following structure from the N-terminus to the C-terminus: FR1-CDR1-FR2-CDR2-FR3-CDR3-FR4, in which FR1 to FR4 refer to framework regions 1 to 4, respectively, and in which CDR1 to CDR3 refer to the complementarity determining regions 1 to 3.

[0054] An “isolated” antibody (or construct) is one that has been identified, separated and/or recovered from a component of its production environment (*e.g.*, natural or recombinant). Preferably, the isolated polypeptide is free of association with all other components from its production environment. Contaminant components of its production environment, such as that resulting from recombinant transfected cells, are materials that would typically interfere with research, diagnostic or therapeutic uses for the antibody, and may include enzymes, hormones, and other proteinaceous or non-proteinaceous solutes. In preferred embodiments, the polypeptide will be purified: (1) to greater than 95% by weight of

antibody as determined by, for example, the Lowry method, and in some embodiments, to greater than 99% by weight; (2) to a degree sufficient to obtain at least 15 residues of N-terminal or internal amino acid sequence by use of a spinning cup sequenator; or (3) to homogeneity by SDS-PAGE under non-reducing or reducing conditions using Coomassie Blue or, preferably, silver stain. Isolated antibody (or construct) includes the antibody *in situ* within recombinant cells since at least one component of the antibody's natural environment will not be present. Ordinarily, however, an isolated polypeptide, antibody, or construct will be prepared by at least one purification step.

[0055] The “variable region” or “variable domain” of an antibody refers to the amino-terminal domains of the heavy or light chain of the antibody. The variable domains of the heavy chain and light chain may be referred to as “V_H” and “V_L”, respectively. These domains are generally the most variable parts of the antibody (relative to other antibodies of the same class) and contain the antigen binding sites. Heavy-chain only antibodies from the *Camelid* species have a single heavy chain variable region, which is referred to as “V_HH”. V_HH is thus a special type of V_H.

[0056] The term “variable” refers to the fact that certain segments of the variable domains differ extensively in sequence among antibodies. The V domain mediates antigen binding and defines the specificity of a particular antibody for its particular antigen. However, the variability is not evenly distributed across the entire span of the variable domains. Instead, it is concentrated in three segments called complementary determining regions (CDRs) or hypervariable regions (HVRs) both in the heavy chain and light chain variable domains. The more highly conserved portions of variable domains are called the framework regions (FR). The variable domains of native heavy and light chains each comprise four FR regions, largely adopting a beta-sheet configuration, connected by three CDRs, which form loops connecting, and in some cases forming part of, the beta-sheet structure. The CDRs in each chain are held together in close proximity by the FR regions and, with the CDRs from the other chain, contribute to the formation of the antigen binding site of antibodies (see Kabat *et al.*, *Sequences of Immunological Interest*, Fifth Edition, National Institute of Health, Bethesda, Md. (1991)). The constant domains are not involved directly in the binding of antibody to an antigen, but exhibit various effector functions, such as participation of the antibody in antibody-dependent cellular toxicity.

[0057] The term “monoclonal antibody” as used herein refers to an antibody obtained from a population of substantially homogeneous antibodies, *i.e.*, the individual antibodies comprising the population are identical except for possible naturally occurring mutations and/or post-translation modifications (*e.g.*, isomerizations, amidations) that may be present in minor amounts. Monoclonal antibodies are highly specific, being directed against a single antigenic site. In contrast to polyclonal antibody preparations which typically include different antibodies directed against different determinants

(epitopes), each monoclonal antibody is directed against a single determinant on the antigen. In addition to their specificity, the monoclonal antibodies are advantageous in that they are synthesized by the hybridoma culture, uncontaminated by other immunoglobulins. The modifier “monoclonal” indicates the character of the antibody as being obtained from a substantially homogeneous population of antibodies, and is not to be construed as requiring production of the antibody by any particular method. For example, the monoclonal antibodies to be used in accordance with the present invention may be made by a variety of techniques, including, for example, the hybridoma method (*e.g.*, Kohler and Milstein., *Nature*, 256:495-97 (1975); Hongo *et al.*, *Hybridoma*, 14 (3): 253-260 (1995), Harlow *et al.*, *Antibodies: A Laboratory Manual*, (Cold Spring Harbor Laboratory Press, 2nd ed. 1988); Hammerling *et al.*, in: *Monoclonal Antibodies and T-Cell Hybridomas* 563-681 (Elsevier, N.Y., 1981)), recombinant DNA methods (see, *e.g.*, U.S. Pat. No. 4,816,567), phage-display technologies (see, *e.g.*, Clackson *et al.*, *Nature*, 352: 624-628 (1991); Marks *et al.*, *J. Mol. Biol.* 222: 581-597 (1992); Sidhu *et al.*, *J. Mol. Biol.* 338(2): 299-310 (2004); Lee *et al.*, *J. Mol. Biol.* 340(5): 1073-1093 (2004); Fellouse, *Proc. Natl. Acad. Sci. USA* 101(34): 12467-12472 (2004); and Lee *et al.*, *J. Immunol. Methods* 284(1-2): 119-132 (2004), and technologies for producing human or human-like antibodies in animals that have parts or all of the human immunoglobulin loci or genes encoding human immunoglobulin sequences (see, *e.g.*, WO 1998/24893; WO 1996/34096; WO 1996/33735; WO 1991/10741; Jakobovits *et al.*, *Proc. Natl. Acad. Sci. USA* 90: 2551 (1993); Jakobovits *et al.*, *Nature* 362: 255-258 (1993); Bruggemann *et al.*, *Year in Immunol.* 7:33 (1993); U.S. Pat. Nos. 5,545,807; 5,545,806; 5,569,825; 5,625,126; 5,633,425; and 5,661,016; Marks *et al.*, *Bio/Technology* 10: 779-783 (1992); Lonberg *et al.*, *Nature* 368: 856-859 (1994); Morrison, *Nature* 368: 812-813 (1994); Fishwild *et al.*, *Nature Biotechnol.* 14: 845-851 (1996); Neuberger, *Nature Biotechnol.* 14: 826 (1996); and Lonberg and Huszar, *Intern. Rev. Immunol.* 13: 65-93 (1995).

[0058] The terms “full-length antibody”, “intact antibody”, or “whole antibody” are used interchangeably to refer to an antibody in its substantially intact form, as opposed to an antibody fragment. Specifically, full-length 4-chain antibodies include those with heavy and light chains including an Fc region. Full-length heavy-chain only antibodies include the heavy chain variable domain (such as V_HH) and an Fc region. The constant domains may be native sequence constant domains (*e.g.*, human native sequence constant domains) or amino acid sequence variants thereof. In some cases, the intact antibody may have one or more effector functions.

[0059] An “antibody fragment” or “antigen-binding fragment” comprises a portion of an intact antibody, preferably the antigen binding and/or the variable region of the intact antibody. Examples of antibody fragments include, but are not limited to Fab, Fab', F(ab')₂ and Fv fragments; diabodies; linear antibodies (see U.S. Pat. No. 5,641,870, Example 2; Zapata *et al.*, *Protein Eng.* 8(10): 1057-1062 (1995));

single-chain antibody (scFv) molecules; single-domain antibodies (such as V_HH), and multispecific antibodies formed from antibody fragments. Papain digestion of antibodies produced two identical antigen-binding fragments, called “Fab” fragments, and a residual “Fc” fragment, a designation reflecting the ability to crystallize readily. The Fab fragment consists of an entire L chain along with the variable domain of the H chain (V_H), and the first constant domain of one heavy chain (C_{H1}). Each Fab fragment is monovalent with respect to antigen binding, *i.e.*, it has a single antigen-binding site. Pepsin treatment of an antibody yields a single large $F(ab')_2$ fragment which roughly corresponds to two disulfide linked Fab fragments having different antigen-binding activity and is still capable of cross-linking antigen. Fab' fragments differ from Fab fragments by having a few additional residues at the carboxy-terminus of the C_{H1} domain including one or more cysteines from the antibody hinge region. Fab'-SH is the designation herein for Fab' in which the cysteine residue(s) of the constant domains bear a free thiol group. $F(ab')_2$ antibody fragments originally were produced as pairs of Fab' fragments which have hinge cysteines between them. Other chemical couplings of antibody fragments are also known.

[0060] The term “constant domain” refers to the portion of an immunoglobulin molecule having a more conserved amino acid sequence relative to the other portion of the immunoglobulin, the variable domain, which contains the antigen-binding site. The constant domain contains the C_{H1} , C_{H2} and C_{H3} domains (collectively, C_H) of the heavy chain and the C_{HL} (or C_L) domain of the light chain.

[0061] The “light chains” of antibodies (immunoglobulins) from any mammalian species can be assigned to one of two clearly distinct types, called kappa (“ κ ”) and lambda (“ λ ”), based on the amino acid sequences of their constant domains.

[0062] “Fv” is the minimum antibody fragment which contains a complete antigen-recognition and -binding site. This fragment consists of a dimer of one heavy- and one light-chain variable region domain in tight, non-covalent association. From the folding of these two domains emanate six hypervariable loops (3 loops each from the H and L chain) that contribute the amino acid residues for antigen binding and confer antigen binding specificity to the antibody. However, even a single variable domain (or half of an Fv comprising only three CDRs specific for an antigen) has the ability to recognize and bind antigen, although at a lower affinity than the entire binding site.

[0063] “Single-chain Fv” also abbreviated as “sFv” or “scFv” are antibody fragments that comprise the V_H and V_L antibody domains connected into a single polypeptide chain. Preferably, the scFv polypeptide further comprises a polypeptide linker between the V_H and V_L domains which enables the scFv to form the desired structure for antigen binding. For a review of the scFv, see Pluckthun in *The Pharmacology of*

Monoclonal Antibodies, vol. 113, Rosenberg and Moore eds., Springer-Verlag, New York, pp. 269-315 (1994).

[0064] The term “diabodies” refers to small antibody fragments prepared by constructing sFv fragments (see preceding paragraph) with short linkers (about 5-10 residues) between the V_H and V_L domains such that inter-chain but not intra-chain pairing of the V domains is achieved, thereby resulting in a bivalent fragment, *i.e.*, a fragment having two antigen-binding sites. Bispecific diabodies are heterodimers of two “crossover” sFv fragments in which the V_H and V_L domains of the two antibodies are present on different polypeptide chains. Diabodies are described in greater detail in, for example, EP 404,097; WO 93/11161; Hollinger *et al.*, *Proc. Natl. Acad. Sci. USA* 90: 6444-6448 (1993).

[0065] The monoclonal antibodies herein specifically include “chimeric” antibodies (immunoglobulins) in which a portion of the heavy and/or light chain is identical with or homologous to corresponding sequences in antibodies derived from a particular species or belonging to a particular antibody class or subclass, while the remainder of the chain(s) is(are) identical with or homologous to corresponding sequences in antibodies derived from another species or belonging to another antibody class or subclass, as well as fragments of such antibodies, so long as they exhibit the desired biological activity (U.S. Pat. No. 4,816,567; Morrison *et al.*, *Proc. Natl. Acad. Sci. USA*, 81:6851-6855 (1984)). “Humanized antibody” is used as a subset of “chimeric antibodies”.

[0066] “Humanized” forms of non-human (*e.g.*, llama or camelid) antibodies are chimeric antibodies that contain minimal sequence derived from non-human immunoglobulin. In some embodiments, a humanized antibody is a human immunoglobulin (recipient antibody) in which residues from an CDR (hereinafter defined) of the recipient are replaced by residues from an CDR of a non-human species (donor antibody) such as mouse, rat, rabbit, camel, llama, alpaca, or non-human primate having the desired specificity, affinity, and/or capacity. In some instances, framework (“FR”) residues of the human immunoglobulin are replaced by corresponding non-human residues. Furthermore, humanized antibodies may comprise residues that are not found in the recipient antibody or in the donor antibody. These modifications may be made to further refine antibody performance, such as binding affinity. In general, a humanized antibody will comprise substantially all of at least one, and typically two, variable domains, in which all or substantially all of the hypervariable loops correspond to those of a non-human immunoglobulin sequence, and all or substantially all of the FR regions are those of a human immunoglobulin sequence, although the FR regions may include one or more individual FR residue substitutions that improve antibody performance, such as binding affinity, isomerization, immunogenicity, *etc.* The number of these amino acid substitutions in the FR is typically no more than 6 in the H chain, and in the L chain, no more than 3. The humanized antibody optionally will also comprise at least a

portion of an immunoglobulin constant region (Fc), typically that of a human immunoglobulin. For further details, see, *e.g.*, Jones *et al.*, *Nature* 321:522-525 (1986); Riechmann *et al.*, *Nature* 332:323-329 (1988); and Presta, *Curr. Op. Struct. Biol.* 2:593-596 (1992). See also, for example, Vaswani and Hamilton, *Ann. Allergy, Asthma & Immunol.* 1:105-115 (1998); Harris, *Biochem. Soc. Transactions* 23:1035-1038 (1995); Hurle and Gross, *Curr. Op. Biotech.* 5:428-433 (1994); and U.S. Pat. Nos. 6,982,321 and 7,087,409.

[0067] A “human antibody” is an antibody that possesses an amino-acid sequence corresponding to that of an antibody produced by a human and/or has been made using any of the techniques for making human antibodies as disclosed herein. This definition of a human antibody specifically excludes a humanized antibody comprising non-human antigen-binding residues. Human antibodies can be produced using various techniques known in the art, including phage-display libraries. Hoogenboom and Winter, *J. Mol. Biol.*, 227:381 (1991); Marks *et al.*, *J. Mol. Biol.*, 222:581 (1991). Also available for the preparation of human monoclonal antibodies are methods described in Cole *et al.*, *Monoclonal Antibodies and Cancer Therapy*, Alan R. Liss, p. 77 (1985); Boerner *et al.*, *J. Immunol.*, 147(1):86-95 (1991). See also van Dijk and van de Winkel, *Curr. Opin. Pharmacol.*, 5: 368-74 (2001). Human antibodies can be prepared by administering the antigen to a transgenic animal that has been modified to produce such antibodies in response to antigenic challenge, but whose endogenous loci have been disabled, *e.g.*, immunized xenomice (see, *e.g.*, U.S. Pat. Nos. 6,075,181 and 6,150,584 regarding XENOMOUSE™ technology). See also, for example, Li *et al.*, *Proc. Natl. Acad. Sci. USA*, 103:3557-3562 (2006) regarding human antibodies generated via a human B-cell hybridoma technology.

[0068] The term “hypervariable region,” “HVR,” or “HV,” when used herein refers to the regions of an antibody variable domain which are hypervariable in sequence and/or form structurally defined loops. Generally, single-domain antibodies comprise three HVRs (or CDRs): HVR1 (or CDR1), HVR2 (or CDR2), and HVR3 (or CDR3). HVR3 (or CDR3) displays the most diversity of the three HVRs, and is believed to play a unique role in conferring fine specificity to antibodies. See, *e.g.*, Hamers-Casterman *et al.*, *Nature* 363:446-448 (1993); Sheriff *et al.*, *Nature Struct. Biol.* 3:733-736 (1996).

[0069] The term “Complementarity Determining Region” or “CDR” are used to refer to hypervariable regions as defined by the Kabat system. See Kabat *et al.*, *Sequences of Proteins of Immunological Interest*, 5th Ed. Public Health Service, National Institutes of Health, Bethesda, Md. (1991).

[0070] A number of HVR delineations are in use and are encompassed herein. The Kabat Complementarity Determining Regions (CDRs) are based on sequence variability and are the most commonly used (Kabat *et al.*, *Sequences of Proteins of Immunological Interest*, 5th Ed. Public Health

Service, National Institutes of Health, Bethesda, Md. (1991)). Chothia refers instead to the location of the structural loops (Chothia and Lesk, *J. Mol. Biol.* 196:901-917 (1987)). The AbM HVRs represent a compromise between the Kabat HVRs and Chothia structural loops, and are used by Oxford Molecular's AbM antibody modeling software. The "contact" HVRs are based on an analysis of the available complex crystal structures. The residues from each of these HVRs are noted below in Table 1.

Table 1. HVR delineations.

Loop	Kabat	AbM	Chothia	Contact
L1	L24-L34	L24-L34	L26-L32	L30-L36
L2	L50-L56	L50-L56	L50-L52	L46-L55
L3	L89-L97	L89-L97	L91-L96	L89-L96
H1	H31-H35B	H26-H35B	H26-H32	H30-H35B
(Kabat Numbering)				
H1	H31-H35	H26-H35	H26-H32	H30-H35
(Chothia Numbering)				
H2	H50-H65	H50-H58	H53-H55	H47-H58
H3	H95-H102	H95-H102	H96-H101	H93-H101

[0071] HVRs may comprise "extended HVRs" as follows: 24-36 or 24-34 (L1), 46-56 or 50-56 (L2) and 89-97 or 89-96 (L3) in the V_L and 26-35 (H1), 50-65 or 49-65 (H2) and 93-102, 94-102, or 95-102 (H3) in the V_H . The variable domain residues are numbered according to Kabat *et al.*, supra, for each of these definitions.

[0072] The amino acid residues of a single-domain antibody (such as V_{HH}) are numbered according to the general numbering for V_H domains given by Kabat *et al.* ("Sequence of proteins of immunological interest", US Public Health Services, NIH Bethesda, Md., Publication No. 91), as applied to V_{HH} domains from Camelids in the article of Riechmann and Muyldermans, *J. Immunol. Methods* 2000 Jun. 23; 240 (1-2): 185-195. According to this numbering, FR1 of a V_{HH} comprises the amino acid residues at positions 1-30, CDR1 of a V_{HH} comprises the amino acid residues at positions 31-35, FR2 of a V_{HH} comprises the amino acids at positions 36-49, CDR2 of a V_{HH} comprises the amino acid residues at positions 50-65, FR3 of a V_{HH} comprises the amino acid residues at positions 66-94, CDR3 of a V_{HH} comprises the amino acid residues at positions 95-102, and FR4 of a V_{HH} comprises the amino acid residues at positions 103-113. In this respect, it should be noted that—as is well known in the art for V_H domains and for V_{HH} domains—the total number of amino acid residues in each of the CDRs may vary and may not correspond to the total number of amino acid residues indicated by the Kabat numbering (that is, one or more positions according to the Kabat numbering may not be occupied in the actual

sequence, or the actual sequence may contain more amino acid residues than the number allowed for by the Kabat numbering).

[0073] The expression “variable-domain residue-numbering as in Kabat” or “amino-acid-position numbering as in Kabat,” and variations thereof, refers to the numbering system used for heavy-chain variable domains or light-chain variable domains of the compilation of antibodies in Kabat *et al.*, supra. Using this numbering system, the actual linear amino acid sequence may contain fewer or additional amino acids corresponding to a shortening of, or insertion into, a FR or HVR of the variable domain. For example, a heavy-chain variable domain may include a single amino acid insert (residue 52a according to Kabat) after residue 52 of H2 and inserted residues (*e.g.* residues 82a, 82b, and 82c, etc. according to Kabat) after heavy-chain FR residue 82. The Kabat numbering of residues may be determined for a given antibody by alignment at regions of homology of the sequence of the antibody with a “standard” Kabat numbered sequence.

[0074] Unless indicated otherwise herein, the numbering of the residues in an immunoglobulin heavy chain is that of the EU index as in Kabat *et al.*, supra. The “EU index as in Kabat” refers to the residue numbering of the human IgG1 EU antibody.

[0075] “Framework” or “FR” residues are those variable-domain residues other than the HVR residues as herein defined.

[0076] A “human consensus framework” or “acceptor human framework” is a framework that represents the most commonly occurring amino acid residues in a selection of human immunoglobulin V_L or V_H framework sequences. Generally, the selection of human immunoglobulin V_L or V_H sequences is from a subgroup of variable domain sequences. Generally, the subgroup of sequences is a subgroup as in Kabat *et al.*, *Sequences of Proteins of Immunological Interest*, 5th Ed. Public Health Service, National Institutes of Health, Bethesda, Md. (1991). Examples include for the V_L, the subgroup may be subgroup kappa I, kappa II, kappa III or kappa IV as in Kabat *et al.*, supra. Additionally, for the V_H, the subgroup may be subgroup I, subgroup II, or subgroup III as in Kabat *et al.* Alternatively, a human consensus framework can be derived from the above in which particular residues, such as when a human framework residue is selected based on its homology to the donor framework by aligning the donor framework sequence with a collection of various human framework sequences. An acceptor human framework “derived from” a human immunoglobulin framework or a human consensus framework may comprise the same amino acid sequence thereof, or it may contain pre-existing amino acid sequence changes. In some embodiments, the number of pre-existing amino acid changes are 10 or less, 9 or less, 8 or less, 7 or less, 6 or less, 5 or less, 4 or less, 3 or less, or 2 or less.

[0077] An “affinity-matured” antibody is one with one or more alterations in one or more CDRs thereof that result in an improvement in the affinity of the antibody for antigen, compared to a parent antibody that does not possess those alteration(s). In some embodiments, an affinity-matured antibody has nanomolar or even picomolar affinities for the target antigen. Affinity-matured antibodies are produced by procedures known in the art. For example, Marks *et al.*, *Bio/Technology* 10:779-783 (1992) describes affinity maturation by V_H- and V_L-domain shuffling. Random mutagenesis of CDR and/or framework residues is described by, for example: Barbas *et al.* *Proc Nat. Acad. Sci. USA* 91:3809-3813 (1994); Schier *et al.* *Gene* 169:147-155 (1995); Yelton *et al.* *J. Immunol.* 155:1994-2004 (1995); Jackson *et al.*, *J. Immunol.* 154(7):3310-9 (1995); and Hawkins *et al.*, *J. Mol. Biol.* 226:889-896 (1992).

[0078] As used herein, the term “specifically binds,” “specifically recognizes,” or is “specific for” refers to measurable and reproducible interactions such as binding between a target and an antigen binding protein (such as an sdAb), which is determinative of the presence of the target in the presence of a heterogeneous population of molecules including biological molecules. For example, an antigen binding protein (such as an sdAb) that specifically binds a target (which can be an epitope) is an antigen binding protein (such as an sdAb) that binds this target with greater affinity, avidity, more readily, and/or with greater duration than it binds other targets. In some embodiments, the extent of binding of an antigen binding protein (such as an sdAb) to an unrelated target is less than about 10% of the binding of the antigen binding protein (such as an sdAb) to the target as measured, *e.g.*, by a radioimmunoassay (RIA). In some embodiments, an antigen binding protein (such as an sdAb) that specifically binds a target has a dissociation constant (K_D) of $\leq 10^{-5}$ M, $\leq 10^{-6}$ M, $\leq 10^{-7}$ M, $\leq 10^{-8}$ M, $\leq 10^{-9}$ M, $\leq 10^{-10}$ M, $\leq 10^{-11}$ M, or $\leq 10^{-12}$ M. In some embodiments, an antigen binding protein specifically binds an epitope on a protein that is conserved among the protein from different species. In some embodiments, specific binding can include, but does not require exclusive binding. Binding specificity of the antibody or antigen-binding domain can be determined experimentally by methods known in the art. Such methods comprise, but are not limited to Western blots, ELISA-, RIA-, ECL-, IRMA-, EIA-, BIACORE™ -tests and peptide scans.

[0079] The term “specificity” refers to selective recognition of an antigen binding protein (such as an sdAb) for a particular epitope of an antigen. Natural antibodies, for example, are monospecific. The term “multispecific” as used herein denotes that an antigen binding protein has polyepitopic specificity (*i.e.*, is capable of specifically binding to two, three, or more, different epitopes on one biological molecule or is capable of specifically binding to epitopes on two, three, or more, different biological molecules). “Bispecific” as used herein denotes that an antigen binding protein has two different antigen-binding specificities. Unless otherwise indicated, the order in which the antigens bound by a bispecific antibody listed is arbitrary. That is, for example, the terms “anti-LAG-3/PD-1,” “anti-PD-1/LAG-3,” “LAG-3×PD-

1” and “PD-1×LAG-3” may be used interchangeably to refer to bispecific antibodies that specifically bind to both LAG-3 and PD-1. The term “monospecific” as used herein denotes an antigen binding protein that has one or more binding sites each of which bind the same epitope of the same antigen.

[0080] The term “valent” as used herein denotes the presence of a specified number of binding sites in an antigen binding protein. A natural antibody for example or a full length antibody has two binding sites and is bivalent. As such, the terms “trivalent”, “tetravalent”, “pentavalent” and “hexavalent” denote the presence of two binding site, three binding sites, four binding sites, five binding sites, and six binding sites, respectively, in an antigen binding protein.

[0081] “Antibody effector functions” refer to those biological activities attributable to the Fc region (a native sequence Fc region or amino acid sequence variant Fc region) of an antibody, and vary with the antibody isotype. Examples of antibody effector functions include: C1q binding and complement dependent cytotoxicity; Fc receptor binding; antibody-dependent cell-mediated cytotoxicity (ADCC); phagocytosis; down regulation of cell surface receptors (*e.g.*, B cell receptors); and B cell activation. “Reduced or minimized” antibody effector function means that which is reduced by at least 50% (alternatively 60%, 65%, 70%, 75%, 80%, 85%, 90%, 95%, 96%, 97%, 98%, 99%) from the wild type or unmodified antibody. The determination of antibody effector function is readily determinable and measurable by one of ordinary skill in the art. In a preferred embodiment, the antibody effector functions of complement binding, complement dependent cytotoxicity and antibody dependent cytotoxicity are affected. In some embodiments, effector function is eliminated through a mutation in the constant region that eliminated glycosylation, *e.g.*, “effectorless mutation.” In one aspect, the effectorless mutation is an N297A or DANA mutation (D265A+N297A) in the C_H2 region. Shields *et al.*, *J. Biol. Chem.* 276 (9): 6591-6604 (2001). Alternatively, additional mutations resulting in reduced or eliminated effector function include: K322A and L234A/L235A (LALA). Alternatively, effector function can be reduced or eliminated through production techniques, such as expression in host cells that do not glycosylate (*e.g.*, *E. coli.*) or in which result in an altered glycosylation pattern that is ineffective or less effective at promoting effector function (*e.g.*, Shinkawa *et al.*, *J. Biol. Chem.* 278(5): 3466-3473 (2003).

[0082] “Antibody-dependent cell-mediated cytotoxicity” or ADCC refers to a form of cytotoxicity in which secreted Ig bound onto Fc receptors (FcRs) present on certain cytotoxic cells (*e.g.*, natural killer (NK) cells, neutrophils and macrophages) enable these cytotoxic effector cells to bind specifically to an antigen-bearing target cell and subsequently kill the target cell with cytotoxins. The antibodies “arm” the cytotoxic cells and are required for killing of the target cell by this mechanism. The primary cells for mediating ADCC, NK cells, express FcγRIII only, whereas monocytes express FcγRI, FcγRII and FcγRIII. Fc expression on hematopoietic cells is summarized in Table 2 on page 464 of Ravetch and

Kinet, *Annu. Rev. Immunol.* 9: 457-92 (1991). To assess ADCC activity of a molecule of interest, an *in vitro* ADCC assay, such as that described in U.S. Pat. No. 5,500,362 or 5,821,337 may be performed. Useful effector cells for such assays include peripheral blood mononuclear cells (PBMC) and natural killer (NK) cells. Alternatively, or additionally, ADCC activity of the molecule of interest may be assessed *in vivo*, e.g., in an animal model such as that disclosed in Clynes *et al.*, *PNAS USA* 95:652-656 (1998).

[0083] The term “Fc region” or “fragment crystallizable region” herein is used to define a C-terminal region of an immunoglobulin heavy chain, including native-sequence Fc regions and variant Fc regions. Although the boundaries of the Fc region of an immunoglobulin heavy chain might vary, the human IgG heavy-chain Fc region is usually defined to stretch from an amino acid residue at position Cys226, or from Pro230, to the carboxyl-terminus thereof. The C-terminal lysine (residue 447 according to the EU numbering system) of the Fc region may be removed, for example, during production or purification of the antibody, or by recombinantly engineering the nucleic acid encoding a heavy chain of the antibody. Accordingly, a composition of intact antibodies may comprise antibody populations with all K447 residues removed, antibody populations with no K447 residues removed, and antibody populations having a mixture of antibodies with and without the K447 residue. Suitable native-sequence Fc regions for use in the antibodies described herein include human IgG1, IgG2 (IgG2A, IgG2B), IgG3 and IgG4.

[0084] “Fc receptor” or “FcR” describes a receptor that binds the Fc region of an antibody. The preferred FcR is a native sequence human FcR. Moreover, a preferred FcR is one which binds an IgG antibody (a gamma receptor) and includes receptors of the FcγRI, FcγRII, and FcγRIII subclasses, including allelic variants and alternatively spliced forms of these receptors, FcγRII receptors include FcγRIIA (an “activating receptor”) and FcγRIIB (an “inhibiting receptor”), which have similar amino acid sequences that differ primarily in the cytoplasmic domains thereof. Activating receptor FcγRIIA contains an immunoreceptor tyrosine-based activation motif (ITAM) in its cytoplasmic domain. Inhibiting receptor FcγRIIB contains an immunoreceptor tyrosine-based inhibition motif (ITIM) in its cytoplasmic domain. (See M. Daéron, *Annu. Rev. Immunol.* 15:203-234 (1997). FcRs are reviewed in Ravetch and Kinet, *Annu. Rev. Immunol.* 9: 457-92 (1991); Capel *et al.*, *Immunomethods* 4: 25-34 (1994); and de Haas *et al.*, *J. Lab. Clin. Med.* 126: 330-41 (1995). Other FcRs, including those to be identified in the future, are encompassed by the term “FcR” herein.

[0085] The term “Fc receptor” or “FcR” also includes the neonatal receptor, FcRn, which is responsible for the transfer of maternal IgGs to the fetus. Guyer *et al.*, *J. Immunol.* 117: 587 (1976) and Kim *et al.*, *J. Immunol.* 24: 249 (1994). Methods of measuring binding to FcRn are known (see, e.g., Ghetie and Ward, *Immunol. Today* 18: (12): 592-8 (1997); Ghetie *et al.*, *Nature Biotechnology* 15 (7):

637-40 (1997); Hinton *et al.*, *J. Biol. Chem.* 279 (8): 6213-6 (2004); WO 2004/92219 (Hinton *et al.*). Binding to FcRn *in vivo* and serum half-life of human FcRn high-affinity binding polypeptides can be assayed, *e.g.*, in transgenic mice or transfected human cell lines expressing human FcRn, or in primates to which the polypeptides having a variant Fc region are administered. WO 2004/42072 (Presta) describes antibody variants which improved or diminished binding to FcRs. See also, *e.g.*, Shields *et al.*, *J. Biol. Chem.* 9(2): 6591-6604 (2001).

[0086] “Complement dependent cytotoxicity” or “CDC” refers to the lysis of a target cell in the presence of complement. Activation of the classical complement pathway is initiated by the binding of the first component of the complement system (C1q) to antibodies (of the appropriate subclass) which are bound to their cognate antigen. To assess complement activation, a CDC assay, *e.g.*, as described in Gazzano-Santoro *et al.*, *J. Immunol. Methods* 202: 163 (1996), may be performed. Antibody variants with altered Fc region amino acid sequences and increased or decreased C1q binding capability are described in U.S. Pat. No. 6,194,551B1 and WO99/51642. The contents of those patent publications are specifically incorporated herein by reference. See, also, Idusogie *et al.* *J. Immunol.* 164: 4178-4184 (2000).

[0087] “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 that reflects a 1:1 interaction between members of a binding pair. Binding affinity can be indicated by K_d , K_{off} , K_{on} , or K_a . The term “ K_{off} ”, as used herein, is intended to refer to the off rate constant for dissociation of an antibody (or antigen-binding domain) from the antibody/antigen complex, as determined from a kinetic selection set up, expressed in units of s^{-1} . The term “ K_{on} ”, as used herein, is intended to refer to the on rate constant for association of an antibody (or antigen-binding domain) to the antigen to form the antibody/antigen complex, expressed in units of $M^{-1}s^{-1}$. The term equilibrium dissociation constant “ K_D ” or “ K_d ”, as used herein, refers to the dissociation constant of a particular antibody-antigen interaction, and describes the concentration of antigen required to occupy one half of all of the antibody-binding domains present in a solution of antibody molecules at equilibrium, and is equal to K_{off}/K_{on} , expressed in units of M. The measurement of K_d presupposes that all binding agents are in solution. In the case where the antibody is tethered to a cell wall, *e.g.*, in a yeast expression system, the corresponding equilibrium rate constant is expressed as EC50, which gives a good approximation of K_d . The affinity constant, K_a , is the inverse of the dissociation constant, K_d , expressed in units of M^{-1} . The dissociation constant (K_D or K_d) is used as an indicator showing affinity of antibodies to antigens. For example, easy analysis is possible by the Scatchard method using antibodies marked with a variety of marker agents, as well as by using BIACORE™ X (made by Amersham Biosciences), which is an over-

the-counter, measuring kit, or similar kit, according to the user's manual and experiment operation method attached with the kit. The K_D value that can be derived using these methods is expressed in units of M (Mols). An antibody or antigen-binding fragment thereof that specifically binds to a target may have a dissociation constant (K_d) of, for example, $\leq 10^{-5}$ M, $\leq 10^{-6}$ M, $\leq 10^{-7}$ M, $\leq 10^{-8}$ M, $\leq 10^{-9}$ M, $\leq 10^{-10}$ M, $\leq 10^{-11}$ M, or $\leq 10^{-12}$ M.

[0088] Half maximal inhibitory concentration (IC_{50}) is a measure of the effectiveness of a substance (such as an antibody) in inhibiting a specific biological or biochemical function. It indicates how much of a particular drug or other substance (inhibitor, such as an antibody) is needed to inhibit a given biological process by half. The values are typically expressed as molar concentration. IC_{50} is comparable to an “ EC_{50} ” for agonist drug or other substance (such as an antibody). EC_{50} also represents the plasma concentration required for obtaining 50% of a maximum effect *in vivo*. As used herein, an “ IC_{50} ” is used to indicate the effective concentration of an antibody needed to neutralize 50% of the antigen bioactivity *in vitro*. IC_{50} or EC_{50} can be measured by bioassays such as inhibition of ligand binding by FACS analysis (competition binding assay), cell based cytokine release assay, or amplified luminescent proximity homogeneous assay (AlphaLISA).

[0089] “Percent (%) amino acid sequence identity” and “homology” with respect to a peptide, polypeptide or antibody sequence are defined as the percentage of amino acid residues in a candidate sequence that are identical with the amino acid residues in the specific peptide or polypeptide sequence, after aligning the sequences and introducing gaps, if necessary, to achieve the maximum percent sequence identity, and not considering any conservative substitutions as part of the sequence identity. Alignment for purposes of determining percent amino acid sequence identity can be achieved in various ways that are within the skill in the art, for instance, using publicly available computer software such as BLAST, BLAST-2, ALIGN or MEGALIGN™ (DNASTAR) software. Those skilled in the art can determine appropriate parameters for measuring alignment, including any algorithms needed to achieve maximal alignment over the full length of the sequences being compared.

[0090] An “isolated” nucleic acid molecule encoding a construct, antibody, or antigen-binding fragment thereof described herein is a nucleic acid molecule that is identified and separated from at least one contaminant nucleic acid molecule with which it is ordinarily associated in the environment in which it was produced. Preferably, the isolated nucleic acid is free of association with all components associated with the production environment. The isolated nucleic acid molecules encoding the polypeptides and antibodies described herein is in a form other than in the form or setting in which it is found in nature. Isolated nucleic acid molecules therefore are distinguished from nucleic acid encoding the polypeptides and antibodies described herein existing naturally in cells. An isolated nucleic acid includes a nucleic acid

molecule contained in cells that ordinarily contain the nucleic acid molecule, but the nucleic acid molecule is present extrachromosomally or at a chromosomal location that is different from its natural chromosomal location.

[0091] The term “control sequences” refers to DNA sequences necessary for the expression of an operably linked coding sequence in a particular host organism. The control sequences that are suitable for prokaryotes, for example, include a promoter, optionally an operator sequence, and a ribosome binding site. Eukaryotic cells are known to utilize promoters, polyadenylation signals, and enhancers.

[0092] Nucleic acid is “operably linked” when it is placed into a functional relationship with another nucleic acid sequence. For example, DNA for a presequence or secretory leader is operably linked to DNA for a polypeptide if it is expressed as a preprotein that participates in the secretion of the polypeptide; a promoter or enhancer is operably linked to a coding sequence if it affects the transcription of the sequence; or a ribosome binding site is operably linked to a coding sequence if it is positioned so as to facilitate translation. Generally, “operably linked” means that the DNA sequences being linked are contiguous, and, in the case of a secretory leader, contiguous and in reading phase. However, enhancers do not have to be contiguous. Linking is accomplished by ligation at convenient restriction sites. If such sites do not exist, the synthetic oligonucleotide adaptors or linkers are used in accordance with conventional practice.

[0093] The term “vector,” as used herein, refers to a nucleic acid molecule capable of propagating another nucleic acid to which it is linked. The term includes the vector as a self-replicating nucleic acid structure as well as the vector incorporated into the genome of a host cell into which it has been introduced. Certain vectors are capable of directing the expression of nucleic acids to which they are operatively linked. Such vectors are referred to herein as “expression vectors.”

[0094] The term “transfected” or “transformed” or “transduced” as used herein refers to a process by which exogenous nucleic acid is transferred or introduced into the host cell. A “transfected” or “transformed” or “transduced” cell is one which has been transfected, transformed or transduced with exogenous nucleic acid. The cell includes the primary subject cell and its progeny.

[0095] The terms “host cell,” “host cell line,” and “host cell culture” are used interchangeably and refer to cells into which exogenous nucleic acid has been introduced, including the progeny of such cells. Host cells include “transformants” and “transformed cells,” which include the primary transformed cell and progeny derived therefrom without regard to the number of passages. Progeny may not be completely identical in nucleic acid content to a parent cell, but may contain mutations. Mutant progeny that have the

same function or biological activity as screened or selected for in the originally transformed cell are included herein.

[0096] The term “pharmaceutical formulation” of “pharmaceutical composition” refers to a preparation that is in such form as to permit the biological activity of the active ingredient to be effective, and that contains no additional components that are unacceptably toxic to a subject to which the formulation would be administered. Such formulations are sterile. A “sterile” formulation is aseptic or free from all living microorganisms and their spores.

[0097] It is understood that embodiments of the invention described herein include “consisting” and/or “consisting essentially of” embodiments.

[0098] Reference to “about” a value or parameter herein includes (and describes) variations that are directed to that value or parameter *per se*. For example, description referring to “about X” includes description of “X”.

[0099] As used herein, reference to “not” a value or parameter generally means and describes “other than” a value or parameter. For example, the method is not used to treat cancer of type X means the method is used to treat cancer of types other than X.

[0100] The term “about X-Y” used herein has the same meaning as “about X to about Y.”

[0101] As used herein and in the appended claims, the singular forms “a,” “or,” and “the” include plural referents unless the context clearly dictates otherwise.

II. Anti-LAG-3 constructs

[0102] One aspect of the present application provides isolated anti-LAG-3 constructs comprising a single-domain antibody (sdAb) that specifically recognizes LAG-3 (i.e., “anti-LAG-3 sdAb”). In some embodiments, the anti-LAG-3 construct is an anti-LAG-3 sdAb, a fusion protein, or an antigen-binding fragment thereof, which specifically binds to LAG-3 and antagonizes its ability to bind MHC class II molecules and/or mediate T cell exhaustion.

(I) Anti-LAG-3 single-domain antibodies

[0103] The isolated anti-LAG-3 constructs described herein comprise an anti-LAG-3 sdAb. In some embodiments, the anti-LAG-3 sdAb specifically recognizes human LAG-3. The complete amino acid sequence of an exemplary human LAG-3 comprises or consists of the amino acid sequence of SEQ ID NO: 350. In some embodiments, the anti-LAG-3 sdAb specifically recognizes an epitope within human LAG-3. In some embodiments, the anti-LAG-3 sdAb specifically recognizes the extracellular domain of

human LAG-3. The amino acid sequence of the extracellular domain of an exemplary human LAG-3 comprises or consists of the amino acid sequence of SEQ ID NO: 351. In some embodiments, the anti-LAG-3 sdAb cross-reacts with a LAG-3 from a non-human mammal. In some embodiments, the anti-LAG-3 sdAb cross-reacts with LAG-3 from a non-human primate, such as cynomolgus monkey.

[0104] In some embodiments, the anti-LAG-3 sdAb cross-reacts with at least one interspecies variant of the LAG-3 protein. In some embodiments, for example, the LAG-3 protein (or fragments thereof) is human LAG-3 and the interspecies variant of the LAG-3 protein (or fragments thereof) is a cynomolgus monkey variant thereof. Cross-reactivity of the anti-LAG-3 sdAbs or constructs thereof may facilitate clinical development of the anti-LAG-3 constructs, for example, by allowing more accurate and responsive dosing in animal studies.

[0105] In some embodiments, there is provided an anti-LAG-3 sdAb comprising a CDR1 comprising the amino acid sequence of any one of SEQ ID NOs: 39-76, or a variant thereof comprising up to about 3 (such as about any of 1, 2, or 3) amino acid substitutions; a CDR2 comprising the amino acid sequence of any one of SEQ ID NOs: 115-152, or a variant thereof comprising up to about 3 (such as about any of 1, 2, or 3) amino acid substitutions; and a CDR3 comprising the amino acid sequence of any one of SEQ ID NOs: 191-228, or a variant thereof comprising up to about 3 (such as about any of 1, 2, or 3) amino acid substitutions. In some embodiments, the K_D of the binding between the anti-LAG-3 sdAb and LAG-3 is about 10^{-7} M to about 10^{-12} M (such as about 10^{-8} M to about 10^{-12} M, or about 10^{-9} M to about 10^{-11} M). In some embodiments, the anti-LAG-3 sdAb cross-reacts with a LAG-3 from a non-human mammal. In some embodiments, the anti-LAG-3 sdAb is camelid, chimeric, human, partially humanized, or fully humanized.

[0106] In some embodiments, the anti-LAG-3 sdAb comprises a CDR3 comprising the amino acid sequence of any one of SEQ ID NOs: 191-228, and the amino acid substitutions are in CDR1 and/or CDR2. Thus, in some embodiments, there is provided an anti-LAG-3 sdAb comprising a CDR1 comprising the amino acid sequence of any one of SEQ ID NOs: 39-76, or a variant thereof comprising up to about 3 (such as about any of 1, 2, or 3) amino acid substitutions; a CDR2 comprising the amino acid sequence of any one of SEQ ID NOs: 115-152, or a variant thereof comprising up to about 3 (such as about any of 1, 2, or 3) amino acid substitutions; and a CDR3 comprising the amino acid sequence of any one of SEQ ID NOs: 191-228. In some embodiments, the K_D of the binding between the anti-LAG-3 sdAb and LAG-3 is about 10^{-7} M to about 10^{-12} M (such as about 10^{-8} M to about 10^{-12} M, or about 10^{-9} M to about 10^{-11} M). In some embodiments, the anti-LAG-3 sdAb cross-reacts with a LAG-3 from a non-human mammal. In some embodiments, the anti-LAG-3 sdAb is camelid, chimeric, human, partially humanized, or fully humanized.

[0107] In some embodiments, there is provided an anti-LAG-3 sdAb comprising a CDR1 comprising the amino acid sequence of any one of SEQ ID NOs: 39-76; a CDR2 comprising the amino acid sequence of any one of SEQ ID NOs: 115-152; and a CDR3 comprising the amino acid sequence of any one of SEQ ID NOs: 191-228; or a variant thereof comprising up to about 3 (such as about any of 1, 2, or 3) amino acid substitutions in the CDR regions. In some embodiments, the amino acid substitutions are in CDR1 and/or CDR2. In some embodiments, there is provided an anti-LAG-3 sdAb comprising a CDR1 comprising the amino acid sequence of any one of SEQ ID NOs: 39-76; a CDR2 comprising the amino acid sequence of any one of SEQ ID NOs: 115-152; and a CDR3 comprising the amino acid sequence of any one of SEQ ID NOs: 191-228; or a variant thereof comprising up to about 3 (such as about any of 1, 2, or 3) amino acid substitutions in the CDR regions, wherein the amino acid substitutions are in CDR1 and/or CDR2. In some embodiments, the K_D of the binding between the anti-LAG-3 sdAb and LAG-3 is about 10^{-7} M to about 10^{-12} M (such as about 10^{-8} M to about 10^{-12} M, or about 10^{-9} M to about 10^{-11} M). In some embodiments, the anti-LAG-3 sdAb cross-reacts with a LAG-3 from a non-human mammal. In some embodiments, the anti-LAG-3 sdAb is camelid, chimeric, human, partially humanized, or fully humanized.

[0108] In some embodiments, there is provided an anti-LAG-3 sdAb comprising a CDR1 comprising the amino acid sequence of any one of SEQ ID NOs: 39-76; a CDR2 comprising the amino acid sequence of any one of SEQ ID NOs: 115-152; and a CDR3 comprising the amino acid sequence of any one of SEQ ID NOs: 191-228. In some embodiments, the K_D of the binding between the anti-LAG-3 sdAb and LAG-3 is about 10^{-7} M to about 10^{-12} M (such as about 10^{-8} M to about 10^{-12} M, or about 10^{-9} M to about 10^{-11} M). In some embodiments, the anti-LAG-3 sdAb cross-reacts with a LAG-3 from a non-human mammal. In some embodiments, the anti-LAG-3 sdAb is camelid, chimeric, human, partially humanized, or fully humanized.

[0109] The sequences of the CDRs noted herein are provided in Table 11. The CDRs can be combined in any combinations to generate a number of anti-LAG-3 sdAbs. A skilled person in the art would readily appreciate that antibody constructs comprising CDRs or variable domain sequences derived from the antibodies described herein, but wherein the CDRs or variable domain sequences are predicted using algorithms other than the Kabat system are within the scope of the present invention.

[0110] In some embodiments, there is provided an anti-LAG-3 sdAb comprising a CDR1 comprising the amino acid sequence of SEQ ID NO: 39, or a variant thereof comprising up to about 3 (such as about any of 1, 2, or 3) amino acid substitutions; a CDR2 comprising the amino acid sequence of SEQ ID NO: 115, or a variant thereof comprising up to about 3 (such as about any of 1, 2, or 3) amino acid substitutions; and a CDR3 comprising the amino acid sequence of SEQ ID NO: 191, or a variant thereof

comprising up to about 3 (such as about any of 1, 2, or 3) amino acid substitutions. In some embodiments, there is provided an anti-LAG-3 sdAb comprising a CDR1 comprising the amino acid sequence of SEQ ID NO: 39; a CDR2 comprising the amino acid sequence of SEQ ID NO: 115; and a CDR3 comprising the amino acid sequence of SEQ ID NO: 191; or a variant thereof comprising up to about 3 (such as about any of 1, 2, or 3) amino acid substitutions in the CDR regions. In some embodiments, the amino acid substitutions are in CDR1 and/or CDR2. In some embodiments, there is provided an anti-LAG-3 sdAb comprising a CDR1 comprising the amino acid sequence of SEQ ID NO: 39; a CDR2 comprising the amino acid sequence of SEQ ID NO: 115; and a CDR3 comprising the amino acid sequence of SEQ ID NO: 191. In some embodiments, the K_D of the binding between the anti-LAG-3 sdAb and LAG-3 is about 10^{-7} M to about 10^{-12} M (such as about 10^{-8} M to about 10^{-12} M, or about 10^{-9} M to about 10^{-11} M). In some embodiments, the anti-LAG-3 sdAb cross-reacts with a LAG-3 from a non-human mammal. In some embodiments, the anti-LAG-3 sdAb is camelid, chimeric, human, partially humanized, or fully humanized.

[0111] In some embodiments, there is provided an anti-LAG-3 sdAb comprising a CDR1 comprising the amino acid sequence of SEQ ID NO: 40, or a variant thereof comprising up to about 3 (such as about any of 1, 2, or 3) amino acid substitutions; a CDR2 comprising the amino acid sequence of SEQ ID NO: 116, or a variant thereof comprising up to about 3 (such as about any of 1, 2, or 3) amino acid substitutions; and a CDR3 comprising the amino acid sequence of SEQ ID NO: 192, or a variant thereof comprising up to about 3 (such as about any of 1, 2, or 3) amino acid substitutions. In some embodiments, there is provided an anti-LAG-3 sdAb comprising a CDR1 comprising the amino acid sequence of SEQ ID NO: 40; a CDR2 comprising the amino acid sequence of SEQ ID NO: 116; and a CDR3 comprising the amino acid sequence of SEQ ID NO: 192; or a variant thereof comprising up to about 3 (such as about any of 1, 2, or 3) amino acid substitutions in the CDR regions. In some embodiments, the amino acid substitutions are in CDR1 and/or CDR2. In some embodiments, there is provided an anti-LAG-3 sdAb comprising a CDR1 comprising the amino acid sequence of SEQ ID NO: 40; a CDR2 comprising the amino acid sequence of SEQ ID NO: 116; and a CDR3 comprising the amino acid sequence of SEQ ID NO: 192. In some embodiments, the K_D of the binding between the anti-LAG-3 sdAb and LAG-3 is about 10^{-7} M to about 10^{-12} M (such as about 10^{-8} M to about 10^{-12} M, or about 10^{-9} M to about 10^{-11} M). In some embodiments, the anti-LAG-3 sdAb cross-reacts with a LAG-3 from a non-human mammal. In some embodiments, the anti-LAG-3 sdAb is camelid, chimeric, human, partially humanized, or fully humanized.

[0112] In some embodiments, there is provided an anti-LAG-3 sdAb comprising a CDR1 comprising the amino acid sequence of SEQ ID NO: 41, or a variant thereof comprising up to about 3 (such as about

any of 1, 2, or 3) amino acid substitutions; a CDR2 comprising the amino acid sequence of SEQ ID NO: 117, or a variant thereof comprising up to about 3 (such as about any of 1, 2, or 3) amino acid substitutions; and a CDR3 comprising the amino acid sequence of SEQ ID NO: 193, or a variant thereof comprising up to about 3 (such as about any of 1, 2, or 3) amino acid substitutions. In some embodiments, there is provided an anti-LAG-3 sdAb comprising a CDR1 comprising the amino acid sequence of SEQ ID NO: 41; a CDR2 comprising the amino acid sequence of SEQ ID NO: 117; and a CDR3 comprising the amino acid sequence of SEQ ID NO: 193; or a variant thereof comprising up to about 3 (such as about any of 1, 2, or 3) amino acid substitutions in the CDR regions. In some embodiments, the amino acid substitutions are in CDR1 and/or CDR2. In some embodiments, there is provided an anti-LAG-3 sdAb comprising a CDR1 comprising the amino acid sequence of SEQ ID NO: 41; a CDR2 comprising the amino acid sequence of SEQ ID NO: 117; and a CDR3 comprising the amino acid sequence of SEQ ID NO: 193. In some embodiments, the K_D of the binding between the anti-LAG-3 sdAb and LAG-3 is about 10^{-7} M to about 10^{-12} M (such as about 10^{-8} M to about 10^{-12} M, or about 10^{-9} M to about 10^{-11} M). In some embodiments, the anti-LAG-3 sdAb cross-reacts with a LAG-3 from a non-human mammal. In some embodiments, the anti-LAG-3 sdAb is camelid, chimeric, human, partially humanized, or fully humanized.

[0113] In some embodiments, there is provided an anti-LAG-3 sdAb comprising a CDR1 comprising the amino acid sequence of SEQ ID NO: 42, or a variant thereof comprising up to about 3 (such as about any of 1, 2, or 3) amino acid substitutions; a CDR2 comprising the amino acid sequence of SEQ ID NO: 118, or a variant thereof comprising up to about 3 (such as about any of 1, 2, or 3) amino acid substitutions; and a CDR3 comprising the amino acid sequence of SEQ ID NO: 194, or a variant thereof comprising up to about 3 (such as about any of 1, 2, or 3) amino acid substitutions. In some embodiments, there is provided an anti-LAG-3 sdAb comprising a CDR1 comprising the amino acid sequence of SEQ ID NO: 42; a CDR2 comprising the amino acid sequence of SEQ ID NO: 118; and a CDR3 comprising the amino acid sequence of SEQ ID NO: 194; or a variant thereof comprising up to about 3 (such as about any of 1, 2, or 3) amino acid substitutions in the CDR regions. In some embodiments, the amino acid substitutions are in CDR1 and/or CDR2. In some embodiments, there is provided an anti-LAG-3 sdAb comprising a CDR1 comprising the amino acid sequence of SEQ ID NO: 42; a CDR2 comprising the amino acid sequence of SEQ ID NO: 118; and a CDR3 comprising the amino acid sequence of SEQ ID NO: 194. In some embodiments, the K_D of the binding between the anti-LAG-3 sdAb and LAG-3 is about 10^{-7} M to about 10^{-12} M (such as about 10^{-8} M to about 10^{-12} M, or about 10^{-9} M to about 10^{-11} M). In some embodiments, the anti-LAG-3 sdAb cross-reacts with a LAG-3 from a non-human mammal. In some

embodiments, the anti-LAG-3 sdAb is camelid, chimeric, human, partially humanized, or fully humanized.

[0114] In some embodiments, there is provided an anti-LAG-3 sdAb comprising a CDR1 comprising the amino acid sequence of SEQ ID NO: 43, or a variant thereof comprising up to about 3 (such as about any of 1, 2, or 3) amino acid substitutions; a CDR2 comprising the amino acid sequence of SEQ ID NO: 119, or a variant thereof comprising up to about 3 (such as about any of 1, 2, or 3) amino acid substitutions; and a CDR3 comprising the amino acid sequence of SEQ ID NO: 195, or a variant thereof comprising up to about 3 (such as about any of 1, 2, or 3) amino acid substitutions. In some embodiments, there is provided an anti-LAG-3 sdAb comprising a CDR1 comprising the amino acid sequence of SEQ ID NO: 43; a CDR2 comprising the amino acid sequence of SEQ ID NO: 119; and a CDR3 comprising the amino acid sequence of SEQ ID NO: 195; or a variant thereof comprising up to about 3 (such as about any of 1, 2, or 3) amino acid substitutions in the CDR regions. In some embodiments, the amino acid substitutions are in CDR1 and/or CDR2. In some embodiments, there is provided an anti-LAG-3 sdAb comprising a CDR1 comprising the amino acid sequence of SEQ ID NO: 43; a CDR2 comprising the amino acid sequence of SEQ ID NO: 119; and a CDR3 comprising the amino acid sequence of SEQ ID NO: 195. In some embodiments, the K_D of the binding between the anti-LAG-3 sdAb and LAG-3 is about 10^{-7} M to about 10^{-12} M (such as about 10^{-8} M to about 10^{-12} M, or about 10^{-9} M to about 10^{-11} M). In some embodiments, the anti-LAG-3 sdAb cross-reacts with a LAG-3 from a non-human mammal. In some embodiments, the anti-LAG-3 sdAb is camelid, chimeric, human, partially humanized, or fully humanized.

[0115] In some embodiments, there is provided an anti-LAG-3 sdAb comprising a CDR1 comprising the amino acid sequence of SEQ ID NO: 44, or a variant thereof comprising up to about 3 (such as about any of 1, 2, or 3) amino acid substitutions; a CDR2 comprising the amino acid sequence of SEQ ID NO: 120, or a variant thereof comprising up to about 3 (such as about any of 1, 2, or 3) amino acid substitutions; and a CDR3 comprising the amino acid sequence of SEQ ID NO: 196, or a variant thereof comprising up to about 3 (such as about any of 1, 2, or 3) amino acid substitutions. In some embodiments, there is provided an anti-LAG-3 sdAb comprising a CDR1 comprising the amino acid sequence of SEQ ID NO: 44; a CDR2 comprising the amino acid sequence of SEQ ID NO: 120; and a CDR3 comprising the amino acid sequence of SEQ ID NO: 196; or a variant thereof comprising up to about 3 (such as about any of 1, 2, or 3) amino acid substitutions in the CDR regions. In some embodiments, the amino acid substitutions are in CDR1 and/or CDR2. In some embodiments, there is provided an anti-LAG-3 sdAb comprising a CDR1 comprising the amino acid sequence of SEQ ID NO: 44; a CDR2 comprising the amino acid sequence of SEQ ID NO: 120; and a CDR3 comprising the amino acid sequence of SEQ ID NO: 196.

NO: 196. In some embodiments, the K_D of the binding between the anti-LAG-3 sdAb and LAG-3 is about 10^{-7} M to about 10^{-12} M (such as about 10^{-8} M to about 10^{-12} M, or about 10^{-9} M to about 10^{-11} M). In some embodiments, the anti-LAG-3 sdAb cross-reacts with a LAG-3 from a non-human mammal. In some embodiments, the anti-LAG-3 sdAb is camelid, chimeric, human, partially humanized, or fully humanized.

[0116] In some embodiments, there is provided an anti-LAG-3 sdAb comprising a CDR1 comprising the amino acid sequence of SEQ ID NO: 45, or a variant thereof comprising up to about 3 (such as about any of 1, 2, or 3) amino acid substitutions; a CDR2 comprising the amino acid sequence of SEQ ID NO: 121, or a variant thereof comprising up to about 3 (such as about any of 1, 2, or 3) amino acid substitutions; and a CDR3 comprising the amino acid sequence of SEQ ID NO: 197, or a variant thereof comprising up to about 3 (such as about any of 1, 2, or 3) amino acid substitutions. In some embodiments, there is provided an anti-LAG-3 sdAb comprising a CDR1 comprising the amino acid sequence of SEQ ID NO: 45; a CDR2 comprising the amino acid sequence of SEQ ID NO: 121; and a CDR3 comprising the amino acid sequence of SEQ ID NO: 197; or a variant thereof comprising up to about 3 (such as about any of 1, 2, or 3) amino acid substitutions in the CDR regions. In some embodiments, the amino acid substitutions are in CDR1 and/or CDR2. In some embodiments, there is provided an anti-LAG-3 sdAb comprising a CDR1 comprising the amino acid sequence of SEQ ID NO: 45; a CDR2 comprising the amino acid sequence of SEQ ID NO: 121; and a CDR3 comprising the amino acid sequence of SEQ ID NO: 197. In some embodiments, the K_D of the binding between the anti-LAG-3 sdAb and LAG-3 is about 10^{-7} M to about 10^{-12} M (such as about 10^{-8} M to about 10^{-12} M, or about 10^{-9} M to about 10^{-11} M). In some embodiments, the anti-LAG-3 sdAb cross-reacts with a LAG-3 from a non-human mammal. In some embodiments, the anti-LAG-3 sdAb is camelid, chimeric, human, partially humanized, or fully humanized.

[0117] In some embodiments, there is provided an anti-LAG-3 sdAb comprising a CDR1 comprising the amino acid sequence of SEQ ID NO: 46, or a variant thereof comprising up to about 3 (such as about any of 1, 2, or 3) amino acid substitutions; a CDR2 comprising the amino acid sequence of SEQ ID NO: 122, or a variant thereof comprising up to about 3 (such as about any of 1, 2, or 3) amino acid substitutions; and a CDR3 comprising the amino acid sequence of SEQ ID NO: 198, or a variant thereof comprising up to about 3 (such as about any of 1, 2, or 3) amino acid substitutions. In some embodiments, there is provided an anti-LAG-3 sdAb comprising a CDR1 comprising the amino acid sequence of SEQ ID NO: 46; a CDR2 comprising the amino acid sequence of SEQ ID NO: 122; and a CDR3 comprising the amino acid sequence of SEQ ID NO: 198; or a variant thereof comprising up to about 3 (such as about any of 1, 2, or 3) amino acid substitutions in the CDR regions. In some embodiments, the amino acid

substitutions are in CDR1 and/or CDR2. In some embodiments, there is provided an anti-LAG-3 sdAb comprising a CDR1 comprising the amino acid sequence of SEQ ID NO: 46; a CDR2 comprising the amino acid sequence of SEQ ID NO: 122; and a CDR3 comprising the amino acid sequence of SEQ ID NO: 198. In some embodiments, the K_D of the binding between the anti-LAG-3 sdAb and LAG-3 is about 10^{-7} M to about 10^{-12} M (such as about 10^{-8} M to about 10^{-12} M, or about 10^{-9} M to about 10^{-11} M). In some embodiments, the anti-LAG-3 sdAb cross-reacts with a LAG-3 from a non-human mammal. In some embodiments, the anti-LAG-3 sdAb is camelid, chimeric, human, partially humanized, or fully humanized.

[0118] In some embodiments, there is provided an anti-LAG-3 sdAb comprising a CDR1 comprising the amino acid sequence of SEQ ID NO: 54, or a variant thereof comprising up to about 3 (such as about any of 1, 2, or 3) amino acid substitutions; a CDR2 comprising the amino acid sequence of SEQ ID NO: 130, or a variant thereof comprising up to about 3 (such as about any of 1, 2, or 3) amino acid substitutions; and a CDR3 comprising the amino acid sequence of SEQ ID NO: 206, or a variant thereof comprising up to about 3 (such as about any of 1, 2, or 3) amino acid substitutions. In some embodiments, there is provided an anti-LAG-3 sdAb comprising a CDR1 comprising the amino acid sequence of SEQ ID NO: 54; a CDR2 comprising the amino acid sequence of SEQ ID NO: 130; and a CDR3 comprising the amino acid sequence of SEQ ID NO: 206; or a variant thereof comprising up to about 3 (such as about any of 1, 2, or 3) amino acid substitutions in the CDR regions. In some embodiments, the amino acid substitutions are in CDR1 and/or CDR2. In some embodiments, there is provided an anti-LAG-3 sdAb comprising a CDR1 comprising the amino acid sequence of SEQ ID NO: 54; a CDR2 comprising the amino acid sequence of SEQ ID NO: 130; and a CDR3 comprising the amino acid sequence of SEQ ID NO: 206. In some embodiments, the K_D of the binding between the anti-LAG-3 sdAb and LAG-3 is about 10^{-7} M to about 10^{-12} M (such as about 10^{-8} M to about 10^{-12} M, or about 10^{-9} M to about 10^{-11} M). In some embodiments, the anti-LAG-3 sdAb cross-reacts with a LAG-3 from a non-human mammal. In some embodiments, the anti-LAG-3 sdAb is camelid, chimeric, human, partially humanized, or fully humanized.

[0119] In some embodiments, there is provided an anti-LAG-3 sdAb comprising a CDR1 comprising the amino acid sequence of SEQ ID NO: 61, or a variant thereof comprising up to about 3 (such as about any of 1, 2, or 3) amino acid substitutions; a CDR2 comprising the amino acid sequence of SEQ ID NO: 137, or a variant thereof comprising up to about 3 (such as about any of 1, 2, or 3) amino acid substitutions; and a CDR3 comprising the amino acid sequence of SEQ ID NO: 213, or a variant thereof comprising up to about 3 (such as about any of 1, 2, or 3) amino acid substitutions. In some embodiments, there is provided an anti-LAG-3 sdAb comprising a CDR1 comprising the amino acid sequence of SEQ

ID NO: 61; a CDR2 comprising the amino acid sequence of SEQ ID NO: 137; and a CDR3 comprising the amino acid sequence of SEQ ID NO: 213; or a variant thereof comprising up to about 3 (such as about any of 1, 2, or 3) amino acid substitutions in the CDR regions. In some embodiments, the amino acid substitutions are in CDR1 and/or CDR2. In some embodiments, there is provided an anti-LAG-3 sdAb comprising a CDR1 comprising the amino acid sequence of SEQ ID NO: 61; a CDR2 comprising the amino acid sequence of SEQ ID NO: 137; and a CDR3 comprising the amino acid sequence of SEQ ID NO: 213. In some embodiments, the K_D of the binding between the anti-LAG-3 sdAb and LAG-3 is about 10^{-7} M to about 10^{-12} M (such as about 10^{-8} M to about 10^{-12} M, or about 10^{-9} M to about 10^{-11} M). In some embodiments, the anti-LAG-3 sdAb cross-reacts with a LAG-3 from a non-human mammal. In some embodiments, the anti-LAG-3 sdAb is camelid, chimeric, human, partially humanized, or fully humanized.

[0120] In some embodiments, there is provided an anti-LAG-3 sdAb comprising a CDR1 comprising the amino acid sequence of SEQ ID NO: 70, or a variant thereof comprising up to about 3 (such as about any of 1, 2, or 3) amino acid substitutions; a CDR2 comprising the amino acid sequence of SEQ ID NO: 146, or a variant thereof comprising up to about 3 (such as about any of 1, 2, or 3) amino acid substitutions; and a CDR3 comprising the amino acid sequence of SEQ ID NO: 222, or a variant thereof comprising up to about 3 (such as about any of 1, 2, or 3) amino acid substitutions. In some embodiments, there is provided an anti-LAG-3 sdAb comprising a CDR1 comprising the amino acid sequence of SEQ ID NO: 70; a CDR2 comprising the amino acid sequence of SEQ ID NO: 146; and a CDR3 comprising the amino acid sequence of SEQ ID NO: 222; or a variant thereof comprising up to about 3 (such as about any of 1, 2, or 3) amino acid substitutions in the CDR regions. In some embodiments, the amino acid substitutions are in CDR1 and/or CDR2. In some embodiments, there is provided an anti-LAG-3 sdAb comprising a CDR1 comprising the amino acid sequence of SEQ ID NO: 70; a CDR2 comprising the amino acid sequence of SEQ ID NO: 146; and a CDR3 comprising the amino acid sequence of SEQ ID NO: 222. In some embodiments, the K_D of the binding between the anti-LAG-3 sdAb and LAG-3 is about 10^{-7} M to about 10^{-12} M (such as about 10^{-8} M to about 10^{-12} M, or about 10^{-9} M to about 10^{-11} M). In some embodiments, the anti-LAG-3 sdAb cross-reacts with a LAG-3 from a non-human mammal. In some embodiments, the anti-LAG-3 sdAb is camelid, chimeric, human, partially humanized, or fully humanized.

[0121] In some embodiments, there is provided an anti-LAG-3 sdAb comprising a CDR1 comprising the amino acid sequence of SEQ ID NO: 72, or a variant thereof comprising up to about 3 (such as about any of 1, 2, or 3) amino acid substitutions; a CDR2 comprising the amino acid sequence of SEQ ID NO: 148, or a variant thereof comprising up to about 3 (such as about any of 1, 2, or 3) amino acid

substitutions; and a CDR3 comprising the amino acid sequence of SEQ ID NO: 224, or a variant thereof comprising up to about 3 (such as about any of 1, 2, or 3) amino acid substitutions. In some embodiments, there is provided an anti-LAG-3 sdAb comprising a CDR1 comprising the amino acid sequence of SEQ ID NO: 72; a CDR2 comprising the amino acid sequence of SEQ ID NO: 148; and a CDR3 comprising the amino acid sequence of SEQ ID NO: 224; or a variant thereof comprising up to about 3 (such as about any of 1, 2, or 3) amino acid substitutions in the CDR regions. In some embodiments, the amino acid substitutions are in CDR1 and/or CDR2. In some embodiments, there is provided an anti-LAG-3 sdAb comprising a CDR1 comprising the amino acid sequence of SEQ ID NO: 72; a CDR2 comprising the amino acid sequence of SEQ ID NO: 148; and a CDR3 comprising the amino acid sequence of SEQ ID NO: 224. In some embodiments, the K_D of the binding between the anti-LAG-3 sdAb and LAG-3 is about 10^{-7} M to about 10^{-12} M (such as about 10^{-8} M to about 10^{-12} M, or about 10^{-9} M to about 10^{-11} M). In some embodiments, the anti-LAG-3 sdAb cross-reacts with a LAG-3 from a non-human mammal. In some embodiments, the anti-LAG-3 sdAb is camelid, chimeric, human, partially humanized, or fully humanized.

[0122] In some embodiments, there is provided an anti-LAG-3 sdAb comprising a CDR1 comprising the amino acid sequence of SEQ ID NO: 73, or a variant thereof comprising up to about 3 (such as about any of 1, 2, or 3) amino acid substitutions; a CDR2 comprising the amino acid sequence of SEQ ID NO: 149, or a variant thereof comprising up to about 3 (such as about any of 1, 2, or 3) amino acid substitutions; and a CDR3 comprising the amino acid sequence of SEQ ID NO: 225, or a variant thereof comprising up to about 3 (such as about any of 1, 2, or 3) amino acid substitutions. In some embodiments, there is provided an anti-LAG-3 sdAb comprising a CDR1 comprising the amino acid sequence of SEQ ID NO: 73; a CDR2 comprising the amino acid sequence of SEQ ID NO: 149; and a CDR3 comprising the amino acid sequence of SEQ ID NO: 225; or a variant thereof comprising up to about 3 (such as about any of 1, 2, or 3) amino acid substitutions in the CDR regions. In some embodiments, the amino acid substitutions are in CDR1 and/or CDR2. In some embodiments, there is provided an anti-LAG-3 sdAb comprising a CDR1 comprising the amino acid sequence of SEQ ID NO: 73; a CDR2 comprising the amino acid sequence of SEQ ID NO: 149; and a CDR3 comprising the amino acid sequence of SEQ ID NO: 225. In some embodiments, the K_D of the binding between the anti-LAG-3 sdAb and LAG-3 is about 10^{-7} M to about 10^{-12} M (such as about 10^{-8} M to about 10^{-12} M, or about 10^{-9} M to about 10^{-11} M). In some embodiments, the anti-LAG-3 sdAb cross-reacts with a LAG-3 from a non-human mammal. In some embodiments, the anti-LAG-3 sdAb is camelid, chimeric, human, partially humanized, or fully humanized.

[0123] In some embodiments, there is provided an anti-LAG-3 sdAb comprising a CDR1 comprising the amino acid sequence of SEQ ID NO: 53, or a variant thereof comprising up to about 3 (such as about any of 1, 2, or 3) amino acid substitutions; a CDR2 comprising the amino acid sequence of SEQ ID NO: 129, or a variant thereof comprising up to about 3 (such as about any of 1, 2, or 3) amino acid substitutions; and a CDR3 comprising the amino acid sequence of SEQ ID NO: 205, or a variant thereof comprising up to about 3 (such as about any of 1, 2, or 3) amino acid substitutions. In some embodiments, there is provided an anti-LAG-3 sdAb comprising a CDR1 comprising the amino acid sequence of SEQ ID NO: 53; a CDR2 comprising the amino acid sequence of SEQ ID NO: 129; and a CDR3 comprising the amino acid sequence of SEQ ID NO: 205; or a variant thereof comprising up to about 3 (such as about any of 1, 2, or 3) amino acid substitutions in the CDR regions. In some embodiments, the amino acid substitutions are in CDR1 and/or CDR2. In some embodiments, there is provided an anti-LAG-3 sdAb comprising a CDR1 comprising the amino acid sequence of SEQ ID NO: 53; a CDR2 comprising the amino acid sequence of SEQ ID NO: 129; and a CDR3 comprising the amino acid sequence of SEQ ID NO: 205. In some embodiments, the K_D of the binding between the anti-LAG-3 sdAb and LAG-3 is about 10^{-7} M to about 10^{-12} M (such as about 10^{-8} M to about 10^{-12} M, or about 10^{-9} M to about 10^{-11} M). In some embodiments, the anti-LAG-3 sdAb cross-reacts with a LAG-3 from a non-human mammal. In some embodiments, the anti-LAG-3 sdAb is camelid, chimeric, human, partially humanized, or fully humanized.

[0124] In some embodiments, there is provided an anti-LAG-3 sdAb comprising a CDR1 comprising the amino acid sequence of SEQ ID NO: 60, or a variant thereof comprising up to about 3 (such as about any of 1, 2, or 3) amino acid substitutions; a CDR2 comprising the amino acid sequence of SEQ ID NO: 136, or a variant thereof comprising up to about 3 (such as about any of 1, 2, or 3) amino acid substitutions; and a CDR3 comprising the amino acid sequence of SEQ ID NO: 212, or a variant thereof comprising up to about 3 (such as about any of 1, 2, or 3) amino acid substitutions. In some embodiments, there is provided an anti-LAG-3 sdAb comprising a CDR1 comprising the amino acid sequence of SEQ ID NO: 60; a CDR2 comprising the amino acid sequence of SEQ ID NO: 136; and a CDR3 comprising the amino acid sequence of SEQ ID NO: 212; or a variant thereof comprising up to about 3 (such as about any of 1, 2, or 3) amino acid substitutions in the CDR regions. In some embodiments, the amino acid substitutions are in CDR1 and/or CDR2. In some embodiments, there is provided an anti-LAG-3 sdAb comprising a CDR1 comprising the amino acid sequence of SEQ ID NO: 60; a CDR2 comprising the amino acid sequence of SEQ ID NO: 136; and a CDR3 comprising the amino acid sequence of SEQ ID NO: 212. In some embodiments, the K_D of the binding between the anti-LAG-3 sdAb and LAG-3 is about 10^{-7} M to about 10^{-12} M (such as about 10^{-8} M to about 10^{-12} M, or about 10^{-9} M to about 10^{-11} M). In some

embodiments, the anti-LAG-3 sdAb cross-reacts with a LAG-3 from a non-human mammal. In some embodiments, the anti-LAG-3 sdAb is camelid, chimeric, human, partially humanized, or fully humanized.

[0125] In some embodiments, there is provided an anti-LAG-3 sdAb comprising a CDR1 comprising the amino acid sequence of SEQ ID NO: 69, or a variant thereof comprising up to about 3 (such as about any of 1, 2, or 3) amino acid substitutions; a CDR2 comprising the amino acid sequence of SEQ ID NO: 145, or a variant thereof comprising up to about 3 (such as about any of 1, 2, or 3) amino acid substitutions; and a CDR3 comprising the amino acid sequence of SEQ ID NO: 221, or a variant thereof comprising up to about 3 (such as about any of 1, 2, or 3) amino acid substitutions. In some embodiments, there is provided an anti-LAG-3 sdAb comprising a CDR1 comprising the amino acid sequence of SEQ ID NO: 69; a CDR2 comprising the amino acid sequence of SEQ ID NO: 145; and a CDR3 comprising the amino acid sequence of SEQ ID NO: 221; or a variant thereof comprising up to about 3 (such as about any of 1, 2, or 3) amino acid substitutions in the CDR regions. In some embodiments, the amino acid substitutions are in CDR1 and/or CDR2. In some embodiments, there is provided an anti-LAG-3 sdAb comprising a CDR1 comprising the amino acid sequence of SEQ ID NO: 69; a CDR2 comprising the amino acid sequence of SEQ ID NO: 145; and a CDR3 comprising the amino acid sequence of SEQ ID NO: 221. In some embodiments, the K_D of the binding between the anti-LAG-3 sdAb and LAG-3 is about 10^{-7} M to about 10^{-12} M (such as about 10^{-8} M to about 10^{-12} M, or about 10^{-9} M to about 10^{-11} M). In some embodiments, the anti-LAG-3 sdAb cross-reacts with a LAG-3 from a non-human mammal. In some embodiments, the anti-LAG-3 sdAb is camelid, chimeric, human, partially humanized, or fully humanized.

[0126] In some embodiments, there is provided an anti-LAG-3 sdAb comprising a CDR1 comprising the amino acid sequence of SEQ ID NO: 71, or a variant thereof comprising up to about 3 (such as about any of 1, 2, or 3) amino acid substitutions; a CDR2 comprising the amino acid sequence of SEQ ID NO: 147, or a variant thereof comprising up to about 3 (such as about any of 1, 2, or 3) amino acid substitutions; and a CDR3 comprising the amino acid sequence of SEQ ID NO: 223, or a variant thereof comprising up to about 3 (such as about any of 1, 2, or 3) amino acid substitutions. In some embodiments, there is provided an anti-LAG-3 sdAb comprising a CDR1 comprising the amino acid sequence of SEQ ID NO: 71; a CDR2 comprising the amino acid sequence of SEQ ID NO: 147; and a CDR3 comprising the amino acid sequence of SEQ ID NO: 223; or a variant thereof comprising up to about 3 (such as about any of 1, 2, or 3) amino acid substitutions in the CDR regions. In some embodiments, the amino acid substitutions are in CDR1 and/or CDR2. In some embodiments, there is provided an anti-LAG-3 sdAb comprising a CDR1 comprising the amino acid sequence of SEQ ID NO: 71; a CDR2 comprising the

amino acid sequence of SEQ ID NO: 147; and a CDR3 comprising the amino acid sequence of SEQ ID NO: 223. In some embodiments, the K_D of the binding between the anti-LAG-3 sdAb and LAG-3 is about 10^{-7} M to about 10^{-12} M (such as about 10^{-8} M to about 10^{-12} M, or about 10^{-9} M to about 10^{-11} M). In some embodiments, the anti-LAG-3 sdAb cross-reacts with a LAG-3 from a non-human mammal. In some embodiments, the anti-LAG-3 sdAb is camelid, chimeric, human, partially humanized, or fully humanized.

[0127] In some embodiments, there is provided an anti-LAG-3 sdAb comprising a V_{HH} domain comprising the amino acid sequence of any one of SEQ ID NOs: 39-76, the amino acid sequence of any one of SEQ ID NOs: 115-152, and the amino acid sequence of any one of SEQ ID NOs: 191-228. In some embodiments, there is provided an anti-LAG-3 sdAb comprising a V_{HH} domain comprising the sequences of SEQ ID NOs: 39, 115 and 191. In some embodiments, there is provided an anti-LAG-3 sdAb comprising a V_{HH} domain comprising the sequences of SEQ ID NOs: 40, 116 and 192. In some embodiments, there is provided an anti-LAG-3 sdAb comprising a V_{HH} domain comprising the sequences of SEQ ID NOs: 41, 117 and 193. In some embodiments, there is provided an anti-LAG-3 sdAb comprising a V_{HH} domain comprising the sequences of SEQ ID NOs: 42, 118 and 194. In some embodiments, there is provided an anti-LAG-3 sdAb comprising a V_{HH} domain comprising the sequences of SEQ ID NOs: 43, 119 and 195. In some embodiments, there is provided an anti-LAG-3 sdAb comprising a V_{HH} domain comprising the sequences of SEQ ID NOs: 44, 120 and 196. In some embodiments, there is provided an anti-LAG-3 sdAb comprising a V_{HH} domain comprising the sequences of SEQ ID NOs: 45, 121 and 197. In some embodiments, there is provided an anti-LAG-3 sdAb comprising a V_{HH} domain comprising the sequences of SEQ ID NOs: 46, 122 and 198. In some embodiments, there is provided an anti-LAG-3 sdAb comprising a V_{HH} domain comprising the sequences of SEQ ID NOs: 54, 130 and 206. In some embodiments, there is provided an anti-LAG-3 sdAb comprising a V_{HH} domain comprising the sequence of SEQ ID NO: 61, 137, 213. In some embodiments, there is provided an anti-LAG-3 sdAb comprising a V_{HH} domain comprising the sequences of SEQ ID NOs: 70, 146 and 222. In some embodiments, there is provided an anti-LAG-3 sdAb comprising a V_{HH} domain comprising the sequences of SEQ ID NOs: 72, 148 and 224. In some embodiments, there is provided an anti-LAG-3 sdAb comprising a V_{HH} domain comprising the sequences of SEQ ID NOs: 73, 149 and 225. In some embodiments, there is provided an anti-LAG-3 sdAb comprising a V_{HH} domain comprising the sequences of SEQ ID NOs: 53, 129 and 205. In some embodiments, there is provided an anti-LAG-3 sdAb comprising a V_{HH} domain comprising the sequences of SEQ ID NOs: 60, 136 and 212. In some embodiments, there is provided an anti-LAG-3 sdAb comprising a V_{HH} domain comprising the sequences of SEQ ID NOs: 69, 145 and 221. In some embodiments, there is provided an anti-LAG-3

sdAb comprising a V_{HH} domain comprising the sequences of SEQ ID NOs: 71, 147 and 223. In some embodiments, the K_D of the binding between the anti-LAG-3 sdAb and LAG-3 is about 10^{-7} M to about 10^{-12} M (such as about 10^{-8} M to about 10^{-12} M, or about 10^{-9} M to about 10^{-11} M). In some embodiments, the anti-LAG-3 sdAb cross-reacts with a LAG-3 from a non-human mammal. In some embodiments, the anti-LAG-3 sdAb is camelid, chimeric, human, partially humanized, or fully humanized.

[0128] In some embodiments, there is provided an anti-LAG-3 sdAb comprising a V_{HH} comprising one, two or three CDRs of the amino acid sequence of SEQ ID NO: 274. In some embodiments, there is provided an anti-LAG-3 sdAb comprising a V_{HH} comprising one, two or three CDRs of the amino acid sequence of SEQ ID NO: 275. In some embodiments, there is provided an anti-LAG-3 sdAb comprising a V_{HH} comprising one, two or three CDRs of the amino acid sequence of SEQ ID NO: 276. In some embodiments, there is provided an anti-LAG-3 sdAb comprising a V_{HH} comprising one, two or three CDRs of the amino acid sequence of SEQ ID NO: 277. In some embodiments, there is provided an anti-LAG-3 sdAb comprising a V_{HH} comprising one, two or three CDRs of the amino acid sequence of SEQ ID NO: 278. In some embodiments, there is provided an anti-LAG-3 sdAb comprising a V_{HH} comprising one, two or three CDRs of the amino acid sequence of SEQ ID NO: 279. In some embodiments, there is provided an anti-LAG-3 sdAb comprising a V_{HH} comprising one, two or three CDRs of the amino acid sequence of SEQ ID NO: 280. In some embodiments, there is provided an anti-LAG-3 sdAb comprising a V_{HH} comprising one, two or three CDRs of the amino acid sequence of SEQ ID NO: 281. In some embodiments, there is provided an anti-LAG-3 sdAb comprising a V_{HH} comprising one, two or three CDRs of the amino acid sequence of SEQ ID NO: 282. In some embodiments, there is provided an anti-LAG-3 sdAb comprising a V_{HH} comprising one, two or three CDRs of the amino acid sequence of SEQ ID NO: 289. In some embodiments, there is provided an anti-LAG-3 sdAb comprising a V_{HH} comprising one, two or three CDRs of the amino acid sequence of SEQ ID NO: 296. In some embodiments, there is provided an anti-LAG-3 sdAb comprising a V_{HH} comprising one, two or three CDRs of the amino acid sequence of SEQ ID NO: 305. In some embodiments, there is provided an anti-LAG-3 sdAb comprising a V_{HH} comprising one, two or three CDRs of the amino acid sequence of SEQ ID NO: 307. In some embodiments, there is provided an anti-LAG-3 sdAb comprising a V_{HH} comprising one, two or three CDRs of the amino acid sequence of SEQ ID NO: 308. In some embodiments, there is provided an anti-LAG-3 sdAb comprising a V_{HH} comprising one, two or three CDRs of the amino acid sequence of SEQ ID NO: 288. In some embodiments, there is provided an anti-LAG-3 sdAb comprising a V_{HH} comprising one, two or three CDRs of the amino acid sequence of SEQ ID NO: 295. In some embodiments, there is provided an anti-LAG-3 sdAb comprising a V_{HH} comprising one, two or three CDRs of the amino acid sequence of SEQ ID NO: 304. In some embodiments, there is provided an anti-LAG-3 sdAb comprising a

V_HH comprising one, two or three CDRs of the amino acid sequence of SEQ ID NO: 306. In some embodiments, there is provided an anti-LAG-3 sdAb comprising a V_HH comprising CDR1, CDR2, and CDR3 of the amino acid sequence of any one of SEQ ID NOs: 274-306. In some embodiments, the K_D of the binding between the anti-LAG-3 sdAb and LAG-3 is about 10⁻⁷ M to about 10⁻¹² M (such as about 10⁻⁸ M to about 10⁻¹² M, or about 10⁻⁹ M to about 10⁻¹¹ M). In some embodiments, the anti-LAG-3 sdAb cross-reacts with a LAG-3 from a non-human mammal. In some embodiments, the anti-LAG-3 sdAb is camelid, chimeric, human, partially humanized, or fully humanized.

[0129] The anti-LAG-3 sdAbs described herein may comprise any suitable sequences for the FR region. In some embodiments, the anti-LAG-3 sdAb comprises a FR1 comprising the amino acid sequence of any one of SEQ ID NOs: 1-38, or a variant thereof comprising up to about 3 (such as about any one of 1, 2, 3, or more) amino acid substitutions. In some embodiments, the anti-LAG-3 sdAb comprises a FR2 comprising the amino acid sequence of any one of SEQ ID NOs: 77-114, or a variant thereof comprising up to about 3 (such as about any one of 1, 2, 3, or more) amino acid substitutions. In some embodiments, the anti-LAG-3 sdAb comprises a FR3 comprising the amino acid sequence of any one of SEQ ID NOs: 153-190, or a variant thereof comprising up to about 3 (such as about any one of 1, 2, 3, or more) amino acid substitutions. In some embodiments, the anti-LAG-3 sdAb comprises a FR4 comprising the amino acid sequence of any one of SEQ ID NOs: 229-266, or a variant thereof comprising up to about 3 (such as about any one of 1, 2, 3, or more) amino acid substitutions. In some embodiments, the anti-LAG-3 sdAb comprises a FR1, a FR2, a FR3, and a FR4 of any one of the anti-LAG-3 sdAbs of Table 11.

[0130] In some embodiments, there is provided an anti-LAG-3 sdAb comprising a V_HH domain comprising the amino acid sequence of any one of SEQ ID NOs: 274-311, or a variant thereof having at least about 80% (such as at least about any of 80%, 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, or 99%) sequence identity to any one of SEQ ID NOs: 289-324. In some embodiments, there is provided an anti-LAG-3 sdAb comprising a V_HH domain comprising the amino acid sequence of any one of SEQ ID NOs: 274-311, or a variant thereof comprising up to about 10 (such as about any of 1, 2, 3, 4, 5, 6, 7, 8, 9, or 10) amino acid substitutions in the V_HH domain. In some embodiments, the anti-LAG-3 sdAb comprising the V_HH domain comprising the amino acid sequence of any one of SEQ ID NOs: 274-311, or a variant thereof comprising up to about 3 (such as about any one of 1, 2, or 3) amino acid substitutions in the CDR1, and/or the CDR2, and/or the CDR3. In some embodiments, the anti-LAG-3 sdAb comprising the V_HH domain comprising the amino acid sequence of any one of SEQ ID NOs: 274-311, or a variant thereof comprising up to about 3 (such as about any one of 1, 2, or 3) amino acid substitutions are in the FR1, and/or the FR2, and/or the FR3, and/or the FR4. In some embodiments, the

anti-LAG-3 sdAb comprising the V_HH domain comprising the amino acid sequence of any one of SEQ ID NOs: 274-311, or a variant thereof comprising amino acid substitutions in both CDRs and FRs. In some embodiments, there is provided an anti-LAG-3 sdAb comprising a V_HH domain comprising the amino acid sequence of any one of SEQ ID NOs: 274-311. In some embodiments, there is provided an anti-LAG-3 sdAb comprising the amino acid sequence of any one of SEQ ID NOs: 274-311. In some embodiments, the K_D of the binding between the anti-LAG-3 sdAb and LAG-3 is about 10⁻⁷ M to about 10⁻¹² M (such as about 10⁻⁸ M to about 10⁻¹² M, or about 10⁻⁹ M to about 10⁻¹¹ M). In some embodiments, the anti-LAG-3 sdAb cross-reacts with a LAG-3 from a non-human mammal. In some embodiments, the anti-LAG-3 sdAb is camelid, chimeric, human, partially humanized, or fully humanized.

[0131] In some embodiments, the anti-LAG-3 sdAb comprises a V_HH domain comprising the amino acid sequence of SEQ ID NO: 288, or a variant thereof having at least about 80% (such as at least about any of 80%, 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, or 99%) sequence identity to SEQ ID NO: 288. In some embodiments, the anti-LAG-3 sdAb comprises one or more amino acid substitutions selected from the group consisting of: (1) at amino acid position 11 (*e.g.*, S or L); (2) at amino acid position 14 (*e.g.*, A or P); (3) at amino acid position 44 (*e.g.*, E or G); (4) at amino acid position 45 (*e.g.*, R or L); (5) at amino acid position 49 (*e.g.*, A or S); (6) at amino acid position 71 (*e.g.*, K or R); (7) at amino acid position 74 (*e.g.*, A or S); (8) at amino acid position 83 (*e.g.*, D or N); (9) at amino acid position 86 (*e.g.*, R or K); (10) at amino acid position 87 (*e.g.*, A or P); (11) at amino acid position 92 (*e.g.*, M or V); (12) at amino acid position 119 (*e.g.*, Q or L); wherein the amino acid positions are based on SEQ ID NO: 288.

[0132] In some embodiments, the anti-LAG-3 sdAb comprises a V_HH domain comprising the amino acid sequence of SEQ ID NO: 295, or a variant thereof having at least about 80% (such as at least about any of 80%, 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, or 99%) sequence identity to SEQ ID NO: 295. In some embodiments, the anti-LAG-3 sdAb comprises one or more amino acid substitutions selected from the group consisting of: (1) at amino acid position 1 (*e.g.*, Q or E); (2) at amino acid position 5 (*e.g.*, A or V); (3) at amino acid position 11 (*e.g.*, S or L); (4) at amino acid position 14 (*e.g.*, A or P); (5) at amino acid position 44 (*e.g.*, E or G); (6) at amino acid position 45 (*e.g.*, R or L); (7) at amino acid position 71 (*e.g.*, K or R); (8) at amino acid position 74 (*e.g.*, A or S); (9) at amino acid position 86 (*e.g.*, R or K); (10) at amino acid position 87 (*e.g.*, A or P); (11) at amino acid position 92 (*e.g.*, M or V); or (12) at amino acid position 117 (*e.g.*, Q or L); wherein the amino acid positions are based on SEQ ID NO: 295.

[0133] In some embodiments, the anti-LAG-3 sdAb comprises a V_HH domain comprising the amino acid sequence of SEQ ID NO: 304, or a variant thereof having at least about 80% (such as at least about

any of 80%, 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, or 99%) sequence identify to SEQ ID NO: 304. In some embodiments, the anti-LAG-3 sdAb comprises one or more amino acid substitutions selected from the group consisting of: (1) at amino acid position 1 (*e.g.*, Q or E); (2) at amino acid position 3 (*e.g.*, Q or H); (3) at amino acid position 5 (*e.g.*, M or V); (4) at amino acid position 11 (*e.g.*, S or L); (5) at amino acid position 14 (*e.g.*, V or P); (6) at amino acid position 44 (*e.g.*, E or G); (7) at amino acid position 45 (*e.g.*, R or L); (8) at amino acid position 49 (*e.g.*, A or S); (8) at amino acid position 71 (*e.g.*, K or R); (9) at amino acid position 74 (*e.g.*, A or S); (10) at amino acid position 86 (*e.g.*, R or K); (11) at amino acid position 87 (*e.g.*, A or P); (12) at amino acid position 92 (*e.g.*, M or V); (13) at amino acid position 94 (*e.g.*, F or Y); or (14) at amino acid position 117 (*e.g.*, Q, L, or I); wherein the amino acid positions are based on SEQ ID NO: 304.

[0134] In some embodiments, the anti-LAG-3 sdAb comprises a V_HH domain comprising the amino acid sequence of SEQ ID NO: 306, or a variant thereof having at least about 80% (such as at least about any of 80%, 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, or 99%) sequence identify to SEQ ID NO: 306. In some embodiments, the anti-LAG-3 sdAb comprises one or more amino acid substitutions selected from the group consisting of: (1) at amino acid position 5 (*e.g.*, A or V); (2) at amino acid position 11 (*e.g.*, S or L); (3) at amino acid position 14 (*e.g.*, A or P); (4) at amino acid position 44 (*e.g.*, E or G); (5) at amino acid position 45 (*e.g.*, R or L); (6) at amino acid position 49 (*e.g.*, A or S); (7) at amino acid position 71 (*e.g.*, K or R); (8) at amino acid position 74 (*e.g.*, A or S); (9) at amino acid position 86 (*e.g.*, R or S); (10) at amino acid position 87 (*e.g.*, A or P); (11) at amino acid position 92 (*e.g.*, M or V); or (12) at amino acid position 121 (*e.g.*, Q or L); wherein the amino acid positions are based on SEQ ID NO: 306.

[0135] In some embodiments, there is provided an anti-LAG-3 sdAb or anti-LAG-3 construct comprising an anti-LAG-3 sdAb that specifically binds to LAG-3 competitively with any one of the anti-LAG-3 sdAbs described herein. In some embodiments, competitive binding may be determined using an ELISA assay. In some embodiments, there is provided an anti-LAG-3 sdAb (or an anti-LAG-3 construct comprising an anti-LAG-3 sdAb) that specifically binds to LAG-3 competitively with an anti-LAG-3 sdAb comprising the amino acid sequence of any one of SEQ ID NOs: 274-311. In some embodiments, there is provided an anti-LAG-3 sdAb (or an anti-LAG-3 construct comprising an anti-LAG-3 sdAb) that specifically binds to LAG-3 competitively with an anti-LAG-3 sdAb comprising a CDR1 comprising the amino acid sequence of any one of SEQ ID NOs: 39-76; a CDR2 comprising the amino acid sequence of any one of SEQ ID NOs: 115-152; and a CDR3 comprising the amino acid sequence of any one of SEQ ID NOs: 191-228. In some embodiments, there is provided an anti-LAG-3 sdAb (or an anti-LAG-3 construct comprising an anti-LAG-3 sdAb) that specifically binds to LAG-3 competitively with any one

of the anti-LAG-3 sdAbs of Table 11. In some embodiments, the K_D of the binding between the competing anti-LAG-3 sdAb and LAG-3 is about 10^{-7} M to about 10^{-12} M (such as about 10^{-8} M to about 10^{-12} M, or about 10^{-9} M to about 10^{-11} M). In some embodiments, the anti-LAG-3 sdAb cross-reacts with a LAG-3 from a non-human mammal. In some embodiments, the competing anti-LAG-3 sdAb is camelid, chimeric, human, partially humanized, or fully humanized.

Single-domain antibodies

[0136] In some embodiments, the anti-LAG-3 construct is a single-domain antibody. Exemplary sdAbs include, but are not limited to, heavy chain variable domains from heavy-chain only antibodies (*e.g.*, V_{HH} (Variable domain of the heavy chain of the Heavy chain antibody) in *Camelidae* or V_{NAR} (Variable domain of the shark New Antigen Receptor) in cartilaginous fish), binding molecules naturally devoid of light chains, single domains (such as V_H or V_L) derived from conventional 4-chain antibodies, humanized heavy-chain only antibodies, human single-domain antibodies produced by transgenic mice or rats expressing human heavy chain segments, and engineered domains and single domain scaffolds other than those derived from antibodies. The sdAbs may be derived from any species including, but not limited to mouse, rat, human, camel, llama, lamprey, fish, shark, goat, rabbit, and bovine. sdAbs contemplated herein also include naturally occurring sdAb molecules from species other than *Camelidae* and sharks.

[0137] In some embodiments, the sdAb is derived from a naturally occurring single-domain antigen binding molecule known as heavy chain antibody devoid of light chains (also referred herein as “heavy chain-only antibodies”, or “HCAb”). Such single domain molecules are disclosed in WO 94/04678 and Hamers-Casterman, C. *et al.* (1993) *Nature* 363:446-448, for example. For clarity reasons, the variable domain derived from a heavy chain molecule naturally devoid of light chain is known herein as a V_{HH} to distinguish it from the conventional V_H of four chain immunoglobulins. Such a V_{HH} molecule can be derived from antibodies raised in *Camelidae* species, for example, camel, llama, vicuna, dromedary, alpaca and guanaco. Other species besides *Camelidae* may produce heavy chain molecules naturally devoid of light chain, and such V_{HH} s are within the scope of the present application.

[0138] In some embodiments, the sdAb is derived from a variable region of the immunoglobulin found in cartilaginous fish. For example, the sdAb can be derived from the immunoglobulin isotype known as Novel Antigen Receptor (NAR) found in the serum of shark. Methods of producing single domain molecules derived from a variable region of NAR (“IgNARs”) are described in WO 03/014161 and Streltsov (2005) *Protein Sci.* 14:2901-2909.

[0139] In some embodiments, the sdAb is recombinant, CDR-grafted, humanized, camelized, de-immunized and/or *in vitro* generated (*e.g.*, selected by phage display). In some embodiments, the amino

acid sequence of the framework regions may be altered by “camelization” of specific amino acid residues in the framework regions. Camelization refers to the replacing or substitution of one or more amino acid residues in the amino acid sequence of a (naturally occurring) V_H domain from a conventional 4-chain antibody by one or more of the amino acid residues that occur at the corresponding position(s) in a V_HH domain of a heavy chain antibody. This can be performed in a manner known *per se*, which will be clear to the skilled person, for example on the basis of the further description herein. Such “camelizing” substitutions are preferably inserted at amino acid positions that form and/or are present at the V_H - V_L interface, and/or at the so-called Camelidae hallmark residues, as defined herein (*see* for example WO 94/04678, Davies and Riechmann FEBS Letters 339: 285-290, 1994; Davies and Riechmann Protein Engineering 9 (6): 531-537, 1996; Riechmann J. Mol. Biol. 259: 957-969, 1996; and Riechmann and Muyldermans J. Immunol. Meth. 231: 25-38, 1999).

[0140] In some embodiments, the sdAb is a human sdAb produced by transgenic mice or rats expressing human heavy chain segments. See, *e.g.*, US20090307787A1, U.S. Pat. No. 8,754,287, US20150289489A1, US20100122358A1, and WO2004049794. In some embodiments, the sdAb is affinity-matured.

[0141] In some embodiments, naturally occurring V_HH domains against a particular antigen or target, can be obtained from (naïve or immune) libraries of Camelid V_HH sequences. Such methods may or may not involve screening such a library using said antigen or target, or at least one part, fragment, antigenic determinant or epitope thereof using one or more screening techniques known *per se*. Such libraries and techniques are for example described in WO 99/37681, WO 01/90190, WO 03/025020 and WO 03/035694. Alternatively, improved synthetic or semi-synthetic libraries derived from (naïve or immune) V_HH libraries may be used, such as V_HH libraries obtained from (naïve or immune) V_HH libraries by techniques such as random mutagenesis and/or CDR shuffling, as for example described in WO 00/43507.

[0142] In some embodiments, the sdAbs are generated from conventional 4-chain antibodies. See, for example, EP 0 368 684, Ward *et al.* (Nature 1989 Oct. 12; 341 (6242): 544-6), Holt *et al.*, Trends Biotechnol., 2003, 21(11):484-490; WO 06/030220; and WO 06/003388.

[0143] Because of the unique properties of sdAbs, using V_HH domains as single antigen-binding proteins or as antigen-binding domains (*i.e.* as part of a larger protein or polypeptide) offers a number of significant advantages over the conventional V_H and V_L , scFv and conventional antibody fragments (such as Fab or (Fab')₂): 1) only a single domain is required to bind an antigen with high affinity, so there is no need to have a second domain, nor to assure that these two domains are present in the correct spatial conformation and configuration (*e.g.* no need to pair the heavy chain and light chain during folding, no

need to use a specially designed linker such as for scFv); 2) V_HH domains and other sdAbs can be expressed from a single gene and require no post-translational folding or modifications; 3) V_HH domains and other sdAbs can be easily engineered into multivalent and/or multispecific formats (such as those described in the present application); 4) V_HH domains and other sdAbs are highly soluble and do not have a tendency to aggregate (as with the mouse-derived “dAbs” described by Ward *et al.*, Nature. 1989 Oct 12;341(6242):544-6); 5) V_HH domains and other sdAbs are highly stable against heat, pH, proteases and other denaturing agents or conditions; 6) V_HH domains and other sdAbs are easy and relatively cheap to prepare (even on a large production scale), such as using microbial fermentation, because there is no need to use mammalian expression systems (required by production of, for example, conventional antibody fragments); 7) V_HH domains and other sdAbs are relatively small (approximately 15 kDa, or 10 times smaller than a conventional IgG) compared to conventional 4-chain antibodies and antigen-binding fragments thereof, thus have high(er) tissue penetration ability, such as for solid tumors and other dense tissues; and 8) V_HH domains and other sdAbs can exhibit so-called “cavity-binding properties” (due to their extended CDR3 loop compared to that of conventional V_H domains) and can therefore access targets and epitopes not accessible to conventional 4-chain antibodies and antigen-binding fragments thereof, for example, it has been shown that V_HH domains and other sdAbs can inhibit enzymes (*see* for example WO1997049805; Transue *et al.*, Proteins. 1998 Sep 1;32(4):515-22; Lauwereys *et al.*, EMBO J. 1998 Jul 1;17(13):3512-20).

LAG-3

[0144] Lymphocyte-activation protein 3 (LAG-3), comprised of 503 amino acids, belongs to the Ig superfamily and contains 4 extracellular Ig-like domains, designated D1 to D4. LAG-3 is a cell surface protein expressed on activated T cells, NK cells, B cells, and plasmacytoid dendritic cells, and plays a role in the function of these lymphocyte subsets that is important but not completely understood.

[0145] The terms “lymphocyte-activation protein 3”, “LAG-3”, “LAG-3 antigen”, and “LAG-3 epitope” are used interchangeably, and include variants, isoforms, species homologs of human LAG-3, and analogs having at least one common epitope with LAG-3.

[0146] An exemplary amino acid sequence of human LAG-3 is disclosed at Genbank Accession Number P18627. In some embodiments, a human LAG-3 may be at least about 90%, 95%, 96%, 97%, 98%, or 99% identical in amino acid sequence to the human LAG-3 of Genbank Accession Number P18627. In some embodiments, a human LAG-3 sequence has no more than about 10 amino acid differences from the human LAG-3 of Genbank Accession Number P18627. In some embodiments, the human LAG-3 may display no more than 5, 4, 3, 2, or 1 amino acid difference from the human LAG-

3 of Genbank Accession Number P18627. In some embodiments, a human LAG-3 sequence may differ from the human LAG-3 of Genbank Accession Number P18627 by having, for example, conserved mutations or mutations in non-conserved regions and the LAG-3 has substantially the same biological function as the human LAG-3 of Genbank Accession Number P18627.

[0147] In some embodiments, the anti-LAG-3 sdAb described herein specifically recognizes a LAG-3 polypeptide having at least about 90% amino acid sequence identity to the human LAG-3 of Genbank Accession Number P18627. In some embodiments, the anti-LAG-3 sdAb described herein specifically recognizes a LAG-3 polypeptide comprising an amino acid sequence of SEQ ID NO: 350 or 351.

[0148] In some embodiments, the anti-LAG-3 sdAb may cross-react with LAG-3 from species other than human, or other proteins which are structurally related to human LAG-3 (*e.g.*, human LAG-3 homologs). In some embodiments, the anti-LAG-3 sdAb is completely specific for human LAG-3 and not exhibit species or other types of cross-reactivity. In some embodiments, the anti-LAG-3 sdAb specifically recognizes a soluble isoform of human LAG-3. In some embodiments, the anti-LAG-3 sdAb specifically recognizes a membrane-bound isoform of human LAG-3 (*e.g.*, SEQ ID NO: 350).

[0149] In some embodiments, the anti-LAG-3 sdAb described herein specifically recognizes the extracellular domain (ECD) of LAG-3. In some embodiments, the anti-LAG-3 sdAb specifically recognizes the N-terminal portion of the LAG-3 ECD. In some embodiments, the anti-LAG-3 sdAb specifically recognizes the C-terminal portion of the LAG-3 ECD. In some embodiments, the anti-LAG-3 sdAb specifically recognizes the middle portion of the LAG-3 ECD. In some embodiments, the ECD of LAG-3 specifically recognized by the anti-LAG-3 sdAb is at least about 95%, 96%, 97%, 98%, or 99% identical in amino acid sequence to the ECD of the human LAG-3 of Genbank Accession Number P18627. In some embodiments, the ECD of LAG-3 specifically recognized by the anti-LAG-3 sdAb is 100% identical in amino acid sequence to the ECD of the human LAG-3 of Genbank Accession Number P18627. In some embodiments, the anti-LAG-3 sdAb specifically recognizes a LAG-3 polypeptide comprising an amino acid sequence of SEQ ID NO: 351.

Antibody affinity

[0150] Binding specificity of the antibody or antigen-binding domain can be determined experimentally by methods known in the art. Such methods comprise, but are not limited to Western blots, ELISA-, RIA-, ECL-, IRMA-, EIA-, BIACORE™-tests and peptide scans.

[0151] In some embodiments, the K_D of the binding between the anti-LAG-3 sdAb and LAG-3 is about 10^{-7} M to about 10^{-12} M, about 10^{-7} M to about 10^{-8} M, about 10^{-8} M to about 10^{-9} M, about 10^{-9} M to about 10^{-10} M, about 10^{-10} M to about 10^{-11} M, about 10^{-11} M to about 10^{-12} M, about 10^{-7} M to about 10^{-12}

M, about 10^{-8} M to about 10^{-12} M, about 10^{-9} M to about 10^{-12} M, about 10^{-10} M to about 10^{-12} M, about 10^{-7} M to about 10^{-11} M, about 10^{-8} M to about 10^{-11} M, about 10^{-9} M to about 10^{-11} M, about 10^{-7} M to about 10^{-10} M, about 10^{-8} M to about 10^{-10} M, or about 10^{-7} M to about 10^{-9} M. In some embodiments, the K_d of the binding between the anti-LAG-3 sdAb and LAG-3 is stronger than about any one of 10^{-7} M, 10^{-8} M, 10^{-9} M, 10^{-10} M, 10^{-11} M, or 10^{-12} M. In some embodiments, the LAG-3 is human LAG-3. In some embodiments, the LAG-3 is cynomolgus monkey LAG-3. In some embodiments, the LAG-3 is the extracellular domain of LAG-3.

[0152] In some embodiments, the K_{on} of the binding between the anti-LAG-3 sdAb and LAG-3 is about 10^3 $M^{-1}s^{-1}$ to about 10^8 $M^{-1}s^{-1}$, about 10^3 $M^{-1}s^{-1}$ to about 10^4 $M^{-1}s^{-1}$, about 10^4 $M^{-1}s^{-1}$ to about 10^5 $M^{-1}s^{-1}$, about 10^5 $M^{-1}s^{-1}$ to about 10^6 $M^{-1}s^{-1}$, about 10^6 $M^{-1}s^{-1}$ to about 10^7 $M^{-1}s^{-1}$, or about 10^7 $M^{-1}s^{-1}$ to about 10^8 $M^{-1}s^{-1}$. In some embodiments, the K_{on} of the binding between the anti-LAG-3 sdAb and LAG-3 is about 10^3 $M^{-1}s^{-1}$ to about 10^5 $M^{-1}s^{-1}$, about 10^4 $M^{-1}s^{-1}$ to about 10^6 $M^{-1}s^{-1}$, about 10^5 $M^{-1}s^{-1}$ to about 10^7 $M^{-1}s^{-1}$, about 10^6 $M^{-1}s^{-1}$ to about 10^8 $M^{-1}s^{-1}$, about 10^4 $M^{-1}s^{-1}$ to about 10^7 $M^{-1}s^{-1}$, or about 10^5 $M^{-1}s^{-1}$ to about 10^8 $M^{-1}s^{-1}$. In some embodiments, the K_{on} of the binding between the anti-LAG-3 sdAb and LAG-3 is no more than about any one of 10^3 $M^{-1}s^{-1}$, 10^4 $M^{-1}s^{-1}$, 10^5 $M^{-1}s^{-1}$, 10^6 $M^{-1}s^{-1}$, 10^7 $M^{-1}s^{-1}$ or 10^8 $M^{-1}s^{-1}$.

[0153] In some embodiments, the K_{off} of the binding between the anti-LAG-3 sdAb and LAG-3 is about 1 s^{-1} to about 10^{-6} s^{-1} , about 1 s^{-1} to about 10^{-2} s^{-1} , about 10^{-2} s^{-1} to about 10^{-3} s^{-1} , about 10^{-3} s^{-1} to about 10^{-4} s^{-1} , about 10^{-4} s^{-1} to about 10^{-5} s^{-1} , about 10^{-5} s^{-1} to about 10^{-6} s^{-1} , about 1 s^{-1} to about 10^{-5} s^{-1} , about 10^{-2} s^{-1} to about 10^{-6} s^{-1} , about 10^{-3} s^{-1} to about 10^{-6} s^{-1} , about 10^{-4} s^{-1} to about 10^{-6} s^{-1} , about 10^{-2} s^{-1} to about 10^{-5} s^{-1} , or about 10^{-3} s^{-1} to about 10^{-5} s^{-1} . In some embodiments, the K_{off} of the binding between the anti-LAG-3 sdAb and LAG-3 is at least about any one of 1 s^{-1} , 10^{-2} s^{-1} , 10^{-3} s^{-1} , 10^{-4} s^{-1} , 10^{-5} s^{-1} or 10^{-6} s^{-1} .

[0154] In some embodiments, the EC_{50} of the anti-LAG-3 sdAb is less than 10 nM in an amplified luminescent proximity homogeneous assay (AlphaLISA). In some embodiments, the EC_{50} of the anti-LAG-3 sdAb is less than 500 nM in an inhibition of ligand binding by FACS analysis (competition binding assay), or cell based cytokine release assay. In some embodiments, the EC_{50} of the anti-LAG-3 sdAb is less than 1 nM (such as about 0.001 nM to about 0.01 nM, about 0.01 nM to about 0.1 nM, about 0.1 nM to about 1 nM, *etc.*), about 1 nM to about 10 nM, about 1 nM to about 5 nM, about 5 nM to about 10 nM, about 10 nM to about 50 nM, about 50 nM to about 100 nM, about 100 nM to about 200 nM, about 200 nM to about 300 nM, about 300 nM to about 400 nM, or about 400 nM to about 500 nM. In some embodiments, the EC_{50} of the binding between the anti-LAG-3 sdAb and LAG-3 is no more than about any one of 0.1 nM, 1 nM, 5 nM, 10 nM, 50 nM, 10 nM, 100 nM, 200 nM, or 500 nM.

Chimeric or humanized antibodies

[0155] In some embodiments, the anti-LAG-3 sdAb provided herein is a chimeric antibody. Certain chimeric antibodies are described, *e.g.*, in U.S. Patent No. 4,816,567; and Morrison et al., *Proc. Natl. Acad. Sci. USA*, 81:6851-6855 (1984)). In some embodiments, a chimeric antibody comprises a non-human variable region (*e.g.*, a variable region derived from a camelid species, such as llama) and a human constant region. In some embodiments, a chimeric antibody is a “class switched” antibody in which the class or subclass has been changed from that of the parent antibody. Chimeric antibodies include antigen-binding fragments thereof.

[0156] In some embodiments, a chimeric antibody is a humanized antibody. Typically, a non-human antibody is humanized to reduce immunogenicity to humans, while retaining the specificity and affinity of the parental non-human antibody. Generally, a humanized antibody comprises one or more variable domains in which HVRs, *e.g.*, CDRs, (or portions thereof) are derived from a non-human antibody, and FRs (or portions thereof) are derived from human antibody sequences. A humanized antibody optionally will also comprise at least a portion of a human constant region. In some embodiments, some FR residues in a humanized antibody are substituted with corresponding residues from a non-human antibody (*e.g.*, the antibody from which the HVR residues are derived), *e.g.*, to restore or improve antibody specificity or affinity.

[0157] Humanized antibodies and methods of making them are reviewed, *e.g.*, in Almagro and Fransson, *Front. Biosci.* 13:1619-1633 (2008), and are further described, *e.g.*, in Riechmann et al., *Nature* 332:323-329 (1988); Queen et al., *Proc. Nat'l Acad. Sci. USA* 86:10029-10033 (1989); US Patent Nos. 5, 821,337, 7,527,791, 6,982,321, and 7,087,409; Kashmiri *et al.*, *Methods* 36:25-34 (2005) (describing SDR (a-CDR) grafting); Padlan, *Mol. Immunol.* 28:489-498 (1991) (describing “resurfacing”); Dall’Acqua et al., *Methods* 36:43-60 (2005) (describing “FR shuffling”); and Osbourn et al., *Methods* 36:61-68 (2005) and Klimka et al., *Br. J. Cancer*, 83:252-260 (2000) (describing the “guided selection” approach to FR shuffling).

[0158] Human framework regions that may be used for humanization include but are not limited to: framework regions selected using the “best-fit” method (*see, e.g.*, Sims et al. *J. Immunol.* 151:2296 (1993)); framework regions derived from the consensus sequence of human antibodies of a particular subgroup of light or heavy chain variable regions (*see, e.g.*, Carter et al. *Proc. Natl. Acad. Sci. USA*, 89:4285 (1992); and Presta et al. *J. Immunol.*, 151:2623 (1993)); human mature (somatically mutated) framework regions or human germline framework regions (*see, e.g.*, Almagro and Fransson, *Front. Biosci.*

13:1619-1633 (2008)); and framework regions derived from screening FR libraries (see, *e.g.*, Baca et al., *J. Biol. Chem.* 272:10678-10684 (1997) and Rosok et al., *J. Biol. Chem.* 271:22611-22618 (1996)).

[0159] In some embodiments, the anti-LAG-3 sdAbs are modified, such as humanized, without diminishing the native affinity of the domain for antigen and while reducing its immunogenicity with respect to a heterologous species. For example, the amino acid residues of the antibody variable domain (V_HH) of an llama antibody can be determined, and one or more of the Camelid amino acids, for example, in the framework regions, are replaced by their human counterpart as found in the human consensus sequence, without that polypeptide losing its typical character, *i.e.* the humanization does not significantly affect the antigen binding capacity of the resulting polypeptide. Humanization of Camelid single-domain antibodies requires the introduction and mutagenesis of a limited amount of amino acids in a single polypeptide chain. This is in contrast to humanization of scFv, Fab', (Fab')₂ and IgG, which requires the introduction of amino acid changes in two chains, the light and the heavy chain and the preservation of the assembly of both chains.

[0160] sdAbs comprising a V_HH domain can be humanized to have human-like sequences. In some embodiments, the FR regions of the V_HH domain used herein comprise at least about any one of 50%, 60%, 70%, 80%, 90%, 95% or more of amino acid sequence homology to human VH framework regions. One exemplary class of humanized V_HH domains is characterized in that the V_HH s carry an amino acid from the group consisting of glycine, alanine, valine, leucine, isoleucine, proline, phenylalanine, tyrosine, tryptophan, methionine, serine, threonine, asparagine, or glutamine at position 45, such as, for example, L45 and a tryptophan at position 103, according to the Kabat numbering. As such, polypeptides belonging to this class show a high amino acid sequence homology to human VH framework regions and said polypeptides might be administered to a human directly without expectation of an unwanted immune response therefrom, and without the burden of further humanization.

[0161] Another exemplary class of humanized Camelid single-domain antibodies has been described in WO 03/035694 and contains hydrophobic FR2 residues typically found in conventional antibodies of human origin or from other species, but compensating this loss in hydrophilicity by the charged arginine residue on position 103 that substitutes the conserved tryptophan residue present in V_H from double-chain antibodies. As such, peptides belonging to these two classes show a high amino acid sequence homology to human V_H framework regions and said peptides might be administered to a human directly without expectation of an unwanted immune response therefrom, and without the burden of further humanization.

Human antibodies

[0162] In some embodiments, the anti-LAG-3 sdAb provided herein is a human antibody (known as human domain antibody, or human DAb). Human antibodies can be produced using various techniques known in the art. Human antibodies are described generally in van Dijk and van de Winkel, *Curr. Opin. Pharmacol.* 5: 368-74 (2001), Lonberg, *Curr. Opin. Immunol.* 20:450-459 (2008), and Chen, *Mol. Immunol.* 47(4):912-21 (2010). Transgenic mice or rats capable of producing fully human single-domain antibodies (or DAb) are known in the art. See, *e.g.*, US20090307787A1, U.S. Pat. No. 8,754,287, US20150289489A1, US20100122358A1, and WO2004049794.

[0163] Human antibodies (*e.g.*, human DABs) may be prepared by administering an immunogen to a transgenic animal that has been modified to produce intact human antibodies or intact antibodies with human variable regions in response to antigenic challenge. Such animals typically contain all or a portion of the human immunoglobulin loci, which replace the endogenous immunoglobulin loci, or which are present extrachromosomally or integrated randomly into the animal's chromosomes. In such transgenic mice, the endogenous immunoglobulin loci have generally been inactivated. For review of methods for obtaining human antibodies from transgenic animals, see Lonberg, *Nat. Biotech.* 23:1117-1125 (2005). See also, *e.g.*, U.S. Patent Nos. 6,075,181 and 6,150,584 describing XENOMOUSE™ technology; U.S. Patent No. 5,770,429 describing HUMAB® technology; U.S. Patent No. 7,041,870 describing K-M MOUSE® technology, and U.S. Patent Application Publication No. US 2007/0061900, describing VELOCIMOUSE® technology). Human variable regions from intact antibodies generated by such animals may be further modified, *e.g.*, by combining with a different human constant region.

[0164] Human antibodies (*e.g.*, human DABs) can also be made by hybridoma-based methods. Human myeloma and mouse-human heteromyeloma cell lines for the production of human monoclonal antibodies have been described (See, *e.g.*, Kozbor *J. Immunol.*, 133: 3001 (1984); Brodeur et al., *Monoclonal Antibody Production Techniques and Applications*, pp. 51-63 (Marcel Dekker, Inc., New York, 1987); and Boerner et al., *J. Immunol.*, 147: 86 (1991)). Human antibodies generated via human B-cell hybridoma technology are also described in Li et al., *Proc. Natl. Acad. Sci. USA*, 103:3557-3562 (2006). Additional methods include those described, for example, in U.S. Patent No. 7,189,826 (describing production of monoclonal human IgM antibodies from hybridoma cell lines) and Ni, *Xiandai Mianyixue*, 26(4):265-268 (2006) (describing human-human hybridomas). Human hybridoma technology (Trioma technology) is also described in Vollmers and Brandlein, *Histology and Histopathology*, 20(3):927-937 (2005) and Vollmers and Brandlein, *Methods and Findings in Experimental and Clinical Pharmacology*, 27(3):185-91 (2005).

[0165] Human antibodies (*e.g.*, human DABs) may also be generated by isolating Fv clone variable domain sequences selected from human-derived phage display libraries. Such variable domain sequences may then be combined with a desired human constant domain. Techniques for selecting human antibodies from antibody libraries are described below.

[0166] One technique for obtaining V_HH sequences directed against a particular antigen or target involves suitably immunizing a transgenic mammal that is capable of expressing heavy chain antibodies (*i.e.* so as to raise an immune response and/or heavy chain antibodies directed against said antigen or target), obtaining a suitable biological sample from said transgenic mammal that contains (nucleic acid sequences encoding) said V_HH sequences (such as a blood sample, serum sample or sample of B-cells), and then generating V_HH sequences directed against said antigen or target, starting from said sample, using any suitable technique known *per se* (such as any of the methods described herein or a hybridoma technique). For example, for this purpose, the heavy chain antibody-expressing mice and the further methods and techniques described in WO 02/085945, WO 04/049794 and WO 06/008548 and Janssens *et al.*, *Proc. Natl. Acad. Sci. USA* 2006 Oct. 10; 103(41):15130-5 can be used. For example, such heavy chain antibody expressing mice can express heavy chain antibodies with any suitable (single) variable domain, such as (single) variable domains from natural sources (*e.g.* human (single) variable domains, Camelid (single) variable domains or shark (single) variable domains), as well as for example synthetic or semi-synthetic (single) variable domains.

Library-derived antibodies

[0167] Antibodies of the present application may be isolated by screening combinatorial libraries for antibodies with the desired activity or activities. For example, a variety of methods are known in the art for generating phage display libraries and screening such libraries for antibodies possessing the desired binding characteristics. Such methods are reviewed, *e.g.*, in Hoogenboom *et al.* in *Methods in Molecular Biology* 178:1-37 (O'Brien *et al.*, ed., Human Press, Totowa, NJ, 2001) and further described, *e.g.*, in the McCafferty *et al.*, *Nature* 348:552-554; Clackson *et al.*, *Nature* 352: 624-628 (1991); Marks *et al.*, *J. Mol. Biol.* 222: 581-597 (1992); Marks and Bradbury, in *Methods in Molecular Biology* 248:161-175 (Lo, ed., Human Press, Totowa, NJ, 2003); Sidhu *et al.*, *J. Mol. Biol.* 338(2): 299-310 (2004); Lee *et al.*, *J. Mol. Biol.* 340(5): 1073-1093 (2004); Fellouse, *Proc. Natl. Acad. Sci. USA* 101(34): 12467-12472 (2004); and Lee *et al.*, *J. Immunol. Methods* 284(1-2): 119-132(2004). Methods for constructing single-domain antibody libraries have been described, for example, see U.S. Pat. NO. 7371849.

[0168] In certain phage display methods, repertoires of V_H and V_L genes are separately cloned by polymerase chain reaction (PCR) and recombined randomly in phage libraries, which can then be

screened for antigen-binding phage as described in Winter et al., *Ann. Rev. Immunol.*, 12: 433-455 (1994). Repertoires of V_HH genes can be similarly cloned by PCR, recombined randomly in phage libraries, and screened for antigen-binding phage. Phage typically display antibody fragments, either as scFv fragments or as Fab fragments. Libraries from immunized sources provide high-affinity antibodies to the immunogen without the requirement of constructing hybridomas. Alternatively, the naive repertoire can be cloned (e.g., from human) to provide a single source of antibodies to a wide range of non-self and also self-antigens without any immunization as described by Griffiths et al., *EMBO J*, 12: 725-734 (1993). Finally, naive libraries can also be made synthetically by cloning unrearranged V-gene segments from stem cells, and using PCR primers containing random sequence to encode the highly variable CDR3 regions and to accomplish rearrangement *in vitro*, as described by Hoogenboom and Winter, *J. Mol. Biol.*, 227: 381-388 (1992). Patent publications describing human antibody phage libraries include, for example: US Patent No. 5,750,373, and US Patent Publication Nos. 2005/0079574, 2005/0119455, 2005/0266000, 2007/0117126, 2007/0160598, 2007/0237764, 2007/0292936, and 2009/0002360.

[0169] Antibodies or antibody fragments isolated from human antibody libraries are considered human antibodies or human antibody fragments herein.

Biological activities

[0170] The biological activity of anti-LAG-3 sdAb described herein can be determined by measuring its half maximal effective concentration (EC₅₀), which is a measure of the effectiveness of an antibody in binding to its target, or half maximal inhibitory concentration (IC₅₀), which is a measure of the effectiveness of an antibody in inhibiting a specific biological or biochemical function (such as inhibiting the binding between LAG-3 and MHC class II molecules). For example, here EC₅₀ can be used to indicate the effective concentration of an anti-LAG-3 sdAb needed to bind 50% LAG-3 on cell surface, IC₅₀ can be used to indicate the effective concentration of anti-LAG-3 sdAb needed to neutralize 50% of LAG-3 bioactivity *in vitro*. EC₅₀ also represents the plasma concentration required for obtaining 50% of a maximum effect *in vivo*. EC₅₀ or IC₅₀ can be measured by assays known in the art, for example, bioassays such as FACS binding analysis, inhibition of ligand binding by FACS analysis (competition binding assay), cell-based cytokine release assay, or amplified luminescent proximity homogeneous assay (AlphaLISA).

[0171] For example, the blockade of ligand binding can be studied using flow cytometry (also *see* Example 1). CHO cells expressing human LAG-3 can be dissociated from adherent culture flasks and mixed with varying concentrations of anti-LAG-3 sdAb for test, and a constant concentration of labeled-MHC class II protein. An anti-LAG-3 antibody positive control can be employed, such as BMS-986016

(Bristol-Myers Squibb). The mixture is equilibrated for 30 minutes at room temperature, washed three times with FACS buffer (PBS containing 1% BSA). Then, an antibody specifically recognizing the labeled MHC class II of constant concentration is added and incubated for 15 minutes at room temperature. Cells are washed with FACS buffer and analyzed by flow cytometry. Data can be analyzed with Prism (GraphPad Software, San Diego, CA) using non-linear regression to calculate IC_{50} . The results from the competition assay can demonstrate the ability of anti-LAG-3 sdAbs in inhibiting the interaction between MHC class II and LAG-3.

[0172] The biological activity of anti-LAG-3 sdAb can be tested using a LAG-3-blockade assay via a luciferase reporter (also see Example 4). LAG-3 blockade reporter assay was performed using Promega LAG-3 blockade reporter assay kit (Promega, Cat#CS194819), according to the vendor's protocol. Briefly, Thaw-and-Use MHC-II APC Cells (including TCR Activating Antigen) can be plated overnight and then incubated with a serial dilution of anti-LAG-3 antibodies or anti-LAG-3 sdAb-Fc fusion proteins, followed by addition of Thaw-and-Use LAG-3 Effector cells. After 6 hours of induction at 37°C and 5% CO₂, BIO-GLO™ Luciferase Assay Reagent can be added, and luminescence can be determined. The results can demonstrate the ability of anti-LAG-3 sdAbs in inhibiting the interaction between MHC class II and LAG-3.

[0173] In some embodiments, the anti-LAG-3 sdAb blocks or antagonizes signals transduced by the LAG-3 receptor. In some embodiments, the anti-LAG-3 sdAb can bind to an epitope on LAG-3 so as to inhibit LAG-3 from interacting with MHC class II molecules. In some embodiments, the anti-LAG-3 sdAb can reduce the binding of LAG-3 to MHC class II molecules by at least about any one of 5%, 10%, 20%, 25%, 30%, 40%, 50%, 60%, 70%, 75%, 80%, 90%, 95%, 99% or 99.9%.

(II) Fusion constructs

[0174] The present application further provides anti-LAG-3 fusion constructs comprising any one of the anti-LAG-3 sdAbs described herein and a second polypeptide, such as a second antibody or antigen binding fragment thereof, or an Fc fragment of an immunoglobulin. In some embodiments, the anti-LAG-3 construct comprises two or more polypeptides other than the anti-LAG-3 sdAb. The additional polypeptide(s) may or may not change or otherwise influence the biological properties of the anti-LAG-3 sdAb, and may or may not add further functionality to the anti-LAG-3 sdAb. In some embodiments, the second polypeptide confers one or more desired properties or functionalities to the anti-LAG-3 sdAb.

[0175] In some embodiments, the anti-LAG-3 construct comprises a second antibody or antigen binding fragment thereof (such as sdAb, scFv, Fab, full-length antibody, etc.) that specifically recognizes a second epitope. In some embodiments, the second epitope is from LAG-3. In some embodiments, the

second epitope is not from LAG-3. In some embodiments, the second antibody specifically recognizes the same epitope on LAG-3 as the anti-LAG-3 sdAb described herein. In some embodiments, the second antibody specifically recognizes a different epitope on LAG-3 as the anti-LAG-3 sdAb described herein.

[0176] In some embodiments, there is provided an anti-LAG-3 construct comprising a plurality (such as 2, 3, 4, or more) of anti-LAG-3 sdAbs described herein. In some embodiments, the plurality of the anti-LAG-3 sdAbs are fused to each other via a linker (such as a peptide linker). The plurality of the anti-LAG-3 sdAbs can be the same or different.

[0177] In some embodiments, the anti-LAG-3 construct comprises a second polypeptide that enhances the half-life, solubility, and/or absorption, reduces immunogenicity or toxicity, eliminates or attenuates undesirable side effects, confers other advantageous properties to and/or reduces other undesired properties of the anti-LAG-3 construct, compared to the anti-LAG-3 sdAb alone. Some non-limiting examples of such polypeptides include serum proteins, such as human serum albumin (HSA; *see e.g.* WO 00/27435) or haptenic molecules (*e.g.* haptens that are recognized by circulating antibodies, *see e.g.* WO 98/22141). It was shown that linking fragments of immunoglobulins (such as V_H domains) to serum albumin or fragments thereof may increase antibody half-life (*see e.g.* WO 00/27435 and WO 01/077137). Thus, in some embodiments, the anti-LAG-3 construct comprises an anti-LAG-3 sdAb fused to serum albumin or a fragment thereof, optionally via a suitable linker (such as peptide linker). In some embodiments, the serum albumin comprises at least domain III (*see* PCT/EP2007/002817). The anti-LAG-3 sdAb-HSA fusion protein can be of any suitable format, such as (sdAb)_n-HSA (n is an integer of at least 1), sdAb-HSA-sdAb, *etc.*

Anti-LAG-3 heavy chain-only antibody (HCAb)

[0178] In some embodiments, the anti-LAG-3 construct is a heavy chain-only antibody (HCAb) comprising an anti-LAG-3 sdAb described herein. In some embodiments, the anti-LAG-3 sdAb is fused to one or more C_H2 and/or C_H3 domains, *e.g.*, an Fc fragment. In some embodiments, C_H2 and/or C_H3 domains are derived from human immunoglobulins. In some embodiments, the anti-LAG-3 sdAb is fused to the C_H2 and/or C_H3 domains via a peptide linker. The C_H2 and/or C_H3 domains may increase the half-life of the anti-LAG-3 construct *in vivo*.

[0179] Thus, in some embodiments, there is provided an isolated anti-LAG-3 HCAb comprising an anti-LAG-3 sdAb described herein fused to an Fc fragment of an immunoglobulin, such as IgA, IgD, IgE, IgG, or IgM. In some embodiments, the anti-LAG-3 HCAb comprises an Fc fragment of IgG, such as IgG1, IgG2, IgG3, or IgG4. In some embodiments, the Fc fragment is a human Fc, such as human IgG1 (hIgG1) Fc, hIgG2 Fc, or hIgG4 Fc. In some embodiments, the Fc fragment is effectorless, with reduced,

minimized, or eliminated antibody effector functions such as ADCC, CDC, and/or ADCP (antibody-dependent cellular phagocytosis). In some embodiments, the effectorless Fc comprises an N297A or DANA mutation (D265A+N297A) in the C_H2 region. In some embodiments, the effectorless Fc comprises K322A and L234A/L235A (LALA) mutations. In some embodiments, the Fc fragment is an effectorless IgG1 Fc, such as effectorless hIgG1 Fc. In some embodiments, the Fc fragment is a human IgG4 Fc (S228P). In some embodiments, the anti-LAG-3 HCAb is monomeric. In some embodiments, the anti-LAG-3 HCAb is dimeric. In some embodiments, the anti-LAG-3 HCAb is multispecific and multivalent (such as bispecific and bivalent), *e.g.*, comprising two or more different anti-LAG-3 sdAbs described herein. In some embodiments, the anti-LAG-3 HCAb is monospecific and multivalent (*e.g.*, bivalent), *e.g.*, comprising two or more copies of the same anti-LAG-3 sdAb.

[0180] In some embodiments, the anti-LAG-3 sdAb and the Fc fragment are fused to each other via a peptide linker. In some embodiments, the peptide linker is a human IgG1 hinge (SEQ ID NO: 352). In some embodiments, the peptide linker is a mutated human IgG1 hinge (SEQ ID NO: 353). In some embodiments, the peptide linker is a human IgG4 hinge. In some embodiments, the peptide linker is a hIgG2 hinge.

[0181] Thus, in some embodiments, there is provided an isolated anti-LAG-3 HCAb comprising an sdAb specifically recognizing LAG-3, wherein the anti-LAG-3 sdAb comprises a CDR1 comprising the amino acid sequence of any one of SEQ ID NOs: 39-76, or a variant thereof comprising up to about 3 (such as about any of 1, 2, or 3) amino acid substitutions; a CDR2 comprising the amino acid sequence of any one of SEQ ID NOs: 115-152, or a variant thereof comprising up to about 3 (such as about any of 1, 2, or 3) amino acid substitutions; and a CDR3 comprising the amino acid sequence of any one of SEQ ID NOs: 191-228, or a variant thereof comprising up to about 3 (such as about any of 1, 2, or 3) amino acid substitutions, and wherein the anti-LAG-3 sdAb is fused to an Fc fragment of an immunoglobulin. In some embodiments, there is provided an anti-LAG-3 HCAb comprising an sdAb specifically recognizing LAG-3, wherein the sdAb comprises: (1) a CDR1 comprising the amino acid sequence of SEQ ID NO: 53; a CDR2 comprising the amino acid sequence of SEQ ID NO: 129; and a CDR3 comprising the amino acid sequence of SEQ ID NO: 205; (2) a CDR1 comprising the amino acid sequence of SEQ ID NO: 60; a CDR2 comprising the amino acid sequence of SEQ ID NO: 136; and a CDR3 comprising the amino acid sequence of SEQ ID NO: 212; (3) a CDR1 comprising the amino acid sequence of SEQ ID NO: 69; a CDR2 comprising the amino acid sequence of SEQ ID NO: 145; and a CDR3 comprising the amino acid sequence of SEQ ID NO: 221; or (4) a CDR1 comprising the amino acid sequence of SEQ ID NO: 71; a CDR2 comprising the amino acid sequence of SEQ ID NO: 147; and a CDR3 comprising the amino acid sequence of SEQ ID NO: 223, and wherein the anti-LAG-3 sdAb is fused to an Fc fragment of an

immunoglobulin. In some embodiments, there is provided an isolated anti-LAG-3 HCAb comprising an sdAb specifically recognizing LAG-3, wherein the sdAb comprises a V_HH domain comprising the amino acid sequence of any one of SEQ ID NOs: 274-311, or a variant thereof having at least about 80% (such as at least about any of 80%, 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, or 99%) sequence identity to any one of SEQ ID NOs: 274-311, and wherein the anti-LAG-3 sdAb is fused to an Fc fragment of an immunoglobulin. In some embodiments, there is provided an isolated anti-LAG-3 HCAb comprising an sdAb specifically recognizing LAG-3, wherein the sdAb comprises a V_HH domain comprising the amino acid sequence of any one of SEQ ID NOs: 274-311, and wherein the anti-LAG-3 sdAb is fused to an Fc fragment of an immunoglobulin. In some embodiments, the anti-LAG-3 sdAb is fused to the Fc fragment via a peptide linker. In some embodiments, the anti-LAG-3 HCAb is monomeric. In some embodiments, the Fc fragment is a human IgG1 Fc, effectorless human IgG1 Fc, hIgG2 Fc, human IgG4 Fc, or hIgG4 Fc (S228P).

[0182] In some embodiments, there is provided an isolated anti-LAG-3 HCAb comprising two sdAbs specifically recognizing LAG-3, wherein each anti-LAG-3 sdAb comprises a CDR1 comprising the amino acid sequence of any one of SEQ ID NOs: 39-76, or a variant thereof comprising up to about 3 (such as about any of 1, 2, or 3) amino acid substitutions; a CDR2 comprising the amino acid sequence of any one of SEQ ID NOs: 115-152, or a variant thereof comprising up to about 3 (such as about any of 1, 2, or 3) amino acid substitutions; and a CDR3 comprising the amino acid sequence of any one of SEQ ID NOs: 191-228, or a variant thereof comprising up to about 3 (such as about any of 1, 2, or 3) amino acid substitutions, and wherein the C-terminus of each anti-LAG-3 sdAb is fused to the N-terminus of an Fc fragment of an immunoglobulin. In some embodiments, there is provided an isolated anti-LAG-3 HCAb comprising two sdAbs specifically recognizing LAG-3, wherein each anti-LAG-3 sdAb comprises: (1) a CDR1 comprising the amino acid sequence of SEQ ID NO: 53; a CDR2 comprising the amino acid sequence of SEQ ID NO: 129; and a CDR3 comprising the amino acid sequence of SEQ ID NO: 205; (2) a CDR1 comprising the amino acid sequence of SEQ ID NO: 60; a CDR2 comprising the amino acid sequence of SEQ ID NO: 136; and a CDR3 comprising the amino acid sequence of SEQ ID NO: 212; (3) a CDR1 comprising the amino acid sequence of SEQ ID NO: 69; a CDR2 comprising the amino acid sequence of SEQ ID NO: 145; and a CDR3 comprising the amino acid sequence of SEQ ID NO: 221; or (4) a CDR1 comprising the amino acid sequence of SEQ ID NO: 71; a CDR2 comprising the amino acid sequence of SEQ ID NO: 147; and a CDR3 comprising the amino acid sequence of SEQ ID NO: 223, and wherein the C-terminus of each anti-LAG-3 sdAb is fused to the N-terminus of an Fc fragment of an immunoglobulin. In some embodiments, the two anti-LAG-3 sdAbs are the same. In some embodiments, the two anti-LAG-3 sdAbs are different. In some embodiments, the anti-LAG-3 sdAbs are fused to the Fc

fragment via a peptide linker. In some embodiments, the Fc fragment is a human IgG1 Fc, effectorless human IgG1 Fc, hIgG2 Fc, human IgG4 Fc, or hIgG4 Fc (S228P).

[0183] In some embodiments, there is provided an isolated anti-LAG-3 HCAb comprising the amino acid sequence of any one of SEQ ID NOs: 312-349, or a variant thereof having at least about 80% (such as at least about any of 80%, 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, or 99%) sequence identity to any one of SEQ ID NOs: 312-349. In some embodiments, there is provided a polypeptide comprising the amino acid sequence of any one of SEQ ID NOs: 312-349.

[0184] Also provided are isolated anti-LAG-3 HCABs that specifically binds to LAG-3 competitively with any one of the isolated anti-LAG-3 HCABs, anti-LAG-3 sdAbs, or anti-LAG-3 constructs described herein.

Multivalent and/or multispecific constructs

[0185] The present application provides multivalent and multispecific anti-LAG-3 constructs. Multispecific anti-LAG-3 constructs are also referred herein as “anti-LAG-3 multispecific antigen binding proteins (MABPs).” In some embodiments, the anti-LAG-3 constructs are bispecific, which are also referred herein as “anti-LAG-3 bispecific antigen binding proteins (BABPs).”

[0186] In some embodiments, there is provided an isolated anti-LAG-3 construct (*e.g.*, MABP or BABP) comprising: (a) a first antigen binding portion comprising an anti-LAG-3 sdAb comprising a CDR1 comprising the amino acid sequence of any one of SEQ ID NOs: 39-76, or a variant thereof comprising up to about 3 (such as about any of 1, 2, or 3) amino acid substitutions; a CDR2 comprising the amino acid sequence of any one of SEQ ID NOs: 115-152, or a variant thereof comprising up to about 3 (such as about any of 1, 2, or 3) amino acid substitutions; and a CDR3 comprising the amino acid sequence of any one of SEQ ID NOs: 191-228, or a variant thereof comprising up to about 3 (such as about any of 1, 2, or 3) amino acid substitutions, and (b) a second antigen binding portion that specifically binds a second epitope (*e.g.*, immune checkpoint molecule, such as PD-1), wherein the first antigen binding portion and the second antigen binding portion are fused to each other. In some embodiments, there is provided an isolated anti-LAG-3 construct (*e.g.*, MABP or BABP) comprising: (a) a first antigen binding portion comprising an anti-LAG-3 sdAb comprising: (1) a CDR1 comprising the amino acid sequence of SEQ ID NO: 53; a CDR2 comprising the amino acid sequence of SEQ ID NO: 129; and a CDR3 comprising the amino acid sequence of SEQ ID NO: 205; (2) a CDR1 comprising the amino acid sequence of SEQ ID NO: 60; a CDR2 comprising the amino acid sequence of SEQ ID NO: 136; and a CDR3 comprising the amino acid sequence of SEQ ID NO: 212; (3) a CDR1 comprising the amino acid sequence of SEQ ID NO: 69; a CDR2 comprising the amino acid sequence of SEQ ID NO: 145; and a

CDR3 comprising the amino acid sequence of SEQ ID NO: 221; or (4) a CDR1 comprising the amino acid sequence of SEQ ID NO: 71; a CDR2 comprising the amino acid sequence of SEQ ID NO: 147; and a CDR3 comprising the amino acid sequence of SEQ ID NO: 223, and (b) a second antigen binding portion that specifically binds a second epitope (*e.g.*, immune checkpoint molecule, such as PD-1), wherein the first antigen binding portion and the second antigen binding portion are fused to each other. In some embodiments, the second antigen binding portion specifically recognizes an immune checkpoint molecule, such as PD-1, 4-1BB, PD-L1, TIM-3, TIGIT, CTLA-4, VISTA, B7-1, B7-H3, CD47, OX40 or GITR. In some embodiments, the second antigen binding portion comprises a heavy chain comprising the V_H and a light chain comprising the V_L . In some embodiments, the first antigen binding portion is fused to the second antigen binding portion at the N-terminus of the heavy chain, the N-terminus of the light chain, the N-terminus of the Fc region, the C-terminus of the heavy chain, or the C-terminus of the light chain. In some embodiments, the second antigen binding portion comprises a Fab or an scFv. In some embodiments, the first antigen binding portion is fused to the second antigen binding portion at the C-terminus of the Fab or scFv. In some embodiments, the second antigen binding portion comprises a full-length 4-chain antibody. In some embodiments, the first antigen binding portion is fused to the second antigen binding portion via a peptide linker. In some embodiments, the peptide linker is no more than about 30 (such as no more than about any one of 25, 20, or 15) amino acids long. In some embodiments, the peptide linker comprises the amino acid sequence of any one of SEQ ID NOs: 352-353 and 366-372. In some embodiments, the N-terminus or the C-terminus of the anti-LAG-3 sdAb is fused to a peptide sequence. In some embodiments, the peptide sequence comprises the amino acid sequence of any one of SEQ ID NOs: 352-353 and 366-372. In some embodiments, the second antigen binding fragment comprises an Fc region, such as an IgG1 Fc, effectorless IgG1 Fc, IgG2 Fc, IgG4 Fc, or IgG4 Fc (S228P).

[0187] In some embodiments, the anti-LAG-3 construct is an anti-LAG-3 MABP comprising a second antigen binding portion that specifically recognizes an immune checkpoint molecule. As used herein, the term "immune checkpoint molecules" refer molecules in the immune system that generally act to maintain self-tolerance or modulate the duration and amplitude of physiological immune responses to minimize collateral tissue damage. Immune checkpoint inhibitors can inhibit an immune system checkpoint by stimulating the activity of a stimulatory checkpoint molecule, or inhibiting the activity of an inhibitory checkpoint molecule in the pathway. Stimulatory checkpoint molecules are molecules, such as proteins, that stimulate or positively regulate the immune system. Inhibitory checkpoint molecules are molecules, such as proteins, that inhibit or negatively regulate the immune system. Immune system checkpoint molecules include, but are not limited to, cytotoxic T-lymphocyte antigen 4 (CTLA-4), programmed cell death 1 protein (PD-1), programmed cell death 1 ligand 1 (PD-L1), programmed cell death 1 ligand 2

(PD-L2), lymphocyte activation gene 3 (LAG3), B7-1, B7-H3, T cell membrane protein 3 (TIM3), B- and T-lymphocyte attenuator (BTLA), T cell immunoreceptor with Ig and ITIM domains (TIGIT), V-domain immunoglobulin (Ig)-containing suppressor of T-cell activation (VISTA), tumor necrosis factor receptor superfamily member 4 (TNFRSF4, also known as CD134 or OX40), tumor necrosis factor receptor superfamily member 9 (TNFRSF9, also known as 4-1BB or CD137), cluster of differentiation 47 (CD47 or IAP) and glucocorticoid-induced tumor necrosis factor receptor (GITR). Any of the known antibodies against the immune checkpoint molecules may be used in an anti-LAG-3 MABP.

[0188] In some embodiments, there is provided an isolated anti-LAG-3 construct (*e.g.*, MABP or BABP) comprising: (a) a first antigen binding portion comprising an anti-LAG-3 sdAb comprising a CDR1 comprising the amino acid sequence of any one of SEQ ID NOs: 39-76, or a variant thereof comprising up to about 3 (such as about any of 1, 2, or 3) amino acid substitutions; a CDR2 comprising the amino acid sequence of any one of SEQ ID NOs: 115-152, or a variant thereof comprising up to about 3 (such as about any of 1, 2, or 3) amino acid substitutions; and a CDR3 comprising the amino acid sequence of any one of SEQ ID NOs: 191-228, or a variant thereof comprising up to about 3 (such as about any of 1, 2, or 3) amino acid substitutions, and (b) a second antigen binding portion that specifically binds an immune checkpoint molecule (*e.g.*, PD-1), wherein the first antigen binding portion and the second antigen binding portion are fused to each other. In some embodiments, there is provided an isolated anti-LAG-3 construct (*e.g.*, MABP or BABP) comprising: (a) a first antigen binding portion comprising an anti-LAG-3 sdAb comprising: (1) a CDR1 comprising the amino acid sequence of SEQ ID NO: 53; a CDR2 comprising the amino acid sequence of SEQ ID NO: 129; and a CDR3 comprising the amino acid sequence of SEQ ID NO: 205; (2) a CDR1 comprising the amino acid sequence of SEQ ID NO: 60; a CDR2 comprising the amino acid sequence of SEQ ID NO: 136; and a CDR3 comprising the amino acid sequence of SEQ ID NO: 212; (3) a CDR1 comprising the amino acid sequence of SEQ ID NO: 69; a CDR2 comprising the amino acid sequence of SEQ ID NO: 145; and a CDR3 comprising the amino acid sequence of SEQ ID NO: 221; or (4) a CDR1 comprising the amino acid sequence of SEQ ID NO: 71; a CDR2 comprising the amino acid sequence of SEQ ID NO: 147; and a CDR3 comprising the amino acid sequence of SEQ ID NO: 223, and (b) a second antigen binding portion that specifically binds an immune checkpoint molecule (*e.g.*, PD-1), wherein the first antigen binding portion and the second antigen binding portion are fused to each other. In some embodiments, the second antigen binding portion comprises a heavy chain comprising the V_H and a light chain comprising the V_L . In some embodiments, the first antigen binding portion is fused to the second antigen binding portion at the N-terminus of the heavy chain, the N-terminus of the light chain, the N-terminus of the Fc region, the C-terminus of the heavy chain, or the C-terminus of the light chain. In some embodiments, the second antigen binding

portion comprises a Fab or an scFv. In some embodiments, the first antigen binding portion is fused to the second antigen binding portion at the C-terminus of the Fab or scFv. In some embodiments, the second antigen binding portion comprises a full-length 4-chain antibody. In some embodiments, the first antigen binding portion is fused to the second antigen binding portion chemically. In some embodiments, the first antigen binding portion is fused to the second antigen binding portion via a peptide linker. In some embodiments, the peptide linker is no more than about 30 (such as no more than about any one of 25, 20, or 15) amino acids long. In some embodiments, the peptide linker comprises the amino acid sequence of any one of SEQ ID NOs: 352-353 and 366-372. In some embodiments, the N-terminus or the C-terminus of the anti-LAG-3 sdAb is fused to a peptide sequence. In some embodiments, the peptide sequence comprises the amino acid sequence of any one of SEQ ID NOs: 352-353 and 366-372. In some embodiments, the second antigen binding fragment comprises an Fc region, such as an IgG1 Fc, effectorless IgG1 Fc, IgG2 Fc, IgG4 Fc, or IgG4 Fc (S228P).

[0189] In some embodiments, the isolated anti-LAG-3 construct comprises an anti-LAG-3 sdAb described herein fused to a second antibody or antigen binding fragment thereof. In some embodiments, the anti-LAG-3 construct comprises two or more antibodies or antigen binding fragments thereof fused to the anti-LAG-3 sdAb. In some embodiments, the second antibody specifically recognizes an antigen other than LAG-3, or a second epitope on LAG-3. In some embodiments, the second antibody is a full-length antibody, a Fab, a Fab', a (Fab')₂, an Fv, an scFv, an scFv-scFv, a minibody, a diabody, or an sdAb. In some embodiments, the second antibody comprises a heavy chain variable domain (V_H) and a light chain variable domain (V_L).

[0190] In some embodiments, the anti-LAG-3 construct is monospecific. In some embodiments, the anti-LAG-3 construct is multispecific (such as bispecific). Multispecific molecules are molecules that have binding specificities for at least two different epitopes (*e.g.*, bispecific antibodies have binding specificities for two epitopes). Multispecific molecules with more than two valencies and/or specificities are also contemplated. For example, trispecific antibodies can be prepared. Tutt *et al. J. Immunol.* 147: 60 (1991).

[0191] In some embodiments, the anti-LAG-3 construct is monovalent. In some embodiments, the anti-LAG-3 construct is multivalent (*e.g.*, bivalent) and monospecific. In some embodiments, the anti-LAG-3 construct comprises an anti-LAG-3 sdAb described herein and a second antibody (such as a full-length antibody, sdAb, or an antigen binding fragment comprising a V_H and a V_L) specifically recognizing the same LAG-3 epitope as the anti-LAG-3 sdAb. In some embodiments, the second antibody comprises the same CDRs and/or the same V_HH as the anti-LAG-3 sdAb. For example, the anti-LAG-3 construct may comprise two or more anti-LAG-3 sdAbs described herein, wherein the two or more anti-LAG-3

sdAbs are the same. In some embodiments, the two or more anti-LAG-3 sdAbs are fused to each other via a peptide linker. The monospecific or multispecific anti-LAG-3 construct comprising two or more anti-LAG-3 sdAbs may have increase avidity compared to that of a single anti-LAG-3 sdAb described herein.

[0192] In some embodiments, the anti-LAG-3 construct is multivalent and multispecific (*e.g.*, bispecific). In some embodiments, the anti-LAG-3 construct comprises an anti-LAG-3 sdAb described herein and a second antibody (such as a full-length antibody, sdAb, or an antigen binding fragment comprising a V_H and a V_L) specifically recognizing a second antigen other than LAG-3, or a different LAG-3 epitope from that recognized by the anti-LAG-3 sdAb.

[0193] Techniques for making multispecific antibodies include, but are not limited to, recombinant co-expression of two immunoglobulin heavy chain-light chain pairs having different specificities (see Milstein and Cuello, *Nature* 305: 537 (1983)), WO 93/08829, and Traunecker et al., *EMBO J.* 10: 3655 (1991)), and “knob-in-hole” engineering (*see, e.g.*, U.S. Patent No. 5,731,168). Multi-specific antibodies may also be made by engineering electrostatic steering effects for making antibody Fc-heterodimeric molecules (WO 2009/089004A1); cross-linking two or more antibodies or fragments (*see, e.g.*, US Patent No. 4,676,980, and Brennan et al., *Science*, 229: 81 (1985)); using leucine zippers to produce bi-specific antibodies (*see, e.g.*, Kostelny et al., *J. Immunol.*, 148(5):1547-1553 (1992)); using “diabody” technology for making bispecific antibody fragments (*see, e.g.*, Hollinger et al., *Proc. Natl. Acad. Sci. USA*, 90:6444-6448 (1993)); and using single-chain Fv (sFv) dimers (*see, e.g.*, Gruber et al., *J. Immunol.*, 152:5368 (1994)); and preparing trispecific antibodies as described, *e.g.*, in Tutt et al. *J. Immunol.* 147: 60 (1991); and creating polypeptides comprising tandem single-domain antibodies (*see, e.g.*, U.S. Patent Application No. 20110028695; and Conrath et al. *J. Biol. Chem.*, 2001; 276(10):7346-50). Engineered antibodies with three or more functional antigen binding sites, including “Octopus antibodies,” are also included herein (*see, e.g.*, US 2006/0025576A1).

[0194] In some embodiments, there is provided an isolated anti-LAG-3 construct comprising an anti-LAG-3 sdAb described herein fused to a full-length antibody or antigen binding fragment thereof that comprises a V_H and a V_L. The full-length antibody or antigen binding fragment recognizes an antigen other than LAG-3, or an epitope from LAG-3 that is different from the epitope recognized by the anti-LAG-3 sdAb, thereby conferring a broadened targeting capability. Due to the small size of the sdAb, in some embodiments, the anti-LAG-3 MABP (*e.g.*, anti-LAG-3 BABPs) described herein can have similar molecular weight and pharmacokinetic properties compared to those of the full-length antibody or antigen binding fragment component. For example, an anti-LAG-3 MABP can be designed by fusing one or more anti-LAG-3 sdAbs to a monoclonal antibody with proven clinical efficacy and safety to provide increased clinical benefits and desirable pharmacokinetic properties without impeding the expressibility of the

multispecific construct. In some embodiments, the anti-LAG-3 sdAb is fused to the full-length antibody or antigen binding fragment by a peptide linker.

[0195] The anti-LAG-3 MABPs (*e.g.*, anti-LAG-3 BABPs) described herein can be adopted to target a variety of disease-related epitope or antigen combinations besides LAG-3, such as LAG-3 with the combination of immune checkpoint molecules, cell surface antigens (such as tumor antigens), or pro-inflammatory molecules, thereby providing agents that are useful for treating a variety of diseases and conditions, such as cancer. The anti-LAG-3 MABP (*e.g.*, anti-LAG-3 BABPs) can be of any format, such as those disclosed in PCT/CN2017/093644, which is incorporated herein by reference in its entirety.

[0196] Thus, in some embodiments, there is provided an isolated anti-LAG-3 construct (*e.g.*, MABP or BABP) comprising: (a) a first antigen binding portion comprising an anti-LAG-3 sdAb comprising a CDR1 comprising the amino acid sequence of any one of SEQ ID NOs: 39-76, or a variant thereof comprising up to about 3 (such as about any of 1, 2, or 3) amino acid substitutions; a CDR2 comprising the amino acid sequence of any one of SEQ ID NOs: 115-152, or a variant thereof comprising up to about 3 (such as about any of 1, 2, or 3) amino acid substitutions; and a CDR3 comprising the amino acid sequence of any one of SEQ ID NOs: 191-228, or a variant thereof comprising up to about 3 (such as about any of 1, 2, or 3) amino acid substitutions, and (b) a second antigen binding portion comprising a V_H and a V_L , wherein the V_H and V_L together form an antigen-binding site that specifically binds a second epitope (*e.g.*, immune checkpoint molecule, such as PD-1), wherein the first antigen binding portion and the second antigen binding portion are fused to each other. In some embodiments, there is provided an isolated anti-LAG-3 construct (*e.g.*, MABP or BABP) comprising: (a) a first antigen binding portion comprising an anti-LAG-3 sdAb comprising: (1) a CDR1 comprising the amino acid sequence of SEQ ID NO: 53; a CDR2 comprising the amino acid sequence of SEQ ID NO: 129; and a CDR3 comprising the amino acid sequence of SEQ ID NO: 205; (2) a CDR1 comprising the amino acid sequence of SEQ ID NO: 60; a CDR2 comprising the amino acid sequence of SEQ ID NO: 136; and a CDR3 comprising the amino acid sequence of SEQ ID NO: 212; (3) a CDR1 comprising the amino acid sequence of SEQ ID NO: 69; a CDR2 comprising the amino acid sequence of SEQ ID NO: 145; and a CDR3 comprising the amino acid sequence of SEQ ID NO: 221; or (4) a CDR1 comprising the amino acid sequence of SEQ ID NO: 71; a CDR2 comprising the amino acid sequence of SEQ ID NO: 147; and a CDR3 comprising the amino acid sequence of SEQ ID NO: 223, and (b) a second antigen binding portion comprising a V_H and a V_L , wherein the V_H and V_L together form an antigen-binding site that specifically binds a second epitope (*e.g.*, immune checkpoint molecule, such as PD-1), wherein the first antigen binding portion and the second antigen binding portion are fused to each other. In some embodiments, the second epitope is from an immune checkpoint molecule, such as PD-1, 4-1BB, PD-L1, TIM-3, TIGIT, CTLA-4, VISTA,

B7-1, B7-H3, CD47, OX40 or GITR. In some embodiments, the second antigen binding portion comprises a heavy chain comprising the V_H and a light chain comprising the V_L . In some embodiments, the first antigen binding portion is fused to the second antigen binding portion at the N-terminus of the heavy chain, the N-terminus of the light chain, the N-terminus of the Fc region, the C-terminus of the heavy chain, or the C-terminus of the light chain. In some embodiments, the second antigen binding portion comprises a Fab or an scFv. In some embodiments, the first antigen binding portion is fused to the second antigen binding portion at the C-terminus of the Fab or scFv. In some embodiments, the second antigen binding portion comprises a full-length 4-chain antibody. In some embodiments, the second antigen binding portion comprises an anti-PD-1 full-length antibody or antigen binding fragment thereof. In some embodiments, the anti-PD-1 full-length antibody or antigen binding fragment thereof (*e.g.*, Fab, scFv) comprises: (i) a V_H comprising HC-CDR1, HC-CDR2, and HC-CDR3 of the amino acid sequence of SEQ ID NO: 356, and a V_L comprising LC-CDR1, LC-CDR2, and LC-CDR3 of the amino acid sequence of SEQ ID NO: 357; or (ii) a V_H comprising HC-CDR1, HC-CDR2, and HC-CDR3 of the amino acid sequence of SEQ ID NO: 373, and a V_L comprising LC-CDR1, LC-CDR2, and LC-CDR3 of the amino acid sequence of SEQ ID NO: 374. In some embodiments, the anti-PD-1 full-length antibody comprises: (i) a heavy chain comprising the amino acid sequence of SEQ ID NO: 356, and a light chain comprising the amino acid sequence of SEQ ID NO: 357; or (ii) a heavy chain comprising the amino acid sequence of SEQ ID NO: 373, and a light chain comprising the amino acid sequence of SEQ ID NO: 374. In some embodiments, the anti-PD-1 full-length antibody is pembrolizumab or PD1-BM-min. In some embodiments, the first antigen binding portion is fused to the second antigen binding portion chemically. In some embodiments, the first antigen binding portion is fused to the second antigen binding portion via a peptide linker. In some embodiments, the peptide linker is no more than about 30 (such as no more than about any one of 25, 20, or 15) amino acids long. In some embodiments, the peptide linker comprises the amino acid sequence of any one of SEQ ID NOs: 352-353 and 366-372. In some embodiments, the N-terminus or the C-terminus of the anti-LAG-3 sAb is fused to a peptide sequence. In some embodiments, the peptide sequence comprises the amino acid sequence of any one of SEQ ID NOs: 352-353 and 366-372. In some embodiments, the second antigen binding fragment comprises an Fc region, such as an IgG1 Fc, effectorless IgG1 Fc, IgG2 Fc, IgG4 Fc, or IgG4 Fc (S228P).

[0197] In some embodiments, the isolated anti-LAG-3 MABP (*e.g.*, BABP) comprises at least two antigen binding portions that can specifically bind at least two different epitopes. Some of the at least two antigen binding portions may be identical, so long as the MABP has binding sites for two different epitopes. The anti-LAG-3 MABPs (*e.g.*, BABPs) can be symmetric or asymmetric. For example, the anti-LAG-3 MABP (*e.g.*, BABP) may comprise one to eight copies of the first antigen binding portion

comprising the anti-LAG-3 sdAb described herein, and one or two copies of the second antigen binding portion comprising a V_H and a V_L . In some embodiments, the anti-LAG-3 MABP (*e.g.*, BABP) comprises two different antigen binding portions that each comprise a V_H domain and a V_L domain that together form a different antigen binding site. For example, the second antigen binding portion can be a bispecific antibody. In some embodiments, the second antigen binding portion is a monospecific full-length antibody or antigen binding fragment thereof, such as Fab or scFv.

[0198] In some embodiments, the anti-LAG-3 MABP (*e.g.*, BABP) comprises any one of 1, 2, 3, 4, 5, 6, 7, 8, or more different antigen binding portions that each comprises an anti-LAG-3 sdAb described herein. In some embodiments, two identical anti-LAG-3 sdAbs are fused to each other, which are further fused to the second antigen binding portion. In some embodiments, two different anti-LAG-3 sdAbs are fused to each other, which are further fused to the second antigen binding portion.

[0199] The isolated anti-LAG-3 MABP (*e.g.*, BABP) may have any suitable number of valencies for LAG-3 and/or the second epitope (*e.g.*, PD-1), and any suitable number of specificity. In some embodiments, the anti-LAG-3 MABP (*e.g.*, BABP) is bivalent, trivalent, tetravalent, pentavalent, hexavalent, or of higher valencies for LAG-3. In some embodiments, the anti-LAG-3 MABP (*e.g.*, BABP) is bivalent, trivalent, tetravalent, pentavalent, hexavalent, or of higher valencies for the second epitope (*e.g.*, PD-1). In some embodiments, the anti-LAG-3 MABP is bispecific (*e.g.*, PD-1 $\times$ LAG-3 BABP). Exemplary BABPs are depicted in FIGs. 7-16. In some embodiments, the MABP is trispecific. In some embodiments, the MABP is tetraspecific. In some embodiments, the MABP has more than four specificities.

[0200] In some embodiments, the anti-LAG-3 MABP (*e.g.*, BABP) comprises: (a) one or more copies (such as 2) of a first antigen binding portion comprising an anti-LAG-3 sdAb, and (b) a single copy of a second antigen binding portion comprising a V_H and a V_L , wherein the V_H and V_L together form an antigen-binding site that specifically binds a second epitope (*e.g.*, PD-1), wherein each copy of the first antigen binding portion is fused to the second antigen binding portion. In some embodiments, there is provided an anti-LAG-3 MABP (*e.g.*, BABP) comprising: (a) a plurality (such as 2, 3, 4, 5, 6, 7, 8, or more) of identical or different anti-LAG-3 sdAbs, and (b) a plurality (such as 2, 3, 4, 5, 6, or more) of a second antigen binding portion comprising a V_H and a V_L , wherein the V_H and V_L together form an antigen-binding site that specifically binds a second epitope (*e.g.*, PD-1), and, wherein the anti-LAG-3 sdAbs are fused to each other, and/or to the second antigen binding portion. In some embodiments, there is provided an anti-LAG-3 MABP (*e.g.*, BABP) comprising: (a) a plurality (such as 2, 3, or 4) of identical or different anti-LAG-3 sdAbs, and (b) two copies of a second antigen binding portion each comprising a V_H and a V_L , wherein the V_H and V_L together form an antigen-binding site that specifically

binds a second epitope (*e.g.*, PD-1), and wherein the anti- LAG-3 sdAbs are fused to each other, and/or to the second antigen binding portion. In some embodiments, one or more of the anti-LAG-3 sdAb is each further fused to another identical or different anti-LAG-3 sdAb.

[0201] In some embodiments, the anti-LAG-3 MABP (*e.g.*, BABP) comprises: (a) a single copy of a first antigen binding portion comprising an anti-LAG-3 sdAb, and (b) two copies of a second antigen binding portion each comprising a V_H and a V_L, wherein the V_H and V_L together form an antigen-binding site that specifically binds a second epitope, wherein the first antigen binding portion is fused to one of the two copies of the second antigen binding portion. In some embodiments, the anti-PD-1 MABP (*e.g.*, BABP) comprises: (a) two copies of a first antigen binding portion each comprising an anti-LAG-3 sdAb, (b) two copies of a second antigen binding portion each comprising a V_H and a V_L, wherein the V_H and V_L together form an antigen-binding site that specifically binds a second epitope, wherein one copy of the first antigen binding portion is fused to each copy of the second antigen binding portion. In some embodiments, one or more of the anti-LAG-3 sdAbs are each further fused to another identical or different anti-LAG-3 sdAb.

[0202] In some embodiments, the anti-LAG-3 construct comprises an anti-LAG-3 sdAb described herein fused to a second antibody, wherein the second antibody is a full-length antibody consisting of two heavy chains and two light chains (such as full-length antibody specifically recognizing PD-1). In some embodiments, the anti-LAG-3 sdAb and the full-length antibody are fused to each other via a linker, such as a peptide linker. In some embodiments, the N-terminus or the C-terminus of the anti-LAG-3 sdAb is fused to a peptide sequence.

[0203] Thus, in some embodiments, there is provided an isolated anti-LAG-3 construct comprising an anti-LAG-3 sdAb and a full-length antibody consisting of two heavy chains and two light chains (such as a full-length antibody specifically recognizing PD-1), wherein the anti-LAG-3 sdAb comprises a CDR1 comprising the amino acid sequence of any one of SEQ ID NOs: 39-76, or a variant thereof comprising up to about 3 (such as about any of 1, 2, or 3) amino acid substitutions; a CDR2 comprising the amino acid sequence of any one of SEQ ID NOs: 115-152, or a variant thereof comprising up to about 3 (such as about any of 1, 2, or 3) amino acid substitutions; and a CDR3 comprising the amino acid sequence of any one of SEQ ID NOs: 191-228, or a variant thereof comprising up to about 3 (such as about any of 1, 2, or 3) amino acid substitutions. In some embodiments, there is provided an isolated anti-LAG-3 construct comprising an anti-LAG-3 sdAb and a full-length antibody consisting of two heavy chains and two light chains (such as a full-length antibody specifically recognizing PD-1), wherein the anti-LAG-3 sdAb comprises: (1) a CDR1 comprising the amino acid sequence of SEQ ID NO: 53; a CDR2 comprising the amino acid sequence of SEQ ID NO: 129; and a CDR3 comprising the amino acid sequence of SEQ ID

NO: 205; (2) a CDR1 comprising the amino acid sequence of SEQ ID NO: 60; a CDR2 comprising the amino acid sequence of SEQ ID NO: 136; and a CDR3 comprising the amino acid sequence of SEQ ID NO: 212; (3) a CDR1 comprising the amino acid sequence of SEQ ID NO: 69; a CDR2 comprising the amino acid sequence of SEQ ID NO: 145; and a CDR3 comprising the amino acid sequence of SEQ ID NO: 221; or (4) a CDR1 comprising the amino acid sequence of SEQ ID NO: 71; a CDR2 comprising the amino acid sequence of SEQ ID NO: 147; and a CDR3 comprising the amino acid sequence of SEQ ID NO: 223. In some embodiments, the Fc fragment of the full-length antibody is hIgG1 Fc, effectorless hIgG1 Fc, hIgG2 Fc, hIgG4 Fc, or hIgG4 Fc (S228P). In some embodiments, the full-length antibody is an activator of a stimulatory immune checkpoint molecule. In some embodiments, the full-length antibody is an immune checkpoint inhibitor, such as an inhibitor of TIGIT, TIM-3, CTLA-4, or PD-1. In some embodiments, the full-length antibody is pembrolizumab, PD1-BM-min or nivolumab. In some embodiments, the anti-LAG-3 sdAb and the full-length antibody are fused to each other via a peptide linker. In some embodiments, the peptide linker comprises the amino acid sequence of any one of SEQ ID NOs: 352-353 and 366-372. In some embodiments, the N-terminus or the C-terminus of the anti-LAG-3 sdAb is fused to a peptide sequence. In some embodiments, the peptide sequence comprises the amino acid sequence of any one of SEQ ID NOs: 352-353 and 366-372.

[0204] The multispecific anti-LAG3 construct may be of any suitable format. Exemplary bispecific anti-LAG3 constructs are shown in FIGs. 7-16.

[0205] In some embodiments, the anti-LAG-3 construct comprises two anti-LAG-3 sdAbs. In some embodiments, the N-terminus of each anti-LAG-3 sdAb is fused to the C-terminus of a heavy chain of the full-length antibody (exemplified as FIG. 8). In some embodiments, the C-terminus of each anti-LAG-3 sdAb is fused to the N-terminus of a heavy chain of the full-length antibody (exemplified as FIG. 7). In some embodiments, the N-terminus of each anti-LAG-3 sdAb is fused to the C-terminus of a light chain of the full-length antibody (exemplified as FIG. 10). In some embodiments, the C-terminus of each anti-LAG-3 sdAb is fused to the N-terminus of a light chain of the full-length antibody (exemplified as FIG. 9). In some embodiments, the C-terminus of each anti-LAG-3 sdAb is fused to the N-terminus of a C_H2 domain of the full-length antibody, and the N-terminus of each anti-LAG-3 sdAb is fused to the C-terminus of a C_H1 domain of the full-length antibody (exemplified as FIG. 13). In some embodiments, the N-terminus of each anti-LAG-3 sdAb is fused to the C-terminus of an scFv, and the C-terminus of each anti-LAG-3 sdAb is fused to the N-terminus of an C_H2 domain of a Fc region (exemplified as FIG. 14).

[0206] In some embodiments, the anti-LAG-3 construct comprises four anti-LAG-3 sdAbs. In some embodiments, the C-terminus of a first anti-LAG-3 sdAb is fused to the N-terminus of a heavy chain of the full-length antibody, and the C-terminus of a second anti-LAG-3 sdAb is fused to the N-terminus of a

light chain of the full-length antibody (exemplified as FIG. 11). In some embodiments, the C-terminus of a first anti-LAG-3 sdAb is fused to the N-terminus of a heavy chain of the full-length antibody, and the C-terminus of a second anti-LAG-3 sdAb is fused to the N-terminus of the first anti-LAG-3 sdAb (exemplified as FIG. 12). In some embodiments, the N-terminus of a first anti-LAG-3 sdAb is fused to the C-terminus of a C_{H1} domain of a Fab, and the C-terminus of the first anti-LAG-3 sdAb is fused to the N-terminus of a second C_{H1} domain; and the N-terminus of a second anti-LAG-3 sdAb is fused to the C-terminus of a C_L domain of a Fab, and the C-terminus of the second anti-LAG-3 sdAb is fused to the N-terminus of a second C_L domain (exemplified as FIG. 15). In some embodiments, the N-terminus of a first anti-LAG-3 sdAb is fused to the C-terminus of an scFv, and the C-terminus of the first anti-LAG-3 sdAb is fused to the N-terminus of a C_{H1} domain; and the C-terminus of a second anti-LAG-3 sdAb is fused to the N-terminus of a second C_L domain (exemplified as FIG. 16).

a) Fusion polypeptides

[0207] The first antigen binding portion comprising an anti-LAG-3 sdAb described herein and the second antigen binding portion comprising a V_H and a V_L of the anti-LAG-3 MABP (*e.g.*, BABP) are fused (*i.e.*, covalently linked) to each other. Thus, the anti-LAG-3 MABPs (*e.g.*, BABPs) of the present application comprise one or more fusion polypeptides. Each fusion polypeptide may comprise the first antigen binding portion comprising an anti-LAG-3 sdAb described herein, and a polypeptide from the second antigen binding portion.

[0208] The first antigen binding portion comprising an anti-LAG-3 sdAb described herein and the second antigen binding portion comprising a V_H and a V_L may be linked directly by a single chemical bond (such as peptide bond) or via a peptide linker. The first antigen binding portion comprising an anti-LAG-3 sdAb may be fused at either the N-terminus or the C-terminus of any one (including each) polypeptide of the second antigen binding portion, or may be fused at an internal position of any one (including each) polypeptide of the second antigen binding portion, such as at the N-terminus of the Fc region in the heavy chain of the second antigen binding portion. The fusion polypeptides may be obtained either recombinantly or chemically. In some embodiments, the C-terminus of the first antigen binding portion comprising an anti-LAG-3 sdAb is fused to the N-terminus of any (including each) polypeptide of the second antigen binding portion via a chemical bond (such as peptide bond) or a peptide linker. In some embodiments, the N-terminus of the first antigen binding portion comprising an anti-LAG-3 sdAb is fused to the C-terminus of any (including each) polypeptide of the second antigen binding portion via a chemical bond (such as peptide bond) or a peptide linker. In some embodiments, the first antigen binding portion comprising an anti-LAG-3 sdAb is fused to the second antigen binding portion via a chemical bond that is not a peptide bond involving the main chain chemical groups of amino acids.

[0209] In some embodiments, the second antigen binding portion comprises a single-chain antibody fragment comprising the V_H and V_L . In some embodiments, the second antigen binding portion comprises an scFv. In some embodiments, the anti-LAG-3 MABP (*e.g.*, BABP) comprises a fusion polypeptide comprising in the N-terminus to C-terminus direction: the first antigen binding portion comprising the anti-LAG-3 sdAb described herein, an optional peptide linker, the V_H domain and the V_L domain. In some embodiments, the anti-LAG-3 MABP (*e.g.*, BABP) comprises a fusion polypeptide comprising in the N-terminus to C-terminus direction: the first antigen binding portion comprising the anti-LAG-3 sdAb described herein, an optional peptide linker, the V_L domain and the V_H domain. In some embodiments, the anti-LAG-3 MABP (*e.g.*, BABP) comprises a fusion polypeptide comprising in the N-terminus to C-terminus direction: the V_H domain, the V_L domain, an optional peptide linker, and the first antigen binding portion comprising the anti-LAG-3 sdAb described herein. In some embodiments, anti-LAG-3 MABP (*e.g.*, BABP) comprises a fusion polypeptide comprising in the N-terminus to C-terminus direction: the V_L domain, the V_H domain, an optional peptide linker, and the first antigen binding portion comprising the anti-LAG-3 sdAb described herein.

[0210] In some embodiments, the second antigen binding portion comprises a heavy chain comprising the V_H domain, and a light chain comprising the V_L domain. In some embodiments, the heavy chain further comprises one or more heavy chain constant domains, such as C_{H1} , C_{H2} , C_{H3} , and C_{H4} , and/or an antibody hinge region (HR). In some embodiments, the light chain further comprises a light chain constant domain (C_L), such as the lambda C_L domain or kappa C_L domain. In some embodiments, the N-terminus of the first antigen binding portion comprising the anti-LAG-3 sdAb described herein is fused to the C-terminus of the heavy chain. In some embodiments, the C-terminus of the first antigen binding portion comprising the anti-LAG-3 sdAb is fused to the N-terminus of the heavy chain. In some embodiments, the N-terminus of the first antigen binding portion comprising the anti-LAG-3 sdAb is fused to the C-terminus of the light chain. In some embodiments, the C-terminus of the first antigen binding portion comprising the anti-LAG-3 sdAb is fused to the N-terminus of the light chain. In some embodiments, the anti-LAG-3 MABP (*e.g.*, BABP) comprises a first polypeptide comprising from the N-terminus to the C-terminus: the heavy chain, an optional peptide linker, and the first antigen binding portion comprising the anti-LAG-3 sdAb and an optional peptide sequence; and a second polypeptide comprising the light chain. In some embodiments, the anti-LAG-3 MABP (*e.g.*, BABP) comprises a first polypeptide comprising from the N-terminus to the C-terminus: the first antigen binding portion comprising an optional peptide sequence and the anti-LAG-3 sdAb, an optional peptide linker, and the heavy chain; and a second polypeptide comprising the light chain. In some embodiments, the anti-LAG-3 MABP (*e.g.*, BABP) comprises a first polypeptide comprising from the N-terminus to the C-terminus: the

light chain, an optional peptide linker, and the first antigen binding portion comprising the anti-LAG-3 sdAb and an optional peptide sequence; and a second polypeptide comprising the heavy chain. In some embodiments, the anti-LAG-3 MABP (*e.g.*, BABP) comprises a first polypeptide comprising from the N-terminus to the C-terminus: the first antigen binding portion comprising an optional peptide sequence and the anti-LAG-3 sdAb, an optional peptide linker, and the light chain; and a second polypeptide comprising the heavy chain. In some embodiments, the anti-LAG-3 MABP (*e.g.*, BABP) comprises two identical first polypeptides and two identical second polypeptides described herein.

[0211] In some embodiments, the second antigen binding portion comprises a full-length antibody consisting of two heavy chains and two light chains. In some embodiments, the full-length antibody is a full-length monoclonal antibody consisting of two identical heavy chains and two identical light chains. In some embodiments, the anti-LAG-3 MABP (*e.g.*, BABP) comprises two identical first polypeptides each comprising from the N-terminus to the C-terminus the heavy chain, an optional peptide linker, the first antigen binding portion comprising the anti-LAG-3 sdAb and an optional peptide sequence; and two identical second polypeptides each comprising the light chain (*e.g.*, FIG. 8). In some embodiments, the anti-LAG-3 MABP (*e.g.*, BABP) comprises two identical first polypeptides each comprising from the N-terminus to the C-terminus: the first antigen binding portion comprising an optional peptide sequence and the anti-LAG-3 sdAb, an optional peptide linker, and the heavy chain; and two identical second polypeptides each comprising the light chain (*e.g.*, FIG. 7). In some embodiments, the anti-LAG-3 MABP (*e.g.*, BABP) comprises two identical first polypeptides each comprising from the N-terminus to the C-terminus: the light chain, an optional peptide linker, and the first antigen binding portion comprising the anti-LAG-3 sdAb and an optional peptide sequence; and two identical second polypeptides each comprising the heavy chain (*e.g.*, FIG. 10). In some embodiments, the anti-LAG-3 MABP (*e.g.*, BABP) comprises two identical first polypeptides each comprising from the N-terminus to the C-terminus: the first antigen binding portion comprising an optional peptide sequence and the anti-LAG-3 sdAb, an optional peptide linker, and the light chain; and two identical second polypeptides comprising the heavy chain (*e.g.*, FIG. 9).

[0212] In some embodiments, the anti-LAG-3 MABP (*e.g.*, BABP) comprises: (a) a full-length antibody consisting of a first and a second heavy chains and a first and a second light chains, wherein the full-length antibody specifically recognizes a first epitope (*e.g.*, PD-1); (b) a first anti-LAG-3 sdAb; (c) a second anti-LAG-3 sdAb; (d) a third anti-LAG-3 sdAb; and (e) a fourth anti-LAG-3 sdAb; wherein the C-terminus of the first anti-LAG-3 sdAb is fused to the N-terminus of the first light chain, wherein the C-terminus of the second anti-LAG-3 sdAb is fused to the N-terminus of the second light chain, wherein the C-terminus of the third anti-LAG-3 sdAb is fused to the N-terminus of the first heavy chain, and wherein

the C-terminus of the fourth anti-LAG-3 sdAb is fused to the N-terminus of the second heavy chain. In some embodiments, the four anti-LAG-3 sdAbs are different. In some embodiments, the four anti-LAG-3 sdAbs are identical. In some embodiments, the anti-LAG-3 MABP (*e.g.*, BABP) comprises two identical first polypeptides each comprising from the N-terminus to the C-terminus: an optional peptide sequence, the third or the fourth anti-LAG-3 sdAb, an optional peptide linker, and the heavy chain; and two identical second polypeptides each comprising an optional peptide sequence, the first or the second anti-LAG-3 sdAb, an optional peptide linker, and the light chain. See, for example, FIG. 11.

[0213] In some embodiments, the anti-LAG-3 MABP (*e.g.*, BABP) comprises: (a) a full-length antibody consisting of two heavy chains and two light chains, wherein the full-length antibody specifically recognizes a first epitope (*e.g.*, PD-1); (b) a first anti-LAG-3 sdAb; (c) a second anti-LAG-3 sdAb; (d) a third anti-LAG-3 sdAb; and (e) a fourth anti-LAG-3 sdAb; wherein the C-terminus of the first anti-LAG-3 sdAb is fused to the N-terminus of the second anti-LAG-3 sdAb, and the C-terminus of the second anti-LAG-3 sdAb is fused to the N-terminus of one heavy chain, and wherein the C-terminus of the third anti-LAG-3 sdAb is fused to the N-terminus of the fourth anti-LAG-3 sdAb, and the C-terminus of the fourth anti-LAG-3 sdAb is fused to the N-terminus of the other heavy chain. In some embodiments, the four anti-LAG-3 sdAbs are different. In some embodiments, the four anti-LAG-3 sdAbs are identical. In some embodiments, the anti-LAG-3 MABP (*e.g.*, BABP) comprises two identical first polypeptides each comprising from the N-terminus to the C-terminus: an optional peptide sequence, the first or the third anti-LAG-3 sdAb, an optional peptide linker, the second or the fourth anti-LAG-3 sdAb, an optional peptide linker, and the heavy chain; and two identical second polypeptides each comprising the light chain. See, for example, FIG. 12.

[0214] In some embodiments, the anti-LAG-3 MABP (*e.g.*, BABP) comprises: (a) a full-length antibody consisting of two heavy chains and two light chains, wherein the full-length antibody specifically recognizes a first epitope (*e.g.*, PD-1); (b) a first anti-LAG-3 sdAb; and (c) a second anti-LAG-3 sdAb, wherein the N-terminus of the first or the second anti-LAG-3 sdAb is fused to the C-terminus of the C_{H1} region of the heavy chain, and the C-terminus of the first or the second anti-LAG-3 sdAb is fused to the N-terminus of the C_{H2} region of the heavy chain. In some embodiments, the two anti-LAG-3 sdAbs are identical. In some embodiments, the two anti-LAG-3 sdAbs are different. In some embodiments, the anti-LAG-3 MABP (*e.g.*, BABP) comprises two identical first polypeptides each comprising from the N-terminus to the C-terminus: V_H-C_{H1}-an optional peptide linker-anti-LAG-3 sdAb-C_{H2}-C_{H3}; and two identical second polypeptides each comprising the light chain. See, for example, FIG. 13.

[0215] In some embodiments, the anti-LAG-3 MABP (*e.g.*, BABP) comprises: (a) a first scFv that specifically recognizes a first epitope (*e.g.*, PD-1); (b) a second scFv that specifically recognizes a second epitope (*e.g.*, PD-1); (c) an Fc region; (d) a first anti-LAG-3 sdAb; and (e) a second anti-LAG-3 sdAb, wherein the N-terminus of each anti-LAG-3 sdAb is fused to the C-terminus of an scFv and the C-terminus of the anti-LAG-3 sdAb is fused to the N-terminus of the Fc region. In some embodiments, the two anti-LAG-3 sdAbs are identical. In some embodiments, the two anti-LAG-3 sdAbs are different. In some embodiments, the two scFvs are identical. In some embodiments, the two scFvs are different. In some embodiments, the anti-LAG-3 MABP (*e.g.*, BABP) comprises two identical polypeptides each comprising from the N-terminus to the C-terminus: scFv-an optional peptide linker-anti-LAG-3 sdAb-CH₂-CH₃, such as V_H-V_L-an optional peptide linker-anti-LAG-3 sdAb-CH₂-CH₃, or V_L-V_H-an optional peptide linker-anti-LAG-3 sdAb-CH₂-CH₃. See, for example, FIG. 14.

[0216] In some embodiments, the anti-LAG-3 MABP (*e.g.*, BABP) comprises: (a) a first Fab that specifically recognizes a first epitope (*e.g.*, PD-1); (b) a second Fab that specifically recognizes a second epitope (*e.g.*, PD-1); (c) an Fc region; (d) a first Fab-like domain comprising a first anti-LAG-3 sdAb and a second anti-LAG-3 sdAb; (e) a second Fab-like domain comprising a third anti-LAG-3 sdAb and a fourth anti-LAG-3 sdAb, wherein the N-termini of each Fab-like domain are fused to the C-termini of a Fab and one of the two C-termini of the Fab-like domain is fused to the N-terminus of the Fc region. In some embodiments, the four anti-LAG-3 sdAbs are identical. In some embodiments, the four anti-LAG-3 sdAbs are different. In some embodiments, the two Fabs are identical. In some embodiments, the two Fabs are different. In some embodiments, the anti-LAG-3 MABP (*e.g.*, BABP) comprises two identical first polypeptides each comprising from the N-terminus to the C-terminus: V_H-C_{H1}-an optional peptide linker-anti-LAG-3 sdAb-C_{H1}-C_{H2}-C_{H3}; and two identical second polypeptides each comprising from the N-terminus to the C-terminus: V_L-C_L-an optional peptide linker-anti-LAG-3 sdAb-C_L. See, for example, FIG. 15.

[0217] In some embodiments, the anti-LAG-3 MABP (*e.g.*, BABP) comprises: (a) a first scFv that specifically recognizes a first epitope (*e.g.*, PD-1); (b) a second scFv that specifically recognizes a second epitope (*e.g.*, PD-1); (c) an Fc region; (d) a first Fab-like domain comprising a first anti-LAG-3 sdAb and a second anti-LAG-3 sdAb; (e) a second Fab-like domain comprising a third anti-LAG-3 sdAb and a fourth anti-LAG-3 sdAb, wherein one of the two N-termini of each Fab-like domain is fused to the C-terminus of an scFv and one of the two C-termini of the Fab-like domain is fused to the N-terminus of the Fc region. In some embodiments, the four anti-LAG-3 sdAbs are identical. In some embodiments, the four anti-LAG-3 sdAbs are different. In some embodiments, the two scFvs are identical. In some embodiments, the two scFvs are different. In some embodiments, the anti-LAG-3 MABP (*e.g.*, BABP)

comprises two identical first polypeptides each comprising from the N-terminus to the C-terminus: scFv- an optional peptide linker-anti-LAG-3 sdAb-C_H1-C_H2-C_H3; and two identical second polypeptides each comprising from the N-terminus to the C-terminus: anti-LAG-3 sdAb-C_L. In some embodiments, the scFv comprises from the N-terminus to the C-terminus: V_H-V_L or V_L-V_H. See, for example, FIG. 16.

[0218] The anti-LAG-3 MABPs (*e.g.*, BABPs) described herein may comprise one or more peptide linkers situated between the first antigen binding portion and the second antigen binding portion. In some embodiments, the peptide linker between the heavy chain polypeptide of the second antigen binding portion and the first antigen binding portion is the same as the peptide linker between the light chain polypeptide of the second antigen binding portion and the first antigen binding portion. In some embodiments, the peptide linker between the heavy chain polypeptide of the second antigen binding portion and the first antigen binding portion is different from the peptide linker between the light chain polypeptide of the second antigen binding portion and the first antigen binding portion. In some embodiments, the first antigen binding portion and the second antigen binding portion are directly fused to each other without a peptide linker disposed therebetween. The peptide linker between the two or more anti-LAG-3 sdAbs may be the same as or different from that between the anti-LAG-3 sdAb and the second antigen binding portion. Any of the peptide linkers described below in the “Peptide linkers” section above can be employed in any of the anti-LAG-3 MABPs (*e.g.*, BABPs) described herein.

b) Second antigen binding portion comprising V_H and V_L

[0219] The anti-LAG-3 MABPs (*e.g.*, BABPs) comprise at least one second antigen binding portion comprising a V_H and a V_L. Such antigen binding portion can be a full-length conventional antibody consisting of two heavy chains and two light chains, or an antigen binding fragment derived therefrom, *e.g.*, Fab, scFv.

[0220] In some embodiments, the second antigen binding portion is an antigen binding fragment comprising a heavy chain comprising the V_H domain and a light chain comprising the V_L domain. Exemplary antigen binding fragments contemplated herein include, but are not limited to, Fab, Fab', F(ab')₂, and Fv fragments; diabodies; linear antibodies; single-chain antibody molecules (such as scFv); and multispecific antibodies formed from antibody fragments.

[0221] In some embodiments, the second antigen binding portion comprises an Fc region, such as a human Fc region. In some embodiments, the Fc region is derived from an IgG molecule, such as any one of the IgG1, IgG2, IgG3, or IgG4 subclass. In some embodiments, the Fc region is capable of mediating an antibody effector function, such as ADCC and/or CDC. For example, antibodies of subclass IgG1, IgG2, and IgG3 with wildtype Fc sequences usually show complement activation including C1q and C3

binding, whereas IgG4 does not activate the complement system and does not bind C1q and/or C3. In some embodiments, the Fc region comprises a modification that reduces binding affinity of the Fc region to an Fc receptor. In some embodiments, the Fc region is an IgG1 Fc. In some embodiments, the IgG1 Fc comprises one or mutations in positions 233-236, such as L234A and/or L235A. In some embodiments, the Fc region is an effectorless IgG1 Fc. In some embodiments, the Fc region is an IgG4 Fc. In some embodiments, the IgG4 Fc comprises a mutation in positions 327, 330 and/or 331. See, for example, Armour KL *et al.*, *Eur J. Immunol.* 1999; 29: 2613; and Shields RL *et al.*, *J. Biol. Chem.* 2001; 276: 6591. In some embodiments, the Fc region comprises a P329G mutation. In some embodiments, the Fc region is an IgG4 Fc (S228P). In some embodiments, the Fc region comprises an amino acid sequence of any one of SEQ ID NOs: 363-365.

[0222] In some embodiments, the Fc region comprises a modification that promotes heterodimerization of two non-identical heavy chains. Such modified Fc regions may be of particular interest for anti-LAG-3 MABPs (*e.g.*, BABPs) described herein having an asymmetric design. In some embodiments, said modification is a knob-into-hole modification, comprising a knob modification in one of the heavy chains or heavy chain fusion polypeptides and a hole modification in the other one of the two heavy chains or heavy chain fusion polypeptides. In one embodiment, the Fc region comprises a modification within the interface between the two heavy chains in the CH3 domain, wherein i) in the CH3 domain of one heavy chain, an amino acid residue is replaced with an amino acid residue having a larger side chain volume, thereby generating a protuberance ("knob") within the interface in the CH3 domain of one heavy chain which is positionable in a cavity ("hole") within the interface in the CH3 domain of the other heavy chain, and ii) in the CH3 domain of the other heavy chain, an amino acid residue is replaced with an amino acid residue having a smaller side chain volume, thereby generating a cavity ("hole") within the interface in the second CH3 domain within which a protuberance ("knob") within the interface in the first CH3 domain is positionable. Examples of knob-into-hole modifications have been described, for example, in US 2011/0287009, US2007/0178552, WO 96/027011, WO 98/050431, and Zhu *et al.*, 1997, *Protein Science* 6:781-788. Other modifications to the Fc region that promote heterodimerization are also contemplated herein. For example, electrostatic steering effects can be engineered into the Fc region to provide Fc-heterodimeric molecules (see, *e.g.*, US4676980, and Brennan *et al.*, *Science*, 229: 81 (1985)). In some embodiments, the Fc region comprises a modification that inhibits Fab arm exchange. For example, the S228P mutation in IgG4 Fc prevents Fab arm exchange.

[0223] In some embodiments, the second antigen binding portion comprises a kappa light chain constant region. In some embodiments, the second antigen binding portion comprises a lambda light

chain constant region. In some embodiments, the second antigen binding portion comprises a heavy chain constant region.

[0224] In some embodiments, the second antigen binding portion is a full-length antibody consisting of two heavy chains and two light chains. In some embodiments, the second antigen binding portion comprises a monoclonal antibody consisting of two heavy chains and two light chains (also referred herein as “4-chain antibody”). In some embodiments, the second antigen binding portion comprises a multispecific (*e.g.*, bispecific) full-length antibody consisting of two heavy chains and two light chains. In some embodiments, the second antigen binding portion comprises a full-length antibody of human IgG1 subclass, of effectorless hIgG1 subclass, or of human IgG1 subclass with the mutations L234A and L235A. In some embodiments, the second antigen binding portion comprises a full-length antibody of human IgG2 subclass. In some embodiments, the second antigen binding portion comprises a full-length antibody of human IgG3 subclass. In some embodiments, the second antigen binding portion comprises a full-length antibody of human IgG4 subclass or, of human IgG4 subclass with the additional mutation S228P.

[0225] Any full-length 4-chain antibody known in the art or antigen binding fragments derived therefrom can be used as the second antigen binding portion of the anti-LAG-3 MABP (*e.g.* BABP) described herein. Antibodies or antibody fragments with proven clinical efficacy, safety, and pharmacokinetics profile are of particular interest. In some embodiments, the antibody or antibody fragment known in the art is further engineered, such as humanized or mutagenized to select for a variant with a suitable affinity, prior to fusion with the first antigen binding portion to provide the anti-LAG-3 MABP (*e.g.*, BABP). In some embodiments, the second antigen binding portion comprises the V_H and V_L domains of a monoclonal antibody or antibody fragment known in the art, and modified heavy chain constant region and/or light chain constant region. In some embodiments, the second antigen binding portion comprises the monoclonal antibody known in the art and a modified Fc region, such as an IgG4 Fc with an S228P mutation, or an effectorless IgG1 Fc. In some embodiments, the second antigen binding portion comprises a human, humanized, or chimeric full-length antibody or antibody fragments.

c) Exemplary anti-LAG-3 MABPs

[0226] In some embodiments, the anti-LAG-3 construct is an anti-LAG-3 MABP comprising a second antigen binding portion that specifically recognizes PD-1. In some embodiments, the second antigen binding portion comprises an anti-PD-1 sdAb. In some embodiments, the second antigen binding portion comprises a full-length anti-PD-1 antibody consisting of two heavy chains and two light chains.

[0227] PD-1 is an inhibitory member of the CD28 receptor family, which also includes CD28, CTLA-4, ICOS and BTLA. PD-1 contains a membrane proximal immunoreceptor tyrosine inhibitory motif (ITIM) and a membrane distal tyrosine-based switch motif (ITSM) (Thomas, M.L. (1995) J Exp Med 181:1953-6; Vivier, E and Daeron, M (1997) Immunol Today 18:286-91). Although structurally similar to CTLA-4, PD-1 lacks the MYPPPY motif that is critical for B7-1 and B7-2 binding.

[0228] PD-1 regulates T-cell activation and tolerance, and thus antagonistic anti-PD-1 antibodies can be useful for overcoming tolerance. PD-1 has been defined as a receptor for B7-4. B7-4 can inhibit immune cell activation upon binding to an inhibitory receptor on an immune cell. Engagement of the PD-1/PD-L1 pathway results in inhibition of T-cell effector function, cytokine secretion and proliferation (Turnis *et al.*, OncoImmunology 1(7):1172-1174, 2012). High levels of PD-1 are associated with exhausted or chronically stimulated T cells. Moreover, increased PD-1 expression correlates with reduced survival in cancer patients. Agents for down modulating PD-1, B7-4, and the interaction between B7-4 and PD-1 inhibitory signal in an immune cell can result in enhancement of the immune response.

[0229] In some embodiments, there is provided an anti-LAG-3 construct comprising an anti-LAG-3 sdAb described herein fused to an anti-PD-1 sdAb. In some embodiments, the anti-LAG-3 sdAb is fused to the anti-PD-1 sdAb via a linker (such as a peptide linker). In some embodiments, the anti-LAG-3 construct comprises a plurality (such as 2, 3, 4, or more) of anti-LAG-3 sdAbs, which can be the same or different. In some embodiments, the anti-LAG-3 construct comprises a plurality (such as 2, 3, 4, or more) of anti-PD-1 sdAbs, which can be the same or different. The anti-PD-1 sdAb can be derived from any known anti-PD-1 sdAbs, such as those disclosed in PCT/CN2018/071729, the contents of which are incorporated herein by reference in their entirety. The anti-LAG-3 construct can be of any suitable format, for example, from N- to C-terminus: (anti-LAG-3 sdAb)-L₁-(anti-PD-1 sdAb) or (anti-PD-1 sdAb)-L₁-(anti-LAG-3 sdAb), wherein L₁ is a chemical bond or a linker, such as peptide linker.

[0230] Exemplary 4-chain anti-PD-1 antibodies that can be used for making multispecific anti-LAG_3 constructs described herein include, but are not limited to, pembrolizumab (*e.g.*, KEYTRUDA®), nivolumab (*e.g.*, OPDIVO®), PD1-BM-min, including biosimilars thereof, derivatives thereof, and antibodies having the same sequences.

[0231] In some embodiments, the second antigen binding portion comprises an anti-PD-1 full-length antibody or antigen binding fragment thereof (*e.g.*, Fab, scFv). In some embodiments, the anti-PD-1 full-length antibody or antigen binding fragment thereof (*e.g.*, Fab, scFv) comprises: (i) a V_H comprising HC-CDR1, HC-CDR2, and HC-CDR3 of the amino acid sequence of SEQ ID NO: 356, and a V_L comprising LC-CDR1, LC-CDR2, and LC-CDR3 of the amino acid sequence of SEQ ID NO: 357; or (ii) a V_H

comprising HC-CDR1, HC-CDR2, and HC-CDR3 of the amino acid sequence of SEQ ID NO: 373, and a V_L comprising LC-CDR1, LC-CDR2, and LC-CDR3 of the amino acid sequence of SEQ ID NO: 374. In some embodiments, the anti-PD-1 full-length antibody comprises: (i) a heavy chain comprising the amino acid sequence of SEQ ID NO: 356, and a light chain comprising the amino acid sequence of SEQ ID NO: 357; or (ii) a heavy chain comprising the amino acid sequence of SEQ ID NO: 373, and a light chain comprising the amino acid sequence of SEQ ID NO: 374. In some embodiments, the anti-PD-1 full-length antibody is pembrolizumab (*e.g.*, KEYTRUDA[®]) or PD1-BM-min.

[0232] In some embodiments, the anti-LAG-3 MABP (*e.g.*, BABP) comprises (a) a first antigen binding portion comprising an anti-LAG-3 sdAb described herein, and (b) a second antigen binding portion comprising a V_H and a V_L, wherein the V_H and V_L together form an antigen-binding site that specifically binds PD-1, wherein the first antigen binding portion and the second antigen binding portion are fused to each other (herein after referred to as “LAG-3×PD-1 MABP” or “LAG-3×PD-1 BABP”). Exemplary anti-LAG-3/PD-1 BABPs are shown in FIGs. 7-14 and Table 7.

[0233] In some embodiments, there is provided an anti-LAG-3 BABP comprising: (a) a first polypeptide comprising from N-terminus to C-terminus: anti-LAG-3 sdAb-V_H-C_H1-C_H2-C_H3; and (b) a second polypeptide comprising from N-terminus to C-terminus: V_L-C_L, wherein V_H and V_L forms an antigen binding site that specifically binds a second epitope (*e.g.*, an immune checkpoint molecule, such as PD-1), and wherein the anti-LAG-3 sdAb comprises a CDR1 comprising the amino acid sequence of any one of SEQ ID NOs: 39-76, or a variant thereof comprising up to about 3 (such as about any of 1, 2, or 3) amino acid substitutions; a CDR2 comprising the amino acid sequence of any one of SEQ ID NOs: 115-152, or a variant thereof comprising up to about 3 (such as about any of 1, 2, or 3) amino acid substitutions; and a CDR3 comprising the amino acid sequence of any one of SEQ ID NOs: 191-228, or a variant thereof comprising up to about 3 (such as about any of 1, 2, or 3) amino acid substitutions. In some embodiments, the anti-LAG-3 sdAb comprises: (1) a CDR1 comprising the amino acid sequence of SEQ ID NO: 53; a CDR2 comprising the amino acid sequence of SEQ ID NO: 129; and a CDR3 comprising the amino acid sequence of SEQ ID NO: 205; (2) a CDR1 comprising the amino acid sequence of SEQ ID NO: 60; a CDR2 comprising the amino acid sequence of SEQ ID NO: 136; and a CDR3 comprising the amino acid sequence of SEQ ID NO: 212; (3) a CDR1 comprising the amino acid sequence of SEQ ID NO: 69; a CDR2 comprising the amino acid sequence of SEQ ID NO: 145; and a CDR3 comprising the amino acid sequence of SEQ ID NO: 221; or (4) a CDR1 comprising the amino acid sequence of SEQ ID NO: 71; a CDR2 comprising the amino acid sequence of SEQ ID NO: 147; and a CDR3 comprising the amino acid sequence of SEQ ID NO: 223. In some embodiments, the anti-LAG-3 sdAb comprises a V_HH domain comprising the amino acid sequence of any one of SEQ ID NO: 274-311.

In some embodiments, the anti-LAG-3 sdAb comprises a V_HH domain comprising the amino acid sequence of any one of SEQ ID NO: 288, 295, 304 or 306. In some embodiments, V_H and V_L form an antigen binding site that specifically binds PD-1. In some embodiments, the V_H and V_L domains are derived from pembrolizumab or PD1-BM-min. In some embodiments, the V_H comprises HC-CDR1, HC-CDR2, and HC-CDR3 of the amino acid sequence of SEQ ID NO: 356, and V_L comprises LC-CDR1, LC-CDR2, and LC-CDR3 of the amino acid sequence of SEQ ID NO: 357. In some embodiments, the V_H comprises HC-CDR1, HC-CDR2, and HC-CDR3 of the amino acid sequence of SEQ ID NO: 373, and V_L comprises LC-CDR1, LC-CDR2, and LC-CDR3 of the amino acid sequence of SEQ ID NO: 374. In some embodiments, the C_H3 and anti-LAG-3 sdAb are fused to each other via a peptide linker, such as a peptide linker comprising the amino acid sequence of any one of SEQ ID NOs: 352-353 and 366-372. In some embodiments, the N-terminus of the anti-LAG-3 sdAb is fused to a peptide sequence. In some embodiments, the peptide sequence comprises the amino acid sequence of any one of SEQ ID NOs: 352-353 and 366-372. In some embodiments, the C_H2 and C_H3 domains are derived from IgG1 Fc, effectorless IgG1 Fc, IgG2 Fc, IgG4 Fc, or IgG4 Fc (S228P).

[0234] In some embodiments, there is provided an anti-LAG-3 BABP comprising: (a) a first polypeptide comprising from N-terminus to C-terminus: V_H-C_H1-C_H2-C_H3-anti-LAG-3 sdAb; and (b) a second polypeptide comprising from N-terminus to C-terminus: V_L-C_L, wherein V_H and V_L forms an antigen binding site that specifically binds a second epitope (*e.g.*, an immune checkpoint molecule, such as PD-1), and wherein the anti-LAG-3 sdAb comprises a CDR1 comprising the amino acid sequence of any one of SEQ ID NOs: 39-76, or a variant thereof comprising up to about 3 (such as about any of 1, 2, or 3) amino acid substitutions; a CDR2 comprising the amino acid sequence of any one of SEQ ID NOs: 115-152, or a variant thereof comprising up to about 3 (such as about any of 1, 2, or 3) amino acid substitutions; and a CDR3 comprising the amino acid sequence of any one of SEQ ID NOs: 191-228, or a variant thereof comprising up to about 3 (such as about any of 1, 2, or 3) amino acid substitutions. In some embodiments, the anti-LAG-3 sdAb comprises: (1) a CDR1 comprising the amino acid sequence of SEQ ID NO: 53; a CDR2 comprising the amino acid sequence of SEQ ID NO: 129; and a CDR3 comprising the amino acid sequence of SEQ ID NO: 205; (2) a CDR1 comprising the amino acid sequence of SEQ ID NO: 60; a CDR2 comprising the amino acid sequence of SEQ ID NO: 136; and a CDR3 comprising the amino acid sequence of SEQ ID NO: 212; (3) a CDR1 comprising the amino acid sequence of SEQ ID NO: 69; a CDR2 comprising the amino acid sequence of SEQ ID NO: 145; and a CDR3 comprising the amino acid sequence of SEQ ID NO: 221; or (4) a CDR1 comprising the amino acid sequence of SEQ ID NO: 71; a CDR2 comprising the amino acid sequence of SEQ ID NO: 147; and a CDR3 comprising the amino acid sequence of SEQ ID NO: 223. In some embodiments, the anti-LAG-3

sdAb comprises a V_{HH} domain comprising the amino acid sequence of any one of SEQ ID NO: 274-311. In some embodiments, the anti-LAG-3 sdAb comprises a V_{HH} domain comprising the amino acid sequence of any one of SEQ ID NO: 288, 295, 304 or 306. In some embodiments, V_H and V_L form an antigen binding site that specifically binds PD-1. In some embodiments, the V_H and V_L domains are derived from pembrolizumab or PD1-BM-min. In some embodiments, the V_H comprises HC-CDR1, HC-CDR2, and HC-CDR3 of the amino acid sequence of SEQ ID NO: 356, and V_L comprises LC-CDR1, LC-CDR2, and LC-CDR3 of the amino acid sequence of SEQ ID NO: 357. In some embodiments, the V_H comprises HC-CDR1, HC-CDR2, and HC-CDR3 of the amino acid sequence of SEQ ID NO: 373, and V_L comprises LC-CDR1, LC-CDR2, and LC-CDR3 of the amino acid sequence of SEQ ID NO: 374. In some embodiments, the C_{H3} and anti-LAG-3 sdAb are fused to each other via a peptide linker, such as a peptide linker comprising the amino acid sequence of any one of SEQ ID NOs: 352-353 and 366-372. In some embodiments, the C-terminus of the anti-LAG-3 sdAb is fused to a peptide sequence. In some embodiments, the peptide sequence comprises the amino acid sequence of any one of SEQ ID NOs: 352-353 and 366-372. In some embodiments, the C_{H2} and C_{H3} domains are derived from IgG1 Fc, effectorless IgG1 Fc, IgG2 Fc, IgG4 Fc, or IgG4 Fc (S228P).

[0235] In some embodiments, there is provided an anti-LAG-3 BABP comprising: (a) a first polypeptide comprising from N-terminus to C-terminus: V_H - C_{H1} - C_{H2} - C_{H3} ; and (b) a second polypeptide comprising from N-terminus to C-terminus: anti-LAG-3 sdAb- V_L - C_L , wherein V_H and V_L forms an antigen binding site that specifically binds a second epitope (*e.g.*, an immune checkpoint molecule, such as PD-1), and wherein the anti-LAG-3 sdAb comprises a CDR1 comprising the amino acid sequence of any one of SEQ ID NOs: 39-76, or a variant thereof comprising up to about 3 (such as about any of 1, 2, or 3) amino acid substitutions; a CDR2 comprising the amino acid sequence of any one of SEQ ID NOs: 115-152, or a variant thereof comprising up to about 3 (such as about any of 1, 2, or 3) amino acid substitutions; and a CDR3 comprising the amino acid sequence of any one of SEQ ID NOs: 191-228, or a variant thereof comprising up to about 3 (such as about any of 1, 2, or 3) amino acid substitutions. In some embodiments, the anti-LAG-3 sdAb comprises: (1) a CDR1 comprising the amino acid sequence of SEQ ID NO: 53; a CDR2 comprising the amino acid sequence of SEQ ID NO: 129; and a CDR3 comprising the amino acid sequence of SEQ ID NO: 205; (2) a CDR1 comprising the amino acid sequence of SEQ ID NO: 60; a CDR2 comprising the amino acid sequence of SEQ ID NO: 136; and a CDR3 comprising the amino acid sequence of SEQ ID NO: 212; (3) a CDR1 comprising the amino acid sequence of SEQ ID NO: 69; a CDR2 comprising the amino acid sequence of SEQ ID NO: 145; and a CDR3 comprising the amino acid sequence of SEQ ID NO: 221; or (4) a CDR1 comprising the amino acid sequence of SEQ ID NO: 71; a CDR2 comprising the amino acid sequence of SEQ ID NO: 147; and

a CDR3 comprising the amino acid sequence of SEQ ID NO: 223. In some embodiments, the anti-LAG-3 sdAb comprises a V_HH domain comprising the amino acid sequence of any one of SEQ ID NO: 274-311. In some embodiments, the anti-LAG-3 sdAb comprises a V_HH domain comprising the amino acid sequence of any one of SEQ ID NO: 288, 295, 304 or 306. In some embodiments, V_H and V_L form an antigen binding site that specifically binds PD-1. In some embodiments, the V_H and V_L domains are derived from pembrolizumab or PD1-BM-min. In some embodiments, the V_H comprises HC-CDR1, HC-CDR2, and HC-CDR3 of the amino acid sequence of SEQ ID NO: 356, and V_L comprises LC-CDR1, LC-CDR2, and LC-CDR3 of the amino acid sequence of SEQ ID NO: 357. In some embodiments, the V_H comprises HC-CDR1, HC-CDR2, and HC-CDR3 of the amino acid sequence of SEQ ID NO: 373, and V_L comprises LC-CDR1, LC-CDR2, and LC-CDR3 of the amino acid sequence of SEQ ID NO: 374. In some embodiments, the C_H3 and anti-LAG-3 sdAb are fused to each other via a peptide linker, such as a peptide linker comprising the amino acid sequence of any one of SEQ ID NOs: 352-353 and 366-372. In some embodiments, the N-terminus of the anti-LAG-3 sdAb is fused to a peptide sequence. In some embodiments, the peptide sequence comprises the amino acid sequence of any one of SEQ ID NOs: 352-353 and 366-372. In some embodiments, the C_H2 and C_H3 domains are derived from IgG1 Fc, effectorless IgG1 Fc, IgG2 Fc, IgG4 Fc, or IgG4 Fc (S228P).

[0236] In some embodiments, there is provided an anti-LAG-3 BABP comprising: (a) a first polypeptide comprising from N-terminus to C-terminus: V_H-C_H1-C_H2-C_H3; and (b) a second polypeptide comprising from N-terminus to C-terminus: V_L-C_L-anti-LAG-3 sdAb, wherein V_H and V_L forms an antigen binding site that specifically binds a second epitope (*e.g.*, an immune checkpoint molecule, such as PD-1), and wherein the anti-LAG-3 sdAb comprises a CDR1 comprising the amino acid sequence of any one of SEQ ID NOs: 39-76, or a variant thereof comprising up to about 3 (such as about any of 1, 2, or 3) amino acid substitutions; a CDR2 comprising the amino acid sequence of any one of SEQ ID NOs: 115-152, or a variant thereof comprising up to about 3 (such as about any of 1, 2, or 3) amino acid substitutions; and a CDR3 comprising the amino acid sequence of any one of SEQ ID NOs: 191-228, or a variant thereof comprising up to about 3 (such as about any of 1, 2, or 3) amino acid substitutions. In some embodiments, the anti-LAG-3 sdAb comprises: (1) a CDR1 comprising the amino acid sequence of SEQ ID NO: 53; a CDR2 comprising the amino acid sequence of SEQ ID NO: 129; and a CDR3 comprising the amino acid sequence of SEQ ID NO: 205; (2) a CDR1 comprising the amino acid sequence of SEQ ID NO: 60; a CDR2 comprising the amino acid sequence of SEQ ID NO: 136; and a CDR3 comprising the amino acid sequence of SEQ ID NO: 212; (3) a CDR1 comprising the amino acid sequence of SEQ ID NO: 69; a CDR2 comprising the amino acid sequence of SEQ ID NO: 145; and a CDR3 comprising the amino acid sequence of SEQ ID NO: 221; or (4) a CDR1 comprising the amino

acid sequence of SEQ ID NO: 71; a CDR2 comprising the amino acid sequence of SEQ ID NO: 147; and a CDR3 comprising the amino acid sequence of SEQ ID NO: 223. In some embodiments, the anti-LAG-3 sdAb comprises a V_HH domain comprising the amino acid sequence of any one of SEQ ID NO: 274-311. In some embodiments, the anti-LAG-3 sdAb comprises a V_HH domain comprising the amino acid sequence of any one of SEQ ID NO: 288, 295, 304 or 306. In some embodiments, V_H and V_L form an antigen binding site that specifically binds PD-1. In some embodiments, the V_H and V_L domains are derived from pembrolizumab or PD1-BM-min. In some embodiments, the V_H comprises HC-CDR1, HC-CDR2, and HC-CDR3 of the amino acid sequence of SEQ ID NO: 356, and V_L comprises LC-CDR1, LC-CDR2, and LC-CDR3 of the amino acid sequence of SEQ ID NO: 357. In some embodiments, the V_H comprises HC-CDR1, HC-CDR2, and HC-CDR3 of the amino acid sequence of SEQ ID NO: 373, and V_L comprises LC-CDR1, LC-CDR2, and LC-CDR3 of the amino acid sequence of SEQ ID NO: 374. In some embodiments, the C_H3 and anti-LAG-3 sdAb are fused to each other via a peptide linker, such as a peptide linker comprising the amino acid sequence of any one of SEQ ID NOs: 352-353 and 366-372. In some embodiments, the C-terminus of the anti-LAG-3 sdAb is fused to a peptide sequence. In some embodiments, the peptide sequence comprises the amino acid sequence of any one of SEQ ID NOs: 352-353 and 366-372. In some embodiments, the C_H2 and C_H3 domains are derived from IgG1 Fc, effectorless IgG1 Fc, IgG2 Fc, IgG4 Fc, or IgG4 Fc (S228P).

[0237] In some embodiments, there is provided an anti-LAG-3 BABP comprising: (a) a first polypeptide comprising from N-terminus to C-terminus: V_H-C_H1-anti-LAG-3 sdAb-C_H2-C_H3; and (b) a second polypeptide comprising from N-terminus to C-terminus: V_L-C_L, wherein V_H and V_L forms an antigen binding site that specifically binds a second epitope (*e.g.*, an immune checkpoint molecule, such as PD-1), and wherein the anti-LAG-3 sdAb comprises a CDR1 comprising the amino acid sequence of any one of SEQ ID NOs: 39-76, or a variant thereof comprising up to about 3 (such as about any of 1, 2, or 3) amino acid substitutions; a CDR2 comprising the amino acid sequence of any one of SEQ ID NOs: 115-152, or a variant thereof comprising up to about 3 (such as about any of 1, 2, or 3) amino acid substitutions; and a CDR3 comprising the amino acid sequence of any one of SEQ ID NOs: 191-228, or a variant thereof comprising up to about 3 (such as about any of 1, 2, or 3) amino acid substitutions. In some embodiments, the anti-LAG-3 sdAb comprises: (1) a CDR1 comprising the amino acid sequence of SEQ ID NO: 53; a CDR2 comprising the amino acid sequence of SEQ ID NO: 129; and a CDR3 comprising the amino acid sequence of SEQ ID NO: 205; (2) a CDR1 comprising the amino acid sequence of SEQ ID NO: 60; a CDR2 comprising the amino acid sequence of SEQ ID NO: 136; and a CDR3 comprising the amino acid sequence of SEQ ID NO: 212; (3) a CDR1 comprising the amino acid sequence of SEQ ID NO: 69; a CDR2 comprising the amino acid sequence of SEQ ID NO: 145; and a

CDR3 comprising the amino acid sequence of SEQ ID NO: 221; or (4) a CDR1 comprising the amino acid sequence of SEQ ID NO: 71; a CDR2 comprising the amino acid sequence of SEQ ID NO: 147; and a CDR3 comprising the amino acid sequence of SEQ ID NO: 223. In some embodiments, the anti-LAG-3 sdAb comprises a V_HH domain comprising the amino acid sequence of any one of SEQ ID NO: 274-311. In some embodiments, the anti-LAG-3 sdAb comprises a V_HH domain comprising the amino acid sequence of any one of SEQ ID NO: 288, 295, 304 or 306. In some embodiments, V_H and V_L form an antigen binding site that specifically binds PD-1. In some embodiments, the V_H and V_L domains are derived from pembrolizumab or PD1-BM-min. In some embodiments, the V_H comprises HC-CDR1, HC-CDR2, and HC-CDR3 of the amino acid sequence of SEQ ID NO: 356, and V_L comprises LC-CDR1, LC-CDR2, and LC-CDR3 of the amino acid sequence of SEQ ID NO: 357. In some embodiments, the V_H comprises HC-CDR1, HC-CDR2, and HC-CDR3 of the amino acid sequence of SEQ ID NO: 373, and V_L comprises LC-CDR1, LC-CDR2, and LC-CDR3 of the amino acid sequence of SEQ ID NO: 374. In some embodiments, the C_H3 and anti-LAG-3 sdAb are fused to each other via a peptide linker, such as a peptide linker comprising the amino acid sequence of any one of SEQ ID NOs: 352-353 and 366-372. In some embodiments, the C_H2 and C_H3 domains are derived from IgG1 Fc, effectorless IgG1 Fc, IgG2 Fc, IgG4 Fc, or IgG4 Fc (S228P).

[0238] In some embodiments, there is provided an anti-LAG-3 BABP comprising a polypeptide comprising from N-terminus to C-terminus: V_L-V_H-anti-LAG-3 sdAb-C_H2-C_H3, wherein the V_L and V_H together forms an scFv that specifically binds a second epitope (*e.g.*, an immune checkpoint molecule, such as PD-1), and wherein the anti-LAG-3 sdAb comprises a CDR1 comprising the amino acid sequence of any one of SEQ ID NOs: 39-76, or a variant thereof comprising up to about 3 (such as about any of 1, 2, or 3) amino acid substitutions; a CDR2 comprising the amino acid sequence of any one of SEQ ID NOs: 115-152, or a variant thereof comprising up to about 3 (such as about any of 1, 2, or 3) amino acid substitutions; and a CDR3 comprising the amino acid sequence of any one of SEQ ID NOs: 191-228, or a variant thereof comprising up to about 3 (such as about any of 1, 2, or 3) amino acid substitutions. In some embodiments, the anti-LAG-3 sdAb comprises: (1) a CDR1 comprising the amino acid sequence of SEQ ID NO: 53; a CDR2 comprising the amino acid sequence of SEQ ID NO: 129; and a CDR3 comprising the amino acid sequence of SEQ ID NO: 205; (2) a CDR1 comprising the amino acid sequence of SEQ ID NO: 60; a CDR2 comprising the amino acid sequence of SEQ ID NO: 136; and a CDR3 comprising the amino acid sequence of SEQ ID NO: 212; (3) a CDR1 comprising the amino acid sequence of SEQ ID NO: 69; a CDR2 comprising the amino acid sequence of SEQ ID NO: 145; and a CDR3 comprising the amino acid sequence of SEQ ID NO: 221; or (4) a CDR1 comprising the amino acid sequence of SEQ ID NO: 71; a CDR2 comprising the amino acid sequence of SEQ ID NO: 147; and

a CDR3 comprising the amino acid sequence of SEQ ID NO: 223. In some embodiments, the anti-LAG-3 sdAb comprises a V_HH domain comprising the amino acid sequence of any one of SEQ ID NO: 274-311. In some embodiments, the anti-LAG-3 sdAb comprises a V_HH domain comprising the amino acid sequence of any one of SEQ ID NO: 288, 295, 304 or 306. In some embodiments, V_H and V_L form an antigen binding site that specifically binds PD-1. In some embodiments, the V_H and V_L domains are derived from pembrolizumab or PD1-BM-min. In some embodiments, the V_H comprises HC-CDR1, HC-CDR2, and HC-CDR3 of the amino acid sequence of SEQ ID NO: 356, and V_L comprises LC-CDR1, LC-CDR2, and LC-CDR3 of the amino acid sequence of SEQ ID NO: 357. In some embodiments, the V_H comprises HC-CDR1, HC-CDR2, and HC-CDR3 of the amino acid sequence of SEQ ID NO: 373, and V_L comprises LC-CDR1, LC-CDR2, and LC-CDR3 of the amino acid sequence of SEQ ID NO: 374. In some embodiments, the C_H3 and anti-LAG-3 sdAb are fused to each other via a peptide linker, such as a peptide linker comprising the amino acid sequence of any one of SEQ ID NOs: 352-353 and 366-372. In some embodiments, the C_H2 and C_H3 domains are derived from IgG1 Fc, effectorless IgG1 Fc, IgG2 Fc, IgG4 Fc, or IgG4 Fc (S228P).

[0239] In some embodiments, the anti-LAG-3 construct comprises two identical copies of the first polypeptide and two identical copies of the second polypeptide. In some embodiments, there is provided an anti-LAG-3 construct (*e.g.*, BABP) comprising a first polypeptide comprising the amino acid sequence of SEQ ID NO: 358, and a second polypeptide comprising the amino acid sequence of SEQ ID NO: 359. In some embodiments, there is provided an anti-LAG-3 construct (*e.g.*, BABP) comprising a first polypeptide comprising the amino acid sequence of SEQ ID NO: 360, and a second polypeptide comprising the amino acid sequence of SEQ ID NO: 361. In some embodiments, there is provided an anti-LAG-3 construct (*e.g.*, BABP) comprising a first polypeptide comprising the amino acid sequence of SEQ ID NO: 362, and a second polypeptide comprising the amino acid sequence of SEQ ID NO: 363. In some embodiments, there is provided an anti-LAG-3 construct (*e.g.*, BABP) comprising a first polypeptide comprising the amino acid sequence of SEQ ID NO: 364, and a second polypeptide comprising the amino acid sequence of SEQ ID NO: 365. In some embodiments, there is provided an anti-LAG-3 construct (*e.g.*, BABP) comprising a first polypeptide comprising the amino acid sequence of SEQ ID NO: 375, and a second polypeptide comprising the amino acid sequence of SEQ ID NO: 376. In some embodiments, there is provided an anti-LAG-3 construct (*e.g.*, BABP) comprising a first polypeptide comprising the amino acid sequence of SEQ ID NO: 377, and a second polypeptide comprising the amino acid sequence of SEQ ID NO: 378. In some embodiments, there is provided an anti-LAG-3 construct (*e.g.*, BABP) comprising a first polypeptide comprising the amino acid sequence of SEQ ID NO: 379, and a second polypeptide comprising the amino acid sequence of SEQ ID NO: 380. In

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anti-LAG-3 construct (*e.g.*, BABP) comprising a first polypeptide comprising the amino acid sequence of SEQ ID NO: 435, and a second polypeptide comprising the amino acid sequence of SEQ ID NO: 436. In some embodiments, there is provided an anti-LAG-3 construct (*e.g.*, BABP) comprising a first polypeptide comprising the amino acid sequence of SEQ ID NO: 437, and a second polypeptide comprising the amino acid sequence of SEQ ID NO: 438. In some embodiments, there is provided an anti-LAG-3 construct (*e.g.*, BABP) comprising a first polypeptide comprising the amino acid sequence of SEQ ID NO: 439, and a second polypeptide comprising the amino acid sequence of SEQ ID NO: 440. In some embodiments, there is provided an anti-LAG-3 construct (*e.g.*, BABP) comprising a first polypeptide comprising the amino acid sequence of SEQ ID NO: 441, and a second polypeptide comprising the amino acid sequence of SEQ ID NO: 442. In some embodiments, there is provided an anti-LAG-3 construct (*e.g.*, BABP) comprising a first polypeptide comprising the amino acid sequence of SEQ ID NO: 443, and a second polypeptide comprising the amino acid sequence of SEQ ID NO: 444. In some embodiments, there is provided an anti-LAG-3 construct (*e.g.*, BABP) comprising a first polypeptide comprising the amino acid sequence of SEQ ID NO: 445, and a second polypeptide comprising the amino acid sequence of SEQ ID NO: 446. In some embodiments, there is provided a polypeptide comprising the amino acid sequence of any one of SEQ ID NOs: 358-365 and 375-446.

Peptide linkers

[0240] The various domains and components, such as anti-LAG-3 sdAbs, Fc fragment, the first antigen binding portion, and the second antigen binding portion in the anti-LAG3 construct may be fused to each other via a suitable linker, such as a peptide linker. The length, the degree of flexibility and/or other properties of the peptide linker(s) used in the anti-LAG-3 construct may have some influence on properties, including but not limited to the affinity, specificity or avidity for one or more particular antigens or epitopes. For example, longer peptide linkers may be selected to ensure that two adjacent domains do not sterically interfere with one another. In some embodiment, a peptide linker comprises flexible residues (such as glycine and serine) so that the adjacent domains are free to move relative to each other. For example, a glycine-serine doublet can be a suitable peptide linker.

[0241] The peptide linker can be of any suitable length. In some embodiments, the peptide linker is at least about any of 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 25, 30, 35, 40, 50, 75, 100 or more amino acids long. In some embodiments, the peptide linker is no more than about any of 100, 75, 50, 40, 35, 30, 25, 20, 19, 18, 17, 16, 15, 14, 13, 12, 11, 10, 9, 8, 7, 6, 5 or fewer amino acids long. In some embodiments, the length of the peptide linker is any of about 1 amino acid to about 10 amino acids, about 1 amino acid to about 20 amino acids, about 1 amino acid to about 30 amino acids, about 5 amino acids to about 15 amino acids, about 10 amino acids to about 25 amino acids, about 5 amino acids to

about 30 amino acids, about 10 amino acids to about 30 amino acids long, about 30 amino acids to about 50 amino acids, about 50 amino acids to about 100 amino acids, or about 1 amino acid to about 100 amino acids.

[0242] The peptide linker may have a naturally occurring sequence, or a non-naturally occurring sequence. For example, a sequence derived from the hinge region of heavy chain only antibodies may be used as the linker. *See*, for example, WO1996/34103. In some embodiments, the peptide linker is a human IgG1 hinge (SEQ ID NO: 352). In some embodiments, the peptide linker is a mutated human IgG1 hinge (SEQ ID NO: 353). In some embodiments, the peptide linker is a human IgG4 hinge. In some embodiments, the peptide linker is a hIgG2 hinge. In some embodiments, the peptide linker is a flexible linker. Exemplary flexible linkers include glycine polymers (G)_n (SEQ ID NO: 368), glycine-serine polymers (including, for example, (GS)_n (SEQ ID NO: 369), (GSGGS)_n (SEQ ID NO: 370), (GGGS)_n (SEQ ID NO: 371), and (GGGGS)_n (SEQ ID NO: 372), where n is an integer of at least one), glycine-alanine polymers, alanine-serine polymers, and other flexible linkers known in the art. In some embodiments, the peptide linker comprises the amino acid sequence of SEQ ID NO: 366 (GGGGSGGGGS) or 367 (GGGGSGGGSGGGGS).

[0243] In some embodiments, the N-terminus or the C-terminus of the anti-LAG-3 sdAb may be fused to a peptide sequence, which may have any of the sequence features described herein for peptide linkers.

(III) Antibody variants

[0244] In some embodiments, amino acid sequence variants of the anti-LAG-3 construct (*e.g.*, anti-LAG-3 sdAb, anti-LAG-3 sdAb-Fc fusion protein (*e.g.*, HCAb), anti-LAG-3 MABP/BABP) provided herein are contemplated. For example, it may be desirable to improve the binding affinity and/or other biological properties of the antibody. Amino acid sequence variants of an antibody may be prepared by introducing appropriate modifications into the nucleic acid sequence encoding the antibody, or by peptide synthesis. Such modifications include, for example, deletions from, and/or insertions into and/or substitutions of residues within the amino acid sequences of the antibody. Any combination of deletion, insertion, and substitution can be made to arrive at the final construct, provided that the final construct possesses the desired characteristics, *e.g.*, antigen-binding.

a) Substitution, insertion, deletion and variants

[0245] In some embodiments, antibody variants having one or more amino acid substitutions are provided. Sites of interest for substitutional mutagenesis include the HVRs (or CDRs) and FRs. Conservative substitutions are shown in Table 2 under the heading of “Preferred substitutions.” More substantial changes are provided in Table 2 under the heading of “exemplary substitutions,” and as further

described below in reference to amino acid side chain classes. Amino acid substitutions may be introduced into an antibody of interest and the products screened for a desired activity, *e.g.*, retained/improved antigen binding, decreased immunogenicity, or improved ADCC or CDC.

Table 2. Amino acid substitutions

Original Residue	Exemplary Substitutions	Preferred Substitutions
Ala (A)	Val; Leu; Ile	Val
Arg (R)	Lys; Gln; Asn	Lys
Asn (N)	Gln; His; Asp, Lys; Arg	Gln
Asp (D)	Glu; Asn	Glu
Cys (C)	Ser; Ala	Ser
Gln (Q)	Asn; Glu	Asn
Glu (E)	Asp; Gln	Asp
Gly (G)	Ala	Ala
His (H)	Asn; Gln; Lys; Arg	Arg
Ile (I)	Leu; Val; Met; Ala; Phe; Norleucine	Leu
Leu (L)	Norleucine; Ile; Val; Met; Ala; Phe	Ile
Lys (K)	Arg; Gln; Asn	Arg
Met (M)	Leu; Phe; Ile	Leu
Phe (F)	Trp; Leu; Val; Ile; Ala; Tyr	Tyr
Pro (P)	Ala	Ala
Ser (S)	Thr	Thr
Thr (T)	Val; Ser	Ser
Trp (W)	Tyr; Phe	Tyr
Tyr (Y)	Trp; Phe; Thr; Ser	Phe
Val (V)	Ile; Leu; Met; Phe; Ala; Norleucine	Leu

[0246] Amino acids may be grouped according to common side-chain properties: (1) hydrophobic: Norleucine, Met, Ala, Val, Leu, Ile; (2) neutral hydrophilic: Cys, Ser, Thr, Asn, Gln; (3) acidic: Asp, Glu; (4) basic: His, Lys, Arg; (5) residues that influence chain orientation: Gly, Pro; and (6) aromatic: Trp, Tyr, Phe.

[0247] Non-conservative substitutions will entail exchanging a member of one of these classes for another class.

[0248] One type of substitutional variant involves substituting one or more hypervariable region residues of a parent antibody (*e.g.*, a humanized or human antibody). Generally, the resulting variant(s) selected for further study will have modifications (*e.g.*, improvements) in certain biological properties (*e.g.*, increased affinity, reduced immunogenicity) relative to the parent antibody and/or will have

substantially retained certain biological properties of the parent antibody. An exemplary substitutional variant is an affinity matured antibody, which may be conveniently generated, *e.g.*, using phage display-based affinity maturation techniques such as those described herein. Briefly, one or more HVR residues are mutated and the variant antibodies displayed on phage and screened for a particular biological activity (*e.g.* binding affinity).

[0249] Alterations (*e.g.*, substitutions) may be made in HVRs, *e.g.*, to improve antibody affinity. Such alterations may be made in HVR “hotspots,” *i.e.*, residues encoded by codons that undergo mutation at high frequency during the somatic maturation process (*see, e.g.*, Chowdhury, *Methods Mol. Biol.* 207:179-196 (2008)), and/or SDRs (a-CDRs), with the resulting variant VH or VL being tested for binding affinity. Affinity maturation by constructing and reselecting from secondary libraries has been described, *e.g.*, in Hoogenboom et al. in *Methods in Molecular Biology* 178:1-37 (O’Brien et al., ed., Human Press, Totowa, NJ, (2001)). In some embodiments of affinity maturation, diversity is introduced into the variable genes chosen for maturation by any of a variety of methods (*e.g.*, error-prone PCR, chain shuffling, or oligonucleotide-directed mutagenesis). A secondary library is then created. The library is then screened to identify any antibody variants with the desired affinity. Another method to introduce diversity involves HVR-directed approaches, in which several HVR residues (*e.g.*, 4-6 residues at a time) are randomized. HVR residues involved in antigen binding may be specifically identified, *e.g.*, using alanine scanning mutagenesis or modeling. CDR-H3 and CDR-L3 in particular are often targeted.

[0250] In some embodiments, substitutions, insertions, or deletions may occur within one or more HVRs so long as such alterations do not substantially reduce the ability of the antibody to bind antigen. For example, conservative alterations (*e.g.*, conservative substitutions as provided herein) that do not substantially reduce binding affinity may be made in HVRs. Such alterations may be outside of HVR “hotspots” or CDRs. In some embodiments of the variant V_HH sequences provided above, each HVR either is unaltered, or contains no more than one, two or three amino acid substitutions.

[0251] A useful method for identification of residues or regions of an antibody that may be targeted for mutagenesis is called “alanine scanning mutagenesis” as described by Cunningham and Wells (1989) *Science*, 244:1081-1085. In this method, a residue or group of target residues (*e.g.*, charged residues such as Arg, Asp, His, Lys, and Glu) are identified and replaced by a neutral or negatively charged amino acid (*e.g.*, alanine or polyalanine) to determine whether the interaction of the antibody with antigen is affected. Further substitutions may be introduced at the amino acid locations demonstrating functional sensitivity to the initial substitutions. Alternatively, or additionally, a crystal structure of an antigen-antibody complex to identify contact points between the antibody and antigen. Such contact residues and

neighboring residues may be targeted or eliminated as candidates for substitution. Variants may be screened to determine whether they contain the desired properties.

[0252] Amino acid sequence insertions include amino- and/or carboxyl-terminal fusions ranging in length from one residue to polypeptides containing a hundred or more residues, as well as intrasequence insertions of single or multiple amino acid residues. Examples of terminal insertions include an antibody with an N-terminal methionyl residue. Other insertional variants of the antibody molecule include the fusion to the N- or C-terminus of the antibody to an enzyme (*e.g.*, for ADEPT) or a polypeptide which increases the serum half-life of the antibody.

b) Glycosylation variants

[0253] In some embodiments, an isolated anti-LAG-3 construct provided herein is altered to increase or decrease the extent to which the construct is glycosylated. Addition or deletion of glycosylation sites to an antibody may be conveniently accomplished by altering the amino acid sequence such that one or more glycosylation sites is created or removed.

[0254] Where the anti-LAG-3 construct comprises an Fc region (*e.g.*, anti-LAG-3 sdAb-Fc fusion protein (*e.g.*, HCAb), LAG-3× PD-1 MABP), the carbohydrate attached thereto may be altered. Native antibodies produced by mammalian cells typically comprise a branched, biantennary oligosaccharide that is generally attached by an N-linkage to Asn297 of the C_H2 domain of the Fc region. *See, e.g.*, Wright et al. *TIBTECH* 15:26-32 (1997). The oligosaccharide may include various carbohydrates, *e.g.*, mannose, N-acetyl glucosamine (GlcNAc), galactose, and sialic acid, as well as a fucose attached to a GlcNAc in the “stem” of the biantennary oligosaccharide structure. In some embodiments, modifications of the oligosaccharide in an anti-LAG-3 construct of the present application may be made in order to create antibody variants with certain improved properties.

[0255] In some embodiments, anti-LAG-3 construct antibody variants are provided having a carbohydrate structure that lacks fucose attached (directly or indirectly) to an Fc region. For example, the amount of fucose in such antibody may be from 1% to 80%, from 1% to 65%, from 5% to 65% or from 20% to 40%. The amount of fucose is determined by calculating the average amount of fucose within the sugar chain at Asn297, relative to the sum of all glycostructures attached to Asn 297 (*e.g.*, complex, hybrid and high mannose structures) as measured by MALDI-TOF mass spectrometry, as described in WO 2008/077546, for example. Asn297 refers to the asparagine residue located at about position 297 in the Fc region (EU numbering of Fc region residues); however, Asn297 may also be located about ± 3 amino acids upstream or downstream of position 297, *i.e.*, between positions 294 and 300, due to minor sequence variations in antibodies. Such fucosylation variants may have improved ADCC function. *See,*

e.g., US Patent Publication Nos. US 2003/0157108 (Presta, L.); US 2004/0093621 (Kyowa Hakko Kogyo Co., Ltd). Examples of publications related to “defucosylated” or “fucose-deficient” antibody variants include: US 2003/0157108; WO 2000/61739; WO 2001/29246; US 2003/0115614; US 2002/0164328; US 2004/0093621; US 2004/0132140; US 2004/0110704; US 2004/0110282; US 2004/0109865; WO 2003/085119; WO 2003/084570; WO 2005/035586; WO 2005/035778; WO2005/053742; WO2002/031140; Okazaki et al. *J. Mol. Biol.* 336:1239-1249 (2004); Yamane-Ohnuki et al. *Biotech. Bioeng.* 87: 614 (2004). Examples of cell lines capable of producing defucosylated antibodies include Lec13 CHO cells deficient in protein fucosylation (Ripka et al. *Arch. Biochem. Biophys.* 249:533-545 (1986); US Patent Application No. US 2003/0157108 A1, Presta, L; and WO 2004/056312 A1, Adams *et al.*, especially at Example 11), and knockout cell lines, such as alpha-1,6-fucosyltransferase gene, *FUT8*, knockout CHO cells (*see, e.g.*, Yamane-Ohnuki et al. *Biotech. Bioeng.* 87: 614 (2004); Kanda, Y. et al., *Biotechnol. Bioeng.*, 94(4):680-688 (2006); and WO2003/085107).

[0256] Anti-LAG-3 construct variants are further provided with bisected oligosaccharides, *e.g.*, in which a biantennary oligosaccharide attached to the Fc region of the antibody is bisected by GlcNAc. Such antibody variants may have reduced fucosylation and/or improved ADCC function. Examples of such antibody variants are described, *e.g.*, in WO 2003/011878 (Jean-Mairet et al.); US Patent No. 6,602,684 (Umana et al.); and US 2005/0123546 (Umana *et al.*). Antibody variants with at least one galactose residue in the oligosaccharide attached to the Fc region are also provided. Such antibody variants may have improved CDC function. Such antibody variants are described, *e.g.*, in WO 1997/30087 (Patel et al.); WO 1998/58964 (Raju, S.); and WO 1999/22764 (Raju, S.).

c) Fc region variants

[0257] In some embodiments, one or more amino acid modifications may be introduced into the Fc region of the anti-LAG-3 constructs provided herein (*e.g.*, anti-LAG-3 HCAs, or anti-LAG-3 MABPs), thereby generating an Fc region variant. The Fc region variant may comprise a human Fc region sequence (*e.g.*, a human IgG1, IgG2, IgG3 or IgG4 Fc region) comprising an amino acid modification (*e.g.* a substitution) at one or more amino acid positions.

[0258] In some embodiments, the present application contemplates an anti-PD-1 construct (*e.g.*, anti-LAG-3 HCAb, or anti-LAG-3 MABP) variant that possesses some but not all effector functions, which make it a desirable candidate for applications in which the half-life of the anti-LAG-3 construct *in vivo* is important yet certain effector functions (such as complement and ADCC) are unnecessary or deleterious. *In vitro* and/or *in vivo* cytotoxicity assays can be conducted to confirm the reduction/depletion of CDC and/or ADCC activities. For example, Fc receptor (FcR) binding assays can be conducted to ensure that

the antibody lacks Fc γ R binding (hence likely lacking ADCC activity), but retains FcRn binding ability. The primary cells for mediating ADCC, NK cells, express Fc γ RIII only, whereas monocytes express Fc γ RI, Fc γ RII and Fc γ RIII. FcR expression on hematopoietic cells is summarized in Table 2 on page 464 of Ravetch and Kinet, *Annu. Rev. Immunol.* 9:457-492 (1991). Non-limiting examples of *in vitro* assays to assess ADCC activity of a molecule of interest is described in U.S. Patent No. 5,500,362 (*see, e.g.* Hellstrom, I. et al. *Proc. Nat'l Acad. Sci. USA* 83:7059-7063 (1986)) and Hellstrom, I et al., *Proc. Nat'l Acad. Sci. USA* 82:1499-1502 (1985); 5,821,337 (*see* Bruggemann, M. et al., *J. Exp. Med.* 166:1351-1361 (1987)). Alternatively, non-radioactive assays methods may be employed (*see, for example*, ACTITM non-radioactive cytotoxicity assay for flow cytometry (CellTechnology, Inc. Mountain View, CA; and CytoTox 96® non-radioactive cytotoxicity assay (Promega, Madison, WI). Useful effector cells for such assays include peripheral blood mononuclear cells (PBMC) and Natural Killer (NK) cells. Alternatively, or additionally, ADCC activity of the molecule of interest may be assessed *in vivo, e.g.,* in an animal model such as that disclosed in Clynes et al. *Proc. Nat'l Acad. Sci. USA* 95:652-656 (1998). C1q binding assays may also be carried out to confirm that the antibody is unable to bind C1q and hence lacks CDC activity. *See, e.g.,* C1q and C3c binding ELISA in WO 2006/029879 and WO 2005/100402. To assess complement activation, a CDC assay may be performed (*see, for example*, Gazzano-Santoro *et al., J. Immunol. Methods* 202:163 (1996); Cragg, M.S. et al., *Blood* 101:1045-1052 (2003); and Cragg, M.S. and M.J. Glennie, *Blood* 103:2738-2743 (2004)). FcRn binding and *in vivo* clearance/half-life determinations can also be performed using methods known in the art (*see, e.g.,* Petkova, S.B. et al., *Int'l. Immunol.* 18(12):1759-1769 (2006)).

[0259] Antibodies with reduced effector function include those with substitution of one or more of Fc region residues 238, 265, 269, 270, 297, 327 and 329 (U.S. Patent No. 6,737,056). Such Fc mutants include Fc mutants with substitutions at two or more of amino acid positions 265, 269, 270, 297 and 327, including the so-called "DANA" Fc mutant with substitution of residues 265 and 297 to alanine (US Patent No. 7,332,581).

[0260] Certain antibody variants with improved or diminished binding to FcRs are described. (*See, e.g.,* U.S. Patent No. 6,737,056; WO 2004/056312, and Shields et al., *J. Biol. Chem.* 9(2): 6591-6604 (2001).)

[0261] In some embodiments, an anti-LAG-3 construct variant comprises an Fc region with one or more amino acid substitutions which improve ADCC, *e.g.,* substitutions at positions 298, 333, and/or 334 of the Fc region (EU numbering of residues).

[0262] In some embodiments, alterations are made in the Fc region that result in altered (*i.e.*, either improved or diminished) C1q binding and/or Complement Dependent Cytotoxicity (CDC), *e.g.*, as described in US Patent No. 6,194,551, WO 99/51642, and Idusogie et al. *J. Immunol.* 164: 4178-4184 (2000).

[0263] In some embodiments, there is provided an anti-LAG-3 construct (*e.g.*, anti-PD-1 sdAb-Fc fusion protein (*e.g.*, anti-LAG-3 HCAb, or anti-LAG-3 MABP) variant comprising a variant Fc region comprising one or more amino acid substitutions which increase half-life and/or improve binding to the neonatal Fc receptor (FcRn). Antibodies with increased half-lives and improved binding to the neonatal Fc receptor (FcRn), which is responsible for the transfer of maternal IgGs to the fetus (Guyer et al., *J. Immunol.* 117:587 (1976) and Kim et al., *J. Immunol.* 24:249 (1994)), are described in US2005/0014934A1 (Hinton et al.). Those antibodies comprise an Fc region with one or more substitutions therein which improve binding of the Fc region to FcRn. Such Fc variants include those with substitutions at one or more of Fc region residues, *e.g.*, substitution of Fc region residue 434 (US Patent No. 7,371,826).

[0264] See also Duncan & Winter, *Nature* 322:738-40 (1988); U.S. Patent No. 5,648,260; U.S. Patent No. 5,624,821; and WO 94/29351 concerning other examples of Fc region variants.

[0265] Anti-LAG-3 constructs (*e.g.*, anti-LAG-3 HCABs or anti-LAG-3 MABPs) comprising any of the Fc variants described herein, or combinations thereof, are contemplated.

d) Cysteine engineered antibody variants

[0266] In some embodiments, it may be desirable to create cysteine engineered anti-LAG-3 constructs, *e.g.*, “thioMAbs,” in which one or more residues of an antibody are substituted with cysteine residues. In particular embodiments, the substituted residues occur at accessible sites of the antibody. By substituting those residues with cysteine, reactive thiol groups are thereby positioned at accessible sites of the antibody and may be used to conjugate the antibody to other moieties, such as drug moieties or linker-drug moieties, to create an immunoconjugate, as described further herein. In some embodiments, any one or more of the following residues may be substituted with cysteine: A118 (EU numbering) of the heavy chain; and S400 (EU numbering) of the heavy chain Fc region. Cysteine engineered anti-LAG-3 constructs may be generated as described, *e.g.*, in U.S. Patent No. 7,521,541.

e) Antibody derivatives

[0267] In some embodiments, an anti-LAG-3 construct provided herein may be further modified to comprise additional nonproteinaceous moieties that are known in the art and readily available. The

moieties suitable for derivatization of the antibody include but are not limited to water soluble polymers. Non-limiting examples of water soluble polymers include, but are not limited to, polyethylene glycol (PEG), copolymers of ethylene glycol/propylene glycol, carboxymethylcellulose, dextran, polyvinyl alcohol, polyvinyl pyrrolidone, poly-1, 3-dioxolane, poly-1,3,6-trioxane, ethylene/maleic anhydride copolymer, polyaminoacids (either homopolymers or random copolymers), and dextran or poly(n-vinyl pyrrolidone)polyethylene glycol, propylene glycol homopolymers, polypropylene oxide/ethylene oxide co-polymers, polyoxyethylated polyols (*e.g.*, glycerol), polyvinyl alcohol, and mixtures thereof. Polyethylene glycol propionaldehyde may have advantages in manufacturing due to its stability in water. The polymer may be of any molecular weight, and may be branched or unbranched. The number of polymers attached to the antibody may vary, and if more than one polymer are attached, they can be the same or different molecules. In general, the number and/or type of polymers used for derivatization can be determined based on considerations including, but not limited to, the particular properties or functions of the antibody to be improved, whether the antibody derivative will be used in a therapy under defined conditions, *etc.*

[0268] In some embodiments, conjugates of an anti-LAG-3 construct and nonproteinaceous moiety that may be selectively heated by exposure to radiation are provided. In some embodiments, the nonproteinaceous moiety is a carbon nanotube (Kam et al., *Proc. Natl. Acad. Sci. USA* 102: 11600-11605 (2005)). The radiation may be of any wavelength, and includes, but is not limited to, wavelengths that do not harm ordinary cells, but which heat the nonproteinaceous moiety to a temperature at which cells proximal to the antibody-nonproteinaceous moiety are killed.

[0269] In some embodiments, an anti-LAG-3 construct provided herein (*e.g.*, anti-LAG-3 HCAb, or anti-LAG-3 MABP) may be further modified to comprise one or more biologically active protein, polypeptides or fragments thereof. “Bioactive” or “biologically active”, as used herein interchangeably, means showing biological activity in the body to carry out a specific function. For example, it may mean the combination with a particular biomolecule such as protein, DNA, *etc.*, and then promotion or inhibition of the activity of such biomolecule. In some embodiments, the bioactive protein or fragments thereof include proteins and polypeptides that are administered to patients as the active drug substance for prevention of or treatment of a disease or condition, as well as proteins and polypeptides that are used for diagnostic purposes, such as enzymes used in diagnostic tests or *in vitro* assays, as well as proteins and polypeptides that are administered to a patient to prevent a disease such as a vaccine. In some embodiments, the bioactive protein or fragments thereof have immune-stimulatory/immune-regulatory, membrane transport, or enzymatic activities. In some embodiments, the biologically active protein, polypeptides or fragments thereof is an enzyme, a hormone, a growth factor, a cytokine, or a mixture

thereof. In some embodiments, the biologically active protein, polypeptides or fragments can specifically recognize a target peptide (such as antigen, or other proteins).

[0270] In some embodiments, the bioactive protein or fragments thereof that can be comprised within the anti-LAG-3 construct described herein is a protein-binding protein. In some embodiments, the bioactive protein or fragments thereof that can be comprised within the anti-LAG-3 construct described herein is an antibody mimetics, which are small engineered proteins comprising antigen-binding domains reminiscent of antibodies (Geering and Fussenegger, Trends Biotechnol., 33(2):65-79, 2015). These molecules are derived from existing human scaffold proteins and comprise a single polypeptide. Exemplary antibody mimetics that can be comprised within the anti-LAG-3 construct described herein can be, but are not limited to, a Designed ankyrin repeat protein (DARPin; comprising 3-5 fully synthetic ankyrin repeats flanked by N- and C-terminal Cap domains), an avidity multimer (avimer; a high-affinity protein comprising multiple A domains, each domain with low affinity for a target), or an Anticalin (based on the scaffold of lipocalins, with four accessible loops, the sequence of each can be randomized). In some embodiments, the bioactive protein or fragments thereof that can be comprised within the anti-LAG-3 construct described herein is an Armadillo repeat protein (*e.g.*, β -catenin, α -importin, plakoglobin, adenomatous polyposis coli (APC)), which comprises armadillo repeat units (characteristic, repetitive amino acid sequence of about 40 residues in length). Each Armadillo repeat is composed of a pair of alpha helices that form a hairpin structure. Multiple copies of the repeat form what is known as an alpha solenoid structure. Armadillo repeat proteins are able to bind different types of peptides, relying on a constant way of binding of the peptide backbone without requiring specific conserved side chains or interactions with free N- or C-termini of a peptide. The possibility of recognizing a peptide residue by residue, combined with the intrinsic modularity of a repeat protein, makes the armadillo repeat proteins promising candidates for the design of a generic scaffold for peptide binding.

[0271] In some embodiments, the biologically active protein or fragments thereof that can be comprised within the anti-LAG-3 construct described herein is a ligand, such as lymphokines and cellular factors which interact with specific cellular receptor. Lymphokines are low molecular weight proteins which are secreted by T cells when antigens or lectins stimulate T cell growth.

III. Pharmaceutical compositions

[0272] Further provided by the present application are pharmaceutical compositions comprising any one of the anti-LAG-3 constructs described herein (*e.g.*, anti-LAG-3 sdAb, anti-LAG-3 HCAb or anti-LAG-3 MABP), and optionally a pharmaceutically acceptable carrier. Pharmaceutical compositions can

be prepared by mixing an anti-LAG-3 construct described herein having the desired degree of purity with optional pharmaceutically acceptable carriers, excipients or stabilizers (Remington's Pharmaceutical Sciences 16th edition, Osol, A. Ed. (1980)), in the form of lyophilized formulations or aqueous solutions.

[0273] The pharmaceutical composition is preferably to be stable, in which the anti-LAG-3 construct here essentially retains its physical and chemical stability and integrity upon storage. Various analytical techniques for measuring protein stability are available in the art and are reviewed in *Peptide and Protein Drug Delivery*, 247-301, Vincent Lee Ed., Marcel Dekker, Inc., New York, N.Y., Pubs. (1991) and Jones, A. *Adv. Drug Delivery Rev.* 10: 29-90 (1993). Stability can be measured at a selected temperature for a selected time period. For rapid screening, the formulation may be kept at 40°C for 2 weeks to 1 month, at which time stability is measured. Where the formulation is to be stored at 2-8°C, generally the formulation should be stable at 30°C or 40°C for at least 1 month, and/or stable at 2-8°C for at least 2 years. Where the formulation is to be stored at 30°C, generally the formulation should be stable for at least 2 years at 30°C, and/or stable at 40°C for at least 6 months. For example, the extent of aggregation during storage can be used as an indicator of protein stability. In some embodiments, the stable formulation of anti-LAG-3 construct described herein may comprise less than about 10% (preferably less than about 5%) of the anti-LAG-3 construct present as an aggregate in the formulation.

[0274] Acceptable carriers, excipients, or stabilizers are nontoxic to recipients at the dosages and concentrations employed, and include buffers, antioxidants including ascorbic acid, methionine, Vitamin E, sodium metabisulfite; preservatives, isotonicifiers (e.g. sodium chloride), stabilizers, metal complexes (e.g. Zn-protein complexes); chelating agents such as EDTA and/or non-ionic surfactants.

[0275] Examples of physiologically acceptable carriers 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) polypeptide; proteins, such as serum albumin, gelatin, or immunoglobulins; hydrophilic polymers such as polyvinylpyrrolidone; amino acids such as glycine, glutamine, asparagine, 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 counterions such as sodium; metal complexes (e.g. Zn-protein complexes); and/or nonionic surfactants such as TWEEN™, polyethylene glycol (PEG), and PLURONICS™ or polyethylene glycol (PEG).

[0276] Buffers are used to control the pH in a range which optimizes the therapeutic effectiveness, especially if stability is pH dependent. Buffers are preferably present at concentrations ranging from about 50 mM to about 250 mM. Suitable buffering agents for use in the present application include both organic and inorganic acids and salts thereof. For example, citrate, phosphate, succinate, tartrate, fumarate, gluconate, oxalate, lactate, acetate. Additionally, buffers may comprise histidine and trimethylamine salts such as Tris.

[0277] Preservatives are added to retard microbial growth, and are typically present in a range from 0.2%-1.0% (w/v). The addition of a preservative may, for example, facilitate the production of a multi-use (multiple-dose) formulation. Suitable preservatives for use in the present application include octadecyldimethylbenzyl ammonium chloride; hexamethonium chloride; benzalkonium halides (*e.g.*, chloride, bromide, iodide), benzethonium chloride; thimerosal, phenol, butyl or benzyl alcohol; alkyl parabens such as methyl or propyl paraben; catechol; resorcinol; cyclohexanol, 3-pentanol, and m-cresol.

[0278] Tonicity agents, sometimes known as “stabilizers” are present to adjust or maintain the tonicity of liquid in a composition. When used with large, charged biomolecules such as proteins and antibodies, they are often termed “stabilizers” because they can interact with the charged groups of the amino acid side chains, thereby lessening the potential for inter and intra-molecular interactions. Tonicity agents can be present in any amount between 0.1% to 25% by weight, preferably 1% to 5%, taking into account the relative amounts of the other ingredients. Preferred tonicity agents include polyhydric sugar alcohols, preferably trihydric or higher sugar alcohols, such as glycerin, erythritol, arabitol, xylitol, sorbitol and mannitol.

[0279] Additional excipients include agents which can serve as one or more of the following: (1) bulking agents, (2) solubility enhancers, (3) stabilizers and (4) agents preventing denaturation or adherence to the container wall. Such excipients include: polyhydric sugar alcohols (enumerated above); amino acids such as alanine, glycine, glutamine, asparagine, histidine, arginine, lysine, ornithine, leucine, 2-phenylalanine, glutamic acid, threonine, etc.; organic sugars or sugar alcohols such as sucrose, lactose, lactitol, trehalose, stachyose, mannose, sorbose, xylose, ribose, ribitol, myoinisitol, myoinisitol, galactose, galactitol, glycerol, cyclitols (*e.g.*, inositol), polyethylene glycol; sulfur containing reducing agents, such as urea, glutathione, thiocetic acid, sodium thioglycolate, thioglycerol, α -monothioglycerol and sodium thio sulfate; low molecular weight proteins such as human serum albumin, bovine serum albumin, gelatin or other immunoglobulins; hydrophilic polymers such as polyvinylpyrrolidone; monosaccharides (*e.g.*, xylose, mannose, fructose, glucose; disaccharides (*e.g.*, lactose, maltose, sucrose); trisaccharides such as raffinose; and polysaccharides such as dextrin or dextran.

[0280] Non-ionic surfactants or detergents (also known as “wetting agents”) are present to help solubilize the therapeutic agent as well as to protect the therapeutic protein against agitation-induced aggregation, which also permits the formulation to be exposed to shear surface stress without causing denaturation of the active therapeutic protein or antibody. Non-ionic surfactants are present in a range of about 0.05 mg/ml to about 1.0 mg/ml, preferably about 0.07 mg/ml to about 0.2 mg/ml.

[0281] Suitable non-ionic surfactants include polysorbates (20, 40, 60, 65, 80, etc.), polyoxamers (184, 188, etc.), PLURONIC® polyols, TRITON®, polyoxyethylene sorbitan monoethers (TWEEN®-20, TWEEN®-80, etc.), laurmacrogol 400, polyoxyl 40 stearate, polyoxyethylene hydrogenated castor oil 10, 50 and 60, glycerol monostearate, sucrose fatty acid ester, methyl cellulose and carboxymethyl cellulose. Anionic detergents that can be used include sodium lauryl sulfate, dioctyl sodium sulfosuccinate and dioctyl sodium sulfonate. Cationic detergents include benzalkonium chloride or benzethonium chloride.

[0282] In order for the pharmaceutical compositions to be used for *in vivo* administration, they must be sterile. The pharmaceutical composition may be rendered sterile by filtration through sterile filtration membranes. The pharmaceutical compositions herein generally are placed into a container having a sterile access port, for example, an intravenous solution bag or vial having a stopper pierceable by a hypodermic injection needle.

[0283] The route of administration is in accordance with known and accepted methods, such as by single or multiple bolus or infusion over a long period of time in a suitable manner, *e.g.*, injection or infusion by subcutaneous, intravenous, intraperitoneal, intramuscular, intra-arterial, intralesional or intraarticular routes, topical administration, inhalation or by sustained release or extended-release means.

[0284] Sustained-release preparations may be prepared. Suitable examples of sustained-release preparations include semi-permeable matrices of solid hydrophobic polymers containing the antagonist, which matrices are in the form of shaped articles, *e.g.* films, or microcapsules. Examples of sustained-release matrices include polyesters, hydrogels (for example, poly(2-hydroxyethyl-methacrylate), or poly(vinylalcohol)), polylactides (U.S. Pat. No. 3,773,919), copolymers of L-glutamic acid and ethyl-L-glutamate, non-degradable ethylene-vinyl acetate, degradable lactic acid-glycolic acid copolymers such as the LUPRON DEPOT™ (injectable microspheres composed of lactic acid-glycolic acid copolymer and leuprolide acetate), and poly-D-(-)-3-hydroxybutyric acid.

[0285] The pharmaceutical compositions herein may also contain more than one active compound as necessary for the particular indication being treated, preferably those with complementary activities that do not adversely affect each other. Alternatively, or in addition, the composition may comprise a

cytotoxic agent, chemotherapeutic agent, cytokine, immunosuppressive agent, or growth inhibitory agent. Such molecules are suitably present in combination in amounts that are effective for the purpose intended.

[0286] The active ingredients may also be entrapped in microcapsules prepared, for example, by coacervation techniques or by interfacial polymerization, for example, hydroxymethylcellulose or gelatin-microcapsules and poly-(methylmethacrylate) microcapsules, respectively, in colloidal drug delivery systems (for example, liposomes, albumin microspheres, microemulsions, nano-particles and nanocapsules) or in macroemulsions. Such techniques are disclosed in *Remington's Pharmaceutical Sciences* 18th edition.

[0287] In some embodiments, the pharmaceutical composition is contained in a single-use vial, such as a single-use sealed vial. In some embodiments, the pharmaceutical composition is contained in a multi-use vial. In some embodiments, the pharmaceutical composition is contained in bulk in a container. In some embodiments, the pharmaceutical composition is cryopreserved.

IV. Methods of treating LAG-3-related diseases

[0288] The anti-LAG-3 constructs (*e.g.*, anti-LAG-3 sdAb, anti-LAG-3 HCAb, or anti-LAG-3 MABP) and compositions (such as pharmaceutical compositions) thereof are useful for a variety of applications, such as in diagnosis, molecular assays, and therapy.

[0289] One aspect of the invention provides a method of treating a LAG-3 related disease or a condition in an individual in need thereof, comprising administering to the individual an effective amount of a pharmaceutical composition comprising any one of the anti-LAG-3 constructs described herein. In some embodiments, the LAG-3 related disease is cancer. In some embodiments, the LAG-3-related disease is an immune-related disease. In some embodiments, immune-related disease is associated with a T cell dysfunctional disorder. In some embodiments, the T cell dysfunctional disorder is characterized by T cell anergy or decreased ability to secrete cytokines, proliferate or execute cytolytic activity. In some embodiments, the T cell dysfunctional disorder is characterized by T cell exhaustion. In some embodiments, the T cells are CD4+ and CD8+ T cells. In some embodiments, an anti-LAG-3 construct described herein is used for increasing, enhancing, or stimulating an immune response or function in a subject in need thereof. In some embodiments, the LAG-3-related disease (*e.g.*, cancer, immune-related disease) is partially resistant to immune checkpoint molecule mono-blockade (*e.g.*, partially resistant to anti- PD-1 antibody monotherapy treatment).

[0290] In some embodiments, there is provided a method of treating a LAG-3-related disease in an individual, comprising administering to the individual an effective amount of a pharmaceutical composition comprising an isolated anti-LAG-3 construct comprising an anti-LAG-3 sdAb, wherein the

anti-LAG-3 sdAb comprises a CDR1 comprising the amino acid sequence of any one of SEQ ID NOs: 39-76, or a variant thereof comprising up to about 3 (such as about any of 1, 2, or 3) amino acid substitutions; a CDR2 comprising the amino acid sequence of any one of SEQ ID NOs: 115-152, or a variant thereof comprising up to about 3 (such as about any of 1, 2, or 3) amino acid substitutions; and a CDR3 comprising the amino acid sequence of any one of SEQ ID NOs: 191-228, or a variant thereof comprising up to about 3 (such as about any of 1, 2, or 3) amino acid substitutions; and a pharmaceutically acceptable carrier. In some embodiments, there is provided a method of treating a LAG-3-related disease in an individual, comprising administering to the individual an effective amount of a pharmaceutical composition comprising an isolated anti-LAG-3 construct comprising an anti-LAG-3 sdAb, wherein the anti-LAG-3 sdAb comprises: (1) a CDR1 comprising the amino acid sequence of SEQ ID NO: 53; a CDR2 comprising the amino acid sequence of SEQ ID NO: 129; and a CDR3 comprising the amino acid sequence of SEQ ID NO: 205; (2) a CDR1 comprising the amino acid sequence of SEQ ID NO: 60; a CDR2 comprising the amino acid sequence of SEQ ID NO: 136; and a CDR3 comprising the amino acid sequence of SEQ ID NO: 212; (3) a CDR1 comprising the amino acid sequence of SEQ ID NO: 69; a CDR2 comprising the amino acid sequence of SEQ ID NO: 145; and a CDR3 comprising the amino acid sequence of SEQ ID NO: 221; or (4) a CDR1 comprising the amino acid sequence of SEQ ID NO: 71; a CDR2 comprising the amino acid sequence of SEQ ID NO: 147; and a CDR3 comprising the amino acid sequence of SEQ ID NO: 223, and a pharmaceutically acceptable carrier. In some embodiments, the anti-LAG-3 sdAb comprises a V_HH domain comprising the amino acid sequence of any one of SEQ ID NOs: 274-311. In some embodiments, the anti-LAG-3 sdAb comprises a V_HH domain comprising the amino acid sequence of any one of SEQ ID NOs: 288, 295, 304, or 306.

[0291] In some embodiments, there is provided a method of treating cancer (*e.g.*, colon cancer) in an individual, comprising administering to the individual an effective amount of a pharmaceutical composition comprising an isolated anti-LAG-3 construct comprising an anti-LAG-3 sdAb, wherein the anti-LAG-3 sdAb comprises a CDR1 comprising the amino acid sequence of any one of SEQ ID NOs: 39-76, or a variant thereof comprising up to about 3 (such as about any of 1, 2, or 3) amino acid substitutions; a CDR2 comprising the amino acid sequence of any one of SEQ ID NOs: 115-152, or a variant thereof comprising up to about 3 (such as about any of 1, 2, or 3) amino acid substitutions; and a CDR3 comprising the amino acid sequence of any one of SEQ ID NOs: 191-228, or a variant thereof comprising up to about 3 (such as about any of 1, 2, or 3) amino acid substitutions; and a pharmaceutically acceptable carrier. In some embodiments, there is provided a method of treating cancer (*e.g.*, colon cancer) in an individual, comprising administering to the individual an effective amount of a pharmaceutical composition comprising an isolated anti-LAG-3 construct comprising an anti-LAG-3

sdAb, wherein the anti-LAG-3 sdAb comprises: (1) a CDR1 comprising the amino acid sequence of SEQ ID NO: 53; a CDR2 comprising the amino acid sequence of SEQ ID NO: 129; and a CDR3 comprising the amino acid sequence of SEQ ID NO: 205; (2) a CDR1 comprising the amino acid sequence of SEQ ID NO: 60; a CDR2 comprising the amino acid sequence of SEQ ID NO: 136; and a CDR3 comprising the amino acid sequence of SEQ ID NO: 212; (3) a CDR1 comprising the amino acid sequence of SEQ ID NO: 69; a CDR2 comprising the amino acid sequence of SEQ ID NO: 145; and a CDR3 comprising the amino acid sequence of SEQ ID NO: 221; or (4) a CDR1 comprising the amino acid sequence of SEQ ID NO: 71; a CDR2 comprising the amino acid sequence of SEQ ID NO: 147; and a CDR3 comprising the amino acid sequence of SEQ ID NO: 223, and a pharmaceutically acceptable carrier. In some embodiments, the anti-LAG-3 sdAb comprises a V_HH domain comprising the amino acid sequence of any one of SEQ ID NOs: 274-311. In some embodiments, the anti-LAG-3 sdAb comprises a V_HH domain comprising the amino acid sequence of any one of SEQ ID NOs: 288, 295, 304, or 306.

[0292] In some embodiments, there is provided a method of treating a LAG-3-related disease (*e.g.*, cancer, such as colon cancer) in an individual comprising administering to the individual an effective amount of a pharmaceutical composition comprising an isolated anti-LAG-3 HCAb comprising an sdAb specifically recognizing LAG-3, wherein the anti-LAG-3 sdAb comprises a CDR1 comprising the amino acid sequence of any one of SEQ ID NOs: 39-76, or a variant thereof comprising up to about 3 (such as about any of 1, 2, or 3) amino acid substitutions; a CDR2 comprising the amino acid sequence of any one of SEQ ID NOs: 115-152, or a variant thereof comprising up to about 3 (such as about any of 1, 2, or 3) amino acid substitutions; and a CDR3 comprising the amino acid sequence of any one of SEQ ID NOs: 191-228, or a variant thereof comprising up to about 3 (such as about any of 1, 2, or 3) amino acid substitutions, wherein the anti-LAG-3 sdAb is fused to an Fc fragment of an immunoglobulin; and a pharmaceutically acceptable carrier. In some embodiments, there is provided a method of treating a LAG-3-related disease (*e.g.*, cancer, such as colon cancer) in an individual comprising administering to the individual an effective amount of a pharmaceutical composition comprising an isolated anti-LAG-3 construct comprising an anti-LAG-3 sdAb, wherein the anti-LAG-3 sdAb comprises: (1) a CDR1 comprising the amino acid sequence of SEQ ID NO: 53; a CDR2 comprising the amino acid sequence of SEQ ID NO: 129; and a CDR3 comprising the amino acid sequence of SEQ ID NO: 205; (2) a CDR1 comprising the amino acid sequence of SEQ ID NO: 60; a CDR2 comprising the amino acid sequence of SEQ ID NO: 136; and a CDR3 comprising the amino acid sequence of SEQ ID NO: 212; (3) a CDR1 comprising the amino acid sequence of SEQ ID NO: 69; a CDR2 comprising the amino acid sequence of SEQ ID NO: 145; and a CDR3 comprising the amino acid sequence of SEQ ID NO: 221; or (4) a CDR1 comprising the amino acid sequence of SEQ ID NO: 71; a CDR2 comprising the amino acid sequence of

SEQ ID NO: 147; and a CDR3 comprising the amino acid sequence of SEQ ID NO: 223, and a pharmaceutically acceptable carrier. In some embodiments, the anti-LAG-3 sdAb comprises a V_HH domain comprising the amino acid sequence of any one of SEQ ID NOs: 274-311. In some embodiments, the anti-LAG-3 sdAb comprises a V_HH domain comprising the amino acid sequence of any one of SEQ ID NOs: 288, 295, 304, or 306. In some embodiments, the anti-LAG-3 sdAb is fused to the Fc fragment via a peptide linker. In some embodiments, the anti-LAG-3 HCAb is monomeric. In some embodiments, the anti-LAG-3 HCAb is dimeric. In some embodiments, the Fc fragment is a human IgG1 Fc, effectorless human IgG1 Fc, hIgG2 Fc, human IgG4 Fc, or hIgG4 Fc (S228P).

[0293] In some embodiments, there is provided a method of treating a LAG-3-related disease (*e.g.*, cancer, such as colon cancer) in an individual, comprising administering to the individual an effective amount of a pharmaceutical composition comprising an isolated anti-LAG-3 construct (*e.g.*, MABP or BABP) comprising: (a) a first antigen binding portion comprising an anti-LAG-3 sdAb comprising a CDR1 comprising the amino acid sequence of any one of SEQ ID NOs: 39-76, or a variant thereof comprising up to about 3 (such as about any of 1, 2, or 3) amino acid substitutions; a CDR2 comprising the amino acid sequence of any one of SEQ ID NOs: 115-152, or a variant thereof comprising up to about 3 (such as about any of 1, 2, or 3) amino acid substitutions; and a CDR3 comprising the amino acid sequence of any one of SEQ ID NOs: 191-228, or a variant thereof comprising up to about 3 (such as about any of 1, 2, or 3) amino acid substitutions, and (b) a second antigen binding portion that specifically binds a second epitope (*e.g.*, an immune checkpoint inhibitor, such as PD-1), wherein the first antigen binding portion and the second antigen binding portion are fused to each other; and a pharmaceutically acceptable carrier. In some embodiments, there is provided a method of treating a LAG-3-related disease (*e.g.*, cancer, such as colon cancer) in an individual, comprising administering to the individual an effective amount of a pharmaceutical composition comprising an isolated anti-LAG-3 construct (*e.g.*, MABP or BABP) comprising: (a) a first antigen binding portion comprising an anti-LAG-3 sdAb comprising: (1) a CDR1 comprising the amino acid sequence of SEQ ID NO: 53; a CDR2 comprising the amino acid sequence of SEQ ID NO: 129; and a CDR3 comprising the amino acid sequence of SEQ ID NO: 205; (2) a CDR1 comprising the amino acid sequence of SEQ ID NO: 60; a CDR2 comprising the amino acid sequence of SEQ ID NO: 136; and a CDR3 comprising the amino acid sequence of SEQ ID NO: 212; (3) a CDR1 comprising the amino acid sequence of SEQ ID NO: 69; a CDR2 comprising the amino acid sequence of SEQ ID NO: 145; and a CDR3 comprising the amino acid sequence of SEQ ID NO: 221; (4) a CDR1 comprising the amino acid sequence of SEQ ID NO: 71; a CDR2 comprising the amino acid sequence of SEQ ID NO: 147; and a CDR3 comprising the amino acid sequence of SEQ ID NO: 223, (5) a CDR1 comprising the amino acid sequence of SEQ ID NO: 43; a CDR2 comprising the

amino acid sequence of SEQ ID NO: 119; and a CDR3 comprising the amino acid sequence of SEQ ID NO: 195, and (b) a second antigen binding portion that specifically binds a second epitope (*e.g.*, an immune checkpoint inhibitor, such as PD-1), wherein the first antigen binding portion and the second antigen binding portion are fused to each other; and a pharmaceutically acceptable carrier. In some embodiments, the second epitope is from an immune checkpoint molecule, such as PD-1, 4-1BB, PD-L1, TIM-3, TIGIT, CTLA-4, VISTA, B7-1, B7-H3, CD47, OX40 or GITR. In some embodiments, the first antigen binding portion is fused to the second antigen binding portion at the N-terminus of the heavy chain, the N-terminus of the light chain, the N-terminus of the Fc region, the C-terminus of the heavy chain, or the C-terminus of the light chain. In some embodiments, the second antigen binding portion comprises a Fab or an scFv. In some embodiments, the second antigen binding portion comprises a full-length 4-chain antibody consisting of two heavy chains and two light chains. In some embodiments, the second antigen binding portion comprises an anti-PD-1 full-length antibody (*e.g.*, pembrolizumab or PD1-BM-min) or antigen binding fragment thereof. In some embodiments, the anti-PD-1 full-length antibody or antigen binding fragment thereof (*e.g.*, Fab, scFv) comprises a V_H comprising HC-CDR1, HC-CDR2, and HC-CDR3 of the amino acid sequence of SEQ ID NO: 356, and a V_L comprising LC-CDR1, LC-CDR2, and LC-CDR3 of the amino acid sequence of SEQ ID NO: 357. In some embodiments, the V_H comprises HC-CDR1, HC-CDR2, and HC-CDR3 of the amino acid sequence of SEQ ID NO: 373, and V_L comprises LC-CDR1, LC-CDR2, and LC-CDR3 of the amino acid sequence of SEQ ID NO: 374. In some embodiments, the anti-PD-1 full-length antibody comprises a heavy chain comprising the amino acid sequence of SEQ ID NO: 356, and a light chain comprising the amino acid sequence of SEQ ID NO: 357. In some embodiments, the anti-PD-1 full-length antibody comprises a heavy chain comprising the amino acid sequence of SEQ ID NO: 373, and a light chain comprising the amino acid sequence of SEQ ID NO: 374. In some embodiments, the first antigen binding portion is fused to the second antigen binding portion via a peptide linker. In some embodiments, the second antigen binding fragment comprises an Fc region, such as an IgG1 Fc, effectorless IgG1 Fc, IgG2 Fc, IgG4 Fc, or IgG4 Fc (S228P). In some embodiments, the anti-LAG-3 construct comprises a first polypeptide comprising the amino acid sequence of SEQ ID NO: 358, and a second polypeptide comprising the amino acid sequence of SEQ ID NO: 359. In some embodiments, the anti-LAG-3 construct comprises a first polypeptide comprising the amino acid sequence of SEQ ID NO: 362, and a second polypeptide comprising the amino acid sequence of SEQ ID NO: 363. In some embodiments, the anti-LAG-3 construct comprises a first polypeptide comprising the amino acid sequence of SEQ ID NO: 381, and a second polypeptide comprising the amino acid sequence of SEQ ID NO: 382.

[0294] In some embodiments, the method of treating cancer has one or more of the following biological activities: (1) killing cancer cells (including bystander killing); (2) inhibiting proliferation of cancer cells; (3) inducing immune response in a tumor; (4) reducing tumor size; (5) alleviating one or more symptoms in an individual having cancer; (6) inhibiting tumor metastasis; (7) prolonging survival; (8) prolonging time to cancer progression; and (9) preventing, inhibiting, or reducing the likelihood of the recurrence of a cancer. In some embodiments, the method of killing cancer cells mediated by the pharmaceutical composition described herein can achieve a tumor cell death rate of at least about any of 40%, 50%, 60%, 70%, 80%, 90%, 95%, or more. In some embodiments, the method of reducing tumor size mediated by the pharmaceutical composition described herein can reduce at least about 10% (including for example at least about any of 20%, 30%, 40%, 60%, 70%, 80%, 90%, or 100%) of the tumor size. In some embodiments, the method of inhibiting tumor metastasis mediated by the pharmaceutical composition described herein can inhibit at least about 10% (including for example at least about any of 20%, 30%, 40%, 60%, 70%, 80%, 90%, or 100%) of the metastasis. In some embodiments, the method of prolonging survival of an individual (such as a human) mediated by the pharmaceutical composition described herein can prolongs the survival of the individual by at least any of 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 18, or 24 months. In some embodiments, the method of prolonging time to cancer progression mediated by the pharmaceutical composition described herein can prolongs the time to cancer progression by at least any of 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, or 12 weeks. In some embodiments, the method of treating immune-related disease can increase, enhance, or stimulate an immune response or function in a subject. In some embodiments, the immune response or function is increased, enhanced, and/or stimulated by activating effector cells (*e.g.*, T cells, *e.g.*, CD8+ and/or CD4+ T cells), expanding (increasing) an effector cell population, and/or killing target cells (*e.g.*, target tumor cells) in the subject. In some embodiments, the CD4 and/or CD8 T cells in the individual have increased or enhanced priming, activation, proliferation, cytokine release and/or cytolytic activity relative to prior to the administration of the pharmaceutical composition comprising the anti-LAG-3 construct described herein.

[0295] The methods described herein are suitable for treating a variety of cancers, including both solid cancer and liquid cancer. The methods are applicable to cancers of all stages, including early stage cancer, non-metastatic cancer, primary cancer, advanced cancer, locally advanced cancer, metastatic cancer, or cancer in remission. The methods described herein may be used as a first therapy, second therapy, third therapy, or combination therapy with other types of cancer therapies known in the art, such as surgery, radiation, chemotherapy, immunotherapy, hormone therapy, or a combination thereof. In some embodiments, the method is used to treat an individual who has previously been treated. In some embodiments, the cancer has been refractory to prior therapy. In some embodiments, the method is used

to treat an individual who has not previously been treated. In some embodiments, the cancer is partially resistant to immune checkpoint inhibitor monotherapy (e.g., partially resistant to anti-PD-1 antibody monotherapy treatment).

[0296] Dosages and desired drug concentrations of pharmaceutical compositions of the present application may vary depending on the particular use envisioned. The determination of the appropriate dosage or route of administration is well within the skill of an ordinary artisan. Animal experiments provide reliable guidance for the determination of effective doses for human therapy. Interspecies scaling of effective doses can be performed following the principles laid down by Mordenti, J. and Chappell, W. "The Use of Interspecies Scaling in Toxicokinetics," In *Toxicokinetics and New Drug Development*, Yacobi *et al.*, Eds, Pergamon Press, New York 1989, pp. 42-46.

[0297] When *in vivo* administration of the anti-LAG-3 construct described herein (such as anti-LAG-3 sdAb, anti-LAG-3 HCAB, anti-LAG-3 MABP) are used, normal dosage amounts may vary from about 10 ng/kg up to about 100 mg/kg of mammal body weight depending upon the route of administration. It is within the scope of the present application that different formulations will be effective for different treatments and different disorders, and that administration intended to treat a specific organ or tissue may necessitate delivery in a manner different from that to another organ or tissue. Moreover, dosages may be administered by one or more separate administrations, or by continuous infusion. For repeated administrations over several days or longer, depending on the condition, the treatment is sustained until a desired suppression of disease symptoms occurs. However, other dosage regimens may be useful. The progress of this therapy is easily monitored by conventional techniques and assays.

[0298] In some embodiments, the pharmaceutical composition is administered for a single time (e.g. bolus injection). In some embodiments, the pharmaceutical composition is administered for multiple times (such as any of 2, 3, 4, 5, 6, or more times). If multiple administrations, they may be performed by the same or different routes and may take place at the same site or at alternative sites. The pharmaceutical composition may be administered daily to once per year. The interval between administrations can be about any one of 24 h to a year. Intervals can also be irregular (e.g. following tumor progression). In some embodiments, there is no break in the dosing schedule. The optimal dosage and treatment regime for a particular patient can readily be determined by one skilled in the art of medicine by monitoring the patient for signs of disease and adjusting the treatment accordingly.

[0299] The pharmaceutical compositions of the present application, including but not limited to reconstituted and liquid formulations, are administered to an individual in need of treatment, preferably a human, in accord with known methods, such as intravenous administration as a bolus or by continuous

infusion over a period of time, by intramuscular, intraperitoneal, intracerebrospinal, subcutaneous, intravenous (i.v.), intra-articular, intrasynovial, intrathecal, oral, topical, or inhalation routes. A reconstituted formulation can be prepared by dissolving a lyophilized anti-LAG-3 construct described herein in a diluent such that the protein is dispersed throughout. Exemplary pharmaceutically acceptable (safe and non-toxic for administration to a human) diluents suitable for use in the present application include, but are not limited to, sterile water, bacteriostatic water for injection (BWFI), a pH buffered solution (*e.g.* phosphate-buffered saline), sterile saline solution, Ringer's solution or dextrose solution, or aqueous solutions of salts and/or buffers.

[0300] In some embodiments, the pharmaceutical compositions are administered to the individual by subcutaneous (*i.e.* beneath the skin) administration. For such purposes, the pharmaceutical compositions may be injected using a syringe. However, other devices for administration of the pharmaceutical compositions are available such as injection devices; injector pens; auto-injector devices, needleless devices; and subcutaneous patch delivery systems. In some embodiments, the pharmaceutical compositions are administered to the individual intravenously. In some embodiments, the pharmaceutical composition is administered to an individual by infusion, such as intravenous infusion. Infusion techniques for immunotherapy are known in the art (see, *e.g.*, Rosenberg *et al.*, New Eng. J. of Med. 319: 1676 (1988)).

V. Methods of preparation

[0301] The anti-LAG-3 construct described herein (such as anti-LAG-3 sdAb, anti-LAG-3 HCAb, anti-LAG-3 MABP) may be prepared using any methods known in the art or as described herein. Also *see* Examples 1-3. In some embodiments, there is provided a method of producing an anti-LAG-3 construct, comprising: (a) culturing a host cell comprising an isolated nucleic acid or vector encoding the anti-LAG-3 construct described herein under conditions effective to express the encoded anti-LAG-3 construct; and (b) obtaining the expressed anti-LAG-3 construct from said host cell. In some embodiments, the method of step (a) further comprises producing a host cell comprising the isolated nucleic acid or vector encoding the anti-LAG-3 construct described herein.

[0302] Methods of preparing sdAbs have been described. *See*, for example, Els Pardon *et al.*, *Nature Protocol*, 2014; 9(3): 674. sdAbs (such as V_HHs) may be obtained using methods known in the art such as by immunizing a *Camelid* species (such as camel or llama) and obtaining hybridomas therefrom, or by cloning a library of single-domain antibodies using molecular biology techniques known in the art and subsequent selection by ELISA with individual clones of unselected libraries or by using phage display.

[0303] For recombinant production of the sdAbs, the nucleic acids encoding the single-domain antibodies are isolated and inserted into a replicable vector for further cloning (amplification of the DNA) or for expression. DNA encoding the single-domain antibody is 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 antibody). Many vectors are available. The choice of vector depends in part on the host cell to be used. Generally, preferred host cells are of either prokaryotic or eukaryotic (generally mammalian) origin. In some embodiments, the isolated nucleic acid encoding the anti-LAG-3 construct described herein comprises the nucleic acid sequence of any one of SEQ ID NOs: 267-273.

1. Recombinant production in prokaryotic cells

a) Vector construction

[0304] Polynucleic acid sequences encoding the antibodies of the present application can be obtained using standard recombinant techniques. Desired polynucleic acid sequences may be isolated and sequenced from antibody producing cells such as hybridoma cells. Alternatively, polynucleotides can be synthesized using nucleotide synthesizer or PCR techniques. Once obtained, sequences encoding the polypeptides are inserted into a recombinant vector capable of replicating and expressing heterologous polynucleotides in prokaryotic hosts. Many vectors that are available and known in the art can be used for the purpose of the present invention. Selection of an appropriate vector will depend mainly on the size of the nucleic acids to be inserted into the vector and the particular host cell to be transformed with the vector. Each vector contains various components, depending on its function (amplification or expression of heterologous polynucleotide, or both) and its compatibility with the particular host cell in which it resides. The vector components generally include, but are not limited to: an origin of replication, a selection marker gene, a promoter, a ribosome binding site (RBS), a signal sequence, the heterologous nucleic acid insert and a transcription termination sequence.

[0305] In general, plasmid vectors containing replicon and control sequences which are derived from species compatible with the host cell are used in connection with these hosts. The vector ordinarily carries a replication site, as well as marking sequences which are capable of providing phenotypic selection in transformed cells. For example, *E. coli* is typically transformed using pBR322, a plasmid derived from an *E. coli* species. pBR322 contains genes encoding ampicillin (Amp) and tetracycline (Tet) resistance and thus provides easy means for identifying transformed cells. pBR322, its derivatives, or other microbial plasmids or bacteriophage may also contain, or be modified to contain, promoters which can be used by

the microbial organism for expression of endogenous proteins. Examples of pBR322 derivatives used for expression of particular antibodies are described in detail in Carter *et al.*, U.S. Pat. No. 5,648,237.

[0306] In addition, phage vectors containing replicon and control sequences that are compatible with the host microorganism can be used as transforming vectors in connection with these hosts. For example, bacteriophage such as GEM™-11 may be utilized in making a recombinant vector which can be used to transform susceptible host cells such as *E. coli* LE392.

[0307] The expression vector of the present application may comprise two or more promoter-cistron pairs, encoding each of the polypeptide components. A promoter is an untranslated regulatory sequence located upstream (5') to a cistron that modulates its expression. Prokaryotic promoters typically fall into two classes, inducible and constitutive. Inducible promoter is a promoter that initiates increased levels of transcription of the cistron under its control in response to changes in the culture condition, *e.g.* the presence or absence of a nutrient or a change in temperature.

[0308] A large number of promoters recognized by a variety of potential host cells are well known. The selected promoter can be operably linked to cistron DNA encoding the light or heavy chain by removing the promoter from the source DNA via restriction enzyme digestion and inserting the isolated promoter sequence into the vector of the present application. Both the native promoter sequence and many heterologous promoters may be used to direct amplification and/or expression of the target genes. In some embodiments, heterologous promoters are utilized, as they generally permit greater transcription and higher yields of expressed target gene as compared to the native target polypeptide promoter.

[0309] Promoters suitable for use with prokaryotic hosts include the PhoA promoter, the -galactamase and lactose promoter systems, a tryptophan (*trp*) promoter system and hybrid promoters such as the *tac* or the *trc* promoter. However, other promoters that are functional in bacteria (such as other known bacterial or phage promoters) are suitable as well. Their nucleic acid sequences have been published, thereby enabling a skilled worker operably to ligate them to cistrons encoding the target light and heavy chains (Siebenlist *et al.* (1980) *Cell* 20: 269) using linkers or adaptors to supply any required restriction sites.

[0310] In one aspect, each cistron within the recombinant vector comprises a secretion signal sequence component that directs translocation of the expressed polypeptides across a membrane. In general, the signal sequence may be a component of the vector, or it may be a part of the target polypeptide DNA that is inserted into the vector. The signal sequence selected for the purpose of this invention should be one that is recognized and processed (*i.e.* cleaved by a signal peptidase) by the host cell. For prokaryotic host cells that do not recognize and process the signal sequences native to the heterologous polypeptides, the signal sequence is substituted by a prokaryotic signal sequence selected, for example, from the group

consisting of the alkaline phosphatase, penicillinase, Ipp, or heat-stable enterotoxin II (STII) leaders, LamB, PhoE, PelB, OmpA and MBP. In some embodiments of the present application, the signal sequences used in both cistrons of the expression system are STII signal sequences or variants thereof.

[0311] In some embodiments, the production of the anti-LAG-3 construct according to the present application can occur in the cytoplasm of the host cell, and therefore does not require the presence of secretion signal sequences within each cistron. In some embodiments, polypeptide components, such as the polypeptide encoding the V_H domain of the second antigen binding portion optionally fused to the first antigen binding portion, and the polypeptide encoding the V_L domain of the second antigen binding portion optionally fused to the first antigen binding portion, are expressed, folded and assembled to form functional antibodies within the cytoplasm. Certain host strains (*e.g.*, the *E. coli* *trxB*⁻ strains) provide cytoplasm conditions that are favorable for disulfide bond formation, thereby permitting proper folding and assembly of expressed protein subunits. Proba and Pluckthun *Gene*, 159:203 (1995).

[0312] The present invention provides an expression system in which the quantitative ratio of expressed polypeptide components can be modulated in order to maximize the yield of secreted and properly assembled the antibodies of the present application. Such modulation is accomplished at least in part by simultaneously modulating translational strengths for the polypeptide components. One technique for modulating translational strength is disclosed in Simmons *et al.*, U.S. Pat. No. 5,840,523. It utilizes variants of the translational initiation region (TIR) within a cistron. For a given TIR, a series of amino acid or nucleic acid sequence variants can be created with a range of translational strengths, thereby providing a convenient means by which to adjust this factor for the desired expression level of the specific chain. TIR variants can be generated by conventional mutagenesis techniques that result in codon changes which can alter the amino acid sequence, although silent changes in the nucleic acid sequence are preferred. Alterations in the TIR can include, for example, alterations in the number or spacing of Shine-Dalgarno sequences, along with alterations in the signal sequence. One method for generating mutant signal sequences is the generation of a “codon bank” at the beginning of a coding sequence that does not change the amino acid sequence of the signal sequence (*i.e.*, the changes are silent). This can be accomplished by changing the third nucleotide position of each codon; additionally, some amino acids, such as leucine, serine, and arginine, have multiple first and second positions that can add complexity in making the bank. This method of mutagenesis is described in detail in Yansura *et al.* (1992) *METHODS: A Companion to Methods in Enzymol.* 4:151-158.

[0313] Preferably, a set of vectors is generated with a range of TIR strengths for each cistron therein. This limited set provides a comparison of expression levels of each chain as well as the yield of the desired protein products under various TIR strength combinations. TIR strengths can be determined by

quantifying the expression level of a reporter gene as described in detail in Simmons *et al.* U.S. Pat. No. 5,840,523. Based on the translational strength comparison, the desired individual TIRs are selected to be combined in the expression vector constructs of the present application.

b) Prokaryotic host cells

[0314] Prokaryotic host cells suitable for expressing the antibodies of the present application include Archaeobacteria and Eubacteria, such as Gram-negative or Gram-positive organisms. Examples of useful bacteria include *Escherichia* (e.g., *E. coli*), *Bacilli* (e.g., *B. subtilis*), Enterobacteria, *Pseudomonas* species (e.g., *P. aeruginosa*), *Salmonella typhimurium*, *Serratia marcescans*, *Klebsiella*, *Proteus*, *Shigella*, *Rhizobia*, *Vitreoscilla*, or *Paracoccus*. In some embodiments, gram-negative cells are used. In some embodiments, *E. coli* cells are used as hosts for the invention. Examples of *E. coli* strains include strain W3110 (Bachmann, *Cellular and Molecular Biology*, vol. 2 (Washington, D.C.: American Society for Microbiology, 1987), pp. 1190-1219; ATCC Deposit No. 27,325) and derivatives thereof, including strain 33D3 having genotype W3110 AfhuA (AtonA) ptr3 lac Iq lacL8 AompT A(nmpc-fepE) degP41 kan^R (U.S. Pat. No. 5,639,635). Other strains and derivatives thereof, such as *E. coli* 294 (ATCC 31,446), *E. coli* B, *E. coli* 1776 (ATCC 31,537) and *E. coli* RV308 (ATCC 31,608) are also suitable. These examples are illustrative rather than limiting. Methods for constructing derivatives of any of the above-mentioned bacteria having defined genotypes are known in the art and described in, for example, Bass *et al.*, *Proteins*, 8:309-314 (1990). It is generally necessary to select the appropriate bacteria taking into consideration replicability of the replicon in the cells of a bacterium. For example, *E. coli*, *Serratia*, or *Salmonella* species can be suitably used as the host when well known plasmids such as pBR322, pBR325, pACYC177, or pKN410 are used to supply the replicon.

[0315] Typically the host cell should secrete minimal amounts of proteolytic enzymes, and additional protease inhibitors may desirably be incorporated in the cell culture.

c) Protein production

[0316] Host cells are transformed with the above-described expression vectors and cultured in conventional nutrient media modified as appropriate for inducing promoters, selecting transformants, or amplifying the genes encoding the desired sequences. Transformation means introducing DNA into the prokaryotic host so that the DNA is replicable, either as an extrachromosomal element or by chromosomal integrant. Depending on the host cell used, transformation is done using standard techniques appropriate to such cells. The calcium treatment employing calcium chloride is generally used for bacterial cells that contain substantial cell-wall barriers. Another method for transformation employs polyethylene glycol/DMSO. Yet another technique used is electroporation.

[0317] Host cells are transformed with the above-described expression vectors and cultured in conventional nutrient media modified as appropriate for inducing promoters, selecting transformants, or amplifying the genes encoding the desired sequences. Transformation means introducing DNA into the prokaryotic host so that the DNA is replicable, either as an extrachromosomal element or by chromosomal integrant. Depending on the host cell used, transformation is done using standard techniques appropriate to such cells. The calcium treatment employing calcium chloride is generally used for bacterial cells that contain substantial cell-wall barriers. Another method for transformation employs polyethylene glycol/DMSO. Yet another technique used is electroporation.

[0318] Prokaryotic cells used to produce the antibodies of the present application are grown in media known in the art and suitable for culture of the selected host cells. Examples of suitable media include luria broth (LB) plus necessary nutrient supplements. In some embodiments, the media also contains a selection agent, chosen based on the construction of the expression vector, to selectively permit growth of prokaryotic cells containing the expression vector. For example, ampicillin is added to media for growth of cells expressing ampicillin resistant gene.

[0319] Any necessary supplements besides carbon, nitrogen, and inorganic phosphate sources may also be included at appropriate concentrations introduced alone or as a mixture with another supplement or medium such as a complex nitrogen source. Optionally the culture medium may contain one or more reducing agents selected from the group consisting of glutathione, cysteine, cystamine, thioglycollate, dithioerythritol and dithiothreitol. The prokaryotic host cells are cultured at suitable temperatures. For *E. coli* growth, for example, the preferred temperature ranges from about 20°C. to about 39°C., more preferably from about 25°C. to about 37°C., even more preferably at about 30°C. The pH of the medium may be any pH ranging from about 5 to about 9, depending mainly on the host organism. For *E. coli*, the pH is preferably from about 6.8 to about 7.4, and more preferably about 7.0.

[0320] If an inducible promoter is used in the expression vector of the present application, protein expression is induced under conditions suitable for the activation of the promoter. In one aspect of the present application, PhoA promoters are used for controlling transcription of the polypeptides. Accordingly, the transformed host cells are cultured in a phosphate-limiting medium for induction. Preferably, the phosphate-limiting medium is the C.R.A.P medium (see, *e.g.*, Simmons *et al.*, *J. Immunol. Methods* (2002), 263:133-147). A variety of other inducers may be used, according to the vector construct employed, as is known in the art.

[0321] The expressed anti-LAG-3 construct of the present application are secreted into and recovered from the periplasm of the host cells. Protein recovery typically involves disrupting the microorganism,

generally by such means as osmotic shock, sonication or lysis. Once cells are disrupted, cell debris or whole cells may be removed by centrifugation or filtration. The proteins may be further purified, for example, by affinity resin chromatography. Alternatively, proteins can be transported into the culture media and isolated therein. Cells may be removed from the culture and the culture supernatant being filtered and concentrated for further purification of the proteins produced. The expressed polypeptides can be further isolated and identified using commonly known methods such as polyacrylamide gel electrophoresis (PAGE) and Western blot assay.

[0322] Alternatively, protein production is conducted in large quantity by a fermentation process. Various large-scale fed-batch fermentation procedures are available for production of recombinant proteins. Large-scale fermentations have at least 1000 liters of capacity, preferably about 1,000 to 100,000 liters of capacity. These fermentors use agitator impellers to distribute oxygen and nutrients, especially glucose (the preferred carbon/energy source). Small scale fermentation refers generally to fermentation in a fermentor that is no more than approximately 100 liters in volumetric capacity, and can range from about 1 liter to about 100 liters.

[0323] During the fermentation process, induction of protein expression is typically initiated after the cells have been grown under suitable conditions to a desired density, *e.g.*, an OD₅₅₀ of about 180-220, at which stage the cells are in the early stationary phase. A variety of inducers may be used, according to the vector construct employed, as is known in the art and described above. Cells may be grown for shorter periods prior to induction. Cells are usually induced for about 12-50 hours, although longer or shorter induction time may be used.

[0324] To improve the production yield and quality of the antibodies of the present application, various fermentation conditions can be modified. For example, to improve the proper assembly and folding of the secreted polypeptides, additional vectors overexpressing chaperone proteins, such as Dsb proteins (DsbA, DsbB, DsbC, DsbD and or DsbG) or FkpA (a peptidylprolyl *cis*,*trans*-isomerase with chaperone activity) can be used to co-transform the host prokaryotic cells. The chaperone proteins have been demonstrated to facilitate the proper folding and solubility of heterologous proteins produced in bacterial host cells. Chen *et al.* (1999) *J Bio Chem* 274:19601-19605; Georgiou *et al.*, U.S. Pat. No. 6,083,715; Georgiou *et al.*, U.S. Pat. No. 6,027,888; Bothmann and Pluckthun (2000) *J. Biol. Chem.* 275:17100-17105; Ramm and Pluckthun (2000) *J. Biol. Chem.* 275:17106-17113; Arie *et al.* (2001) *Mol. Microbiol.* 39:199-210.

[0325] To minimize proteolysis of expressed heterologous proteins (especially those that are proteolytically sensitive), certain host strains deficient for proteolytic enzymes can be used for the present

invention. For example, host cell strains may be modified to effect genetic mutation(s) in the genes encoding known bacterial proteases such as Protease III, OmpT, DegP, Tsp, Protease I, Protease Mi, Protease V, Protease VI and combinations thereof. Some *E. coli* protease-deficient strains are available and described in, for example, Joly *et al.* (1998), *supra*; Georgiou *et al.*, U.S. Pat. No. 5,264,365; Georgiou *et al.*, U.S. Pat. No. 5,508,192; Hara *et al.*, *Microbial Drug Resistance*, 2:63-72 (1996).

[0326] *E. coli* strains deficient for proteolytic enzymes and transformed with plasmids overexpressing one or more chaperone proteins may be used as host cells in the expression system encoding the antibodies of the present application.

d) Protein purification

[0327] The anti-LAG-3 construct produced herein are further purified to obtain preparations that are substantially homogeneous for further assays and uses. Standard protein purification methods known in the art can be employed. The following procedures are exemplary of suitable purification procedures: fractionation on immunoaffinity or ion-exchange columns, ethanol precipitation, reverse phase HPLC, chromatography on silica or on a cation-exchange resin such as DEAE, chromatofocusing, SDS-PAGE, ammonium sulfate precipitation, and gel filtration using, for example, Sephadex G-75.

[0328] In some embodiments, Protein A immobilized on a solid phase is used for immunoaffinity purification of the antibodies comprising an Fc region of the present application. Protein A is a 411(D cell wall protein from *Staphylococcus aureus* which binds with a high affinity to the Fc region of antibodies. Lindmark *et al* (1983) *J. Immunol. Meth.* 62:1-13. The solid phase to which Protein A is immobilized is preferably a column comprising a glass or silica surface, more preferably a controlled pore glass column or a silicic acid column. In some applications, the column has been coated with a reagent, such as glycerol, in an attempt to prevent nonspecific adherence of contaminants. The solid phase is then washed to remove contaminants non-specifically bound to the solid phase. Finally the antibodies of interest are recovered from the solid phase by elution.

2. Recombinant production in eukaryotic cells

[0329] For eukaryotic expression, the vector components generally include, but are not limited to, one or more of the following, a signal sequence, an origin of replication, one or more marker genes, and enhancer element, a promoter, and a transcription termination sequence.

a) Signal sequence component

[0330] A vector for use in a eukaryotic host may also an insert that encodes a signal sequence or other polypeptide having a specific cleavage site at the N-terminus of the mature protein or polypeptide. The

heterologous signal sequence selected preferably is one that is recognized and processed (*i.e.*, cleaved by a signal peptidase) by the host cell. In mammalian cell expression, mammalian signal sequences as well as viral secretory leaders, for example, the herpes simplex gD signal, are available.

[0331] The DNA for such precursor region is ligated in reading frame to DNA encoding the antibodies of the present application.

b) Origin of replication

[0332] Generally, the origin of replication component is not needed for mammalian expression vectors (the SV40 origin may typically be used only because it contains the early promoter).

c) Selection gene component

[0333] Expression and cloning vectors may contain a selection gene, also termed a selectable marker. Typical selection genes encode proteins that (a) confer resistance to antibiotics or other toxins, *e.g.*, ampicillin, neomycin, methotrexate, or tetracycline, (b) complement auxotrophic deficiencies, or (c) supply critical nutrients not available from complex media, *e.g.*, the gene encoding D-alanine racemase for *Bacilli*.

[0334] One example of a selection scheme utilizes a drug to arrest growth of a host cell. Those cells that are successfully transformed with a heterologous gene produce a protein conferring drug resistance and thus survive the selection regimen. Examples of such dominant selection use the drugs neomycin, mycophenolic acid and hygromycin.

[0335] Another example of suitable selectable markers for mammalian cells are those that enable the identification of cells competent to take up nucleic acid encoding the antibodies of the present application, such as DHFR, thymidine kinase, metallothionein-I and -II, preferably primate metallothionein genes, adenosine deaminase, ornithine decarboxylase, etc.

[0336] For example, cells transformed with the DHFR selection gene are first identified by culturing all of the transformants in a culture medium that contains methotrexate (Mtx), a competitive antagonist of DHFR. An appropriate host cell when wild-type DHFR is employed is the Chinese hamster ovary (CHO) cell line deficient in DHFR activity (*e.g.*, ATCC CRL-9096).

[0337] Alternatively, host cells (particularly wild-type hosts that contain endogenous DHFR) transformed or co-transformed with the polypeptide encoding-DNA sequences, wild-type DHFR protein, and another selectable marker such as aminoglycoside 3'-phosphotransferase (APH) can be selected by cell growth in medium containing a selection agent for the selectable marker such as an aminoglycosidic antibiotic, *e.g.*, kanamycin, neomycin, or G418. *See* U.S. Pat. No. 4,965,199.

d) Promoter component

[0338] Expression and cloning vectors usually contain a promoter that is recognized by the host organism and is operably linked to the nucleic acid encoding the desired polypeptide sequences. Virtually all eukaryotic genes have an AT-rich region located approximately 25 to 30 bases upstream from the site where transcription is initiated. Another sequence found 70 to 80 bases upstream from the start of the transcription of many genes is a CNCAAT region where N may be any nucleotide. At the 3' end of most eukaryotic is an AATAAA sequence that may be the signal for addition of the poly A tail to the 3' end of the coding sequence. All of these sequences may be inserted into eukaryotic expression vectors.

[0339] Other promoters suitable for use with prokaryotic hosts include the phoA promoter, -lactamase and lactose promoter systems, alkaline phosphatase promoter, a tryptophan (trp) promoter system, and hybrid promoters such as the tac promoter. However, other known bacterial promoters are suitable. Promoters for use in bacterial systems also will contain a Shine-Dalgarno (S.D.) sequence operably linked to the DNA encoding the antibodies.

[0340] Polypeptide transcription from vectors in mammalian host cells is controlled, for example, by promoters obtained from the genomes of viruses such as polyoma virus, fowlpox virus, adenovirus (such as Adenovirus 2), bovine papilloma virus, avian sarcoma virus, cytomegalovirus, a retrovirus, hepatitis-B virus and most preferably Simian Virus 40 (SV40), from heterologous mammalian promoters, *e.g.*, the actin promoter or an immunoglobulin promoter, from heat-shock promoters, provided such promoters are compatible with the host cell systems.

[0341] The early and late promoters of the SV40 virus are conveniently obtained as an SV40 restriction fragment that also contains the SV40 viral origin of replication. The immediate early promoter of the human cytomegalovirus is conveniently obtained as a HindIII E restriction fragment. A system for expressing DNA in mammalian hosts using the bovine papilloma virus as a vector is disclosed in U.S. Pat. No. 4,419,446. A modification of this system is described in U.S. Pat. No. 4,601,978. See also Reyes *et al.*, *Nature* 297:598-601 (1982) on expression of human-interferon cDNA in mouse cells under the control of a thymidine kinase promoter from herpes simplex virus. Alternatively, the Rous Sarcoma Virus long terminal repeat can be used as the promoter.

e) Enhancer element component

[0342] Transcription of a DNA encoding the antibodies of the present application by higher eukaryotes is often increased by inserting an enhancer sequence into the vector. Many enhancer sequences are now known from mammalian genes (globin, elastase, albumin, α -fetoprotein, and insulin). Typically, however, one will use an enhancer from a eukaryotic cell virus. Examples include the SV40 enhancer on the late

side of the replication origin (100-270 bp), the cytomegalovirus early promoter enhancer, the polyoma enhancer on the late side of the replication origin, and adenovirus enhancers. See also Yaniv, *Nature* 297:17-18 (1982) on enhancing elements for activation of eukaryotic promoters. The enhancer may be spliced into the vector at a position 5' or 3' to the polypeptide encoding sequence, but is preferably located at a site 5' from the promoter.

f) Transcription termination component

[0343] Expression vectors used in eukaryotic host cells (yeast, fungi, insect, plant, animal, human, or nucleated cells from other multicellular organisms) will also contain sequences necessary for the termination of transcription and for stabilizing the mRNA. Such sequences are commonly available from the 5' and, occasionally 3', untranslated regions of eukaryotic or viral DNAs or cDNAs. These regions contain nucleotide segments transcribed as polyadenylated fragments in the untranslated portion of the polypeptide-encoding mRNA. One useful transcription termination component is the bovine growth hormone polyadenylation region. See WO94/11026 and the expression vector disclosed therein.

g) Selection and transformation of host cells

[0344] Suitable host cells for cloning or expressing the DNA in the vectors herein include higher eukaryote cells described herein, including vertebrate host cells. Propagation of vertebrate cells in culture (tissue culture) has become a routine procedure. Examples of useful mammalian host cell lines are monkey kidney CV1 line transformed by SV40 (COS-7, ATCC CRL 1651); human embryonic kidney line (293 or 293 cells subcloned for growth in suspension culture, Graham *et al.*, *J. Gen Virol.* 36:59 (1977)); baby hamster kidney cells (BHK, ATCC CCL 10); Chinese hamster ovary cells/-DHFR (CHO, Urlaub *et al.*, *Proc. Natl. Acad. Sci. USA* 77:4216 (1980)); mouse sertoli cells (TM4, Mather, *Biol. Reprod.* 23:243-251 (1980)); monkey kidney cells (CV1 ATCC CCL 70); African green monkey kidney cells (VERO-76, ATCC CRL-1587); human cervical carcinoma cells (HELA, ATCC CCL 2); canine kidney cells (MDCK, ATCC CCL 34); buffalo rat liver cells (BRL 3A, ATCC CRL 1442); human lung cells (W138, ATCC CCL 75); human liver cells (Hep G2, HB 8065); mouse mammary tumor (MMT 060562, ATCC CCL51); TR1 cells (Mather *et al.*, *Annals N.Y. Acad. Sci.* 383:44-68 (1982)); MRC 5 cells; FS4 cells; and a human hepatoma line (Hep G2).

[0345] Host cells are transformed with the above-described expression or cloning vectors for antibodies production and cultured in conventional nutrient media modified as appropriate for inducing promoters, selecting transformants, or amplifying the genes encoding the desired sequences.

h) Culturing the host cells

[0346] The host cells used to produce the antibodies of the present application may be cultured in a variety of media. Commercially available media such as Ham's F10 (Sigma), Minimal Essential Medium ((MEM), (Sigma), RPMI-1640 (Sigma), and Dulbecco's Modified Eagle's Medium ((DMEM), Sigma) are suitable for culturing the host cells. In addition, any of the media described in Ham *et al.*, *Meth. Enz.* 58:44 (1979), Barnes *et al.*, *Anal. Biochem.* 102:255 (1980), U.S. Pat. No. 4,767,704; 4,657,866; 4,927,762; 4,560,655; or 5,122,469; WO 90/03430; WO 87/00195; or U.S. Pat. Re. 30,985 may be used as culture media for the host cells. Any of these media may be supplemented as necessary with hormones and/or other growth factors (such as insulin, transferrin, or epidermal growth factor), salts (such as sodium chloride, calcium, magnesium, and phosphate), buffers (such as HEPES), nucleotides (such as adenosine and thymidine), antibiotics (such as GENTAMYCIN™ drug), trace elements (defined as inorganic compounds usually present at final concentrations in the micromolar range), and glucose or an equivalent energy source. Any other necessary supplements may also be included at appropriate concentrations that would be known to those skilled in the art. The culture conditions, such as temperature, pH, and the like, are those previously used with the host cell selected for expression, and will be apparent to the ordinarily skilled artisan.

i) Protein purification

[0347] When using recombinant techniques, the antibodies can be produced intracellularly, in the periplasmic space, or directly secreted into the medium. If the antibody is produced intracellularly, as a first step, the particulate debris, either host cells or lysed fragments, are removed, for example, by centrifugation or ultrafiltration. Carter *et al.*, *Bio/Technology* 10:163-167 (1992) describe a procedure for isolating antibodies which are secreted to the periplasmic space of *E. coli*. Briefly, cell paste is thawed in the presence of sodium acetate (pH 3.5), EDTA, and phenylmethylsulfonylfluoride (PMSF) over about 30 min. Cell debris can be removed by centrifugation. Where the antibody is secreted into the medium, supernatants from such expression systems are generally first concentrated using a commercially available protein concentration filter, for example, an Amicon or Millipore Pellicon ultrafiltration unit. A protease inhibitor such as PMSF may be included in any of the foregoing steps to inhibit proteolysis and antibiotics may be included to prevent the growth of adventitious contaminants.

[0348] The protein composition prepared from the cells can be purified using, for example, hydroxylapatite chromatography, gel electrophoresis, dialysis, and affinity chromatography, with affinity chromatography being the preferred purification technique. The suitability of protein A as an affinity ligand depends on the species and isotype of any immunoglobulin Fc domain that is present in the

antibody. Protein A can be used to purify the antibodies that are based on human immunoglobulins containing 1, 2, or 4 heavy chains (Lindmark *et al.*, *J. Immunol. Meth.* 62:1-13 (1983)). Protein G is recommended for all mouse isotypes and for human 3 (Guss *et al.*, *EMBO J.* 5:1567-1575 (1986)). The matrix to which the affinity ligand is attached is most often agarose, but other matrices are available. Mechanically stable matrices such as controlled pore glass or poly(styrene-divinyl)benzene allow for faster flow rates and shorter processing times than can be achieved with agarose. Where the antibody comprises a C_H3 domain, the Bakerbond ABXTMresin (J. T. Baker, Phillipsburg, N.J.) is useful for purification. Other techniques for protein purification such as fractionation on an ion-exchange column, ethanol precipitation, Reverse Phase HPLC, chromatography on silica, chromatography on heparin SEPHAROSE™ chromatography on an anion or cation exchange resin (such as a polyaspartic acid column), chromatofocusing, SDS-PAGE, and ammonium sulfate precipitation are also available depending on the antibody to be recovered.

[0349] Following any preliminary purification step(s), the mixture comprising the antibody of interest and contaminants may be subjected to low pH hydrophobic interaction chromatography using an elution buffer at a pH between about 2.5-4.5, preferably performed at low salt concentrations (*e.g.*, from about 0-0.25M salt).

3. Polyclonal antibodies

[0350] Polyclonal antibodies are generally raised in animals by multiple subcutaneous (s.c.) or intraperitoneal (i.p.) injections of the relevant antigen and an adjuvant. It may be useful to conjugate the relevant antigen to a protein that is immunogenic in the species to be immunized, *e.g.*, keyhole limpet hemocyanin (KLH), serum albumin, bovine thyroglobulin, or soybean trypsin inhibitor, using a bifunctional or derivatizing agent, *e.g.*, maleimidobenzoyl sulfo succinimide ester (conjugation through cysteine residues), N-hydroxysuccinimide (through lysine residues), glutaraldehyde, succinic anhydride, SOCl₂, or R¹N=C=NR, where R and R¹ are independently lower alkyl groups. Examples of adjuvants which may be employed include Freund's complete adjuvant and MPL-TDM adjuvant (monophosphoryl Lipid A, synthetic trehalose dicorynomycolate). The immunization protocol may be selected by one skilled in the art without undue experimentation.

[0351] The animals are immunized against the antigen, immunogenic conjugates, or derivatives by combining, *e.g.*, 100 µg or 5 µg or the protein or conjugate (for rabbits or mice, respectively) with 3 volumes of Freund's complete adjuvant and injecting the solution intradermally at multiple sites. One month later, the animals are boosted with 1/5 to 1/10 the original amount of peptide or conjugate in Freund's complete adjuvant by subcutaneous injection at multiple sites. Seven to fourteen days later, the

animals are bled and the serum is assayed for antibody titer. Animals are boosted until the titer plateaus. Conjugates also can be made in recombinant cell culture as protein fusions. Also, aggregating agents such as alum are suitable to enhance the immune response. Also see Example 1 for immunization in Camels.

4. Monoclonal antibodies

[0352] Monoclonal antibodies are obtained from a population of substantially homogeneous antibodies, *i.e.*, the individual antibodies comprising the population are identical except for possible naturally occurring mutations and/or post-translational modifications (*e.g.*, isomerizations, amidations) that may be present in minor amounts. Thus, the modifier “monoclonal” indicates the character of the antibody as not being a mixture of discrete antibodies. For example, the monoclonal antibodies may be made using the hybridoma method first described by Kohler *et al.*, *Nature*, 256:495 (1975), or may be made by recombinant DNA methods (U.S. Pat. No. 4,816,567). In the hybridoma method, a mouse or other appropriate host animal, such as a hamster or a llama, is immunized as hereinabove described to elicit lymphocytes that produce or are capable of producing antibodies that will specifically bind the protein 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, *Monoclonal Antibodies: Principles and Practice*, pp. 59-103 (Academic Press, 1986). Also see Example 1 for immunization in Camels.

[0353] The immunizing agent will typically include the antigenic protein or a fusion variant thereof. Generally either peripheral blood lymphocytes (“PBLs”) are used if cells of human origin are desired, or spleen cells or lymph node cells are used if non-human mammalian sources are desired. The lymphocytes are then fused with an immortalized cell line using a suitable fusing agent, such as polyethylene glycol, to form a hybridoma cell. Goding, *Monoclonal Antibodies: Principles and Practice*, Academic Press (1986), pp. 59-103.

[0354] Immortalized cell lines are usually transformed mammalian cells, particularly myeloma cells of rodent, bovine and human origin. Usually, rat or mouse myeloma cell lines are employed. 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 are substances that prevent the growth of HGPRT-deficient cells.

[0355] Preferred immortalized myeloma cells are those 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, preferred are murine myeloma lines, such as those derived from MOPC-21 and MPC-11 mouse tumors available from the Salk Institute Cell Distribution Center, San Diego, Calif. USA, and SP-2 cells (and derivatives thereof, *e.g.*, X63-Ag8-653) available from the American Type Culture Collection, Manassas, Va. USA. Human myeloma and mouse-human heteromyeloma cell lines also have been described for the production of human monoclonal antibodies (Kozbor, *J. Immunol.*, 133:3001 (1984); Brodeur *et al.*, *Monoclonal Antibody Production Techniques and Applications*, pp. 51-63 (Marcel Dekker, Inc., New York, 1987)).

[0356] Culture medium in which hybridoma cells are growing is assayed for production of monoclonal antibodies directed against the antigen. Preferably, the binding specificity of monoclonal antibodies produced by hybridoma cells is determined by immunoprecipitation or by an *in vitro* binding assay, such as radioimmunoassay (RIA) or enzyme-linked immunosorbent assay (ELISA).

[0357] The culture medium in which the hybridoma cells are cultured can be assayed for the presence of monoclonal antibodies directed against the desired antigen. Preferably, the binding affinity and specificity of the monoclonal antibody can be determined by immunoprecipitation or by an *in vitro* binding assay, such as radioimmunoassay (RIA) or enzyme-linked assay (ELISA). Such techniques and assays are known in the art. For example, binding affinity may be determined by the Scatchard analysis of Munson *et al.*, *Anal. Biochem.*, 107:220 (1980).

[0358] 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, *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 tumors in a mammal.

[0359] 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.

[0360] Monoclonal antibodies may also be made by recombinant DNA methods, such as those described in U.S. Pat. No. 4,816,567, and as described above. DNA encoding the monoclonal antibodies is readily isolated and sequenced using conventional procedures (*e.g.*, by using oligonucleotide probes that are capable of binding specifically to genes encoding the heavy and light chains of murine antibodies). The hybridoma cells serve as a preferred source of such DNA. Once isolated, the DNA may be placed into expression vectors, which are then transfected into host cells such as *E. coli* cells, simian COS cells, Chinese hamster ovary (CHO) cells, or myeloma cells that do not otherwise produce immunoglobulin

protein, in order to synthesize monoclonal antibodies in such recombinant host cells. Review articles on recombinant expression in bacteria of DNA encoding the antibody include Skerra *et al.*, *Curr. Opinion in Immunol.*, 5:256-262 (1993) and Pliickthun, *Immunol. Revs.* 130:151-188 (1992).

[0361] In a further embodiment, antibodies can be isolated from antibody phage libraries generated using the techniques described in McCafferty *et al.*, *Nature*, 348:552-554 (1990). Clackson *et al.*, *Nature*, 352:624-628 (1991) and Marks *et al.*, *J. Mol. Biol.*, 222:581-597 (1991) describe the isolation of murine and human antibodies, respectively, using phage libraries. Subsequent publications describe the production of high affinity (nM range) human antibodies by chain shuffling (Marks *et al.*, *Bio/Technology*, 10:779-783 (1992)), as well as combinatorial infection and in vivo recombination as a strategy for constructing very large phage libraries (Waterhouse *et al.*, *Nucl. Acids Res.*, 21:2265-2266 (1993)). Thus, these techniques are viable alternatives to traditional monoclonal antibody hybridoma techniques for isolation of monoclonal antibodies.

[0362] The DNA also may be modified, for example, by substituting the coding sequence for human heavy- and light-chain constant domains in place of the homologous murine sequences (U.S. Pat. No. 4,816,567; Morrison, *et al.*, *Proc. Natl Acad. Sci. USA*, 81:6851 (1984)), or by covalently joining to the immunoglobulin coding sequence all or part of the coding sequence for a non-immunoglobulin polypeptide. Typically such non-immunoglobulin polypeptides are substituted for the constant domains of an antibody, or they are substituted for the variable domains of one antigen-combining site of an antibody to create a chimeric bivalent antibody comprising one antigen-combining site having specificity for an antigen and another antigen-combining site having specificity for a different antigen.

[0363] The monoclonal antibodies described herein may be monovalent, the preparation of which is well known in the art. For example, one method involves recombinant expression of immunoglobulin light chain and a modified heavy chain. The heavy chain is truncated generally at any point in the Fc region so as to prevent heavy chain crosslinking. Alternatively, the relevant cysteine residues may be substituted with another amino acid residue or are deleted so as to prevent crosslinking. *In vitro* methods are also suitable for preparing monovalent antibodies. Digestion of antibodies to produce fragments thereof, particularly Fab fragments, can be accomplished using routine techniques known in the art.

[0364] Chimeric or hybrid antibodies also may be prepared *in vitro* using known methods in synthetic protein chemistry, including those involving crosslinking agents. For example, immunotoxins may be constructed using a disulfide-exchange reaction or by forming a thioether bond. Examples of suitable reagents for this purpose include iminothiolate and methyl-4-mercaptobutyrimidate.

[0365] Also, *see*, Example 1 for monoclonal sdAb production.

5. Humanized antibodies

[0366] Humanized forms of non-human (*e.g.*, murine) antibodies are chimeric immunoglobulins, immunoglobulin chains or fragments thereof (such as Fv, Fab, Fab', F(ab')₂ or other antigen-binding subsequences of antibodies) which contain minimal sequence derived from non-human immunoglobulin. Humanized antibodies include human immunoglobulins (recipient antibody) in which residues from a CDR of the recipient are replaced by residues from a CDR of a non-human species (donor antibody) such as mouse, rat, rabbit, camelid, or llama having the desired specificity, affinity, and capacity. In some instances, Fv framework residues of the human immunoglobulin are replaced by corresponding non-human residues. Humanized antibodies can also comprise residues that are found neither in the recipient antibody nor in the imported CDR or framework sequences. In general, the humanized antibody can comprise substantially all of at least one, and typically two, variable domains, in which all or substantially all of the CDR regions correspond to those of a non-human immunoglobulin, and all or substantially all of the FR regions are those of a human immunoglobulin consensus sequence. In some embodiments, the humanized antibody will comprise at least a portion of an immunoglobulin constant region (Fc), typically that of a human immunoglobulin. *See, e.g.*, Jones *et al.*, *Nature*, 321: 522-525 (1986); Riechmann *et al.*, *Nature*, 332: 323-329 (1988); Presta, *Curr. Op. Struct. Biol.*, 2:593-596 (1992).

[0367] Methods for humanizing non-human antibodies are well known in the art. Generally, a humanized antibody has one or more amino acid residues introduced into it from a source which is non-human. These non-human amino acid residues are often referred to as "import" residues, which are typically taken from an "import" variable domain. Humanization can be essentially performed following the method of Winter and co-workers, Jones *et al.*, *Nature* 321:522-525 (1986); Riechmann *et al.*, *Nature* 332:323-327 (1988); Verhoeven *et al.*, *Science* 239:1534-1536 (1988), or through substituting rodent CDRs or CDR sequences for the corresponding sequences of a human antibody. Accordingly, such "humanized" antibodies are chimeric antibodies (U.S. Pat. No. 4,816,567), wherein substantially less than an intact human variable domain has been substituted by the corresponding sequence from a non-human species. In practice, humanized antibodies are typically human antibodies in which some CDR residues and possibly some FR residues are substituted by residues from analogous sites in rodent antibodies.

[0368] The choice of human variable domains, both light and heavy, to be used in making the humanized antibodies is very important to reduce antigenicity. According to the so-called "best-fit" method, the sequence of the variable domain of a rodent antibody is screened against the entire library of known human variable-domain sequences. The human sequence which is closest to that of the rodent is then accepted as the human framework (FR) for the humanized antibody. Sims *et al.*, *J. Immunol.*, 151:2296 (1993); Chothia *et al.*, *J. Mol. Biol.*, 196:901 (1987). Another method uses a particular

framework derived from the consensus sequence of all human antibodies of a particular subgroup of light or heavy chains. The same framework may be used for several different humanized antibodies. Carter *et al.*, *Proc. Natl. Acad. Sci. USA*, 89:4285 (1992); Presta *et al.*, *J. Immunol.*, 151:2623 (1993).

[0369] It is further important that antibodies be humanized with retention of high affinity for the antigen and other favorable biological properties. To achieve this goal, according to a preferred method, humanized antibodies are prepared by a process of analysis of the parental sequences and various conceptual humanized products using three-dimensional models of the parental and humanized sequences. Three-dimensional immunoglobulin models are commonly available and are familiar to those skilled in the art. Computer programs are available which illustrate and display probable three-dimensional conformational structures of selected candidate immunoglobulin sequences. Inspection of these displays permits analysis of the likely role of the residues in the functioning of the candidate immunoglobulin sequence, *i.e.*, the analysis of residues that influence the ability of the candidate immunoglobulin to bind its antigen. In this way, FR residues can be selected and combined from the recipient and import sequences so that the desired antibody characteristic, such as increased affinity for the target antigen(s), is achieved. In general, the CDR residues are directly and most substantially involved in influencing antigen binding.

[0370] In some embodiments, the sdAbs are modified, such as humanized, without diminishing the native affinity of the domain for antigen and while reducing its immunogenicity with respect to a heterologous species. For example, the amino acid residues of the antibody variable domain (V_HH) of an llama antibody can be determined, and one or more of the Camelidae amino acids, for example, in the framework regions, are replaced by their human counterpart as found in the human consensus sequence, without that polypeptide losing its typical character, *i.e.* the humanization does not significantly affect the antigen binding capacity of the resulting polypeptide. Humanization of Camelidae sdAbs requires the introduction and mutagenesis of a limited amount of amino acids in a single polypeptide chain. This is in contrast to humanization of scFv, Fab', (Fab')₂ and IgG, which requires the introduction of amino acid changes in two chains, the light and the heavy chain and the preservation of the assembly of both chains.

6. Human antibodies

[0371] As an alternative to humanization, human antibodies can be generated. For example, it is now possible to produce transgenic animals (*e.g.*, mice) that are capable, upon immunization, of producing a full repertoire of human antibodies in the absence of endogenous immunoglobulin production. For example, it has been described that the homozygous deletion of the antibody heavy-chain joining region (J_H) gene in chimeric and germ-line mutant mice results in complete inhibition of endogenous antibody

production. Transfer of the human germ-line immunoglobulin gene array into such germ-line mutant mice will result in the production of human antibodies upon antigen challenge. See, *e.g.*, Jakobovits *et al.*, *PNAS USA*, 90:2551 (1993); Jakobovits *et al.*, *Nature*, 362:255-258 (1993); Bruggemann *et al.*, *Year in Immunol.*, 7:33 (1993); U.S. Patent Nos. 5,545,806, 5,569,825, 5,591,669; 5,545,807; and WO 97/17852. Transgenic mice or rats capable of producing fully human sdAbs are known in the art. See, *e.g.*, US20090307787A1, U.S. Pat. No. 8,754,287, US20150289489A1, US20100122358A1, and WO2004049794.

[0372] Alternatively, phage display technology can be used to produce human antibodies and antibody fragments *in vitro*, from immunoglobulin variable (V) domain gene repertoires from unimmunized donors. McCafferty *et al.*, *Nature* 348:552-553 (1990); Hoogenboom and Winter, *J. Mol. Biol.* 227: 381 (1991). According to this technique, antibody V domain genes are cloned in-frame into either a major or minor coat protein gene of a filamentous bacteriophage, such as M13 or fd, and displayed as functional antibody fragments on the surface of the phage particle. Because the filamentous particle contains a single-stranded DNA copy of the phage genome, selections based on the functional properties of the antibody also result in selection of the gene encoding the antibody exhibiting those properties. Thus, the phage mimics some of the properties of the B-cell. Phage display can be performed in a variety of formats, reviewed in, *e.g.*, Johnson, Kevin S, and Chiswell, David J., *Curr. Opin Struct. Biol.* 3:564-571 (1993). Several sources of V-gene segments can be used for phage display. Clackson *et al.*, *Nature* 352:624-628 (1991) isolated a diverse array of anti-oxazolone antibodies from a small random combinatorial library of V genes derived from the spleens of immunized mice. A repertoire of V genes from unimmunized human donors can be constructed and antibodies to a diverse array of antigens (including self-antigens) can be isolated essentially following the techniques described by Marks *et al.*, *J. Mol. Biol.* 222:581-597 (1991), or Griffith *et al.*, *EMBO J.* 12:725-734 (1993). See also, U.S. Pat. Nos. 5,565,332 and 5,573,905.

[0373] The techniques of Cole *et al.* and Boerner *et al.* are also available for the preparation of human monoclonal antibodies (Cole *et al.*, *Monoclonal Antibodies and Cancer Therapy*, Alan R. Liss, p. 77 (1985) and Boerner *et al.*, *J. Immunol.*, 147(1): 86-95 (1991)). Similarly, human antibodies can be made by introducing human immunoglobulin loci into transgenic animals, *e.g.*, mice in which the endogenous immunoglobulin genes have been partially or completely inactivated. Upon challenge, human antibody production is observed that closely resembles that seen in humans in all respects, including gene rearrangement, assembly, and antibody repertoire. This approach is described, for example, in U.S. Patent Nos. 5,545,807; 5,545,806; 5,569,825; 5,625,126; 5,633,425; and 5,661,016, and Marks *et al.*, *Bio/Technology*, 10: 779-783 (1992); Lonberg *et al.*, *Nature*, 368: 856-859 (1994); Morrison, *Nature*, 368: 812-813 (1994); Fishwild *et al.*, *Nature Biotechnology*, 14: 845-851 (1996); Neuberger, *Nature*

Biotechnology, 14: 826 (1996); Lonberg and Huszar, *Intern. Rev. Immunol.*, 13: 65-93 (1995). For example, in some embodiments, human antibodies (*e.g.*, human DABs) can be generated by immunization of human HCAB mice. For example, HCAB (*e.g.*, sdAb-Fc fusion proteins) can be produced by immunizing a transgenic mouse in which endogenous murine antibody expression has been eliminated and human transgenes have been introduced. HCAB mice are disclosed in US8,883,150, US8,921,524, US8,921,522, US8,507,748, US8,502,014, US2014/0356908, US2014/0033335, US2014/0037616, US2014/0356908, US2013/0344057, US2013/0323235, US2011/0118444, and US2009/0307787, all of which are incorporated herein by reference for all they disclose regarding heavy chain only antibodies and their production in transgenic mice. The HCAB mice are immunized and the resulting primed spleen cells fused with a murine myeloma cells to form hybridomas. The resultant HCAB can then be made fully human by replacing the murine CH2 and CH3 regions with human sequences.

[0374] Finally, human antibodies may also be generated by *in vitro* activated B cells (see U.S. Patents 5,567,610 and 5,229,275) or by using various techniques known in the art, including phage display libraries (Hoogenboom and Winter, *J. Mol. Biol.*, 227:381 (1991); Marks *et al.*, *J. Mol. Biol.*, 222:581 (1991).

VI. Articles of manufacture and kits

[0375] Further provided are kits and articles of manufacture comprising any of the isolated anti-LAG-3 constructs (such as anti-LAG-3 sdAb, anti-LAG-3 HCAB, anti-LAG-3 MABP), isolated nucleic acids or vectors encoding thereof, or isolated host cells comprising the isolated nucleic acids or vectors encoding the anti-LAG-3 constructs described herein. In some embodiments, a kit is provided which comprises any one of the pharmaceutical compositions described herein and preferably provides instructions for its use.

[0376] The kits of the present application are in suitable packaging. Suitable packaging includes, but is not limited to, vials, bottles, jars, flexible packaging (*e.g.*, sealed Mylar or plastic bags), and the like. Kits may optionally provide additional components such as buffers and interpretative information. The present application thus also provides articles of manufacture, which include vials (such as sealed vials), bottles, jars, flexible packaging, and the like.

[0377] The article of manufacture can comprise a container and a label or package insert on or associated with the container. Suitable containers include, for example, bottles, vials, syringes, *etc.* The containers may be formed from a variety of materials such as glass or plastic. Generally, the container holds a composition which is effective for treating a disease or disorder (such as cancer) described herein, and may have a sterile access port (for example the container may be an intravenous solution bag or a vial

having a stopper pierceable by a hypodermic injection needle). The label or package insert indicates that the composition is used for treating the particular condition in an individual. The label or package insert will further comprise instructions for administering the composition to the individual. The label may indicate directions for reconstitution and/or use. The container holding the pharmaceutical composition may be a multi-use vial, which allows for repeat administrations (*e.g.* from 2-6 administrations) of the reconstituted formulation. Package insert refers to instructions customarily included in commercial packages of therapeutic products that contain information about the indications, usage, dosage, administration, contraindications and/or warnings concerning the use of such therapeutic products. Additionally, the article of manufacture may further comprise a second container comprising a pharmaceutically-acceptable buffer, such as bacteriostatic water for injection (BWI), phosphate-buffered saline, Ringer's solution and dextrose solution. It may further include other materials desirable from a commercial and user standpoint, including other buffers, diluents, filters, needles, and syringes.

[0378] The kits or article of manufacture may include multiple unit doses of the pharmaceutical composition and instructions for use, packaged in quantities sufficient for storage and use in pharmacies, for example, hospital pharmacies and compounding pharmacies.

EXAMPLES

[0379] The examples below are intended to be purely exemplary of the invention and should therefore not be considered to limit the invention in any way. The following examples and detailed description are offered by way of illustration and not by way of limitation.

Example 1: Generation of anti-LAG-3 sdAbs

Immunization

[0380] Camels were immunized with recombinant LAG-3-His and LAG-3-Fc (Acrobiosystems and R&D Systems) proteins and CHO-K1 cells expressing human LAG-3 (CHO-huLAG-3 cells), all under current animal welfare regulations. For immunization, the antigen was formulated as an emulsion with CFA (Complete Freund's adjuvant; primary immunization) or IFA (incomplete Freund's adjuvant; boost immunizations). The antigen was administered subcutaneously at the neck. Each animal received 5 injections of 100 µg of LAG-3-His in CFA emulsion, and 5 subsequent injections of LAG-3-Fc in IFA emulsion, at two-week intervals. Subsequently, two boosts with CHO-huLAG-3 cells and two boosts with LAG-3 protein were performed. At various time points during the immunization schedule, 10 mL blood samples were collected from the animals and sera were prepared. The induction of an antigen specific

humoral immune response was verified using the serum samples in an ELISA-based assay with immobilized LAG-3-Fc protein or Fc protein as a control (FIG. 1 and FIG. 2). Although antibodies against Fc protein were detected, the observed antibody binding to LAG-3-Fc was even stronger, thereby showing an adequate elicitation of response against LAG-3 protein. In particular, heavy chain immunoglobulins (HCAbs) against LAG-3 protein were detected. Five days after the last immunization, a blood sample of 300 mL was collected from each animal. Peripheral blood lymphocytes (PBLs), as the genetic source of the camel HCAbs, were isolated from this 300 mL blood sample using a FICOLL[®]-Paque gradient (Amersham Biosciences), yielding 1×10^9 PBLs.

Library construction

[0381] RNA extracted from PBLs was used as starting material for RT-PCR to amplify sdAb encoding gene fragments. These fragments were cloned into an in-house phagemid vector. The vector coded for a C-terminal His-Tag in frame with the sdAb coding sequence. The library size is around 6×10^8 bp. The library phage was prepared according to a standard protocol, filter sterilized, and stored at 4°C for further use.

Selections and high-throughput screening

[0382] Selections were carried out with on above libraries using solid panning as well as cell-based panning. Only a single round of selection was performed for both conditions. Each selection output was analyzed for enrichment factor (# of phage present in eluate relative to control), as well as diversity and percentage of LAG-3 positive clones (ELISA). Based on these parameters the best selections were chosen for further analysis. To this end, the output from each selection was recloned as a pool into a soluble expression vector for high-throughput screening. The vector coded for a C-terminal His-Tag in frame with the sdAb coding sequence. Colonies were picked and grown in 96 deep well plates (in 1 mL volume); sdAb expression and release into supernatant was induced by adding IPTG and 0.1% Triton to the colonies.

[0383] The contents in the supernatant were analyzed for their ability to bind to LAG-3 protein (by ELISA) and LAG-3-expressing CHO-K1 stable cell line (by FACS). The positive binders were sequenced and the unique clones were selected for further characterization.

[0384] The unique clones were grown in 2XYT medium; sdAb expression and release into supernatant was induced by adding IPTG to the clones. The supernatants containing unique binders were analyzed for their ability to inhibit the interaction between MHC II and LAG-3. To this end, A375 melanoma cells, which express MHC II, were incubated with the sdAb-containing supernatant and biotinylated LAG-3-Fc, followed by incubation with fluorophore-labelled streptavidin. A shift in mean fluorescent intensity

(MFI) as compared to samples without anti-LAG-3 sdAb blockade represents an inhibition of the MHC II/LAG-3 interaction.

[0385] All potential inhibitors were selected for K_D analysis by surface plasmon resonance (SPR) on a BIACORE™ T200 instrument. The dissociation phase was used to calculate the k_{off} values for each individual sdAb.

Example 2: Preparation and *in vitro* evaluation of non-humanized anti-LAG-3 sdAb-Fc fusion proteins

Production of sdAb-Fc fusion protein

[0386] The anti-LAG-3 sdAb-Fc fusion protein constructs were generated by fusion of anti-LAG-3 sdAbs with a human IgG4 Fc region. The IgG4 Fc referred here carries a S228P mutation to prevent arm exchange. Maxipreps of the constructs were prepared for CHO-K1 cell transient expression and purification. The expressed anti-LAG-3 sdAb-Fc fusion proteins were purified by chromatography through a column containing Protein A agarose resins. Protein purity was determined by SEC-HPLC. An anti-LAG-3 monoclonal antibody generated by Bristol-Myers Squibb, BMS-986016, was produced according to the sequence in a published patent (*See* WO/2015/116539, SEQ ID NOs: 17 and 18; incorporated herein as SEQ ID NOs: 354 and 355 in the present application) on a human IgG4 backbone.

Target protein binding and cross-species reaction test by surface plasmon resonance (SPR)

[0387] BIACORE™ T200 instrument was utilized to determine the affinity constant (K_D) of each anti-LAG-3 antibody (sdAb-Fc fusion protein or mAb format) by SPR. Briefly, anti-LAG-3 antibody was immobilized on the sensor chip through capture antibody (Jackson ImmunoResearch Cat.No.115-005-071) at an appropriate density. Human or cynomolgus LAG-3 protein (Acrobiosystem, 772-6BGF2-D5 and 2187-71HF1-DZ, respectively) was injected at no less than 5 different concentrations. The data of dissociation rate constant (k_{off}) and association rate constant (k_{on}) were obtained using BIACORE™ T200 evaluation software and the affinity constant (K_D) were calculated from the ratio of k_{off} to k_{on} . The kinetics data of several exemplary anti-LAG-3 antibodies were summarized in Table 2.

Table 2. Affinity determination of non-humanized anti-LAG-3 sdAb-Fc fusion proteins against LAG-3

Target	Construct	k_{on} (1/Ms)	k_{off} (1/s)	K_D (M)
Human LAG-3-His	AS20592-Fc	2.1E+06	2.2E-04	1.1E-10
	AS20594-Fc	2.2E+06	2.4E-04	1.1E-10
	AS20601-Fc	4.4E+06	1.8E-04	4.2E-11
	AS20846-Fc	2.2E+06	1.5E-04	6.7E-11
	BMS-986016	1.9E+06	1.1E-03	5.9E-10

Cynomolgus LAG-3-Fc	AS20592-Fc	4.5E+06	4.2E-04	9.3E-11
	AS20594-Fc	5.3E+06	2.8E-04	5.2E-11
	AS20601-Fc	1.2E+07	3.5E-03	2.9E-10
	AS20846-Fc	5.4E+06	2.2E-04	4.2E-11
	BMS-986016	1.1E+07	2.0E-02	1.7E-09

Epitope binning test

[0388] Epitope binning test was performed on an OCTET® RED96 instrument (ForteBio). All measurements were performed at 30°C. An sdAb-Fc fusion protein of interest was immobilized onto the biosensors using amine coupling method. Human LAG-3 protein was diluted in PBST buffer (1x PBS, pH 7.4, and 0.05% Tween-20) as used as analyte 1. A mixture of human LAG-3 (at the same concentration as in analyte 1) and a second LAG-3 binding entity (which could be a second sdAb-Fc fusion protein or benchmark antibody BMS-986016) was used as analyte 2. The coated biosensors were first dipped into analyte 1, and after regeneration and equilibration, into analyte 2. The sensorgrams of analyte 1 (i.e., curve for human LAG-3 alone) and analyte 2 (i.e., curves for human LAG-3 + second LAG-3 binding entity) were compared to determine binding competition by the sdAb-Fc fusion protein of interest. Binning results are shown in FIG. 3. None of the 4 non-humanized sdAb-Fc fusion proteins tested competes with BMS-986016 for binding to human LAG-3 protein, while the 4 sdAb-Fc fusion proteins compete with one another, indicating that the sdAbs bind against a new epitope on LAG-3 different from the one targeted by the benchmark antibody BMS-986016.

CHO-huLAG-3 cell binding and inhibition of ligand binding by FACS analysis

[0389] To determine cell binding EC₅₀, CHO-K1 cells expressing human or cynomolgus LAG-3 were harvested and incubated with anti-LAG-3 sdAb-Fc fusion proteins at gradient concentrations, followed by fluorophore-labeled secondary antibodies against human Fc. For blocking assay, anti-LAG-3 sdAb-Fc protein at gradient concentrations and biotinylated LAG-3-Fc protein at a fixed concentration were co-incubated with MHC II-expressing A375 melanoma cells. The binding of LAG-3-Fc to A375 cells was detected with fluorophore-labeled streptavidin. The samples were then analyzed with flow cytometry. The binding and blocking curves were shown in FIG. 4 and FIG. 5, respectively. EC₅₀ of binding and blocking capabilities of the anti-LAG-3 sdAb-Fc fusion proteins were summarized in Table 3. The anti-LAG-3 sdAb-Fc fusion proteins had similar binding capabilities to human LAG-3 expressed on CHO cells, as compared to the positive control BMS-986016. In the cell blocking assay, although EC₅₀ of the blocking curves of all anti-LAG-3 sdAb-Fc fusion proteins are higher than that of BMS-986016, it is notable that their spans of blocking curves are greater than that of BMS-986016, indicating that binding

of sdAb-Fc fusion proteins against its specific epitope may block LAG-3's interaction with MHC II more completely than BMS-986016 does.

Table 3. Binding and blocking of ligand binding data of non-humanized anti-LAG-3 sdAb-Fc fusion proteins against LAG-3

	Binding Assay EC50 (nM)		Blocking Assay (Human)	
	Human	Cynomolgus	EC50 (nM)	Span
AS20592-Fc	0.686	1.829	5.544	233.2
AS20594-Fc	0.390	1.024	6.584	328.2
AS20601-Fc	0.494	1.607	7.992	260.0
AS20846-Fc	0.462	1.230	3.723	354.9
BMS-986016	0.590	17.55	2.634	181.3

Example 3: Humanization, production and characterization of anti-LAG-3 sdAb-Fc fusion proteins

Humanization of anti-LAG-3 sdAbs

[0390] Protein sequences of sdAb AS20592, AS20594, AS20601 and AS20846 were aligned with the 5 closest human germline sequences sharing the highest degree of homology. The best human germline sequences were selected as human acceptors, respectively. Homology models were then constructed. According to the model analysis data, residues potentially critical for antigen binding or antibody scaffold formation were left unaltered while the rest were selected for conversion into human counterparts. Initially a panel of four to six sequence optimized variants was generated (stage 1). These variants were analyzed for a number of parameters and the results obtained were used to design a second set of sdAbs (stage 2). The humanized sdAbs are indicated with "VH" in their names.

Production of humanized anti-LAG-3 sdAb-Fc fusion proteins

[0391] Among humanized variants, AS20592VH10, AS20594VH10, AS20601VH4 and AS20846VH12 were selected for production and characterization according to affinity and small scale production level. The humanized anti-LAG-3 sdAb-Fc fusion protein constructs were generated by fusing humanized anti-LAG-3 sdAbs with the human IgG4 Fc region. Maxipreps of the constructs were prepared for HEK293 cell transient expression and purification. The expressed humanized anti-LAG-3 sdAb-Fc fusion proteins were purified by chromatography through a column containing Protein A agarose resins. Protein purity was determined by SEC-HPLC. Expression results were summarized in Table 4.

Table 4. Expression of humanized anti-LAG-3 sdAb-Fc fusion proteins

Construct	Transient expression (mg/L)	Purity (%)	Endotoxin level (EU/mg)
AS20592VH10-Fc	96.0	99.56	0.3

AS20594VH10-Fc	18.7	97.94	0.7
AS20601VH4-Fc	55.0	95.50	0.8
AS20846VH12-Fc	24.8	99.00	0.8

Target protein binding and cross-species reaction test by surface plasmon resonance (SPR)

[0392] BIACORE™ T200 instrument was utilized to determine affinity constant (K_D) of each anti-LAG-3 antibody (sdAb-Fc fusion protein or mAAb format) by SPR. Briefly, for measuring the affinity against human LAG-3, anti-LAG-3 antibody was immobilized on the sensor chip through capture antibody (Jackson ImmunoResearch Cat.No.115-005-071) at an appropriate density. Human LAG-3 protein (Acrobiosystem, 772-6BGF2-D5) was injected at no less than 5 different concentrations. For measuring the affinity against cynomolgus LAG-3, the cynomolgus LAG-3 protein (Acrobiosystem, 2187-71HF1-DZ) was immobilized on the sensor chip at an appropriate density and anti-LAG-3 antibodies were injected at no less than 5 different concentrations. The data of dissociation rate constant (k_{off}) and association rate constant (k_{on}) were obtained using BIACORE™ T200 evaluation software and the affinity constant (K_D) were calculated from the ratio of k_{off} to k_{on} . The kinetics data of several exemplary anti-LAG-3 antibodies were summarized in Table 5.

Table 5. Affinity determination of humanized anti-LAG-3 sdAb-Fc fusion proteins

Target	Construct	k_{on} (1/Ms)	k_{off} (1/s)	K_D (M)
Human LAG-3-His	AS20592VH10-Fc	1.4E+06	6.5E-04	4.7E-10
	AS20594VH10-Fc	1.6E+06	7.9E-04	4.9E-10
	AS20601VH4-Fc	3.2E+06	3.3E-04	1.0E-10
	AS20846VH12-Fc	1.3E+06	6.0E-04	4.6E-10
	BMS-986016	9.2E+05	7.0E-04	7.6E-10
Cynomolgus LAG-3-Fc	AS20592VH10-Fc	3.7E+04	1.5E-03	3.9E-08
	AS20594VH10-Fc	6.5E+04	3.3E-03	5.1E-08
	AS20601VH4-Fc	9.2E+04	7.0E-03	7.6E-08
	AS20846VH12-Fc	3.1E+04	1.5E-03	4.9E-08
	BMS-986016	7.4E+04	7.5E-02	1.0E-06

LAG-3 blockade reporter assay

[0393] LAG-3 blockade reporter assay was performed using Promega LAG-3 blockade reporter assay kit (Promega, Cat#CS194819), according to the vendor's protocol. Briefly, Thaw-and-Use MHC-II APC Cells (including TCR Activating Antigen) were plated overnight and then incubated with a serial dilution of anti-LAG-3 antibodies or anti-LAG-3 sdAb-Fc fusion proteins, followed by addition of Thaw-and-Use LAG-3 Effector cells. After 6 hours of induction at 37°C and 5% CO₂, BIO-GLO™ Luciferase Assay Reagent was added, and luminescence was determined. Four-parameter logistic curve analysis was performed with GraphPad Prism 6 software. Data curves are shown in FIG. 6 and summarized in Table 6.

AS20592VH10-Fc, AS20601VH4-Fc and AS20846VH12-Fc have comparable or superior functions to BMS-986016 in the LAG-3 blockade reporter assay. Notably, the signal spans, which are indicative of the level of inhibition, of the sdAb-Fc fusion proteins were all greater than that of the benchmark antibody BMS-986016, consistent with the findings in the previously described ligand blocking test.

Table 6. LAG-3 blockade reporter assay for humanized anti-LAG-3 sdAb-Fc fusion proteins

Construct	EC50 (nM)	Signal span (% activation)
AS20592VH10-Fc	0.89	91.96
AS20601VH4-Fc	3.98	92.66
AS20846VH12-Fc	3.13	83.30
BMS-986016	3.82	76.84

Example 4: Generation and characterization of PD-1×LAG-3 BABPs

Construction of PD-1×LAG-3 BABPs

[0394] This example describes the construction of PD-1×LAG-3 BABPs.

[0395] BABPs can be constructed by fusing an anti-LAG-3 sdAb to a full-length antibody, or to a scFv or Fab region derived from the full-length antibody containing an Fc region at the C-terminus, such as anti-PD-1 antibodies, *e.g.*, KEYTRUDA® (Pembrolizumab), OPDIVO® (nivolumab), or PD1-BM-min. The anti-LAG-3 sdAb can be connected to the full-length antibody (or scFv or Fab region derived from the full-length antibody with Fc region at the C-terminus) via a linker (such as 9-amino acid Gly/Ser linker (9GS linker), human IgG1 (hIgG1) hinge, or mutated hIgG1 hinge), or without a linker. In addition, a short peptide sequence can be fused to the N-terminus (*see* FIGs. 7, 9, 11 and 12) or C-terminus (*see* FIGs. 8 and 10) of an anti-LAG-3 sdAb. The BABP can be of any one of the configurations exemplified in FIGs. 7-16. For example, anti-LAG-3 sdAb can be fused to at least one of the heavy chains, at least one of the light chains, or both the heavy chains and light chains, via N- or C-terminus (*see* FIGs. 7-10).

[0396] As shown in Table 7 below, exemplary BABPs were generated. The anti-LAG-3 sdAb (AS20592VH10, AS20594VH10, AS20601VH4 or AS20846VH12) were fused to the N-terminus of the heavy chain, the C terminus of the heavy chain, the N-terminus of the light chain, or the C-terminus of the light chain of a full-length anti-PD-1 antibody (Pembrolizumab or PD1-BM-min) via a mutated human IgG1 (hIgG1) hinge as the linker, or without a linker. In some BABP constructs, an additional short peptide was added to the N-termini of the sdAbs.

Table 7. Exemplary PD-1×LAG-3 constructs

BABP	anti-LAG-3 sdAb	anti-PD-1 full-length	Location of sdAb	Fusion
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		antibody		format
BLP-1	AS20592	PD1-BM-min	N-terminus of heavy chain	FIG. 7
BLP-2	AS20594	PD1-BM-min	N-terminus of heavy chain	FIG. 7
BLP-3	AS20601	PD1-BM-min	N-terminus of heavy chain	FIG. 7
BLP-4	AS20846	PD1-BM-min	N-terminus of heavy chain	FIG. 7
BLP-5	AS20592VH10	PD1-BM-min	N-terminus of heavy chain	FIG. 7
BLP-6	AS20592VH10	PD1-BM-min	C-terminus of heavy chain	FIG. 8
BLP-7	AS20592VH10	PD1-BM-min	N-terminus of light chain	FIG. 9
BLP-8	AS20592VH10	PD1-BM-min	C-terminus of light chain	FIG. 10
BLP-9	AS20594VH10	PD1-BM-min	N-terminus of heavy chain	FIG. 7
BLP-10	AS20594VH10	PD1-BM-min	C-terminus of heavy chain	FIG. 8
BLP-11	AS20594VH10	PD1-BM-min	N-terminus of light chain	FIG. 9
BLP-12	AS20594VH10	PD1-BM-min	C-terminus of light chain	FIG. 10
BLP-13	AS20846VH12	PD1-BM-min	N-terminus of heavy chain	FIG. 7
BLP-14	AS20846VH12	PD1-BM-min	C-terminus of heavy chain	FIG. 8
BLP-15	AS20846VH12	PD1-BM-min	N-terminus of light chain	FIG. 9
BLP-16	AS20846VH12	PD1-BM-min	C-terminus of light chain	FIG. 10
BLP-17	AS20601VH4	PD1-BM-min	N-terminus of heavy chain	FIG. 7
BLP-18	AS20601VH4	PD1-BM-min	C-terminus of heavy chain	FIG. 8
BLP-19	AS20601VH4	PD1-BM-min	N-terminus of light chain	FIG. 9
BLP-20	AS20601VH4	PD1-BM-min	C-terminus of light chain	FIG. 10
BLP-21	AS20846VH12	Pembrolizumab	N-terminus of heavy chain	FIG. 7
BLP-22	AS20846VH12	Pembrolizumab	N-terminus of light chain	FIG. 9
BLP-23	AS20601VH4	Pembrolizumab	N-terminus of heavy chain	FIG. 7
BLP-24	AS20601VH4	Pembrolizumab	N-terminus of light chain	FIG. 9
BLP-25	AS20592VH10	PD1-BM-min	N-terminus of heavy chain	FIG. 7
BLP-26	AS20592VH10	PD1-BM-min	N-terminus of light chain	FIG. 9
BLP-27	AS20594VH10	PD1-BM-min	N-terminus of heavy chain	FIG. 7
BLP-28	AS20594VH10	PD1-BM-min	N-terminus of light chain	FIG. 9
BLP-29	AS20846VH12	PD1-BM-min	N-terminus of heavy chain	FIG. 7
BLP-30	AS20846VH12	PD1-BM-min	N-terminus of light chain	FIG. 9
BLP-31	AS20601VH4	PD1-BM-min	N-terminus of heavy chain	FIG. 7
BLP-32	AS20601VH4	PD1-BM-min	N-terminus of light chain	FIG. 9

BLP-33	AS20592VH10	PD1-BM-min	N-terminus of heavy chain	FIG. 7
BLP-34	AS20592VH10	PD1-BM-min	N-terminus of light chain	FIG. 9
BLP-35	AS20594VH10	PD1-BM-min	N-terminus of heavy chain	FIG. 7
BLP-36	AS20594VH10	PD1-BM-min	N-terminus of light chain	FIG. 9
BLP-37	AS20846VH12	PD1-BM-min	N-terminus of heavy chain	FIG. 7
BLP-38	AS20846VH12	PD1-BM-min	N-terminus of light chain	FIG. 9
BLP-39	AS20601VH4	PD1-BM-min	N-terminus of heavy chain	FIG. 7
BLP-40	AS20601VH4	PD1-BM-min	N-terminus of light chain	FIG. 9

[0397] Anti-LAG-3 sdAbs AS20592, AS20594, AS20601 and AS20846 were each fused to heavy chain N-terminus of an anti-PD-1 antibody named PD1-BM-min, to generate PD-1×LAG-3 proof-of-concept (POC) BABPs BLP-1, BLP-2, BLP-3 and BLP-4, respectively, via a mutated human IgG1 (hIgG1) hinge as the linker. The exemplary structures of PD-1×LAG-3 POC BABPs are shown in FIG. 7.

[0398] Humanized anti-LAG-3 sdAbs AS20592VH10, AS20594VH10, AS20601VH4 and AS20846VH12 were each fused to the N-terminus of the heavy chain, the C terminus of the heavy chain, the N-terminus of the light chain, or the C-terminus of the light chain of PD1-BM-min to generate PD-1×LAG-3 BABPs, via a mutated human IgG1 (hIgG1) hinge as the linker, or without a linker.

Affinity determination of PD-1×LAG-3 BABPs

[0399] Affinities of the exemplary PD-1×LAG-3 BABPs with human PD-1 or human LAG-3 were assessed as described in Example 3 above. Results are summarized in Table 8.

Table 8. Affinity determination of PD-1×LAG-3 BABPs

Affinity (K_D , M)	Human PD-1	Human LAG-3
Experiment 1		
BLP-1	1.8E-09	4.4E-10
BLP-2	4.4E-09	4.4E-10
BLP-3	2.0E-09	5.9E-10
BLP-4	4.5E-09	1.3E-10
PD1-BM-min	1.4E-09	/
AS20592-Fc	/	1.1E-10
AS20594-Fc	/	1.1E-10
AS20601-Fc	/	4.2E-11
AS20846-Fc	/	6.7E-11
Experiment 2		
BLP-34	3.1E-09	3.7E-09
BLP-15	4.4E-09	7.6E-10
BLP-38	2.5E-09	1.7E-09
BLP-19	4.0E-09	1.4E-09
PD1-BM-min	2.3E-09	/
AS20592VH10-Fc	/	4.7E-10

AS20601VH4-Fc	/	1.0E-10
AS20846VH12-Fc	/	4.6E-10
BMS-986016	/	7.6E-10

Target cell binding and ligand inhibition

[0400] The binding affinities of the exemplary PD-1×LAG-3 BABPs to PD-1 or LAG-3 expressed on CHO cells and blockade of ligand binding to the cells (*i.e.* PD-L1 binding to CHO-PD-1 cells or LAG-3-Fc binding to MHC II-expressing A375 melanoma cells) were assessed as described in Example 3 above. Results are summarized in Table 9.

Table 9. Binding and blocking data of PD-1×LAG-3 BABPs

EC50 (nM)	PD-1		LAG-3	
	Binding	Blocking	Binding	Blocking
Experiment 1				
BLP-1	7.906	6.658	0.883	1.797
BLP-2	4.292	3.196	0.360	0.825
BLP-3	4.64	4.376	0.194	0.922
BLP-4	3.512	2.578	0.543	0.778
PD1-BM-min	2.44	1.718	/	/
AS20592-Fc	/	/	1.244	0.781
AS20594-Fc	/	/	0.304	1.058
AS20601-Fc	/	/	0.229	0.844
AS20846-Fc	/	/	0.342	0.802
Experiment 2				
BLP-34	12.48	13.78	3.118	37.10
BLP-15	13.67	18.48	10.260	29.41
BLP-38	8.513	12.44	4.632	17.31
BLP-19	14.59	21.02	13.510	44.61
PD1-BM-min	6.269	9.565	/	/
AS20592VH10-Fc	/	/	10.540	47.40
AS20601VH4-Fc	/	/	14.490	49.78
AS20846VH12-Fc	/	/	12.430	48.83
BMS-986016	/	/	3.973	24.32

In vitro functional assays

[0401] *In vitro* activities of the exemplary PD-1×LAG-3 BABPs were analyzed by PD-1 cell based reporter assay (FIG. 17A) and LAG-3 blockade reporter assay (Promega, FIG. 17B). Results are summarized in Table 10.

Table 10. *In vitro* functional assays for PD-1×LAG-3 BABPs

EC50 (nM)	PD-1	LAG-3
Experiment 1		
BLP-1	7.623	4.597
BLP-2	5.138	2.039

BLP-3	5.917	1.451
BLP-4	5.943	1.848
PD1-BM-min	1.343	/
Pembrolizumab	1.146	/
AS20592-Fc	/	2.329
AS20594-Fc	/	1.667
AS20601-Fc	/	1.342
AS20846-Fc	/	1.992
BMS-986016	/	3.294
Experiment 2		
BLP-34	2.773	15.830
BLP-15	3.322	5.116
BLP-38	1.253	3.834
BLP-19	3.648	1.570
PD1-BM-min	1.209	/
Pembrolizumab	1.596	/
AS20592VH10-Fc	/	0.891
AS20601VH4-Fc	/	3.979
AS20846VH12-Fc	/	3.125
BMS-986016	/	3.820

In vivo anti-tumor efficacy

[0402] The *in vivo* anti-tumor activity of BLP-4 was evaluated in syngeneic MC38 colon cancer model established in C57BL/6 mice having human PD-1 and human LAG-3 double knock-in. Animals started to receive treatment with BLP-4, PD-BM-min, AS20846-Fc or combination of PD1-BM-min and AS20846-Fc when the tumor size reached around 120 mm³. IgG4 was used as a negative control. Although PD-1 blockade was effective in this model, PD1-BM-min alone at tested dosage failed to delay the tumor growth in 2 out of 7 mice. In contrast, treatment with BLP-4, as well as the combination of PD1-BM-min and AS20846-Fc fusion protein, induced tumor regression in all 7 mice, showing superior efficacy to PD-1 blockade alone (FIGs. 18A and 18B).

SEQUENCE LISTING

Table 11. Anti-LAG-3 sdAb SEQ ID NOs

	SEQ ID NO:	FR1	SEQ ID NO:	CDR1	SEQ ID NO:	FR2	SEQ ID NO:	CDR2	SEQ ID NO:	FR3	SEQ ID NO:	CDR3	SEQ ID NO:	FR4
AS20410	1	QVQLA ESGGGS VHAGG SLRLSC VVS	39	EYTF SSDS MG	77	WFRQ APGK EREG VA	115	AIDSD GSTSY AGSVK G	153	RFTISKDN AENTLYL QMNSLKP EDTAMYY CAT	191	SPLWL LSRLD PADFE Y	229	WGQGT QVTVSS
AS20592	2	EVQLVE SGGGSV QAGGSL RLSCAA S	40	GYIIS SYC MG	78	WFRQ APGK EREG VA	116	AIDSD GGTSY ADSVK G	154	RFTISKDN AKNTLYL QMDSLKP EDTAMYY CAA	192	DFCWV DEDRH LYEYN S	230	WGQGT QVTVSS
AS20594	3	QVQLA ESGGGS VQAGG SLRLSC AAS	41	GYTI SSYC MG	79	WFRQ APGK EREG VA	117	AIDSD GSTSY ADSVK G	155	RFTISKDN AKNTLYL QMNSLKP EDTAMYY CAA	193	DFCWV DDFYE YNY	231	WGQGT QVTVSS
AS20601	4	EVQLAE SGGGSV QAGGSL RLSCAA S	42	GYT YGG YCM G	80	WFRQ APGK EREG VA	118	TINSD GSTNY ADSVK G	156	RFTISKDN AKNTLYL QMNSLSP EDTAMYY CAV	194	DLAPC WVAG PIEAA DFGY	232	WGQGT QVTVSS
AS20846	5	QVHLM ESGGGS VQVGG SLRLSC AAS	43	GYT VSSY CMG	81	WFRQ APGK EREG VA	119	AIDSD GSVSY ADSVK G	157	RFTISKDN AKNTLYL QMNSLKP EDTAMYF CAA	195	DLCW VDQDQ GEYNT	233	WGQGT QVTVSS
AS20916	6	EVQLVE SGGGLV QAGGSL RLSCTA S	44	GLTF DDY AMG	82	WFRQ APGK EREG VS	120	CISWS GGSTY YTDSV KG	158	RFTISRDN AKNTLYL QMNSLKP EDTAMYY CAA	196	DLFCP PPDDT TGWG MDPPE HNY	234	WGQGT QVTVSS
AS21317	7	QVQLV ESGGGS VQAGG SLRLSC AAS	45	GYTL SSYC MG	83	WFRQ APGK EREG VA	121	AIDSD GSTSY ADSVK G	159	RFTISKDN AKNTLFL QMNGLKP EDTAVYY CAP	197	LCWV DYPLP SGPY	235	WGQGT QVTVSS
AS20592M 6	8	EVQLVE SGGGSV QAGGSL RLSCAA S	46	GYIIS SYC MG	84	WFRQ APGK EREG VA	122	AIDSSG GTSYA DSVKG	160	RFTISKDN AKNTLYL QMDSLKP EDTAMYY CAA	198	DFCWV DEDRH LYEYQ S	236	WGQGT QVTVSS

AS20592V H4	9	EVQLVE SGGGLV QPGGSL RLSCAA S	47	GYIIS SYC MG	85	WFRQ APGK GLEG VS	123	AIDSD GGTSY ADSVK G	161	RFTISRDN SKNTLYL QMNSLRA EDTAVYY CAA	199	DFCWV DEDRH LYEYN S	237	WGQGT LTVSS
AS20592V H5	10	EVQLVE SGGGLV QPGGSL RLSCAA S	48	GYIIS SYC MG	86	WFRQ APGK GLEG VA	124	AIDSD GGTSY ADSVK G	162	RFTISRDN SKNTLYL QMNSLRA EDTAVYY CAA	200	DFCWV DEDRH LYEYN S	238	WGQGT LTVSS
AS20592V H6	11	EVQLVE SGGGLV QPGGSL RLSCAA S	49	GYIIS SYC MG	87	WFRQ APGK GLEG VA	125	AIDSD GGTSY ADSVK G	163	RFTISRDN SKNTLYL QMNSLRA EDTAMYY CAA	201	DFCWV DEDRH LYEYN S	239	WGQGT LTVSS
AS20592V H7	12	EVQLVE SGGGLV QPGGSL RLSCAA S	50	GYIIS SYC MG	88	WFRQ APGK GREG VA	126	AIDSD GGTSY ADSVK G	164	RFTISRDN SKNTLYL QMNSLRA EDTAMYY CAA	202	DFCWV DEDRH LYEYN S	240	WGQGT LTVSS
AS20592V H8	13	EVQLVE SGGGLV QPGGSL RLSCAA S	51	GYIIS SYC MG	89	WFRQ APGK EREG VA	127	AIDSD GGTSY ADSVK G	165	RFTISRDN SKNTLYL QMNSLRA EDTAMYY CAA	203	DFCWV DEDRH LYEYN S	241	WGQGT LTVSS
AS20592V H9	14	EVQLVE SGGGLV QPGGSL RLSCAA S	52	GYIIS SYC MG	90	WFRQ APGK EREG VA	128	AIDSD GGTSY ADSVK G	166	RFTISKDN SKNTLYL QMNSLRA EDTAMYY CAA	204	DFCWV DEDRH LYEYN S	242	WGQGT LTVSS
AS20592V H10	15	EVQLVE SGGGLV QPGGSL RLSCAA S	53	GYIIS SYC MG	91	WFRQ APGK GLEG VA	129	AIDSD GGTSY ADSVK G	167	RFTISKDN SKNTLYL QMNSLRA EDTAMYY CAA	205	DFCWV DEDRH LYEYN S	243	WGQGT LTVSS
AS20592V H10M1	16	EVQLVE SGGGLV QPGGSL RLSCAA S	54	GYIIS SYC MG	92	WFRQ APGK GLEG VA	130	AIDSSG GTSYA DSVKG	168	RFTISKDN SKNTLYL QMNSLRA EDTAMYY CAA	206	DFCWV DEDRH LYEYN S	244	WGQGT LTVSS
AS20594V H5	17	EVQLVE SGGGLV QPGGSL RLSCAA S	55	GYTI SSYC MG	93	WFRQ APGK GLEG VA	131	AIDSD GSTSY ADSVK G	169	RFTISRDN SKNTLYL QMNSLRA EDTAVYY CAA	207	DFCWV DDFYE YNY	245	WGQGT LTVSS

AS20594V H6	18	EVQLVE SGGGLV QPGGSL RLSCAA S	56	GYTI SSYC MG	94	WFRQ APGK GLEG VA	132	AIDSD GSTSY ADSVK G	170	RFTISRDN SKNTLYL QMNSLRA EDTAMYY CAA	208	DFCWV DDFYE YNY	246	WGQGT LTVSS
AS20594V H7	19	EVQLVE SGGGLV QPGGSL RLSCAA S	57	GYTI SSYC MG	95	WFRQ APGK GREG VA	133	AIDSD GSTSY ADSVK G	171	RFTISRDN SKNTLYL QMNSLRA EDTAMYY CAA	209	DFCWV DDFYE YNY	247	WGQGT LTVSS
AS20594V H8	20	EVQLVE SGGGLV QPGGSL RLSCAA S	58	GYTI SSYC MG	96	WFRQ APGK EREG VA	134	AIDSD GSTSY ADSVK G	172	RFTISRDN SKNTLYL QMNSLRA EDTAMYY CAA	210	DFCWV DDFYE YNY	248	WGQGT LTVSS
AS20594V H9	21	EVQLVE SGGGLV QPGGSL RLSCAA S	59	GYTI SSYC MG	97	WFRQ APGK EREG VA	135	AIDSD GSTSY ADSVK G	173	RFTISKDN SKNTLYL QMNSLRA EDTAMYY CAA	211	DFCWV DDFYE YNY	249	WGQGT LTVSS
AS20594V H10	22	EVQLVE SGGGLV QPGGSL RLSCAA S	60	GYTI SSYC MG	98	WFRQ APGK GLEG VA	136	AIDSD GSTSY ADSVK G	174	RFTISKDN SKNTLYL QMNSLRA EDTAVYY CAA	212	DFCWV DDFYE YNY	250	WGQGT LTVSS
AS20594V H10M1	23	EVQLVE SGGGLV QPGGSL RLSCAA S	61	GYTI SSYC MG	99	WFRQ APGK GLEG VA	137	AIDSSG STSYA DSVKG	175	RFTISKDN SKNTLYL QMNSLRA EDTAVYY CAA	213	DFCWV DDFYE YNY	251	WGQGT LTVSS
AS20846V H4	24	EVQLVE SGGGLV QPGGSL RLSCAA S	62	GYT VSSY CMG	100	WFRQ APGK GLEG VS	138	AIDSD GSVSY ADSVK G	176	RFTISRDN SKNTLYL QMNSLRA EDTAVYY CAA	214	DLCW VDQDQ GEYNT	252	WGQGT LTVSS
AS20846V H5	25	EVQLVE SGGGLV QPGGSL RLSCAA S	63	GYT VSSY CMG	101	WFRQ APGK GLEG VS	139	AIDSD GSVSY ADSVK G	177	RFTISRDN SKNTLYL QMNSLRA EDTAVYF CAA	215	DLCW VDQDQ GEYNT	253	WGQGT LTVSS
AS20846V H6	26	EVQLVE SGGGLV QPGGSL RLSCAA S	64	GYT VSSY CMG	102	WFRQ APGK GLEG VA	140	AIDSD GSVSY ADSVK G	178	RFTISRDN SKNTLYL QMNSLRA EDTAVYF CAA	216	DLCW VDQDQ GEYNT	254	WGQGT LTVSS

AS20846V H7	27	EVQLVE SGGGLV QPGGSL RLSCAA S	65	GYT VSSY CMG	103	WFRQ APGK GLEG VA	141	AIDSD GSVSY ADSVK G	179	RFTISRDN SKNTLYL QMNSLRA EDTAMYF CAA	217	DLCW VDQDQ GEYNT	255	WGQGT LTVSS
AS20846V H8	28	EVQLVE SGGGLV QPGGSL RLSCAA S	66	GYT VSSY CMG	104	WFRQ APGK GREG VA	142	AIDSD GSVSY ADSVK G	180	RFTISRDN SKNTLYL QMNSLRA EDTAMYF CAA	218	DLCW VDQDQ GEYNT	256	WGQGT LTVSS
AS20846V H9	29	EVQLVE SGGGLV QPGGSL RLSCAA S	67	GYT VSSY CMG	105	WFRQ APGK EREG VA	143	AIDSD GSVSY ADSVK G	181	RFTISRDN SKNTLYL QMNSLRA EDTAMYF CAA	219	DLCW VDQDQ GEYNT	257	WGQGT LTVSS
AS20846V H10	30	EVQLVE SGGGLV QPGGSL RLSCAA S	68	GYT VSSY CMG	106	WFRQ APGK EREG VA	144	AIDSD GSVSY ADSVK G	182	RFTISKDN SKNTLYL QMNSLRA EDTAMYF CAA	220	DLCW VDQDQ GEYNT	258	WGQGT LTVSS
AS20846V H12	31	EVQLVE SGGGLV QPGGSL RLSCAA S	69	GYT VSSY CMG	107	WFRQ APGK GREG VS	145	AIDSD GSVSY ADSVK G	183	RFTISKDN SKNTLYL QMNSLRA EDTAVYF CAA	221	DLCW VDQDQ GEYNT	259	WGQGT LTVSS
AS20846V H12M1	32	EVQLVE SGGGLV QPGGSL RLSCAA S	70	GYT VSSY CMG	108	WFRQ APGK GREG VS	146	AIDSSG SVSYA DSVKG	184	RFTISKDN SKNTLYL QMNSLRA EDTAVYF CAA	222	DLCW VDQDQ GEYNT	260	WGQGT LTVSS
AS20601V H4	33	EVQLVE SGGGLV QPGGSL RLSCAA S	71	GYT YGG YCM G	109	WFRQ APGK GLEG VS	147	TINSD GSTNY ADSVK G	185	RFTISRDN SKNTLYL QMNSLRA EDTAVYY CAV	223	DLAPC WVAG PIEAA DFGY	261	WGQGT LTVSS
AS20601V H4.M6	34	EVQLVE SGGGLV QPGGSL RLSCAA S	72	GYT YGG YCM G	110	WFRQ APGK GLEG VS	148	TIASEG STNYA DSVKG	186	RFTISRDN SKNTLYL QMNSLRA EDTAVYY CAV	224	DLAPC WVAG PIEAA DFGY	262	WGQGT LTVSS
AS20601V H4.M7	35	EVQLVE SGGGLV QPGGSL RLSCAA S	73	GYT YGG YCM G	111	WFRQ APGK GLEG VS	149	TIASSG STNYA DSVKG	187	RFTISRDN SKNTLYL QMNSLRA EDTAVYY CAV	225	DLAPC WVAG PIEAA DFGY	263	WGQGT LTVSS

AS20601V H11	36	EVQLVE SGGGLV QPGGSL RLSCAA S	74	GYT YGG YCM G	112	WFRQ APGK GREG VS	150	TINSD GSTNY ADSVK G	188	RFTISRDN AKNTLYL QMNSLRP EDTAVYY CAV	226	DLAPC WVAG PIEAA DFGY	264	WGQGT LTVSS
AS20601V H11.M6	37	EVQLVE SGGGLV QPGGSL RLSCAA S	75	GYT YGG YCM G	113	WFRQ APGK GREG VS	151	TIASEG STNYA DSVKG	189	RFTISRDN AKNTLYL QMNSLRP EDTAVYY CAV	227	DLAPC WVAG PIEAA DFGY	265	WGQGT LTVSS
AS20601V H11.M7	38	EVQLVE SGGGLV QPGGSL RLSCAA S	76	GYT YGG YCM G	114	WFRQ APGK GREG VS	152	TIASSG STNYA DSVKG	190	RFTISRDN AKNTLYL QMNSLRP EDTAVYY CAV	228	DLAPC WVAG PIEAA DFGY	266	WGQGT LTVSS

SEQ ID NO: 267 (AS20410 sdAb nucleic acid sequence)

CAGGTGCAACTGGCGGAGTCTGGGGGAGGCTCGGTGCATGCTGGAGGGTCTCTGAGACTCT
CCTGTGTAGTTTCTGAATATACCTTCAGTAGTGATTCTATGGGCTGGTTCCGCCAGGCTCCAG
GGAAGGAGCGCGAGGGGGTTCGAGCTATTGATAGTGATGGTAGCACAAGCTACGCAGGCTC
CGTGAAGGGGCGATTACCATCTCCAAAGACAACGCCGAGAACAACACTCTGTATCTGCAAATG
AACAGCCTGAAACCTGAGGACACTGCCATGTACTACTGTGCGACAAGCCCTCTGTGGCTGCT
ATCCAGGCTCGACCCAGCTGACTTTGAATACTGGGGCCAGGGGACCCAGGTCACCGTCTCCT
CA

SEQ ID NO: 268 (AS20592 sdAb nucleic acid sequence)

GAGGTGCAACTGGTGGAGTCTGGGGGAGGCTCGGTGCAGGCTGGAGGGTCTCTGAGACTCT
CCTGTGCAGCCTCTGGATACATCATCAGTAGCTACTGCATGGGCTGGTTCCGCCAGGCTCCA
GGGAAGGAGCGCGAGGGGGTTCGAGCTATTGATAGTGATGGTGGCACAAGCTACGCAGACT
CCGTGAAGGGCCGATTACCATCTCCAAAGACAACGCCAAGAACAACACTCTGTATCTGCAAATG
GACAGCCTGAAACCTGAGGACACTGCCATGTACTACTGTGCGGCAGATTTTGTGGGTAGA
TGAGGATCGCCACCTCTATGAGTATAACTCCTGGGGCCAGGGGACCCAGGTCACCGTCTCCT
CA

SEQ ID NO: 269 (AS20594 sdAb nucleic acid sequence)

CAGGTGCAGCTGGCGGAGTCTGGGGGAGGCTCGGTGCAGGCTGGAGGGTCTCTGAGACTCT
CCTGTGCAGCCTCTGGATACACCATCAGTAGCTACTGCATGGGCTGGTTCCGCCAGGCTCCA
GGGAAGGAGCGCGAGGGGGTTCGAGCTATTGATAGTGATGGTAGCACAAGCTACGCAGACT
CCGTGAAGGGCCGATTACCATCTCCAAAGACAACGCCAAGAACAACACTCTGTATCTGCAAATG
AACAGCCTGAAACCTGAGGACACTGCCATGTACTACTGTGCGGCAGATTTTGTGGGTGGA
CGACTTCTATGAGTATAACTACTGGGGCCAGGGGACCCAGGTCACCGTCTCCTCA

SEQ ID NO: 270 (AS20601 sdAb nucleic acid sequence)

GAGGTGCAACTGGCGGAGTCTGGGGGAGGCTCGGTGCAGGCTGGAGGGTCTCTGAGACTCT
CCTGTGCAGCCTCTGGATACACCTACGGTGGTACTGCATGGGCTGGTTCCGCCAGGCTCCA
GGGAAGGAGCGCGAGGGGGTTCGCAACTATTAATAGTGATGGTAGCACAACACTACGCAGACT
CCGTGAAGGGCCGATTACCATCTCCAAAGACAACGCCAAGAACAACACTCTGTATCTGCAAATG
AACAGCCTGAGCCCTGAGGACACTGCCATGTACTACTGCGCGGTGGATCTCGCCCCATGTTG
GGTAGCTGGTCCCATCGAGGCCGCTGACTTTGGTTACTGGGGCCAGGGGACCCAGGTCACCG
TCTCCTCA

SEQ ID NO: 271 (AS20846 sdAb nucleic acid sequence)

CAGGTGCACCTGATGGAGTCTGGGGGAGGCTCGGTGCAGGTTGGAGGGTCTCTGAGACTCTC
CTGTGCAGCCTCTGGATACACCGTCAGTAGCTACTGCATGGGCTGGTTCCGCCAGGCTCCAG
GGAAGGAGCGCGAGGGGGTTCGAGCTATTGATAGTGATGGTAGCGTAAGTTATGCAGACTC
CGTGAAGGGCCGATTACCATCTCCAAAGACAACGCCAAGAACAACACTCTGTATCTGCAAATG
AACAGCCTGAAACCTGAGGACACTGCCATGTACTTCTGTGCGGCAGATCTTTGCTGGGTGGA
CCAGGACCAGGGAGAATATAACACCTGGGGCCAGGGGACCCAGGTACCGTCTCCTCA

SEQ ID NO: 272 (AS20916 sdAb nucleic acid sequence)

GAGGTGCAGCTGGTGGAGTCTGGGGGAGGCTTGGTGCAGGCAGGGGGGTCTCTGAGACTCT
CCTGTACAGCCTCTGGATTGACTTTTGATGATTATGCCATGGGCTGGTTCCGCCAGGCTCCAG
GGAAGGAGCGCGAGGGGGTCTCATGTATTAGCTGGAGTGGTGGTAGCACATACTATACCGA
CTCCGTGAAGGGCCGATTACCATCTCCAGAGACAACGCCAAGAACAACGCTGTATCTGCAA
ATGAACAGCCTGAAACCTGAGGACACGGCCATGTATTACTGTGCGGCAGATTTGTTCTGTCC
CCCACCTGATGATACTACGGGTTGGGGTATGGACCCTCCCGAGCATAACTACTGGGGCCAGG
GGACCCAGGTCACCGTCTCCTCA

SEQ ID NO: 273 (AS21317 sdAb nucleic acid sequence)

CAGGTTTCAGCTGGTGGAGTCTGGGGGAGGCTCGGTGCAGGCTGGAGGGTCTCTGAGACTTTC
CTGTGCAGCCTCTGGATACACCTTGAGTAGCTACTGCATGGGCTGGTTCCGCCAGGCTCCAG
GGAAGGAGCGCGAGGGGGTTCGAGCTATTGATAGTGATGGTAGCACAAGCTACGCAGACTC
CGTGAAGGGCCGATTACCATCTCCAAAGACAACGCCAAGAACAACACTCTGTTTCTGCAAATGA
ACGGCCTGAAACCTGAGGACACTGCGGTGTACTACTGTGCTCCACTATGCTGGGTTGATTAC
CCATTGCCGTCGGGCCATACTGGGGCCAGGGGACCCAGGTACCGTCTCCTCA

SEQ ID NO: 274 (AS20410 sdAb amino acid sequence; CDRs are underlined)

QVQLAESGGGSVHAGGSLRLSCVVSEYTFSSDSMGWFRQAPGKEREGVAAIDSDGSTSYAGSVK
GRFTISKDNAENTLYLQMNSLKPEDTAMYYCATSPLWLLSRLDPADFEYWGQGTQVTVSS

SEQ ID NO: 275 (AS20592 sdAb amino acid sequence; CDRs are underlined)

EVQLVESGGGSVQAGGSLRLSCAASGYHISSYCMGWFRQAPGKEREGVAAIDSDGGTSYADSVK
GRFTISKDNAKNTLYLQMDSLKPEDTAMYYCAADFCWVDEDRHLYEYNSWGQGTQVTVSS

SEQ ID NO: 276 (AS20594 sdAb amino acid sequence; CDRs are underlined)

QVQLAESGGGSVQAGGSLRLSCAASGYTISSYCMGWFRQAPGKEREGVAAIDSDGSTSYADSVK
GRFTISKDNAKNTLYLQMNSLKPEDTAMYYCAADFCWVDDFYEYNYWGQGTQVTVSS

SEQ ID NO: 277 (AS20601 sdAb amino acid sequence; CDRs are underlined)

EVQLAESGGGSVQAGGSLRLSCAASGYTYGGYCMGWFRQAPGKEREGVATINSDGSTNYADSV
KGRFTISKDNAKNTLYLQMNSLSPEDTAMYYCAVDLAPCWVAGPIEAADFGYWGQGTQVTVSS

SEQ ID NO: 278 (AS20846 sdAb amino acid sequence; CDRs are underlined)

QVHLMESGGGSVQVGGSLRLSCAASGYTVSSYCMGWFRQAPGKEREGVAAIDSDGSVSYADSV
KGRFTISKDNAKNTLYLQMNSLKPEDTAMYFCAADLCWVDQDQGEYNTWGQGTQVTVSS

SEQ ID NO: 279 (AS20916 sdAb amino acid sequence; CDRs are underlined)

EVQLVESGGGLVQAGGSLRLSCTASGLTFDDYAMGWFRQAPGKEREGVSCISWSGGSTYYTDS
VKGRFTISRDNKNTLYLQMNSLKPEDTAMYYCAADLFCPPDDTTGWGMDPPEHNYWGQGT
QVTVSS

SEQ ID NO: 280 (AS21317 sdAb amino acid sequence; CDRs are underlined)
QVQLVESGGGSVQAGGSLRLSCAASGYTLSSYCMGWFRQAPGKEREGVAAIDSDGGTSYADSVK
GRFTISKDNAKNTLFLQMNGLPEDTAVYYCAPLCWVDYPLPSGPYWGQGTQVTVSS

SEQ ID NO: 281 (AS20592M6 sdAb amino acid sequence; CDRs are underlined)
EVQLVESGGGSVQAGGSLRLSCAASGYIISYCMGWFRQAPGKEREGVAAIDSSGGTSYADSVK
GRFTISKDNAKNTLYLQMDSLKPEDTAMYYCAADFCWVDEDRHLYEYQSWGQGTQVTVSS

SEQ ID NO: 282 (AS20592VH4 sdAb amino acid sequence; CDRs are underlined)
EVQLVESGGGLVQPGGSLRLSCAASGYIISYCMGWFRQAPGKGLEGVSAIDSDGGTSYADSVK
GRFTISRDNKNTLYLQMNSLRAEDTAVYYCAADFCWVDEDRHLYEYNSWGQGTQVTVSS

SEQ ID NO: 283 (AS20592VH5 sdAb amino acid sequence; CDRs are underlined)
EVQLVESGGGLVQPGGSLRLSCAASGYIISYCMGWFRQAPGKGLEGVAAIDSDGGTSYADSVK
GRFTISRDNKNTLYLQMNSLRAEDTAVYYCAADFCWVDEDRHLYEYNSWGQGTQVTVSS

SEQ ID NO: 284 (AS20592VH6 sdAb amino acid sequence; CDRs are underlined)
EVQLVESGGGLVQPGGSLRLSCAASGYIISYCMGWFRQAPGKGLEGVAAIDSDGGTSYADSVK
GRFTISRDNKNTLYLQMNSLRAEDTAMYYCAADFCWVDEDRHLYEYNSWGQGTQVTVSS

SEQ ID NO: 285 (AS20592VH7 sdAb amino acid sequence; CDRs are underlined)
EVQLVESGGGLVQPGGSLRLSCAASGYIISYCMGWFRQAPGKGREGVAAIDSDGGTSYADSVK
GRFTISRDNKNTLYLQMNSLRAEDTAMYYCAADFCWVDEDRHLYEYNSWGQGTQVTVSS

SEQ ID NO: 286 (AS20592VH8 sdAb amino acid sequence; CDRs are underlined)
EVQLVESGGGLVQPGGSLRLSCAASGYIISYCMGWFRQAPGKEREGVAAIDSDGGTSYADSVK
GRFTISRDNKNTLYLQMNSLRAEDTAMYYCAADFCWVDEDRHLYEYNSWGQGTQVTVSS

SEQ ID NO: 287 (AS20592VH9 sdAb amino acid sequence; CDRs are underlined)
EVQLVESGGGLVQPGGSLRLSCAASGYIISYCMGWFRQAPGKEREGVAAIDSDGGTSYADSVK
GRFTISKDNKNTLYLQMNSLRAEDTAMYYCAADFCWVDEDRHLYEYNSWGQGTQVTVSS

SEQ ID NO: 288 (AS20592VH10 sdAb amino acid sequence; CDRs are underlined)
EVQLVESGGGLVQPGGSLRLSCAASGYIISYCMGWFRQAPGKGLEGVAAIDSDGGTSYADSVK
GRFTISKDNKNTLYLQMNSLRAEDTAMYYCAADFCWVDEDRHLYEYNSWGQGTQVTVSS

SEQ ID NO: 289 (AS20592VH10M1 sdAb amino acid sequence; CDRs are underlined)
EVQLVESGGGLVQPGGSLRLSCAASGYIISYCMGWFRQAPGKGLEGVAAIDSSGGTSYADSVK
GRFTISKDNKNTLYLQMNSLRAEDTAMYYCAADFCWVDEDRHLYEYNSWGQGTQVTVSS

SEQ ID NO: 290 (AS20594VH5 sdAb amino acid sequence; CDRs are underlined)
EVQLVESGGGLVQPGGSLRLSCAASGYTISSYCMGWFRQAPGKGLEGVAAIDSDGGTSYADSVK
GRFTISRDNKNTLYLQMNSLRAEDTAVYYCAADFCWVDIFYEYNYWGQGTQVTVSS

SEQ ID NO: 291 (AS20594VH6 sdAb amino acid sequence; CDRs are underlined)
EVQLVESGGGLVQPGGSLRLSCAASGYTISSYCMGWFRQAPGKGLEGVAAIDSDGGTSYADSVK
GRFTISRDNKNTLYLQMNSLRAEDTAMYYCAADFCWVDIFYEYNYWGQGTQVTVSS

SEQ ID NO: 292 (AS20594VH7 sdAb amino acid sequence; CDRs are underlined)

EVQLVESGGGLVQPGGSLRLSCAASGYTISSYCMGWFRQAPGKGREGVAAIDSDGSTSYADSVK
GRFTISRDN SKNTLYLQMNSLRAEDTAMYYCAADFCWVDDFYEYNYWGQGLTVTVSS

SEQ ID NO: 293 (AS20594VH8 sdAb amino acid sequence; CDRs are underlined)

EVQLVESGGGLVQPGGSLRLSCAASGYTISSYCMGWFRQAPGKEREGVAAIDSDGSTSYADSVK
GRFTISRDN SKNTLYLQMNSLRAEDTAMYYCAADFCWVDDFYEYNYWGQGLTVTVSS

SEQ ID NO: 294 (AS20594VH9 sdAb amino acid sequence; CDRs are underlined)

EVQLVESGGGLVQPGGSLRLSCAASGYTISSYCMGWFRQAPGKEREGVAAIDSDGSTSYADSVK
GRFTISKDN SKNTLYLQMNSLRAEDTAMYYCAADFCWVDDFYEYNYWGQGLTVTVSS

SEQ ID NO: 295 (AS20594VH10 sdAb amino acid sequence; CDRs are underlined)

EVQLVESGGGLVQPGGSLRLSCAASGYTISSYCMGWFRQAPGKGLEGVAAIDSDGSTSYADSVK
GRFTISKDN SKNTLYLQMNSLRAEDTAVYYCAADFCWVDDFYEYNYWGQGLTVTVSS

SEQ ID NO: 296 (AS20594VH10M1 sdAb amino acid sequence; CDRs are underlined)

EVQLVESGGGLVQPGGSLRLSCAASGYTISSYCMGWFRQAPGKGLEGVAAIDSSGSTSYADSVK
GRFTISKDN SKNTLYLQMNSLRAEDTAVYYCAADFCWVDDFYEYNYWGQGLTVTVSS

SEQ ID NO: 297 (AS20846VH4 sdAb amino acid sequence; CDRs are underlined)

EVQLVESGGGLVQPGGSLRLSCAASGYTVSSYCMGWFRQAPGKGLEGVSAIDSDGSVSYADSV
KGRFTISRDN SKNTLYLQMNSLRAEDTAVYYCAADLCWVDQDQGEYNTWGQGLTVTVSS

SEQ ID NO: 298 (AS20846VH5 sdAb amino acid sequence; CDRs are underlined)

EVQLVESGGGLVQPGGSLRLSCAASGYTVSSYCMGWFRQAPGKGLEGVSAIDSDGSVSYADSV
KGRFTISRDN SKNTLYLQMNSLRAEDTAVYFCAADLCWVDQDQGEYNTWGQGLTVTVSS

SEQ ID NO: 299 (AS20846VH6 sdAb amino acid sequence; CDRs are underlined)

EVQLVESGGGLVQPGGSLRLSCAASGYTVSSYCMGWFRQAPGKGLEGVAAIDSDGSVSYADSV
KGRFTISRDN SKNTLYLQMNSLRAEDTAVYFCAADLCWVDQDQGEYNTWGQGLTVTVSS

SEQ ID NO: 300 (AS20846VH7 sdAb amino acid sequence; CDRs are underlined)

EVQLVESGGGLVQPGGSLRLSCAASGYTVSSYCMGWFRQAPGKGLEGVAAIDSDGSVSYADSV
KGRFTISRDN SKNTLYLQMNSLRAEDTAMYFCAADLCWVDQDQGEYNTWGQGLTVTVSS

SEQ ID NO: 301 (AS20846VH8 sdAb amino acid sequence; CDRs are underlined)

EVQLVESGGGLVQPGGSLRLSCAASGYTVSSYCMGWFRQAPGKGREGVAAIDSDGSVSYADSV
KGRFTISRDN SKNTLYLQMNSLRAEDTAMYFCAADLCWVDQDQGEYNTWGQGLTVTVSS

SEQ ID NO: 302 (AS20846VH9 sdAb amino acid sequence; CDRs are underlined)

EVQLVESGGGLVQPGGSLRLSCAASGYTVSSYCMGWFRQAPGKEREGVAAIDSDGSVSYADSV
KGRFTISRDN SKNTLYLQMNSLRAEDTAMYFCAADLCWVDQDQGEYNTWGQGLTVTVSS

SEQ ID NO: 303 (AS20846VH10 sdAb amino acid sequence; CDRs are underlined)

EVQLVESGGGLVQPGGSLRLSCAASGYTVSSYCMGWFRQAPGKEREGVAAIDSDGSVSYADSV
KGRFTISKDN SKNTLYLQMNSLRAEDTAMYFCAADLCWVDQDQGEYNTWGQGLTVTVSS

SEQ ID NO: 304 (AS20846VH12 sdAb amino acid sequence; CDRs are underlined)

EVQLVESGGGLVQPGGSLRLSCAASGYTVSSYCMGWFRQAPGKGREGVSAIDSDGSVSYADSV
KGRFTISKDN SKNTLYLQMNSLRAEDTAVYFCAADLCWVDQDQGEYNTWGQGLTVTVSS

SEQ ID NO: 305 (AS20846VH12M1 sdAb amino acid sequence; CDRs are underlined)
EVQLVESGGGLVQPGGSLRLSCAASGYTVSSYCMGWFRQAPGKGREGVSAIDSSGSVSYADSV
KGRFTISKDNSKNTLYLQMNSLRAEDTAVYFCAADLCWVDQDQGEYNTWGQGTLLTVSS

SEQ ID NO: 306 (AS20601VH4 sdAb amino acid sequence; CDRs are underlined)
EVQLVESGGGLVQPGGSLRLSCAASGYTYGGYCMGWFRQAPGKGLEGVSTINS DGSTNYADSV
KGRFTISRDN SKNTLYLQMNSLRAEDTAVYYCAVDLAPCWVAGPIEAADFGYWGQGTLLTVSS

SEQ ID NO: 307 (AS20601VH4.M6 sdAb amino acid sequence; CDRs are underlined)
EVQLVESGGGLVQPGGSLRLSCAASGYTYGGYCMGWFRQAPGKGLEGVSTIAS EGSTNYADSV
KGRFTISRDN SKNTLYLQMNSLRAEDTAVYYCAVDLAPCWVAGPIEAADFGYWGQGTLLTVSS

SEQ ID NO: 308 (AS20601VH4.M7 sdAb amino acid sequence; CDRs are underlined)
EVQLVESGGGLVQPGGSLRLSCAASGYTYGGYCMGWFRQAPGKGLEGVSTIAS SGSTNYADSV
KGRFTISRDN SKNTLYLQMNSLRAEDTAVYYCAVDLAPCWVAGPIEAADFGYWGQGTLLTVSS

SEQ ID NO: 309 (AS20601VH11 sdAb amino acid sequence; CDRs are underlined)
EVQLVESGGGLVQPGGSLRLSCAASGYTYGGYCMGWFRQAPGKGREGVSTINS DGSTNYADSV
KGRFTISRDN AKNTLYLQMNSLRPEDTAVYYCAVDLAPCWVAGPIEAADFGYWGQGTLLTVSS

SEQ ID NO: 310 (AS20601VH11.M6 sdAb amino acid sequence; CDRs are underlined)
EVQLVESGGGLVQPGGSLRLSCAASGYTYGGYCMGWFRQAPGKGREGVSTIAS EGSTNYADSV
KGRFTISRDN AKNTLYLQMNSLRPEDTAVYYCAVDLAPCWVAGPIEAADFGYWGQGTLLTVSS

SEQ ID NO: 311 (AS20601VH11.M7 sdAb amino acid sequence; CDRs are underlined)
EVQLVESGGGLVQPGGSLRLSCAASGYTYGGYCMGWFRQAPGKGREGVSTIAS SGSTNYADSV
KGRFTISRDN AKNTLYLQMNSLRPEDTAVYYCAVDLAPCWVAGPIEAADFGYWGQGTLLTVSS

SEQ ID NO: 312 (AS20410 HCAb dimeric form amino acid sequence)
QVQLAESGGGSVHAGGSLRLSCVVSSEYTFSSDSMGWFRQAPGKEREGVAAIDSDGSTSYAGSVK
GRFTISKDNAENTLYLQMNSLKPEDTAMYYCATSPLWLLSRLDPADFEYWGQGTQVTVSSESK
YGPCCPPCPAPEFLGGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSQEDPEVQFNWYVDGVEVHN
AKTKPREEQFNSTYRVVSVLTVLHQDWLNGKEYKCKVSNKGLPSSIEKTISKAKGQPREPQVY
LPDSQEEMTKNQVSLTCLVKGFYPSDIAVEWESNGQPENNYKTTTPVLDSDGSFFLYSRLTVDKS
RWQEGNVFSCSVMEALHNHYTQKSLSLSLGK

SEQ ID NO: 313 (AS20592 HCAb dimeric form amino acid sequence)
EVQLVESGGGSVQAGGSLRLSCAASGYIISYCMGWFRQAPGKEREGVAAIDSDGGSTSYADSVK
GRFTISKDNAKNTLYLQMDSLKPEDTAMYYCAADFCWVDEDRLHYEYNSWGQGTQVTVSSES
KYGPCCPPCPAPEFLGGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSQEDPEVQFNWYVDGVEVH
NAKTKPREEQFNSTYRVVSVLTVLHQDWLNGKEYKCKVSNKGLPSSIEKTISKAKGQPREPQVY
TLPPSQEEMTKNQVSLTCLVKGFYPSDIAVEWESNGQPENNYKTTTPVLDSDGSFFLYSRLTVDK
SRWQEGNVFSCSVMEALHNHYTQKSLSLSLGK

SEQ ID NO: 314 (AS20594 HCAb dimeric form amino acid sequence)
QVQLAESGGGSVQAGGSLRLSCAASGYTISSYCMGWFRQAPGKEREGVAAIDSDGSTSYADSVK
GRFTISKDNAKNTLYLQMNSLKPEDTAMYYCAADFCWVDDFYEYNYWGQGTQVTVSSESKYG
PPCCPPCPAPEFLGGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSQEDPEVQFNWYVDGVEVHNAK
TKPREEQFNSTYRVVSVLTVLHQDWLNGKEYKCKVSNKGLPSSIEKTISKAKGQPREPQVYTLPP

SQEEMTKNQVSLTCLVKGFYPSDIAVEWESNGQPENNYKTTPVLDSGDSFFLYSRLTVDKSRW
QEGNVFSCSVMEALHNHYTQKSLSLGLGK

SEQ ID NO: 315 (AS20601 HCAb dimeric form amino acid sequence)

EVQLAESGGGSVQAGGSLRLSCAASGYTYGGYCMGWFRQAPGKEREGVATINSDGSTNYADSV
KGRFTISKDNAKNTLYLQMNSLSPEDTAMYYCAVDLAPCWVAGPIEAADFGYWGQGTQVTVSS
ESKYGPPCPPCPAPEFLGGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSQEDPEVQFNWYVDGVE
VHNAKTKPREEQFNSTYRVVSVLTVLHQDWLNGKEYKCKVSNKGLPSSIEKTISKAKGQPREPQ
VYTLPPSQEEMTKNQVSLTCLVKGFYPSDIAVEWESNGQPENNYKTTPVLDSGDSFFLYSRLTV
DKSRWQEGNVFSCSVMEALHNHYTQKSLSLGLGK

SEQ ID NO: 316 (AS20846 HCAb dimeric form amino acid sequence)

QVHLMESGGGSVQVGGSLRLSCAASGYTVSSYCMGWFRQAPGKEREGVAAIDSDGSVSYADSV
KGRFTISKDNAKNTLYLQMNSLKPEDTAMYFCAADLCWVDQDQGEYNTWGQGTQVTVSSESK
YGPPCPPCPAPEFLGGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSQEDPEVQFNWYVDGVEVHN
AKTKPREEQFNSTYRVVSVLTVLHQDWLNGKEYKCKVSNKGLPSSIEKTISKAKGQPREPQVYT
LPPSQEEMTKNQVSLTCLVKGFYPSDIAVEWESNGQPENNYKTTPVLDSGDSFFLYSRLTVDKS
RWQEGNVFSCSVMEALHNHYTQKSLSLGLGK

SEQ ID NO: 317 (AS20916 HCAb dimeric form amino acid sequence)

EVQLVESGGGLVQAGGSLRLSCTASGLTFDDYAMGWFRQAPGKEREGVSCISWSGGSTYYTDS
VKGRFTISRDNKNTLYLQMNSLKPEDTAMYYCAADLFCPPDDTTGWGMDDPPEHNYWGQGT
QVTVSSESKYGPCCPPCPAPEFLGGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSQEDPEVQFNWY
VDGVEVHNKTKPREEQFNSTYRVVSVLTVLHQDWLNGKEYKCKVSNKGLPSSIEKTISKAKG
QPREPQVYTLPPSQEEMTKNQVSLTCLVKGFYPSDIAVEWESNGQPENNYKTTPVLDSGDSFFL
YSRLTVDKSRWQEGNVFSCSVMEALHNHYTQKSLSLGLGK

SEQ ID NO: 318 (AS21317 HCAb dimeric form amino acid sequence)

QVQLVESGGGSVQAGGSLRLSCAASGYTLSSYCMGWFRQAPGKEREGVAAIDSDGSTSYADSV
KGRFTISKDNAKNTLYLQMNSLKPEDTAVYYCAADFCWVDYPLPSGPYWGQGTQVTVSSESKY
GPCPPCPAPEFLGGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSQEDPEVQFNWYVDGVEVHN
TKPREEQFNSTYRVVSVLTVLHQDWLNGKEYKCKVSNKGLPSSIEKTISKAKGQPREPQVYTLPP
SQEEMTKNQVSLTCLVKGFYPSDIAVEWESNGQPENNYKTTPVLDSGDSFFLYSRLTVDKSRW
QEGNVFSCSVMEALHNHYTQKSLSLGLGK

SEQ ID NO: 319 (AS20592M6 HCAb dimeric form amino acid sequence)

EVQLVESGGGSVQAGGSLRLSCAASGYIISYCMGWFRQAPGKEREGVAAIDSSGGTSYADSVK
GRFTISKDNAKNTLYLQMDSLKPEDTAMYYCAADFCWVDEDRHLYEYQSWGQGTQVTVSSES
KYGPPCPPCPAPEFLGGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSQEDPEVQFNWYVDGVEVH
NAKTKPREEQFNSTYRVVSVLTVLHQDWLNGKEYKCKVSNKGLPSSIEKTISKAKGQPREPQVY
TLPPSQEEMTKNQVSLTCLVKGFYPSDIAVEWESNGQPENNYKTTPVLDSGDSFFLYSRLTVDK
SRWQEGNVFSCSVMEALHNHYTQKSLSLGLGK

SEQ ID NO: 320 (AS20592VH4 HCAb dimeric form amino acid sequence)

EVQLVESGGGLVQPGGSLRLSCAASGYIISYCMGWFRQAPGKGLEGVSAIDSDGGTSYADSVK
GRFTISRDNKNTLYLQMNSLRAEDTAVYYCAADFCWVDEDRHLYEYNSWGQGLTVTVSSESK
YGPPCPPCPAPEFLGGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSQEDPEVQFNWYVDGVEVHN
AKTKPREEQFNSTYRVVSVLTVLHQDWLNGKEYKCKVSNKGLPSSIEKTISKAKGQPREPQVYT
LPPSQEEMTKNQVSLTCLVKGFYPSDIAVEWESNGQPENNYKTTPVLDSGDSFFLYSRLTVDKS
RWQEGNVFSCSVMEALHNHYTQKSLSLGLGK

SEQ ID NO: 321 (AS20592VH5 HCAb dimeric form amino acid sequence)

EVQLVESGGGLVQPGGSLRLSCAASGYIISYCMGWFRQAPGKGLEGVAAIDSDGGTSYADSVK
GRFTISRDNSKNTLYLQMNSLRAEDTAVYYCAADFCWVDEDRHLYEYNSWGQGLTVTVSSEK
YGPPCPPCPAPEFLGGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSQEDPEVQFNWYVDGVEVHN
AKTKPREEQFNSTYRVVSVLTVLHQDWLNGKEYKCKVSNKGLPSSIEKTISKAKGQPREPQVY
LPPSQEEMTKNQVSLTCLVKGFYPSDIAVEWESNGQPENNYKTTTPVLDSDGSFFLYSRLTVDKS
RWQEGNVFSCSVMEALHNHYTQKSLSLGLGK

SEQ ID NO: 322 (AS20592VH6 HCAb dimeric form amino acid sequence)

EVQLVESGGGLVQPGGSLRLSCAASGYIISYCMGWFRQAPGKGLEGVAAIDSDGGTSYADSVK
GRFTISRDNSKNTLYLQMNSLRAEDTAMYYCAADFCWVDEDRHLYEYNSWGQGLTVTVSSEK
YGPPCPPCPAPEFLGGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSQEDPEVQFNWYVDGVEVHN
AKTKPREEQFNSTYRVVSVLTVLHQDWLNGKEYKCKVSNKGLPSSIEKTISKAKGQPREPQVY
LPPSQEEMTKNQVSLTCLVKGFYPSDIAVEWESNGQPENNYKTTTPVLDSDGSFFLYSRLTVDKS
RWQEGNVFSCSVMEALHNHYTQKSLSLGLGK

SEQ ID NO: 323 (AS20592VH7 HCAb dimeric form amino acid sequence)

EVQLVESGGGLVQPGGSLRLSCAASGYIISYCMGWFRQAPGKGLEGVAAIDSDGGTSYADSVK
GRFTISRDNSKNTLYLQMNSLRAEDTAMYYCAADFCWVDEDRHLYEYNSWGQGLTVTVSSEK
YGPPCPPCPAPEFLGGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSQEDPEVQFNWYVDGVEVHN
AKTKPREEQFNSTYRVVSVLTVLHQDWLNGKEYKCKVSNKGLPSSIEKTISKAKGQPREPQVY
LPPSQEEMTKNQVSLTCLVKGFYPSDIAVEWESNGQPENNYKTTTPVLDSDGSFFLYSRLTVDKS
RWQEGNVFSCSVMEALHNHYTQKSLSLGLGK

SEQ ID NO: 324 (AS20592VH8 HCAb dimeric form amino acid sequence)

EVQLVESGGGLVQPGGSLRLSCAASGYIISYCMGWFRQAPGKEREGLVAAIDSDGGTSYADSVK
GRFTISRDNSKNTLYLQMNSLRAEDTAMYYCAADFCWVDEDRHLYEYNSWGQGLTVTVSSEK
YGPPCPPCPAPEFLGGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSQEDPEVQFNWYVDGVEVHN
AKTKPREEQFNSTYRVVSVLTVLHQDWLNGKEYKCKVSNKGLPSSIEKTISKAKGQPREPQVY
LPPSQEEMTKNQVSLTCLVKGFYPSDIAVEWESNGQPENNYKTTTPVLDSDGSFFLYSRLTVDKS
RWQEGNVFSCSVMEALHNHYTQKSLSLGLGK

SEQ ID NO: 325 (AS20592VH9 HCAb dimeric form amino acid sequence)

EVQLVESGGGLVQPGGSLRLSCAASGYIISYCMGWFRQAPGKEREGLVAAIDSDGGTSYADSVK
GRFTISKDNSKNTLYLQMNSLRAEDTAMYYCAADFCWVDEDRHLYEYNSWGQGLTVTVSSES
KYGPPCPPCPAPEFLGGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSQEDPEVQFNWYVDGVEVH
NAKTKPREEQFNSTYRVVSVLTVLHQDWLNGKEYKCKVSNKGLPSSIEKTISKAKGQPREPQVY
TLPPSQEEMTKNQVSLTCLVKGFYPSDIAVEWESNGQPENNYKTTTPVLDSDGSFFLYSRLTVDK
SRWQEGNVFSCSVMEALHNHYTQKSLSLGLGK

SEQ ID NO: 326 (AS20592VH10 HCAb dimeric form amino acid sequence)

EVQLVESGGGLVQPGGSLRLSCAASGYIISYCMGWFRQAPGKGLEGVAAIDSDGGTSYADSVK
GRFTISKDNSKNTLYLQMNSLRAEDTAMYYCAADFCWVDEDRHLYEYNSWGQGLTVTVSSES
KYGPPCPPCPAPEFLGGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSQEDPEVQFNWYVDGVEVH
NAKTKPREEQFNSTYRVVSVLTVLHQDWLNGKEYKCKVSNKGLPSSIEKTISKAKGQPREPQVY
TLPPSQEEMTKNQVSLTCLVKGFYPSDIAVEWESNGQPENNYKTTTPVLDSDGSFFLYSRLTVDK
SRWQEGNVFSCSVMEALHNHYTQKSLSLGLGK

SEQ ID NO: 327 (AS20592VH10M1 HCAb dimeric form amino acid sequence)

EVQLVESGGGLVQPGGSLRLSCAASGYIISYCMGWFRQAPGKGLEGVAAIDSSGGTSYADSVK
GRFTISKDNSKNTLYLQMNSLRAEDTAMYYCAADFCWVDEDRHLYEYNSWGQGLTVTVSSES
KYGPPCPPAPEFLGGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSQEDPEVQFNWYVDGVEVH
NAKTKPREEQFNSTYRVVSVLTVLHQDWLNGKEYKCKVSNKGLPSSIEKTISKAKGQPREPQVY
TLPPSQEEMTKNQVSLTCLVKGFYPSDIAVEWESNGQPENNYKTPPVLDSDGSFFLYSRLTVDK
SRWQEGNVFSCSVMEALHNHYTQKSLSLGLGK

SEQ ID NO: 328 (AS20594VH5 HCAb dimeric form amino acid sequence)

EVQLVESGGGLVQPGGSLRLSCAASGYTISSYCMGWFRQAPGKGLEGVAAIDSDGTSYADSVK
GRFTISRDNSKNTLYLQMNSLRAEDTAVYYCAADFCWVDDFYEYNYWGQGLTVTVSSESKYGP
PCPPCPAPEFLGGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSQEDPEVQFNWYVDGVEVHNAKT
KPREEQFNSTYRVVSVLTVLHQDWLNGKEYKCKVSNKGLPSSIEKTISKAKGQPREPQVYTLPPS
QEEMTKNQVSLTCLVKGFYPSDIAVEWESNGQPENNYKTPPVLDSDGSFFLYSRLTVDKSRWQ
EGNVFSCSVMEALHNHYTQKSLSLGLGK

SEQ ID NO: 329 (AS20594VH6 HCAb dimeric form amino acid sequence)

EVQLVESGGGLVQPGGSLRLSCAASGYTISSYCMGWFRQAPGKGLEGVAAIDSDGTSYADSVK
GRFTISRDNSKNTLYLQMNSLRAEDTAMYYCAADFCWVDDFYEYNYWGQGLTVTVSSESKYG
PPCPPCPAPEFLGGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSQEDPEVQFNWYVDGVEVHNAK
TKPREEQFNSTYRVVSVLTVLHQDWLNGKEYKCKVSNKGLPSSIEKTISKAKGQPREPQVYTLPP
SQEEMTKNQVSLTCLVKGFYPSDIAVEWESNGQPENNYKTPPVLDSDGSFFLYSRLTVDKSRW
QEGNVFSCSVMEALHNHYTQKSLSLGLGK

SEQ ID NO: 330 (AS20594VH7 HCAb dimeric form amino acid sequence)

EVQLVESGGGLVQPGGSLRLSCAASGYTISSYCMGWFRQAPGKGLEGVAAIDSDGTSYADSVK
GRFTISRDNSKNTLYLQMNSLRAEDTAMYYCAADFCWVDDFYEYNYWGQGLTVTVSSESKYG
PPCPPCPAPEFLGGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSQEDPEVQFNWYVDGVEVHNAK
TKPREEQFNSTYRVVSVLTVLHQDWLNGKEYKCKVSNKGLPSSIEKTISKAKGQPREPQVYTLPP
SQEEMTKNQVSLTCLVKGFYPSDIAVEWESNGQPENNYKTPPVLDSDGSFFLYSRLTVDKSRW
QEGNVFSCSVMEALHNHYTQKSLSLGLGK

SEQ ID NO: 331 (AS20594VH8 HCAb dimeric form amino acid sequence)

EVQLVESGGGLVQPGGSLRLSCAASGYTISSYCMGWFRQAPGKEREGVAAIDSDGTSYADSVK
GRFTISRDNSKNTLYLQMNSLRAEDTAMYYCAADFCWVDDFYEYNYWGQGLTVTVSSESKYG
PPCPPCPAPEFLGGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSQEDPEVQFNWYVDGVEVHNAK
TKPREEQFNSTYRVVSVLTVLHQDWLNGKEYKCKVSNKGLPSSIEKTISKAKGQPREPQVYTLPP
SQEEMTKNQVSLTCLVKGFYPSDIAVEWESNGQPENNYKTPPVLDSDGSFFLYSRLTVDKSRW
QEGNVFSCSVMEALHNHYTQKSLSLGLGK

SEQ ID NO: 332 (AS20594VH9 HCAb dimeric form amino acid sequence)

EVQLVESGGGLVQPGGSLRLSCAASGYTISSYCMGWFRQAPGKEREGVAAIDSDGTSYADSVK
GRFTISKDNSKNTLYLQMNSLRAEDTAMYYCAADFCWVDDFYEYNYWGQGLTVTVSSESKYG
PPCPPCPAPEFLGGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSQEDPEVQFNWYVDGVEVHNAK
TKPREEQFNSTYRVVSVLTVLHQDWLNGKEYKCKVSNKGLPSSIEKTISKAKGQPREPQVYTLPP
SQEEMTKNQVSLTCLVKGFYPSDIAVEWESNGQPENNYKTPPVLDSDGSFFLYSRLTVDKSRW
QEGNVFSCSVMEALHNHYTQKSLSLGLGK

SEQ ID NO: 333 (AS20594VH10 HCAb dimeric form amino acid sequence)

EVQLVESGGGLVQPGGSLRLSCAASGYTISSYCMGWFRQAPGKGLEGVAAIDSDGTSYADSVK
GRFTISKDNSKNTLYLQMNSLRAEDTAVYYCAADFCWVDDFYEYNYWGQGLTVTVSSESKYGP

PCPPCPAPEFLGGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSQEDPEVQFNWYVDGVEVHNAKT
 KPREEQFNSTYRVVSVLTVLHQDWLNGKEYKCKVSNKGLPSSIEKTISKAKGQPREPQVYTLPPS
 QEEMTKNQVSLTCLVKGFYPSDIAVEWESNGQPENNYKTTPPVLDSDGSFFLYSRLTVDKSRWQ
 EGNVFSCSVMHEALHNHYTQKSLSLGLGK

SEQ ID NO: 334 (AS20594VH10M1 HCAb dimeric form amino acid sequence)

EVQLVESGGGLVQPGGSLRLSCAASGYTISYCMGWFRQAPGKGLEGVAAIDSSGTSYADSVK
GRFTISKDNSKNTLYLQMNSLRAEDTAVYYCAADFCWVDDFEYNYWGQGLTVTSSESKYGP
 PCPPCPAPEFLGGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSQEDPEVQFNWYVDGVEVHNAKT
 KPREEQFNSTYRVVSVLTVLHQDWLNGKEYKCKVSNKGLPSSIEKTISKAKGQPREPQVYTLPPS
 QEEMTKNQVSLTCLVKGFYPSDIAVEWESNGQPENNYKTTPPVLDSDGSFFLYSRLTVDKSRWQ
 EGNVFSCSVMHEALHNHYTQKSLSLGLGK

SEQ ID NO: 335 (AS20846VH4 HCAb dimeric form amino acid sequence)

EVQLVESGGGLVQPGGSLRLSCAASGYTVSSYCMGWFRQAPGKGLEGVSAIDSDGSVSYADSV
KGRFTISRDNSKNTLYLQMNSLRAEDTAVYYCAADLCWVDQDQGEYNTWGQGLTVTSSESK
 YGPPCPPCPAPEFLGGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSQEDPEVQFNWYVDGVEVHN
 AKTKPREEQFNSTYRVVSVLTVLHQDWLNGKEYKCKVSNKGLPSSIEKTISKAKGQPREPQVYT
 LPPSQEEMTKNQVSLTCLVKGFYPSDIAVEWESNGQPENNYKTTPPVLDSDGSFFLYSRLTVDKS
 RWQEGNVFSCSVMHEALHNHYTQKSLSLGLGK

SEQ ID NO: 336 (AS20846VH5 HCAb dimeric form amino acid sequence)

EVQLVESGGGLVQPGGSLRLSCAASGYTVSSYCMGWFRQAPGKGLEGVSAIDSDGSVSYADSV
KGRFTISRDNSKNTLYLQMNSLRAEDTAVYFCAADLCWVDQDQGEYNTWGQGLTVTSSESK
 YGPPCPPCPAPEFLGGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSQEDPEVQFNWYVDGVEVHN
 AKTKPREEQFNSTYRVVSVLTVLHQDWLNGKEYKCKVSNKGLPSSIEKTISKAKGQPREPQVYT
 LPPSQEEMTKNQVSLTCLVKGFYPSDIAVEWESNGQPENNYKTTPPVLDSDGSFFLYSRLTVDKS
 RWQEGNVFSCSVMHEALHNHYTQKSLSLGLGK

SEQ ID NO: 337 (AS20846VH6 HCAb dimeric form amino acid sequence)

EVQLVESGGGLVQPGGSLRLSCAASGYTVSSYCMGWFRQAPGKGLEGVAAIDSDGSVSYADSV
KGRFTISRDNSKNTLYLQMNSLRAEDTAVYFCAADLCWVDQDQGEYNTWGQGLTVTSSESK
 YGPPCPPCPAPEFLGGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSQEDPEVQFNWYVDGVEVHN
 AKTKPREEQFNSTYRVVSVLTVLHQDWLNGKEYKCKVSNKGLPSSIEKTISKAKGQPREPQVYT
 LPPSQEEMTKNQVSLTCLVKGFYPSDIAVEWESNGQPENNYKTTPPVLDSDGSFFLYSRLTVDKS
 RWQEGNVFSCSVMHEALHNHYTQKSLSLGLGK

SEQ ID NO: 338 (AS20846VH7 HCAb dimeric form amino acid sequence)

EVQLVESGGGLVQPGGSLRLSCAASGYTVSSYCMGWFRQAPGKGLEGVAAIDSDGSVSYADSV
KGRFTISRDNSKNTLYLQMNSLRAEDTAMYFCAADLCWVDQDQGEYNTWGQGLTVTSSESK
 YGPPCPPCPAPEFLGGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSQEDPEVQFNWYVDGVEVHN
 AKTKPREEQFNSTYRVVSVLTVLHQDWLNGKEYKCKVSNKGLPSSIEKTISKAKGQPREPQVYT
 LPPSQEEMTKNQVSLTCLVKGFYPSDIAVEWESNGQPENNYKTTPPVLDSDGSFFLYSRLTVDKS
 RWQEGNVFSCSVMHEALHNHYTQKSLSLGLGK

SEQ ID NO: 339 (AS20846VH8 HCAb dimeric form amino acid sequence)

EVQLVESGGGLVQPGGSLRLSCAASGYTVSSYCMGWFRQAPGKGREGVAAIDSDGSVSYADSV
KGRFTISRDNSKNTLYLQMNSLRAEDTAMYFCAADLCWVDQDQGEYNTWGQGLTVTSSESK
 YGPPCPPCPAPEFLGGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSQEDPEVQFNWYVDGVEVHN
 AKTKPREEQFNSTYRVVSVLTVLHQDWLNGKEYKCKVSNKGLPSSIEKTISKAKGQPREPQVYT

LPPSQEEMTKNQVSLTCLVKGFYPSDIAVEWESNGQPENNYKTTTPVLDSDGSFFLYSRLTVDKS
RWQEGNVFSCSVMEALHNHYTQKSLSLGLGK

SEQ ID NO: 340 (AS20846VH9 HCAb dimeric form amino acid sequence)

EVQLVESGGGLVQPGGSLRLSCAASGYTVSSYCMGWFRQAPGKEREGVAAIDSDGSVSYADSV
KGRFTISRDNSKNTLYLQMNSLRAEDTAMVFCAADLCWVDQDQGEYNTWGQGTLLTVSSESK
YGPCCPPCPAPEFLGGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSQEDPEVQFNWYVDGVEVHN
AKTKPREEQFNSTYRVVSVLTVLHQDWLNGKEYKCKVSNKGLPSSIEKTISKAKGQPREPQVYT
LPPSQEEMTKNQVSLTCLVKGFYPSDIAVEWESNGQPENNYKTTTPVLDSDGSFFLYSRLTVDKS
RWQEGNVFSCSVMEALHNHYTQKSLSLGLGK

SEQ ID NO: 341 (AS20846VH10 HCAb dimeric form amino acid sequence)

EVQLVESGGGLVQPGGSLRLSCAASGYTVSSYCMGWFRQAPGKEREGVAAIDSDGSVSYADSV
KGRFTISKDNSKNTLYLQMNSLRAEDTAMVFCAADLCWVDQDQGEYNTWGQGTLLTVSSESK
YGPCCPPCPAPEFLGGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSQEDPEVQFNWYVDGVEVHN
AKTKPREEQFNSTYRVVSVLTVLHQDWLNGKEYKCKVSNKGLPSSIEKTISKAKGQPREPQVYT
LPPSQEEMTKNQVSLTCLVKGFYPSDIAVEWESNGQPENNYKTTTPVLDSDGSFFLYSRLTVDKS
RWQEGNVFSCSVMEALHNHYTQKSLSLGLGK

SEQ ID NO: 342 (AS20846VH12 HCAb dimeric form amino acid sequence)

EVQLVESGGGLVQPGGSLRLSCAASGYTVSSYCMGWFRQAPGKGREGVSAIDSDGSVSYADSV
KGRFTISKDNSKNTLYLQMNSLRAEDTAVVFCAADLCWVDQDQGEYNTWGQGTLLTVSSESK
YGPCCPPCPAPEFLGGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSQEDPEVQFNWYVDGVEVHN
AKTKPREEQFNSTYRVVSVLTVLHQDWLNGKEYKCKVSNKGLPSSIEKTISKAKGQPREPQVYT
LPPSQEEMTKNQVSLTCLVKGFYPSDIAVEWESNGQPENNYKTTTPVLDSDGSFFLYSRLTVDKS
RWQEGNVFSCSVMEALHNHYTQKSLSLGLGK

SEQ ID NO: 343 (AS20846VH12M1 HCAb dimeric form amino acid sequence)

EVQLVESGGGLVQPGGSLRLSCAASGYTVSSYCMGWFRQAPGKGREGVSAIDSSGSVSYADSV
KGRFTISKDNSKNTLYLQMNSLRAEDTAVVFCAADLCWVDQDQGEYNTWGQGTLLTVSSESK
YGPCCPPCPAPEFLGGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSQEDPEVQFNWYVDGVEVHN
AKTKPREEQFNSTYRVVSVLTVLHQDWLNGKEYKCKVSNKGLPSSIEKTISKAKGQPREPQVYT
LPPSQEEMTKNQVSLTCLVKGFYPSDIAVEWESNGQPENNYKTTTPVLDSDGSFFLYSRLTVDKS
RWQEGNVFSCSVMEALHNHYTQKSLSLGLGK

SEQ ID NO: 344 (AS20601VH4 HCAb dimeric form amino acid sequence)

EVQLVESGGGLVQPGGSLRLSCAASGYTYGGYCMGWFRQAPGKGLEGVSTINSDGSTNYADSV
KGRFTISRDNSKNTLYLQMNSLRAEDTAVYYCAVDLAPCWVAGPIEAADFGYWGQGTLLTVSS
ESKYGPCCPPCPAPEFLGGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSQEDPEVQFNWYVDGVE
VHNAKTKPREEQFNSTYRVVSVLTVLHQDWLNGKEYKCKVSNKGLPSSIEKTISKAKGQPREPQ
VYTLPPSQEEMTKNQVSLTCLVKGFYPSDIAVEWESNGQPENNYKTTTPVLDSDGSFFLYSRLTV
DKSRWQEGNVFSCSVMEALHNHYTQKSLSLGLGK

SEQ ID NO: 345 (AS20601VH4.M6 HCAb dimeric form amino acid sequence)

EVQLVESGGGLVQPGGSLRLSCAASGYTYGGYCMGWFRQAPGKGLEGVSTIASEGSTNYADSV
KGRFTISRDNSKNTLYLQMNSLRAEDTAVYYCAVDLAPCWVAGPIEAADFGYWGQGTLLTVSS
ESKYGPCCPPCPAPEFLGGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSQEDPEVQFNWYVDGVE
VHNAKTKPREEQFNSTYRVVSVLTVLHQDWLNGKEYKCKVSNKGLPSSIEKTISKAKGQPREPQ
VYTLPPSQEEMTKNQVSLTCLVKGFYPSDIAVEWESNGQPENNYKTTTPVLDSDGSFFLYSRLTV
DKSRWQEGNVFSCSVMEALHNHYTQKSLSLGLGK

SEQ ID NO: 346 (AS20601VH4.M7 HCAb dimeric form amino acid sequence)

EVQLVESGGGLVQPGGSLRLSCAASGYTYGGYCMGWFRQAPGKGLEGVSTIASSGSTNYADSV
KGRFTISRDNKNTLYLQMNSLRAEDTAVYYCAVDLAPCWVAGPIEAADFGYWGQGLVTVSS
ESKYGPPCPPCPAPEFLGGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSQEDPEVQFNWYVDGVE
VHNAKTKPREEQFNSTYRVVSVLTVLHQDWLNGKEYKCKVSNKGLPSSIEKTISKAKGQPREPQ
VYTLPPSQEEMTKNQVSLTCLVKGFYPSDIAVEWESNGQPENNYKTPPVLDSDGSFFLYSRLTV
DKSRWQEGNVFSCSVMEALHNHYTQKSLSLSLGK

SEQ ID NO: 347 (AS20601VH11 HCAb dimeric form amino acid sequence)

EVQLVESGGGLVQPGGSLRLSCAASGYTYGGYCMGWFRQAPGKGREGVSTINS DGSTNYADSV
KGRFTISRDNKNTLYLQMNSLRPEDTAVYYCAVDLAPCWVAGPIEAADFGYWGQGLVTVSS
ESKYGPPCPPCPAPEFLGGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSQEDPEVQFNWYVDGVE
VHNAKTKPREEQFNSTYRVVSVLTVLHQDWLNGKEYKCKVSNKGLPSSIEKTISKAKGQPREPQ
VYTLPPSQEEMTKNQVSLTCLVKGFYPSDIAVEWESNGQPENNYKTPPVLDSDGSFFLYSRLTV
DKSRWQEGNVFSCSVMEALHNHYTQKSLSLSLGK

SEQ ID NO: 348 (AS20601VH11.M6 HCAb dimeric form amino acid sequence)

EVQLVESGGGLVQPGGSLRLSCAASGYTYGGYCMGWFRQAPGKGREGVSTIASEGSTNYADSV
KGRFTISRDNKNTLYLQMNSLRPEDTAVYYCAVDLAPCWVAGPIEAADFGYWGQGLVTVSS
ESKYGPPCPPCPAPEFLGGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSQEDPEVQFNWYVDGVE
VHNAKTKPREEQFNSTYRVVSVLTVLHQDWLNGKEYKCKVSNKGLPSSIEKTISKAKGQPREPQ
VYTLPPSQEEMTKNQVSLTCLVKGFYPSDIAVEWESNGQPENNYKTPPVLDSDGSFFLYSRLTV
DKSRWQEGNVFSCSVMEALHNHYTQKSLSLSLGK

SEQ ID NO: 349 (AS20601VH11.M7 HCAb dimeric form amino acid sequence)

EVQLVESGGGLVQPGGSLRLSCAASGYTYGGYCMGWFRQAPGKGREGVSTIASSGSTNYADSV
KGRFTISRDNKNTLYLQMNSLRPEDTAVYYCAVDLAPCWVAGPIEAADFGYWGQGLVTVSS
ESKYGPPCPPCPAPEFLGGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSQEDPEVQFNWYVDGVE
VHNAKTKPREEQFNSTYRVVSVLTVLHQDWLNGKEYKCKVSNKGLPSSIEKTISKAKGQPREPQ
VYTLPPSQEEMTKNQVSLTCLVKGFYPSDIAVEWESNGQPENNYKTPPVLDSDGSFFLYSRLTV
DKSRWQEGNVFSCSVMEALHNHYTQKSLSLSLGK

SEQ ID NO: 350 (Human LAG-3 full length amino acid sequence)

VPVVWAEQAGAPQLPCSTIPLQDLSLLRRAGVTWQHQPDSGPPAAAPGHPLAPGPHPAAPSSW
GPRPRRYTVLSVGPGLRSGRLPLQPRVQLDERGRQRGDFSLWLRPARRADAGEYRAAVHLRD
RALSCRLRLRLGQASMTASPPGSLRASDWVILNCSFSRPDRPASVHWFRNRGQGRVPVRESPHH
HLAESFLFLPQVSPMDSGPWGCILTYRDGFNVSIMYNLTVLGLEPPTPLTVYAGAGSRVGLPCRL
PAGVGTRSFLLTAKWTPPGGPDLLVTGDNGDFTLRLEDVSQAQAGTYTCHIHLEQQLNATVTL
AIITVTPKSFSGPSGLKLLCEVTPVSGQERFVWSSLDTPSQRFSFSGPWLEAQEAQLLSQPWQCQL
YQGERLLGAAYVFTELSSPGAQRSGRAPGALPAGHLLLFLILGVLSLLLLVTGAFGFHLWRRQW
RPRRFSALEQGIHPPQAQSKIEELEQEPEPEPEPEPEPEPEPEPEPEQL

SEQ ID NO: 351 (Human LAG-3 extracellular domain amino acid sequence)

VPVVWAEQAGAPQLPCSTIPLQDLSLLRRAGVTWQHQPDSGPPAAAPGHPLAPGPHPAAPSSW
GPRPRRYTVLSVGPGLRSGRLPLQPRVQLDERGRQRGDFSLWLRPARRADAGEYRAAVHLRD
RALSCRLRLRLGQASMTASPPGSLRASDWVILNCSFSRPDRPASVHWFRNRGQGRVPVRESPHH
HLAESFLFLPQVSPMDSGPWGCILTYRDGFNVSIMYNLTVLGLEPPTPLTVYAGAGSRVGLPCRL
PAGVGTRSFLLTAKWTPPGGPDLLVTGDNGDFTLRLEDVSQAQAGTYTCHIHLEQQLNATVTL

AIITVTPKSFSGPSGLGKLLCEVTPVSGQERFVWSSLDTPSQRSFSGPWLEAQEAQLLSQPWQCQL
YQGERLLGAAVYFTELSSPGAQRSGRAPGALPAGHL

SEQ ID NO: 352 (Human IgG1 hinge amino acid sequence)
EPKSSDKTHTCPPCP

SEQ ID NO: 353 (Mutated human IgG1 hinge amino acid sequence)
EPKSSDKTHTSPPSP

SEQ ID NO: 354 (BMS-986016 Heavy chain amino acid sequence)
QVQLQQWGAGLLKPSETLSLTCVYGGSFSDYYWNWIRQPPGKGLEWIGEINHRGSTNSNPSLK
SRVTLSDLTSKNQFSLKLRSVTAADTAVYYCAFGYSDYEYNWFDPWGQGTTLTVSSASTKGPS
VFPLAPCSRSTSESTAALGCLVKDYFPEPVTVSWNSGALTSGVHTFPAVLQSSGLYSLSSVTVPS
SSLGTKTYTCNVDHKPSNTKVDKRVESKYGPCCPPCPAPEFLGGPSVFLFPPKPKDTLMISRTPEV
TCVYVDVSDQEDPEVQFNWYVDGVEVHNAKTKPREEQFNSTYRVVSVLTVLHQDWLNGKEYKC
KVSNGKLPSSIEKTISKAKGQPREPQVYTLPPSQEEMTKNQVSLTCLVKGFYPSDIAVEWESNGQ
PENNYKTTTPVLDSGDSFFLYSRLTVDKSRWQEGNVFSCSVMHEALHNHYTQKSLSLSLGK

SEQ ID NO: 355 (BMS-986016 Light chain amino acid sequence)
EIVLTQSPATLSLSPGERATLSCRASQSISSYLAWYQQKPGQAPRLLIYDASNRATGIPARFSGSGS
GTDFTLTISLEPEDFAVYYCQQRSNWPLTFGQGTNLEIKRTVAAPSVFIFPPSDEQLKSGTASV
CLLNNFYPREAKVQWKVDNALQSGNSQESVTEQDSKSTYSLSSTLTLSKADYEKHKVYACEV
THQGLSPVTKSFNRGEC

SEQ ID NO: 356 (pembrolizumab Heavy chain amino acid sequence)
QVQLVQSGVEVKKPGASVKVSKASGYTFTNYYMYWVRQAPGQGLEWMGGINPSNGGTNFNE
KFKNRVTLTDSSTTTAYMELKSLQFDDTAVYYCARRDYRFDMGFDYWGQGTTLTVSSASTKG
PSVFPLAPCSRSTSESTAALGCLVKDYFPEPVTVSWNSGALTSGVHTFPAVLQSSGLYSLSSVTV
PSSSLGTKTYTCNVDHKPSNTKVDKRVESKYGPCCPPCPAPEFLGGPSVFLFPPKPKDTLMISRT
EVTCTVYVDVSDQEDPEVQFNWYVDGVEVHNAKTKPREEQFNSTYRVVSVLTVLHQDWLNGKEY
KCKVSNKGLPSSIEKTISKAKGQPREPQVYTLPPSQEEMTKNQVSLTCLVKGFYPSDIAVEWESN
GQPENNYKTTTPVLDSGDSFFLYSRLTVDKSRWQEGNVFSCSVMHEALHNHYTQKSLSLSLGK

SEQ ID NO: 357 (pembrolizumab Light chain amino acid sequence)
EIVLTQSPATLSLSPGERATLSCRASKGVSTSGSYLHWYQQKPGQAPRLLIYLASYLESVGPAPRF
SGSGSGTDFTLTISLEPEDFAVYYCQHSRDLPLTFGGGKVEIKRTVAAPSVFIFPPSDEQLKSGT
ASVVCLLNNFYPREAKVQWKVDNALQSGNSQESVTEQDSKSTYSLSSTLTLSKADYEKHKVY
ACEVTHQGLSPVTKSFNRGEC

SEQ ID NO: 358 (BLP-21 Heavy chain amino acid sequence)
EVQLVESGGGLVQPGGSLRLSCAASGYTVSSYCMGWFRQAPGKGREGVSAIDSDGSVSYADSV
KGRFTISKDNKNTLYLQMNSLRAEDTAVYFCAADLCWVDQDQGEYNTWGQGTTLTVSSEP
SDKTHTSPPSPQVQLVQSGVEVKKPGASVKVSKASGYTFTNYYMYWVRQAPGQGLEWMGGI
NPSNGGTNFNEKFKNRVTLTDSSTTTAYMELKSLQFDDTAVYYCARRDYRFDMGFDYWGQGT
TTVTVSSASTKGPSVFPLAPCSRSTSESTAALGCLVKDYFPEPVTVSWNSGALTSGVHTFPAVLQ
SGLYSLSSVTVPS
SSLGTKTYTCNVDHKPSNTKVDKRVESKYGPCCPPCPAPEFLGGPSVFLFPP
KPKDTLMISRTPEVTCVYVDVSDQEDPEVQFNWYVDGVEVHNAKTKPREEQFNSTYRVVSVLTV
LHQDWLNGKEYKCKVSNKGLPSSIEKTISKAKGQPREPQVYTLPPSQEEMTKNQVSLTCLVKGF
YPSDIAVEWESNGQPENNYKTTTPVLDSGDSFFLYSRLTVDKSRWQEGNVFSCSVMHEALHNHY
TQKSLSLSLGK

SEQ ID NO: 359 (BLP-21 Light chain amino acid sequence)

EIVLTQSPATLSLSPGERATLSCRASKGVSTSGYSYLHWYQQKPGQAPRLLIYLASYLESGVPARF
SGSGSGTDFTLTISLEPEDFAVYYCQHSDRLPLTFGGGTKVEIKRTVAAPSVFIFPPSDEQLKSGT
ASVVCLLNNFYPREAKVQWKVDNALQSGNSQESVTEQDSKDSTYSLSSTLTLSKADYEKHKVY
ACEVTHQGLSSPVTKSFNRGEC

SEQ ID NO: 360 (BLP-22 Heavy chain amino acid sequence)

QVQLVQSGVEVKKPGASVKVSCKASGYTFTNYYMYWVRQAPGQGLEWMGGINPSNGGTNENE
KFKNRVTLTDSSTTTAYMELKSLQFDDTAVYYCARRDYRFDMGFYWGQGTITVTVSSASTKG
PSVFPLAPCSRSTSESTAALGCLVKDYFPEPVTVSWNSGALTSGVHTFPAVLQSSGLYSLSSVTV
PSSSLGKTYTCNVDHKPSNTKVDKRVESKYGPPCPPAPEFLGGPSVFLFPPKPKDTLMISRT
EVTCTVVDVVSQEDPEVQFNWYVDGVEVHNAKTKPREEQFNSTYRVVSVLTVLHQDWLNGKEY
KCKVSNKGLPSSIEKTISKAKGQPREPQVYTLPPSQEEMTKNQVSLTCLVKGFYPSDIAVEWESN
GQPENNYKTTTPVLDSDGSFFLYSRLTVDKSRWQEGNVFSCSVMEALHNHYTQKSLSLSLGK

SEQ ID NO: 361 (BLP-22 Light chain amino acid sequence)

EVQLVESGGGLVQPGGSLRLSCAASGYTVSSYCMGWFRQAPGKGREGVSAIDSDGSVSYADSV
KGRFTISKDNSKNTLYLQMNSLRAEDTAVYFCAADLCWVDQDQGEYNTWGQGTITVTVSSSEPKS
SDKTHTSPSPSPEIVLTQSPATLSLSPGERATLSCRASKGVSTSGYSYLHWYQQKPGQAPRLLIYLA
SYLESGVPARFSGSGSGTDFTLTISLEPEDFAVYYCQHSDRLPLTFGGGTKVEIKRTVAAPSVFIF
PPSDEQLKSGTASVVCLLNNFYPREAKVQWKVDNALQSGNSQESVTEQDSKDSTYSLSSTLTLS
KADYEKHKVYACEVTHQGLSSPVTKSFNRGEC

SEQ ID NO: 362 (BLP-23 Heavy chain amino acid sequence)

EVQLVESGGGLVQPGGSLRLSCAASGYTYGGYCMGWFRQAPGKGLEGVSTINS DGSTNYADSV
KGRFTISRDNKNTLYLQMNSLRAEDTAVYYCAVDLAPCWVAGPIEAADFGYWGQGTITVTVSS
EPKSSDKTHTSPSPQVQLVQSGVEVKKPGASVKVSCKASGYTFTNYYMYWVRQAPGQGLEW
MGGINPSNGGTNENEKFKNRVTLTDSSTTTAYMELKSLQFDDTAVYYCARRDYRFDMGFYWG
GQGTITVTVSSASTKGPSVFPLAPCSRSTSESTAALGCLVKDYFPEPVTVSWNSGALTSGVHTFPA
VLQSSGLYSLSSVTVTPSSSLGKTYTCNVDHKPSNTKVDKRVESKYGPPCPPAPEFLGGPSVFL
FPPKPKDTLMISRTPEVTCVVDVVSQEDPEVQFNWYVDGVEVHNAKTKPREEQFNSTYRVVSV
LTVLHQDWLNGKEYKCKVSNKGLPSSIEKTISKAKGQPREPQVYTLPPSQEEMTKNQVSLTCLV
KGFYPSDIAVEWESNGQPENNYKTTTPVLDSDGSFFLYSRLTVDKSRWQEGNVFSCSVMEALH
NHYTQKSLSLSLGK

SEQ ID NO: 363 (BLP-23 Light chain amino acid sequence)

EIVLTQSPATLSLSPGERATLSCRASKGVSTSGYSYLHWYQQKPGQAPRLLIYLASYLESGVPARF
SGSGSGTDFTLTISLEPEDFAVYYCQHSDRLPLTFGGGTKVEIKRTVAAPSVFIFPPSDEQLKSGT
ASVVCLLNNFYPREAKVQWKVDNALQSGNSQESVTEQDSKDSTYSLSSTLTLSKADYEKHKVY
ACEVTHQGLSSPVTKSFNRGEC

SEQ ID NO: 364 (BLP-24 Heavy chain amino acid sequence)

QVQLVQSGVEVKKPGASVKVSCKASGYTFTNYYMYWVRQAPGQGLEWMGGINPSNGGTNENE
KFKNRVTLTDSSTTTAYMELKSLQFDDTAVYYCARRDYRFDMGFYWGQGTITVTVSSASTKG
PSVFPLAPCSRSTSESTAALGCLVKDYFPEPVTVSWNSGALTSGVHTFPAVLQSSGLYSLSSVTV
PSSSLGKTYTCNVDHKPSNTKVDKRVESKYGPPCPPAPEFLGGPSVFLFPPKPKDTLMISRT
EVTCTVVDVVSQEDPEVQFNWYVDGVEVHNAKTKPREEQFNSTYRVVSVLTVLHQDWLNGKEY
KCKVSNKGLPSSIEKTISKAKGQPREPQVYTLPPSQEEMTKNQVSLTCLVKGFYPSDIAVEWESN
GQPENNYKTTTPVLDSDGSFFLYSRLTVDKSRWQEGNVFSCSVMEALHNHYTQKSLSLSLGK

SEQ ID NO: 365 (BLP-24 Light chain amino acid sequence)

EVQLVESGGGLVQPGGSLRLSCAASGYTYGGYCMGWFRQAPGKGLEGVSTINSDGSTNYADSV
KGRFTISRDNSKNTLYLQMNSLRAEDTAVYYCAVDLAPCWVAGPIEAADFGYWGQGLVTVSS
 EPKSSDKTHTSPSPSPEIVLTQSPATLSLSPGERATLSCRASKGVSTSGYSYLHWYQQKPGQAPRLLI
 YLASYLESGVPARFSGSGSGTDFTLTISLLEPEDFAVYYCQHSDRLPLTFGGGKVEIKRTVAAPS
 VFIFPPSDEQLKSGTASVVCLLNNFYPRFAKQVQWKVDNALQSGNSQESVTEQDSKSTYLSSTL
 TLSKADYEKHKVYACEVTHQGLSSPVTKSFNRGEC

SEQ ID NO: 366 (Linker peptide (9GS) amino acid sequence)

GGGGSGGGGS

SEQ ID NO: 367 (Linker peptide amino acid sequence)

GGGGSGGGSGGGGS

SEQ ID NO: 368 (Linker peptide amino acid sequence, n is an integer of at least one)

(G)_n

SEQ ID NO: 369 (Linker peptide amino acid sequence, n is an integer of at least one)

(GS)_n

SEQ ID NO: 370 (Linker peptide amino acid sequence, n is an integer of at least one)

(GSGGS)_n

SEQ ID NO: 371 (Linker peptide amino acid sequence, n is an integer of at least one)

(GGGS)_n

SEQ ID NO: 372 (Linker peptide amino acid sequence, n is an integer of at least one)

(GGGGGS)_n

SEQ ID NO: 373 (PD1-BM-min Heavy chain amino acid sequence)

EVQLVESGGGLVQPGGSLRLSCAASGFVFSRYDMAWVRQAPGKGLEWVSFISGGGSNTYYPDTVKG
RFTISRDNSKNTLYLQMNSLRAEDTAVYYCISPYYYAMEYWGQGTITVTVSSASTKGPSVFPLAPCSR
 TSESTAALGCLVKDYFPEPVTVSWNSGALTSGVHTFPAVLQSSGLYSLSSVTVTPSSSLGKTKYTCNV
 DHKPSNTKVDKRVESKYGPCPPCPAPEFLGGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSQEDPEVQ
 FNWYVDGVEVHNAKTKPREEQFNSTYRVVSVLTVLHQDWLNGKEYKCKVSNKGLPSSIEKTISKAK
 GQPREPQVYTLPPSQEEMTKNQVSLTCLVKGFYPSDIAVEWESNGQPENNYKTPPVLDSDGSFFLYS
 RLTVDKSRWQEGNVFSCSVMHEALHNHYTQKSLSLGLGK

SEQ ID NO: 374 (PD1-BM-min Light chain amino acid sequence)

DIQMTQSPSSVSASVGDRVTITCKASQDVDTAVAAYQQKPGKAPKLLIYWASTRHTGVPSRFSGSGS
 GTDFTLTISLQPEDFATYYCQQYSTFPWTFGGGKVEIKRTVAAPSVFIFPPSDEQLKSGTASVVCLLN
 NFYPREAKVQWKVDNALQSGNSQESVTEQDSKSTYLSSTLTLSKADYEKHKVYACEVTHQGLSSP
 VTKSFNRGEC

SEQ ID NO: 375 (BLP-1 Heavy chain amino acid sequence)

EVQLVESGGGSVQAGGSLRLSCAASGYIISYCMGWFRQAPGKEREGVAIDSDGGTSYADSVKGRF
 TISKDNAKNTLYLQMDSLKPEDTAMYYCAADFCWVDEDRHLYEYNSWGQGTQVTVSSSEPKSSDKT
 HTSPSPSPEVQLVESGGGLVQPGGSLRLSCAASGFVFSRYDMAWVRQAPGKGLEWVSFISGGGSNTYY
PDTVKGRFTISRDNKNTLYLQMNSLRAEDTAVYYCISPYYYAMEYWGQGTITVTVSSASTKGPSVFP
 LAPCSRSTSESTAALGCLVKDYFPEPVTVSWNSGALTSGVHTFPAVLQSSGLYSLSSVTVTPSSSLGK

TYTCNV D H K P S N T K V D K R V E S K Y G P P C P P C A P E F L G G P S V F L F P P K P K D T L M I S R T P E V T C V V D V S
Q E D P E V Q F N W Y V D G V E V H N A K T K P R E E Q F N S T Y R V V S V L T V L H Q D W L N G K E Y K C K V S N K G L P S S I E
K T I S K A K G Q P R E P Q V Y T L P P S Q E E M T K N Q V S L T C L V K G F Y P S D I A V E W E S N G Q P E N N Y K T T P P V L D S D
G S F F L Y S R L T V D K S R W Q E G N V F S C S V M H E A L H N H Y T Q K S L S L S L G K

SEQ ID NO: 376 (BLP-1 Light chain amino acid sequence)

D I Q M T Q S P S S V S A S V G D R V T I T C K A S Q D V D T A V A W Y Q Q K P G K A P K L L I Y W A S T R H T G V P S R F S G S G S
G T D F T L T I S S L Q P E D F A T Y Y C Q Q Y S T E P W T F G G G T K V E I K R T V A A P S V F I F P P S D E Q L K S G T A S V V C L L N
N F Y P R E A K V Q W K V D N A L Q S G N S Q E S V T E Q D S K D S T Y S L S S T L T L S K A D Y E K H K V Y A C E V T H Q L G L S S P
V T K S F N R G E C

SEQ ID NO: 377 (BLP-2 Heavy chain amino acid sequence)

Q V Q L A E S G G G S V Q A G G S L R L S C A A S G Y T I S S Y C M G W F R Q A P G K E R E G V A A I D S D G S T S Y A D S V K G R F
T I S K D N A K N T L Y L Q M N S L K P E D T A M Y Y C A A D F C W V D D F Y E Y N Y W G Q G T Q V T V S S E P K S S D K T H T S P
P S P E V Q L V E S G G G L V Q P G G S L R L S C A A S G F V F S R Y D M A W V R Q A P G K G L E W V S F I S G G G S N T Y Y P D T V
K G R F T I S R D N S K N T L Y L Q M N S L R A E D T A V Y Y C I S P Y Y Y A M E Y W G Q G T T V T V S S A S T K G P S V F L A P C
S R S T S E S T A A L G C L V K D Y F P E P V T V S W N S G A L T S G V H T F P A V L Q S S G L Y S L S S V T V P S S L G T K T Y T C
N V D H K P S N T K V D K R V E S K Y G P P C P P C A P E F L G G P S V F L F P P K P K D T L M I S R T P E V T C V V D V S Q E D P E
V Q F N W Y V D G V E V H N A K T K P R E E Q F N S T Y R V V S V L T V L H Q D W L N G K E Y K C K V S N K G L P S S I E K T I S K
A K G Q P R E P Q V Y T L P P S Q E E M T K N Q V S L T C L V K G F Y P S D I A V E W E S N G Q P E N N Y K T T P P V L D S D G S F F L
Y S R L T V D K S R W Q E G N V F S C S V M H E A L H N H Y T Q K S L S L S L G K

SEQ ID NO: 378 (BLP-2 Light chain amino acid sequence)

D I Q M T Q S P S S V S A S V G D R V T I T C K A S Q D V D T A V A W Y Q Q K P G K A P K L L I Y W A S T R H T G V P S R F S G S G S
G T D F T L T I S S L Q P E D F A T Y Y C Q Q Y S T E P W T F G G G T K V E I K R T V A A P S V F I F P P S D E Q L K S G T A S V V C L L N
N F Y P R E A K V Q W K V D N A L Q S G N S Q E S V T E Q D S K D S T Y S L S S T L T L S K A D Y E K H K V Y A C E V T H Q L G L S S P
V T K S F N R G E C

SEQ ID NO: 379 (BLP-3 Heavy chain amino acid sequence)

E V Q L A E S G G G S V Q A G G S L R L S C A A S G Y T Y G G Y C M G W F R Q A P G K E R E G V A T I N S D G S T N Y A D S V K G R
F T I S K D N A K N T L Y L Q M N S L S P E D T A M Y Y C A V D L A P C V W A G P I E A A D F G Y W G Q G T Q V T V S S E P K S S D
K T H T S P P S P E V Q L V E S G G G L V Q P G G S L R L S C A A S G F V F S R Y D M A W V R Q A P G K G L E W V S F I S G G G S N T
Y Y P D T V K G R F T I S R D N S K N T L Y L Q M N S L R A E D T A V Y Y C I S P Y Y Y A M E Y W G Q G T T V T V S S A S T K G P S V
F L A P C S R S T S E S T A A L G C L V K D Y F P E P V T V S W N S G A L T S G V H T F P A V L Q S S G L Y S L S S V T V P S S L G
T K T Y T C N V D H K P S N T K V D K R V E S K Y G P P C P P C A P E F L G G P S V F L F P P K P K D T L M I S R T P E V T C V V D
V S Q E D P E V Q F N W Y V D G V E V H N A K T K P R E E Q F N S T Y R V V S V L T V L H Q D W L N G K E Y K C K V S N K G L P S
S I E K T I S K A K G Q P R E P Q V Y T L P P S Q E E M T K N Q V S L T C L V K G F Y P S D I A V E W E S N G Q P E N N Y K T T P P V L D
S D G S F F L Y S R L T V D K S R W Q E G N V F S C S V M H E A L H N H Y T Q K S L S L S L G K

SEQ ID NO: 380 (BLP-3 Light chain amino acid sequence)

D I Q M T Q S P S S V S A S V G D R V T I T C K A S Q D V D T A V A W Y Q Q K P G K A P K L L I Y W A S T R H T G V P S R F S G S G S
G T D F T L T I S S L Q P E D F A T Y Y C Q Q Y S T E P W T F G G G T K V E I K R T V A A P S V F I F P P S D E Q L K S G T A S V V C L L N
N F Y P R E A K V Q W K V D N A L Q S G N S Q E S V T E Q D S K D S T Y S L S S T L T L S K A D Y E K H K V Y A C E V T H Q L G L S S P
V T K S F N R G E C

SEQ ID NO: 381 (BLP-4 Heavy chain amino acid sequence)

Q V H L M E S G G G S V Q V G G S L R L S C A A S G Y T V S S Y C M G W F R Q A P G K E R E G V A A I D S D G S V S Y A D S V K G R
F T I S K D N A K N T L Y L Q M N S L K P E D T A M Y F C A A D L C W V D Q D Q G E Y N T W G Q G T Q V T V S S E P K S S D K T H
T S P P S P E V Q L V E S G G G L V Q P G G S L R L S C A A S G F V F S R Y D M A W V R Q A P G K G L E W V S F I S G G G S N T Y Y P
D T V K G R F T I S R D N S K N T L Y L Q M N S L R A E D T A V Y Y C I S P Y Y Y A M E Y W G Q G T T V T V S S A S T K G P S V F P L
A P C S R S T S E S T A A L G C L V K D Y F P E P V T V S W N S G A L T S G V H T F P A V L Q S S G L Y S L S S V T V P S S L G T K T
Y T C N V D H K P S N T K V D K R V E S K Y G P P C P P C A P E F L G G P S V F L F P P K P K D T L M I S R T P E V T C V V D V S Q

EDPEVQFNWYVDGVEVHNAKTKPREEQFNSTYRVVSVLTVLHQDWLNGKEYKCKVSNKGLPSSIEK
TISKAKGQPREPQVYTLPPSQEEMTKNQVSLTCLVKGFYPSDIAVEWESNGQPENNYKTTPPVLDSDG
SFFLYSRLTVDKSRWQEGNVFSCSVMHREALHNHYTQKSLSLSLGK

SEQ ID NO: 382 (BLP-4 Light chain amino acid sequence)

DIQMTQSPSSVSASVGDRVTITCKASQD~~VDTAVAWY~~QQKPGKAPKLLIYW~~ASTRHT~~GVPSRFSGSGS
GTDFTLTISLQPEDFATYYCQOYST~~FPWTF~~GGGTKVEIKRTVAAPSVFIFPPSDEQLKSGTASVVCLLN
NFYPREAKVQWKVDNALQSGNSQESVTEQDSKDYSLSSSTLTLSKADYEKHKVYACEVTHQGLSSP
VTKSFNRGEC

SEQ ID NO: 383 (BLP-5 Heavy chain amino acid sequence)

EVQLVESGGGLVQPGGSLRLSCAASGYI~~SSYCMGW~~FRQAPGKGLEGVA~~AIDSDGGTSY~~ADSVKGRF
TISKDNSKNTLYLQMNSLRAEDTAMYYCAAD~~FCWVDEDRHLYEYNS~~WGQGLTVTSSEPKSSDKTH
TSPSPSEVQLVESGGGLVQPGGSLRLSCAAS~~GFVFSRYDMAW~~VVRQAPGKGLEWVSFISGGGSNTYYP
DTVKGRFTISRDNKNTLYLQMNSLRAEDTAVYYCIS~~PYYYAMEY~~WGQGT~~TVTVSS~~ASTKGPSVFPL
APCSRSTSESTAALGCLVKDYFPEPVT~~SVNSGALTSGVHTFPAVLQSSGLYSLSSV~~TVPSSSLGKTKT
YTCNV~~DH~~KPSNTKVDKR~~VESKYGPPCPP~~PAPEFLGGPSVFLFPPKPKDTLMISRTPEVTCVVDVSDQEDPEVQ
EDPEVQFNWYVDGVEVHNAKTKPREEQFNSTYRVVSVLTVLHQDWLNGKEYKCKVSNKGLPSSIEK
TISKAKGQPREPQVYTLPPSQEEMTKNQVSLTCLVKGFYPSDIAVEWESNGQPENNYKTTPPVLDSDG
SFFLYSRLTVDKSRWQEGNVFSCSVMHREALHNHYTQKSLSLSLGK

SEQ ID NO: 384 (BLP-5 Light chain amino acid sequence)

DIQMTQSPSSVSASVGDRVTITCKASQD~~VDTAVAWY~~QQKPGKAPKLLIYW~~ASTRHT~~GVPSRFSGSGS
GTDFTLTISLQPEDFATYYCQOYST~~FPWTF~~GGGTKVEIKRTVAAPSVFIFPPSDEQLKSGTASVVCLLN
NFYPREAKVQWKVDNALQSGNSQESVTEQDSKDYSLSSSTLTLSKADYEKHKVYACEVTHQGLSSP
VTKSFNRGEC

SEQ ID NO: 385 (BLP-6 Heavy chain amino acid sequence)

EVQLVESGGGLVQPGGSLRLSCAAS~~GFVFSRYDMAW~~VVRQAPGKGLEWVSFISGGGSNTYYPDTVKG
RFTISRDNKNTLYLQMNSLRAEDTAVYYCIS~~PYYYAMEY~~WGQGT~~TVTVSS~~ASTKGPSVFPLAPCSR
TSESTAALGCLVKDYFPEPVT~~SVNSGALTSGVHTFPAVLQSSGLYSLSSV~~TVPSSSLGKTKYTCNV
DHKPSNTKVDKR~~VESKYGPPCPP~~PAPEFLGGPSVFLFPPKPKDTLMISRTPEVTCVVDVSDQEDPEVQ
FNWYVDGVEVHNAKTKPREEQFNSTYRVVSVLTVLHQDWLNGKEYKCKVSNKGLPSSIEKTISKAK
GQPREPQVYTLPPSQEEMTKNQVSLTCLVKGFYPSDIAVEWESNGQPENNYKTTPPVLDSDGSFFLYS
RLTVDKSRWQEGNVFSCSVMHREALHNHYTQKSLSLSLGKEPKSSDKTH~~TSPSPSEVQLVESGGGLVQ~~
PGGSLRLSCAASGYI~~SSYCMGW~~FRQAPGKGLEGVA~~AIDSDGGTSY~~ADSVKGRFTISKDNSKNTLYLQ
MNSLRAEDTAMYYCAAD~~FCWVDEDRHLYEYNS~~WGQGLTVTVSS

SEQ ID NO: 386 (BLP-6 Light chain amino acid sequence)

DIQMTQSPSSVSASVGDRVTITCKASQD~~VDTAVAWY~~QQKPGKAPKLLIYW~~ASTRHT~~GVPSRFSGSGS
GTDFTLTISLQPEDFATYYCQOYST~~FPWTF~~GGGTKVEIKRTVAAPSVFIFPPSDEQLKSGTASVVCLLN
NFYPREAKVQWKVDNALQSGNSQESVTEQDSKDYSLSSSTLTLSKADYEKHKVYACEVTHQGLSSP
VTKSFNRGEC

SEQ ID NO: 387 (BLP-7 Heavy chain amino acid sequence)

EVQLVESGGGLVQPGGSLRLSCAAS~~GFVFSRYDMAW~~VVRQAPGKGLEWVSFISGGGSNTYYPDTVKG
RFTISRDNKNTLYLQMNSLRAEDTAVYYCIS~~PYYYAMEY~~WGQGT~~TVTVSS~~ASTKGPSVFPLAPCSR
TSESTAALGCLVKDYFPEPVT~~SVNSGALTSGVHTFPAVLQSSGLYSLSSV~~TVPSSSLGKTKYTCNV
DHKPSNTKVDKR~~VESKYGPPCPP~~PAPEFLGGPSVFLFPPKPKDTLMISRTPEVTCVVDVSDQEDPEVQ
FNWYVDGVEVHNAKTKPREEQFNSTYRVVSVLTVLHQDWLNGKEYKCKVSNKGLPSSIEKTISKAK
GQPREPQVYTLPPSQEEMTKNQVSLTCLVKGFYPSDIAVEWESNGQPENNYKTTPPVLDSDGSFFLYS
RLTVDKSRWQEGNVFSCSVMHREALHNHYTQKSLSLSLGK

SEQ ID NO: 388 (BLP-7 Light chain amino acid sequence)

EVQLVESGGGLVQPGGSLRLSCAASGYIISYCMGWFRQAPGKGLEGVAAIDSDGGTSYADSVKGRF
TISKDNSKNTLYLQMNSLRAEDTAMYYCAADFCWVDEDRHLYEYNSWGQGLTVTVSSSEPKSSDKTH
TSPSPDIQMTQSPSSVSASVGDRVTITCKASQDVDTAVAWYQQKPGKAPKLLIYWASTRHTGVPSRF
SGSGSGTDFTLTISLQPEDFATYYCQQYSTFPWTFGGGTKVEIKRTVAAPSVFIFPPSDEQLKSGTASV
VCLLNNFYPREAKVQWKVDNALQSGNSQESVTEQDSKDYSLSTLTLSKADYEKHKVYACEVTH
QGLSSPVTKSFNRGEC

SEQ ID NO: 389 (BLP-8 Heavy chain amino acid sequence)

EVQLVESGGGLVQPGGSLRLSCAASGFVFSRYDMAWVRQAPGKGLEWVSFISGGGSNTIYPDVTKG
RFTISRDNKNTLYLQMNSLRAEDTAVYYCISPYYYAMEYWGQGLTVTVSSASTKGPSVFPLAPCSRS
TSESTAALGCLVKDYFPEPVTVSWNSGALTSGVHTFPAVLQSSGLYSLSSVTVPSSSLGKTKYTCNV
DHKPSNTKVDKRVESKYGPPCPPCPAPEFLGGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSQEDPEVQ
FNWYVDGVEVHNAKTKPREEQFNSTYRVVSVLTVLHQDWLNGKEYKCKVSNKGLPSSIEKTISKAK
GQPREPQVYTLPPSQEEMTKNQVSLTCLVKGFYPSDIAVEWESNGQPENNYKTTTPVLDSDGSFFLYS
RLTVDKSRWQEGNVFSCVMHEALHNHYTQKSLSLSLGK

SEQ ID NO: 390 (BLP-8 Light chain amino acid sequence)

DIQMTQSPSSVSASVGDRVTITCKASQDVDTAVAWYQQKPGKAPKLLIYWASTRHTGVPSRFSGSGS
GTDFTLTISLQPEDFATYYCQQYSTFPWTFGGGTKVEIKRTVAAPSVFIFPPSDEQLKSGTASVCLLN
NFYPREAKVQWKVDNALQSGNSQESVTEQDSKDYSLSTLTLSKADYEKHKVYACEVTHQGLSSP
VTKSFNRGECEPKSSDKTHTSPSPPEVQLVESGGGLVQPGGSLRLSCAASGYIISYCMGWFRQAPGK
GLEGVAAIDSDGGTSYADSVKGRFTTISKDNSKNTLYLQMNSLRAEDTAMYYCAADFCWVDEDRHLY
EYNSWGQGLTVTVSS

SEQ ID NO: 391 (BLP-9 Heavy chain amino acid sequence)

EVQLVESGGGLVQPGGSLRLSCAASGYTISSYCMGWFRQAPGKGLEGVAAIDSDGTSYADSVKGRF
TISKDNSKNTLYLQMNSLRAEDTAVYYCAADFCWVDDFYEYNYWGQGLTVTVSSSEPKSSDKTHTSP
PSPEVQLVESGGGLVQPGGSLRLSCAASGFVFSRYDMAWVRQAPGKGLEWVSFISGGGSNTIYPDVT
KGRFTISRDNKNTLYLQMNSLRAEDTAVYYCISPYYYAMEYWGQGLTVTVSSASTKGPSVFPLAPC
SRSTSESTAALGCLVKDYFPEPVTVSWNSGALTSGVHTFPAVLQSSGLYSLSSVTVPSSSLGKTKYTC
NVNVDHKPSNTKVDKRVESKYGPPCPPCPAPEFLGGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSQEDPE
VQFNWYVDGVEVHNAKTKPREEQFNSTYRVVSVLTVLHQDWLNGKEYKCKVSNKGLPSSIEKTISK
AKGQPREPQVYTLPPSQEEMTKNQVSLTCLVKGFYPSDIAVEWESNGQPENNYKTTTPVLDSDGSFFL
YSRLTVDKSRWQEGNVFSCVMHEALHNHYTQKSLSLSLGK

SEQ ID NO: 392 (BLP-9 Light chain amino acid sequence)

DIQMTQSPSSVSASVGDRVTITCKASQDVDTAVAWYQQKPGKAPKLLIYWASTRHTGVPSRFSGSGS
GTDFTLTISLQPEDFATYYCQQYSTFPWTFGGGTKVEIKRTVAAPSVFIFPPSDEQLKSGTASVCLLN
NFYPREAKVQWKVDNALQSGNSQESVTEQDSKDYSLSTLTLSKADYEKHKVYACEVTHQGLSSP
VTKSFNRGEC

SEQ ID NO: 393 (BLP-10 Heavy chain amino acid sequence)

EVQLVESGGGLVQPGGSLRLSCAASGFVFSRYDMAWVRQAPGKGLEWVSFISGGGSNTIYPDVTKG
RFTISRDNKNTLYLQMNSLRAEDTAVYYCISPYYYAMEYWGQGLTVTVSSASTKGPSVFPLAPCSRS
TSESTAALGCLVKDYFPEPVTVSWNSGALTSGVHTFPAVLQSSGLYSLSSVTVPSSSLGKTKYTCNV
DHKPSNTKVDKRVESKYGPPCPPCPAPEFLGGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSQEDPEVQ
FNWYVDGVEVHNAKTKPREEQFNSTYRVVSVLTVLHQDWLNGKEYKCKVSNKGLPSSIEKTISKAK
GQPREPQVYTLPPSQEEMTKNQVSLTCLVKGFYPSDIAVEWESNGQPENNYKTTTPVLDSDGSFFLYS
RLTVDKSRWQEGNVFSCVMHEALHNHYTQKSLSLSLGKEPKSSDKTHTSPSPPEVQLVESGGGLVQ
PGGSLRLSCAASGYTISSYCMGWFRQAPGKGLEGVAAIDSDGTSYADSVKGRFTTISKDNSKNTLYLQ
MNSLRAEDTAVYYCAADFCWVDDFYEYNYWGQGLTVTVSS

SEQ ID NO: 394 (BLP-10 Light chain amino acid sequence)

DIQMTQSPSSVSASVGDRVITITCKASQDVDTAVAWYQQKPGKAPKLLIYWASTRHTGVPSRFSGSGS
GTDFTLTISLQPEDFATYYCQQYSTFPWTFGGGTKVEIKRTVAAPSVFIFPPSDEQLKSGTASVVCLLN
NFYPREAKVQWKVDNALQSGNSQESVTEQDSKDYSLSTLTLSKADYEKHKVYACEVTHQGLSSP
VTKSFNRGEC

SEQ ID NO: 395 (BLP-11 Heavy chain amino acid sequence)

EVQLVESGGGLVQPGGSLRLSCAASGFVFSRYDMAWVRQAPGKGLEWVSFISGGGSNTYYPDITVKG
RFTISRDNKNTLYLQMNSLRAEDTAVYYCISPYYYAMEYWGQGTITVTVSSASTKGPSVFPLAPCSRS
TSESTAALGCLVKDYFPEPVTVSWNSGALTSGVHTFPAVLQSSGLYSLSSVTVTPSSSLGKTKYTCNV
DHKPSNTKVDKRVESKYGPPCPPCPAPEFLGGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSQEDPEVQ
FNWYVDGVEVHNAKTKPREEQFNSTYRVVSVLTVLHQDWLNGKEYKCKVSNKGLPSSIEKTISKAK
GQPREPQVYTLPPSQEEMTKNQVSLTCLVKGFYPSDIAVEWESNGQPENNYKTPPVLDSDGSFFLYS
RLTVDKSRWQEGNVFSCSVMEALHNHYTQKSLSLGK

SEQ ID NO: 396 (BLP-11 Light chain amino acid sequence)

EVQLVESGGGLVQPGGSLRLSCAASGYTISYCMGWFRQAPGKGLEGVAIDSDGSTSYADSVKGRF
TISKDNKNTLYLQMNSLRAEDTAVYYCAADFCWVDDFYEYNYWGQGTITVTVSSSEPKSSDKTHTSP
PSPDIQMTQSPSSVSASVGDRVITITCKASQDVDTAVAWYQQKPGKAPKLLIYWASTRHTGVPSRFSGS
GSGTDFTLTISLQPEDFATYYCQQYSTFPWTFGGGTKVEIKRTVAAPSVFIFPPSDEQLKSGTASVVC
LNNFYPREAKVQWKVDNALQSGNSQESVTEQDSKDYSLSTLTLSKADYEKHKVYACEVTHQGL
SSPVTKSFNRGEC

SEQ ID NO: 397 (BLP-12 Heavy chain amino acid sequence)

EVQLVESGGGLVQPGGSLRLSCAASGFVFSRYDMAWVRQAPGKGLEWVSFISGGGSNTYYPDITVKG
RFTISRDNKNTLYLQMNSLRAEDTAVYYCISPYYYAMEYWGQGTITVTVSSASTKGPSVFPLAPCSRS
TSESTAALGCLVKDYFPEPVTVSWNSGALTSGVHTFPAVLQSSGLYSLSSVTVTPSSSLGKTKYTCNV
DHKPSNTKVDKRVESKYGPPCPPCPAPEFLGGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSQEDPEVQ
FNWYVDGVEVHNAKTKPREEQFNSTYRVVSVLTVLHQDWLNGKEYKCKVSNKGLPSSIEKTISKAK
GQPREPQVYTLPPSQEEMTKNQVSLTCLVKGFYPSDIAVEWESNGQPENNYKTPPVLDSDGSFFLYS
RLTVDKSRWQEGNVFSCSVMEALHNHYTQKSLSLGK

SEQ ID NO: 398 (BLP-12 Light chain amino acid sequence)

DIQMTQSPSSVSASVGDRVITITCKASQDVDTAVAWYQQKPGKAPKLLIYWASTRHTGVPSRFSGSGS
GTDFTLTISLQPEDFATYYCQQYSTFPWTFGGGTKVEIKRTVAAPSVFIFPPSDEQLKSGTASVVCLLN
NFYPREAKVQWKVDNALQSGNSQESVTEQDSKDYSLSTLTLSKADYEKHKVYACEVTHQGLSSP
VTKSFNRGECEPKSSDKTHTSPSPPEVQLVESGGGLVQPGGSLRLSCAASGYTISYCMGWFRQAPGK
GLEGVAIDSDGSTSYADSVKGRFTISKDNKNTLYLQMNSLRAEDTAVYYCAADFCWVDDFYEYN
YWGQGTITVTVSS

SEQ ID NO: 399 (BLP-13 Heavy chain amino acid sequence)

EVQLVESGGGLVQPGGSLRLSCAASGYTVSSYCMGWFRQAPGKGREGVSAIDSDGSVSYADSVKGR
FTISKDNKNTLYLQMNSLRAEDTAVYFCAADLCWVDQDQGEYNTWGQGTITVTVSSSEPKSSDKTHT
SPPSPEVQLVESGGGLVQPGGSLRLSCAASGFVFSRYDMAWVRQAPGKGLEWVSFISGGGSNTYYPD
TVKGRFTISRDNKNTLYLQMNSLRAEDTAVYYCISPYYYAMEYWGQGTITVTVSSASTKGPSVFPLA
PCSRSTSESTAALGCLVKDYFPEPVTVSWNSGALTSGVHTFPAVLQSSGLYSLSSVTVTPSSSLGKTKY
TCNVDHKPSNTKVDKRVESKYGPPCPPCPAPEFLGGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSQ
DPEVQFNWYVDGVEVHNAKTKPREEQFNSTYRVVSVLTVLHQDWLNGKEYKCKVSNKGLPSSIEKT
ISKAKGQPREPQVYTLPPSQEEMTKNQVSLTCLVKGFYPSDIAVEWESNGQPENNYKTPPVLDSDGS
FFLYSRLTVDKSRWQEGNVFSCSVMEALHNHYTQKSLSLGK

SEQ ID NO: 400 (BLP-13 Light chain amino acid sequence)

DIQMTQSPSSVSASVGDRVITITCKASQDVDTAVAWYQQKPGKAPKLLIYWASTRHTGVPSRFSGSGS
GTDFTLTISSLQPEDFATYYCQQYSTFPWTFGGGTKEIKRTVAAPSVFIFPPSDEQLKSGTASVVCLLN
NFYPREAKVQWKVDNALQSGNSQESVTEQDSKSTYLSSTLTLSKADYEKHKVYACEVTHQGLSSP
VTKSFNRGEC

SEQ ID NO: 401 (BLP-14 Heavy chain amino acid sequence)

EVQLVESGGGLVQPGGSLRLSCAASGFFVFSRYDMAWVRQAPGKGLEWVSFISGGGSNTYYPDTVKG
RFTISRDN SKNTLYLQMNSLRAEDTAVYYCISPYYYAMEYWGQGTITVTVSSASTKGPSVFPLAPCSRS
TSESTAALGCLVKDYFPEPVTVSWNSGALTSGVHTFPAVLQSSGLYSLSSVTVPSSSLGKTKYTCNV
DHKPSNTKVDKRVESKYGPPCPPCPAPEFLGGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSQEDPEVQ
FNWYVDGVEVHNAKTKPREEQFNSTYRVVSVLTVLHQDWLNGKEYKCKVSNKGLPSSIEKTISKAK
GQPREPQVYTLPPSQEEMTKNQVSLTCLVKGFYPSDIAVEWESNGQPENNYKTTTPVLDSDGSFFLYS
RLTVDKSRWQEGNVFSCSVMEALHNHYTQKSLSLSLGKPKSSDKTHTSPSPSEVQLVESGGGLVQ
PGGSLRLSCAASGYTVSSYCMGWFRQAPGKKGREGVSAIDSDGSVSYADSVKGRFTISKDN SKNTLYL
QMNSLRAEDTAVYFCAADLCWVDQDQGEYNTWGQGTITVTVSS

SEQ ID NO: 402 (BLP-14 Light chain amino acid sequence)

DIQMTQSPSSVSASVGDRVITITCKASQDVDTAVAWYQQKPGKAPKLLIYWASTRHTGVPSRFSGSGS
GTDFTLTISSLQPEDFATYYCQQYSTFPWTFGGGTKEIKRTVAAPSVFIFPPSDEQLKSGTASVVCLLN
NFYPREAKVQWKVDNALQSGNSQESVTEQDSKSTYLSSTLTLSKADYEKHKVYACEVTHQGLSSP
VTKSFNRGEC

SEQ ID NO: 403 (BLP-15 Heavy chain amino acid sequence)

EVQLVESGGGLVQPGGSLRLSCAASGFFVFSRYDMAWVRQAPGKGLEWVSFISGGGSNTYYPDTVKG
RFTISRDN SKNTLYLQMNSLRAEDTAVYYCISPYYYAMEYWGQGTITVTVSSASTKGPSVFPLAPCSRS
TSESTAALGCLVKDYFPEPVTVSWNSGALTSGVHTFPAVLQSSGLYSLSSVTVPSSSLGKTKYTCNV
DHKPSNTKVDKRVESKYGPPCPPCPAPEFLGGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSQEDPEVQ
FNWYVDGVEVHNAKTKPREEQFNSTYRVVSVLTVLHQDWLNGKEYKCKVSNKGLPSSIEKTISKAK
GQPREPQVYTLPPSQEEMTKNQVSLTCLVKGFYPSDIAVEWESNGQPENNYKTTTPVLDSDGSFFLYS
RLTVDKSRWQEGNVFSCSVMEALHNHYTQKSLSLSLGK

SEQ ID NO: 404 (BLP-15 Light chain amino acid sequence)

EVQLVESGGGLVQPGGSLRLSCAASGYTVSSYCMGWFRQAPGKKGREGVSAIDSDGSVSYADSVKGR
FTISKDN SKNTLYLQMNSLRAEDTAVYFCAADLCWVDQDQGEYNTWGQGTITVTVSSPEKSSDKTHT
SPPSPDIQMTQSPSSVSASVGDRVITITCKASQDVDTAVAWYQQKPGKAPKLLIYWASTRHTGVPSRFS
GSGSGTDFTLTISSLQPEDFATYYCQQYSTFPWTFGGGTKEIKRTVAAPSVFIFPPSDEQLKSGTASV
VCLLN NFYPREAKVQWKVDNALQSGNSQESVTEQDSKSTYLSSTLTLSKADYEKHKVYACEVTHQ
GLSSPVTKSFNRGEC

SEQ ID NO: 405 (BLP-16 Heavy chain amino acid sequence)

EVQLVESGGGLVQPGGSLRLSCAASGFFVFSRYDMAWVRQAPGKGLEWVSFISGGGSNTYYPDTVKG
RFTISRDN SKNTLYLQMNSLRAEDTAVYYCISPYYYAMEYWGQGTITVTVSSASTKGPSVFPLAPCSRS
TSESTAALGCLVKDYFPEPVTVSWNSGALTSGVHTFPAVLQSSGLYSLSSVTVPSSSLGKTKYTCNV
DHKPSNTKVDKRVESKYGPPCPPCPAPEFLGGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSQEDPEVQ
FNWYVDGVEVHNAKTKPREEQFNSTYRVVSVLTVLHQDWLNGKEYKCKVSNKGLPSSIEKTISKAK
GQPREPQVYTLPPSQEEMTKNQVSLTCLVKGFYPSDIAVEWESNGQPENNYKTTTPVLDSDGSFFLYS
RLTVDKSRWQEGNVFSCSVMEALHNHYTQKSLSLSLGK

SEQ ID NO: 406 (BLP-16 Light chain amino acid sequence)

DIQMTQSPSSVSASVGDRVITITCKASQDVDTAVAWYQQKPGKAPKLLIYWASTRHTGVPSRFSGSGS
GTDFTLTISSLQPEDFATYYCQQYSTFPWTFGGGTKEIKRTVAAPSVFIFPPSDEQLKSGTASVVCLLN
NFYPREAKVQWKVDNALQSGNSQESVTEQDSKSTYLSSTLTLSKADYEKHKVYACEVTHQGLSSP
VTKSFNRGECEPKSSDKTHTSPSPSEVQLVESGGGLVQPGGSLRLSCAASGYTVSSYCMGWFRQAPG

KGREGVSAIDSDGSVSYADSVKGRFTISKDNSKNTLYLQMNSLRAEDTAVYFCAADLCWVDQDQGE
YNTWGGQGLVTVSS

SEQ ID NO: 407 (BLP-17 Heavy chain amino acid sequence)

EVQLVESGGGLVQPGGSLRLSCAASGYTYGGYCMGWFRQAPGKGLEGVSTINSDGSTNYADSVKGR
 FTISRDNKNTLYLQMNSLRAEDTAVYYCAVDLAPCWWAGPIEAADFGYWGQGLVTVSSSEPKSSDK
 THTSPSPSEVQLVESGGGLVQPGGSLRLSCAASGFVFSRYDMAWVRQAPGKGLEWVSFISGGGSNTY
 YPDTVKGRTISRDNKNTLYLQMNSLRAEDTAVYYCISPYYYAMEYWGQGTITVTVSSASTKGPSVF
 PLAPCSRSTSESTAALGCLVKDYFPEPVTVSWNSGALTSGVHTFPAVLQSSGLYSLSSVTVPSSSLGT
 KTYTCNVDHKPSNTKVDKRVESKYGPPCPPCPAPEFLGGPSVFLFPPKPKDTLMISRTPEVTCVVVDV
 SQEDPEVQFNWYVDGVEVHNAKTKPREEQFNSTYRVVSVLTVLHQDWLNGKEYKCKVSNKGLPSSI
 EKTISKAKGQPREPQVYTLPPSQEEMTKNQVSLTCLVKGFYPSDIAVEWESNGQPENNYKTTTPVLDS
 DGSFFLYSRLTVDKSRWQEGNVFSCSVMEALHNHYTQKSLSLGLK

SEQ ID NO: 408 (BLP-17 Light chain amino acid sequence)

DIQMTQSPSSVSASVGDRVTITCKASQDQVDTAVAWYQQKPGKAPKLLIYWASTRHTGVPSRFSGSGS
 GTDFTLTISLQPEDFATYYCQQYSTFPWTFGGGTKEIKRTVAAPSVFIFPPSDEQLKSGTASVVCLLN
 NFYPREAKVQWKVDNALQSGNSQESVTEQDSKSTYLSSTLTLSKADYEKHKVYACEVTHQGLSSP
 VTKSFNRGEC

SEQ ID NO: 409 (BLP-18 Heavy chain amino acid sequence)

EVQLVESGGGLVQPGGSLRLSCAASGFVFSRYDMAWVRQAPGKGLEWVSFISGGGSNTIYPDTVKG
 RFTISRDNKNTLYLQMNSLRAEDTAVYYCISPYYYAMEYWGQGTITVTVSSASTKGPSVFPLAPCSR
 TSESTAALGCLVKDYFPEPVTVSWNSGALTSGVHTFPAVLQSSGLYSLSSVTVPSSSLGTKTYTCNV
 DHKPSNTKVDKRVESKYGPPCPPCPAPEFLGGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSQEDPEVQ
 FNWYVDGVEVHNAKTKPREEQFNSTYRVVSVLTVLHQDWLNGKEYKCKVSNKGLPSSIEKTISKAK
 GQPREPQVYTLPPSQEEMTKNQVSLTCLVKGFYPSDIAVEWESNGQPENNYKTTTPVLDSGFFLYS
 RLTVDKSRWQEGNVFSCSVMEALHNHYTQKSLSLGLKPEKSSDKTHTSPSPSEVQLVESGGGLVQ
 PGGSLRLSCAASGYTYGGYCMGWFRQAPGKGLEGVSTINSDGSTNYADSVKGRFTISRDNKNTLYL
 QMNSLRAEDTAVYYCAVDLAPCWWAGPIEAADFGYWGQGLVTVSS

SEQ ID NO: 410 (BLP-18 Light chain amino acid sequence)

DIQMTQSPSSVSASVGDRVTITCKASQDQVDTAVAWYQQKPGKAPKLLIYWASTRHTGVPSRFSGSGS
 GTDFTLTISLQPEDFATYYCQQYSTFPWTFGGGTKEIKRTVAAPSVFIFPPSDEQLKSGTASVVCLLN
 NFYPREAKVQWKVDNALQSGNSQESVTEQDSKSTYLSSTLTLSKADYEKHKVYACEVTHQGLSSP
 VTKSFNRGEC

SEQ ID NO: 411 (BLP-19 Heavy chain amino acid sequence)

EVQLVESGGGLVQPGGSLRLSCAASGFVFSRYDMAWVRQAPGKGLEWVSFISGGGSNTIYPDTVKG
 RFTISRDNKNTLYLQMNSLRAEDTAVYYCISPYYYAMEYWGQGTITVTVSSASTKGPSVFPLAPCSR
 TSESTAALGCLVKDYFPEPVTVSWNSGALTSGVHTFPAVLQSSGLYSLSSVTVPSSSLGTKTYTCNV
 DHKPSNTKVDKRVESKYGPPCPPCPAPEFLGGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSQEDPEVQ
 FNWYVDGVEVHNAKTKPREEQFNSTYRVVSVLTVLHQDWLNGKEYKCKVSNKGLPSSIEKTISKAK
 GQPREPQVYTLPPSQEEMTKNQVSLTCLVKGFYPSDIAVEWESNGQPENNYKTTTPVLDSGFFLYS
 RLTVDKSRWQEGNVFSCSVMEALHNHYTQKSLSLGLK

SEQ ID NO: 412 (BLP-19 Light chain amino acid sequence)

EVQLVESGGGLVQPGGSLRLSCAASGYTYGGYCMGWFRQAPGKGLEGVSTINSDGSTNYADSVKGR
 FTISRDNKNTLYLQMNSLRAEDTAVYYCAVDLAPCWWAGPIEAADFGYWGQGLVTVSSSEPKSSDK
 THTSPSPDIQMTQSPSSVSASVGDRVTITCKASQDQVDTAVAWYQQKPGKAPKLLIYWASTRHTGVPS
 RFSGSGSGTDFTLTISLQPEDFATYYCQQYSTFPWTFGGGTKEIKRTVAAPSVFIFPPSDEQLKSGTA
 SVVCLLNFYPREAKVQWKVDNALQSGNSQESVTEQDSKSTYLSSTLTLSKADYEKHKVYACEV
 THQGLSSPVTKSFNRGEC

SEQ ID NO: 413 (BLP-20 Heavy chain amino acid sequence)

EVQLVESGGGLVQPGGSLRLSCAASG**GVFSRYDMAWVRQAPGKGLEWVSFISGGGSNTYYPD**TVKG
RFTISRDN**SKNTLYLQMN**SLRAEDTAVYYCIS**PPYYAMEYWGQGT**TVTVSSASTKGPSVF**PLAPCSR**
TSESTAALGCLVKDYFPEP**TVSWNSGALTSGVHTFPAVLQSSGLYSLSSV**TVTPSSSLG**TKTYTCNV**
DHKPSNTKVDKRVESKYGPPCP**CPAPEFLGGPSVFLFPPKPKDTLMISRTPEVTCVVVDV**SQEDPEVQ
FNWYVDGVEVHNAKTKPREEQFNSTYRVVSVLT**TVLHQDWLNGKEYKCKVSNKGLPSSIEK**TISKAK
GQPREPQVY**TLPPSQEEMTKNQVSLTCLVKGFYPSDIAVEWESNGQPENNYK**TTTPVLDSG**SFFLYS**
RLTVDKSRWQEGNVFSCSV**MHEALHNHYTQKSLSL**SLGK

SEQ ID NO: 414 (BLP-20 Light chain amino acid sequence)

DIQMTQSPSSVSASVGDRVTITC**KASQD**VDTAVAWYQQKPGKAP**KLLIYWASTRHTGVPSR**FSGSGS
GTDFTLT**TISSLPEDFATYYCQQY**STFPWTFGGGT**KVEIKRTVAAPS**VFIFPPSDEQLKSGTASV**VCLLN**
NFYPREAKVQWKVDNALQSGNSQESVTEQDSKDSTY**SLSS**TLT**LSKADYEKHKVYACEVTHQGLSSP**
VTKSFNRGECEPKSSDKTHTSPPSPEVQLVESGGGLVQPGGSLRLSCAASG**YTYGGYCMGWFRQAPG**
KGLEGV**STINSDGSTNYADSVKGRFTISRDN**SKNTLYLQMN**SLRAEDTAVYYCAVDLAPC**WVAG**PIE**
AADEFGYWGQGTLVTVSS

SEQ ID NO: 415 (BLP-25 heavy chain amino acid sequence)

EVQLVESGGGLVQPGGSLRLSCAASG**YIISSYCMGWFRQAPGKGLEGVAAIDSDGGTSYADSVKGRFTIS**
KDNSKNTLYLQMN**SLRAEDTAMYYCAADFCWVDEDRHLYEYNSWGQGT**LVTVSS**EVQLVESGGGLVQPGG**
SLRLSCAASG**VFVSRYDMAWVRQAPGKGLEWVSFISGGGSNTYYPD**TVKG**RFTISRDN**SKNTLYLQMN**SL**
RAEDTAVYYCIS**PPYYAMEYWGQGT**TVTVSSASTKGPSVF**PLAPCSR**STSESTAALGCLVKDYFPEP**TV**
SWNSGALTSGVHTFPAVLQSSGLYSLSSV**TVTPSSSLG**TKTYTCNV**DHKPSNTKVDKRVESKYGPPCP**PC
PAPEFLGGPSVFLFPPKPKDTLMISRTPEVTCVVVDV**SQEDPEVQFNWYVDGVEVHNAKTKPREEQFN**ST
YRVVSVLT**TVLHQDWLNGKEYKCKVSNKGLPSSIEK**TISKAKGQPREPQVY**TLPPSQEEMTKNQVSLTCLV**
KGFYPSDIAVEWESNGQPENNYKTTTPVLDSG**SFFLYSRLTVDKSRWQEGNVFSCSV**MHEALHNHYT**QK**
SLSLSLGK

SEQ ID NO: 416 (BLP-25 light chain amino acid sequence)

DIQMTQSPSSVSASVGDRVTITC**KASQD**VDTAVAWYQQKPGKAP**KLLIYWASTRHTGVPSR**FSGSGSGTD
FTLT**TISSLPEDFATYYCQQY**STFPWTFGGGT**KVEIKRTVAAPS**VFIFPPSDEQLKSGTASV**VCLLN**NFY
PREAKVQWKVDNALQSGNSQESVTEQDSKDSTY**SLSS**TLT**LSKADYEKHKVYACEVTHQGLSSP**VTKSFN
RGEC

SEQ ID NO: 417 (BLP-26 heavy chain amino acid sequence)

EVQLVESGGGLVQPGGSLRLSCAASG**GVFSRYDMAWVRQAPGKGLEWVSFISGGGSNTYYPD**TVKG**RFTIS**
SRDN**SKNTLYLQMN**SLRAEDTAVYYCIS**PPYYAMEYWGQGT**TVTVSSASTKGPSVF**PLAPCSR**STSESTA
ALGCLVKDYFPEP**TVSWNSGALTSGVHTFPAVLQSSGLYSLSSV**TVTPSSSLG**TKTYTCNV**DHKPSNTK
VDKRVESKYGPPCP**CPAPEFLGGPSVFLFPPKPKDTLMISRTPEVTCVVVDV**SQEDPEVQFNWYVDGVE
VHNAKTKPREEQFNSTYRVVSVLT**TVLHQDWLNGKEYKCKVSNKGLPSSIEK**TISKAKGQPREPQVY**TLPP**
SQEEMTKNQVSLTCLVKGFYPSDIAVEWESNGQPENNYKTTTPVLDSG**SFFLYSRLTVDKSRWQEGNVF**
SCSV**MHEALHNHYTQKSLSL**SLGK

SEQ ID NO: 418 (BLP-26 light chain amino acid sequence)

EVQLVESGGGLVQPGGSLRLSCAASG**YIISSYCMGWFRQAPGKGLEGVAAIDSDGGTSYADSVKGRFTIS**
KDNSKNTLYLQMN**SLRAEDTAMYYCAADFCWVDEDRHLYEYNSWGQGT**LVTVSSDIQMTQSPSSVSASVG
DRVTITC**KASQD**VDTAVAWYQQKPGKAP**KLLIYWASTRHTGVPSR**FSGSGSGTDFTLT**TISSLPEDFATY**

YCQQYSTFPWTFGGGKVEIKRTVAAPSVFIFPPSDEQLKSGTASVVCLLNNFYPREAKVQWKVDNALQSGNSQESVTEQDSKDYSLSTLTLSKADYEKHKVYACEVTHQGLSSPVTKSFNRGEC

SEQ ID NO: 419 (BLP-27 heavy chain amino acid sequence)

EVQLVESGGGLVQPGGSLRLSCAASGYTIS¹SYCMGWFRQAPGKGLEGVAAIDSDGSTSYADSVKGRFTIS
KDNSKNTLYLQMNSLRAEDTAVYYCAADFCWVDDFYEYNYWGQGT²LVTVSSEVQLVESGGGLVQPGGSLR
LSCAASGFVFSRYDMAWVRQAPGKGLEWVSFISGGGSNTYYPDTV³KGRFTISRDNKNTLYLQMNSLRAE
DTAVYYCIS⁴PIYYAMEYWGQGT⁵TVTVSSASTKGPSVFPLAPCSRSTSESTAALGCLVKDYFPEPVTVSWN
SGALTSGVHTFPAVLQSSGLYSLSSVTVPSSSLGKTYTCNV⁶DHKPSNTKVDKRVESKYGPPCPPCPAP
EFLGGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSQEDPEVQFNWYVDGVEVHNAKTKPREEQFNSTYRV
VSVLTVLHQDWLNGKEYKCKVSNKGLPSSIEKTI⁷SKAKGQPREPQVYTLPPSQEEMTKNQVSLTCLVKGF
YPSDIAVEWESNGQPENNYKTPPVLDSDGSFFLY⁸SRLTVDKSRWQEGNVFSCSVMHEALHNHYTQKSLS
LSLGK

SEQ ID NO: 420 (BLP-27 light chain amino acid sequence)

DIQMTQSPSSVSASVGDRVTITCKASQD¹VDTA²VAWYQQKPGKAPKLLIYWASTRHTGVP³SRFSGSGSGTD
FTLTIS⁴SLQPEDFATYYCQQYSTFPWTFGGGKVEIKRTVAAPSVFIFPPSDEQLKSGTASVVCLLNNFYP
PREAKVQWKVDNALQSGNSQESVTEQDSKDYSLSTLTLSKADYEKHKVYACEVTHQGLSSPVTKSFN
RGEC

SEQ ID NO: 421 (BLP-28 heavy chain amino acid sequence)

EVQLVESGGGLVQPGGSLRLSCAASGFVFSRYDMAWVRQAPGKGLEWVSFISGGGSNTYYPDTV¹KGRFTI
SRDNKNTLYLQMNSLRAEDTAVYYCIS²PIYYAMEYWGQGT³TVTVSSASTKGPSVFPLAPCSRSTSESTA
ALGCLVKDYFPEPVTVSWNSGALTSGVHTFPAVLQSSGLYSLSSVTVPSSSLGKTYTCNV⁴DHKPSNTK
VDKRVESKYGPPCPPCPAPEFLGGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSQEDPEVQFNWYVDGVE
VHNAKTKPREEQFNSTYRVVSVLTVLHQDWLNGKEYKCKVSNKGLPSSIEKTI⁵SKAKGQPREPQVYTLPP
SQEEMTKNQVSLTCLVKGFYPSDIAVEWESNGQPENNYKTPPVLDSDGSFFLY⁶SRLTVDKSRWQEGNVF
SCSVMHEALHNHYTQKSLSLSLGK

SEQ ID NO: 422 (BLP-28 light chain amino acid sequence)

EVQLVESGGGLVQPGGSLRLSCAASGYTIS¹SYCMGWFRQAPGKGLEGVAAIDSDGSTSYADSVKGRFTIS
KDNSKNTLYLQMNSLRAEDTAVYYCAADFCWVDDFYEYNYWGQGT²LVTVSSDIQMTQSPSSVSASVGDRV
TITCKASQD³VDTA⁴VAWYQQKPGKAPKLLIYWASTRHTGVP⁵SRFSGSGSGTDFTLTIS⁶SLQPEDFATYYCQ
QYSTFPWTFGGGKVEIKRTVAAPSVFIFPPSDEQLKSGTASVVCLLNNFYPREAKVQWKVDNALQSGNS
QESVTEQDSKDYSLSTLTLSKADYEKHKVYACEVTHQGLSSPVTKSFNRGEC

SEQ ID NO: 423 (BLP-29 heavy chain amino acid sequence)

EVQLVESGGGLVQPGGSLRLSCAASGYTVSSYCMGWFRQAPGKGREGVSAIDSDGSVS¹YADSVKGRFTIS
KDNSKNTLYLQMNSLRAEDTAVYFCAADLCWVDQDQGEYNTWGQGT²LVTVSSEVQLVESGGGLVQPGGSL
RLSCAASGFVFSRYDMAWVRQAPGKGLEWVSFISGGGSNTYYPDTV³KGRFTISRDNKNTLYLQMNSLRA
EDTAVYYCIS⁴PIYYAMEYWGQGT⁵TVTVSSASTKGPSVFPLAPCSRSTSESTAALGCLVKDYFPEPVTVSW
NSGALTSGVHTFPAVLQSSGLYSLSSVTVPSSSLGKTYTCNV⁶DHKPSNTKVDKRVESKYGPPCPPCPA
PEFLGGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSQEDPEVQFNWYVDGVEVHNAKTKPREEQFNSTYR
VSVLTVLHQDWLNGKEYKCKVSNKGLPSSIEKTI⁷SKAKGQPREPQVYTLPPSQEEMTKNQVSLTCLVKG
FYPSDIAVEWESNGQPENNYKTPPVLDSDGSFFLY⁸SRLTVDKSRWQEGNVFSCSVMHEALHNHYTQKSLS
LSLGK

SEQ ID NO: 424 (BLP-29 light chain amino acid sequence)

DIQMTQSPSSVSASVGDRVTITCKASQD¹VDTA²VAWYQQKPGKAPKLLIYWASTRHTGVP³SRFSGSGSGTD
FTLTIS⁴SLQPEDFATYYCQQYSTFPWTFGGGKVEIKRTVAAPSVFIFPPSDEQLKSGTASVVCLLNNFYP

PREAKVQWKVDNALQSGNSQESVTEQDSKDYSLSTLTLSKADYEKHKVYACEVTHQGLSSPVTKSFNRGEC

SEQ ID NO: 425 (BLP-30 heavy chain amino acid sequence)

EVQLVESGGGLVQPGGSLRLSCAASGFVFSRYDMAWVRQAPGKGLEWVSFISGGGSNTYYPDTVKGRTISRDN SKNTLYLQMNSLRAEDTAVYYCISPYYYAMEYWGQGTITVTVSSASTKGPSVFPLAPCSRSTSESTAALGCLVKDYFPEPVTVSWNSGALTSGVHTFPAVLQSSGLYSLSSVTVPSSSLGKTYTCNV DHKPSNTKVDKRVESKYGPPCPPCPAPEFLGGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSDQEDPEVQFNWYVDGVEVHNAKTKPREEQFNSTYRVVSVLTVLHQDWLNGKEYKCKVSNKGLPSSIEKTI SKAKGQPREPQVYTLPPSQEEMTKNQVSLTCLVKGFYPSDIAVEWESNGQPENNYKTPPVLDSDGSFFLYSRLTVDKSRWQEGNVFSCSV MHEALHNHYTQKSLSLGLGK

SEQ ID NO: 426 (BLP-30 light chain amino acid sequence)

EVQLVESGGGLVQPGGSLRLSCAASGYTVSSYCMGWFRQAPGKREGVSAIDSDGSVSYADSVKGRFTISRDN SKNTLYLQMNSLRAEDTAVYFCAADLCWVDQDQGEYNTWGQGTITVTVSSDIQMTQSPSSVSASVGDRTITCKASQDQDVTAVAWYQQKPKAPKLLIYWASTRHTGVPSRFSGSGSGTDFTLTIS SLQPEDFATYYCQQYSTFPWTFGGGTKEIKRTVAAPSVFIFPPSDEQLKSGTASVVCLLNNFYPREAKVQWKVDNALQSGNSQESVTEQDSKDYSLSTLTLSKADYEKHKVYACEVTHQGLSSPVTKSFNRGEC

SEQ ID NO: 427 (BLP-31 heavy chain amino acid sequence)

EVQLVESGGGLVQPGGSLRLSCAASGYTYGGYCMGWFRQAPGKGLEGVSTINSDGSTNYADSVKGRFTISRDN SKNTLYLQMNSLRAEDTAVYYCAVDLAPCWVAGPIEAADFGYWGQGTITVTVSSSEVQLVESGGGLVQPGGSLRLSCAASGFVFSRYDMAWVRQAPGKGLEWVSFISGGGSNTYYPDTVKGRTISRDN SKNTLYLQMNSLRAEDTAVYYCISPYYYAMEYWGQGTITVTVSSASTKGPSVFPLAPCSRSTSESTAALGCLVKDYFPEPVTVSWNSGALTSGVHTFPAVLQSSGLYSLSSVTVPSSSLGKTYTCNV DHKPSNTKVDKRVESKYGPPCPPCPAPEFLGGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSDQEDPEVQFNWYVDGVEVHNAKTKPREEQFNSTYRVVSVLTVLHQDWLNGKEYKCKVSNKGLPSSIEKTI SKAKGQPREPQVYTLPPSQEEMTKNQVSLTCLVKGFYPSDIAVEWESNGQPENNYKTPPVLDSDGSFFLYSRLTVDKSRWQEGNVFSCSV MHEALHNHYTQKSLSLGLGK

SEQ ID NO: 428 (BLP-31 light chain amino acid sequence)

DIQMTQSPSSVSASVGDRTITCKASQDQDVTAVAWYQQKPKAPKLLIYWASTRHTGVPSRFSGSGSGTDFTLTIS SLQPEDFATYYCQQYSTFPWTFGGGTKEIKRTVAAPSVFIFPPSDEQLKSGTASVVCLLNNFYPREAKVQWKVDNALQSGNSQESVTEQDSKDYSLSTLTLSKADYEKHKVYACEVTHQGLSSPVTKSFNRGEC

SEQ ID NO: 429 (BLP-32 heavy chain amino acid sequence)

EVQLVESGGGLVQPGGSLRLSCAASGFVFSRYDMAWVRQAPGKGLEWVSFISGGGSNTYYPDTVKGRTISRDN SKNTLYLQMNSLRAEDTAVYYCISPYYYAMEYWGQGTITVTVSSASTKGPSVFPLAPCSRSTSESTAALGCLVKDYFPEPVTVSWNSGALTSGVHTFPAVLQSSGLYSLSSVTVPSSSLGKTYTCNV DHKPSNTKVDKRVESKYGPPCPPCPAPEFLGGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSDQEDPEVQFNWYVDGVEVHNAKTKPREEQFNSTYRVVSVLTVLHQDWLNGKEYKCKVSNKGLPSSIEKTI SKAKGQPREPQVYTLPPSQEEMTKNQVSLTCLVKGFYPSDIAVEWESNGQPENNYKTPPVLDSDGSFFLYSRLTVDKSRWQEGNVFSCSV MHEALHNHYTQKSLSLGLGK

SEQ ID NO: 430 (BLP-32 light chain amino acid sequence)

EVQLVESGGGLVQPGGSLRLSCAASGYTYGGYCMGWFRQAPGKGLEGVSTINSDGSTNYADSVKGRFTISRDN SKNTLYLQMNSLRAEDTAVYYCAVDLAPCWVAGPIEAADFGYWGQGTITVTVSSDIQMTQSPSSVSASVGDRTITCKASQDQDVTAVAWYQQKPKAPKLLIYWASTRHTGVPSRFSGSGSGTDFTLTIS SLQPEDFATYYCQQYSTFPWTFGGGTKEIKRTVAAPSVFIFPPSDEQLKSGTASVVCLLNNFYPREAKVQWKVDNALQSGNSQESVTEQDSKDYSLSTLTLSKADYEKHKVYACEVTHQGLSSPVTKSFNRGEC

SEQ ID NO: 431 (BLP-33 heavy chain amino acid sequence)

EPKSSDKTHTSPPSPEVQLVESGGGLVQPGGSLRLSCAASGYIISSYCMGWFRQAPGKGLEGVAAIDSDG
GTSYADSVKGRFTISKDNSKNTLYLQMNSLRAEDTAMYYCAADFCEWVDEDRHLYEYNSWGQGLTVTSSE
VQLVESGGGLVQPGGSLRLSCAASGFVFSRYDMAWVRQAPGKGLEWVSFISGGGSNTYYPDTVKGRTTIS
RDNSKNTLYLQMNSLRAEDTAVYYCISPYYYAMEYWGQGTITVTVSSASTKGPSVFPLAPCSRSTSESTA
LGCLVKDYFPEPVTVSWNSGALTSGVHTFPAVLQSSGLYSLSSVTVTPSSSLGKTYTCNVDHKPSNTKV
DKRVESKYGPPCPPCPAPEFLGGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSDQEDPEVQFNWYVDGVEV
HNAKTKPREEQFNSTYRVVSVLTVLHQDWLNGKEYKCKVSNKGLPSSIEKTIKAKGQPREPQVYTLPPS
QEEMTKNQVSLTCLVKGFYPSDIAVEWESNGQPENNYKTPPVLDSDGSFFLYSRLTVDKSRWQEGNVFS
CSVMHEALHNHYTQKSLSLGLGK

SEQ ID NO: 432 (BLP-33 light chain amino acid sequence)

DIQMTQSPSSVSASVGRVTITCKASQDQDVTAVAWYQQKPGKAPKLLIYWASTRHTGVPSRFSGSGSGTD
FTLTISLQPEDFATYYCQQYSTFPWTFGGGTKEIKRTVAAPSVFIFPPSDEQLKSGTASVVCCLNNFY
PREAKVQWKVDNALQSGNSQESVTEQDSKDYSLSTLTLSKADYEKHKVYACEVTHQGLSSPVTKSFN
RGE

SEQ ID NO: 433 (BLP-34 heavy chain amino acid sequence)

EVQLVESGGGLVQPGGSLRLSCAASGFVFSRYDMAWVRQAPGKGLEWVSFISGGGSNTYYPDTVKGRTTIS
SRDNSKNTLYLQMNSLRAEDTAVYYCISPYYYAMEYWGQGTITVTVSSASTKGPSVFPLAPCSRSTSESTA
ALGCLVKDYFPEPVTVSWNSGALTSGVHTFPAVLQSSGLYSLSSVTVTPSSSLGKTYTCNVDHKPSNTK
VDRVESKYGPPCPPCPAPEFLGGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSDQEDPEVQFNWYVDGVE
VHNAKTKPREEQFNSTYRVVSVLTVLHQDWLNGKEYKCKVSNKGLPSSIEKTIKAKGQPREPQVYTLPP
SQEEMTKNQVSLTCLVKGFYPSDIAVEWESNGQPENNYKTPPVLDSDGSFFLYSRLTVDKSRWQEGNVF
SCSVMHEALHNHYTQKSLSLGLGK

SEQ ID NO: 434 (BLP-34 light chain amino acid sequence)

EPKSSDKTHTSPPSPEVQLVESGGGLVQPGGSLRLSCAASGYIISSYCMGWFRQAPGKGLEGVAAIDSDG
GTSYADSVKGRFTISKDNSKNTLYLQMNSLRAEDTAMYYCAADFCEWVDEDRHLYEYNSWGQGLTVTSSE
IQMTQSPSSVSASVGRVTITCKASQDQDVTAVAWYQQKPGKAPKLLIYWASTRHTGVPSRFSGSGSGTDF
TLTISLQPEDFATYYCQQYSTFPWTFGGGTKEIKRTVAAPSVFIFPPSDEQLKSGTASVVCCLNNFY
REAKVQWKVDNALQSGNSQESVTEQDSKDYSLSTLTLSKADYEKHKVYACEVTHQGLSSPVTKSFN
RGE

SEQ ID NO: 435 (BLP-35 heavy chain amino acid sequence)

EPKSSDKTHTSPPSPEVQLVESGGGLVQPGGSLRLSCAASGYTIISSYCMGWFRQAPGKGLEGVAAIDSDG
STSYADSVKGRFTISKDNSKNTLYLQMNSLRAEDTAVYYCAADFCEWVDDFYEYNYWGQGLTVTSSEVQL
VESGGGLVQPGGSLRLSCAASGFVFSRYDMAWVRQAPGKGLEWVSFISGGGSNTYYPDTVKGRTTISRDN
SKNTLYLQMNSLRAEDTAVYYCISPYYYAMEYWGQGTITVTVSSASTKGPSVFPLAPCSRSTSESTAALGC
LVKDYFPEPVTVSWNSGALTSGVHTFPAVLQSSGLYSLSSVTVTPSSSLGKTYTCNVDHKPSNTKVDKR
VESKYGPPCPPCPAPEFLGGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSDQEDPEVQFNWYVDGVEVHNA
KTKPREEQFNSTYRVVSVLTVLHQDWLNGKEYKCKVSNKGLPSSIEKTIKAKGQPREPQVYTLPPSQEE
MTKNQVSLTCLVKGFYPSDIAVEWESNGQPENNYKTPPVLDSDGSFFLYSRLTVDKSRWQEGNVFSCSV
MHEALHNHYTQKSLSLGLGK

SEQ ID NO: 436 (BLP-35 light chain amino acid sequence)

DIQMTQSPSSVSASVGRVTITCKASQDQDVTAVAWYQQKPGKAPKLLIYWASTRHTGVPSRFSGSGSGTD
FTLTISLQPEDFATYYCQQYSTFPWTFGGGTKEIKRTVAAPSVFIFPPSDEQLKSGTASVVCCLNNFY
PREAKVQWKVDNALQSGNSQESVTEQDSKDYSLSTLTLSKADYEKHKVYACEVTHQGLSSPVTKSFN
RGE

SEQ ID NO: 437 (BLP-36 heavy chain amino acid sequence)

EVQLVESGGGLVQPGGSLRLSCAASG**FVFSRYDMAVVRQAPGKGLEWVSFISGGGSNTYYP**DTVKG**RFTI**
 SRD**NSKNTLYLQMN**SLRAEDTAVYYCIS**PYYYAMEYWGQGT**TVTVSSASTK**GPSVFPLAPCSRSTSESTA**
 ALGCLVKDYFPEPVT**SVNSGALTSGVHTFPAVLQSSGLYSLSSV**TVPS**SSLG**TKTYTCNV**DHKPSNTK**
 VDKR**VESKYGPPCPPCPAPEFLGGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSQEDPEVQFNWYVDGVE**
 VHN**AKTKPREEQFNSTYRVVSVLTVLHQDWLNGKEYKCKVSNKGLPSSIEK**TI**SKAKGQPREPQVYTLPP**
 SQE**EMTKNQVSLTCLVKGFYPSDIAVEWESNGQPENNYKTPPVLDSDGSFFLYSRLTVDKSRWQEGNVF**
 SCSVMHEALHNHYTQKSLSL**SLGK**

SEQ ID NO: 438 (BLP-36 light chain amino acid sequence)

EPKSSDKTHTSPSP**EVQLVESGGGLVQPGGSLRLSCAASGYTIS**SYCMGWFRQAPGK**GLEGVAAIDSDG**
 STSYADSVK**GRFTISKD**NSKNTLYLQMN**SLRAEDTAVYYCAADFCWVDDFYEYNYWGQGT**LVTVSSDIQ**M**
 TQSPSSVSASVGDRVTITCKASQD**VDTAVAWYQQKPGKAPKLLIYWASTRHTGVP**SRFSGSGSGT**DFTLT**
 ISS**LQPEDFATYYCQQYSTFPWTFGGG**TKVEIKRTVAAPSVFIFPPSDEQLKSGTASV**VCLLNNFYPREA**
 KVQWKVDNALQSGNSQESVTEQDSKDSTYLS**SSTLTLSKADYEKHKVYACEVTHQGLSSPVTKSFNRGEC**

SEQ ID NO: 439 (BLP-37 heavy chain amino acid sequence)

EPKSSDKTHTSPSP**EVQLVESGGGLVQPGGSLRLSCAASGYTVSSYCMGWFRQAPGK**GREGVSAIDSDG
 SVSYADSVK**GRFTISKD**NSKNTLYLQMN**SLRAEDTAVYFCAADLCWVDQDQGEYNTWGQGT**LVTVSS**EVQ**
 LVESGGGLVQPGGSLRLSCAASG**FVFSRYDMAVVRQAPGKGLEWVSFISGGGSNTYYP**DTVKG**RFTI**SRD
 NSKNTLYLQMN**SLRAEDTAVYYCISPYYYAMEYWGQGT**TVTVSSASTK**GPSVFPLAPCSRSTSESTAALG**
 CLVKDYFPEPVT**SVNSGALTSGVHTFPAVLQSSGLYSLSSV**TVPS**SSLG**TKTYTCNV**DHKPSNTKVDK**
 R**ESKYGPPCPPCPAPEFLGGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSQEDPEVQFNWYVDGVEVHN**
 AKTKPREEQFNSTYRVVSVLTVLHQDWLNGKEYKCKVSNKGLPSSIEKTI**SKAKGQPREPQVYTLPPSQE**
 EMTKNQVSLTCLVKGFYPSDIAVEWESNGQPENNYKTPPVLDSDGSFFLYSRLTVDKSRWQEGNVFSCS
 VMHEALHNHYTQKSLSL**SLGK**

SEQ ID NO: 440 (BLP-37 light chain amino acid sequence)

DIQMTQSPSSVSASVGDRVTITCKASQD**VDTAVAWYQQKPGKAPKLLIYWASTRHTGVP**SRFSGSGSGT**D**
 FTLTIS**SLQPEDFATYYCQQYSTFPWTFGGG**TKVEIKRTVAAPSVFIFPPSDEQLKSGTASV**VCLLNNFY**
 PREAKVQWKVDNALQSGNSQESVTEQDSKDSTYLS**SSTLTLSKADYEKHKVYACEVTHQGLSSPVTKSFN**
 RGEC

SEQ ID NO: 441 (BLP-38 heavy chain amino acid sequence)

EVQLVESGGGLVQPGGSLRLSCAASG**FVFSRYDMAVVRQAPGKGLEWVSFISGGGSNTYYP**DTVKG**RFTI**
 SRD**NSKNTLYLQMN**SLRAEDTAVYYCIS**PYYYAMEYWGQGT**TVTVSSASTK**GPSVFPLAPCSRSTSESTA**
 ALGCLVKDYFPEPVT**SVNSGALTSGVHTFPAVLQSSGLYSLSSV**TVPS**SSLG**TKTYTCNV**DHKPSNTK**
 VDKR**VESKYGPPCPPCPAPEFLGGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSQEDPEVQFNWYVDGVE**
 VHN**AKTKPREEQFNSTYRVVSVLTVLHQDWLNGKEYKCKVSNKGLPSSIEK**TI**SKAKGQPREPQVYTLPP**
 SQE**EMTKNQVSLTCLVKGFYPSDIAVEWESNGQPENNYKTPPVLDSDGSFFLYSRLTVDKSRWQEGNVF**
 SCSVMHEALHNHYTQKSLSL**SLGK**

SEQ ID NO: 442 (BLP-38 light chain amino acid sequence)

EPKSSDKTHTSPSP**EVQLVESGGGLVQPGGSLRLSCAASGYTVSSYCMGWFRQAPGK**GREGVSAIDSDG
 SVSYADSVK**GRFTISKD**NSKNTLYLQMN**SLRAEDTAVYFCAADLCWVDQDQGEYNTWGQGT**LVTVSSDIQ**M**
 MTQSPSSVSASVGDRVTITCKASQD**VDTAVAWYQQKPGKAPKLLIYWASTRHTGVP**SRFSGSGSGT**DFTLT**
 TIS**SLQPEDFATYYCQQYSTFPWTFGGG**TKVEIKRTVAAPSVFIFPPSDEQLKSGTASV**VCLLNNFYPRE**
 AKVQWKVDNALQSGNSQESVTEQDSKDSTYLS**SSTLTLSKADYEKHKVYACEVTHQGLSSPVTKSFNRGE**
 C

SEQ ID NO: 443 (BLP-39 heavy chain amino acid sequence)

EPKSSDKTHTSPSPSEVQLVESGGGLVQPGGSLRLSCAASGYTYGGYCMGWFRQAPGKGLEGVSTINSDG
 STNYADSVKGRFTISRDN SKNTLYLQMNSLRAEDTAVYYCAVDLAPCWVAGPIEAADF¹GYWGQGT²LVTVS
 SEVQLVESGGGLVQPGGSLRLSCAASGFVFSRYDMAWVRQAPGKGLEWVSFISGGGSNTIYPDTVKGRFT
 ISRDNSKNTLYLQMNSLRAEDTAVYYCISPYYYAMEYWGQGT³TVTVSSASTKGPSVFPLAPCSRSTSEST
 AALGCLVKDYFPEPVTVSWNSGALTSGVHTFPAVLQSSGLYSLSSVTVTPSSSLG⁴TKTYTCNV⁵DHKPSNT
 KVDKRVESKYGPPCPPCPAPEFLGGPSVFLFPPKPKDTLMISRTPEVTCVVDVDSQEDPEVQFNWYVDGV
 EVHNAKTKPREEQFNSTYRVVSVLTVLHQDWLNGKEYKCKVSNKGLPSSIEK⁶TIKAKGQPREPQVYTLPP
 PSQEEMTKNQVSLTCLVKGFYPSDIAVEWESNGQPENNYK⁷TPPVLDSDGSFFLYSRLTVDKSRWQEGNV
 FSCVMHEALHNHYTQKSLSL⁸SLGK

SEQ ID NO: 444 (BLP-39 light chain amino acid sequence)

DIQMTQSPSSVSASVGDRVTITCKASQD¹VD²TAVAWYQQKPGKAPKLLIYWASTRHTGVP³SRFSGSGSGTD
 FTLT⁴ISSLPEDFATYYCQQYSTFPWTFGGGT⁵KVEIKRTVAAPSVFIFPPSDEQLKSGTASVVCLLN⁶NFY
 PREAKVQWKVDNALQSGNSQESVTEQDSK⁷DSTYLSSTLTLSKADYEKHKVYACEVTHQGLSSPVTKSFN
 RGEC

SEQ ID NO: 445 (BLP-40 heavy chain amino acid sequence)

EVQLVESGGGLVQPGGSLRLSCAASGFVFSRYDMAWVRQAPGKGLEWVSFISGGGSNTIYPDTVKGRFTI
 SRDN SKNTLYLQMNSLRAEDTAVYYCISPYYYAMEYWGQGT¹TVTVSSASTKGPSVFPLAPCSRSTSESTA
 ALGCLVKDYFPEPVTVSWNSGALTSGVHTFPAVLQSSGLYSLSSVTVTPSSSLG²TKTYTCNV³DHKPSNTK
 VDKRVESKYGPPCPPCPAPEFLGGPSVFLFPPKPKDTLMISRTPEVTCVVDVDSQEDPEVQFNWYVDGVE
 VHNAKTKPREEQFNSTYRVVSVLTVLHQDWLNGKEYKCKVSNKGLPSSIEK⁴TIKAKGQPREPQVYTLPP
 SQEEMTKNQVSLTCLVKGFYPSDIAVEWESNGQPENNYK⁵TPPVLDSDGSFFLYSRLTVDKSRWQEGNVF
 SCSVMHEALHNHYTQKSLSL⁶SLGK

SEQ ID NO: 446 (BLP-40 light chain amino acid sequence)

EPKSSDKTHTSPSPSEVQLVESGGGLVQPGGSLRLSCAASGYTYGGYCMGWFRQAPGKGLEGVSTINSDG
 STNYADSVKGRFTISRDN SKNTLYLQMNSLRAEDTAVYYCAVDLAPCWVAGPIEAADF¹GYWGQGT²LVTVS
 SDIQMTQSPSSVSASVGDRVTITCKASQD³VD⁴TAVAWYQQKPGKAPKLLIYWASTRHTGVP⁵SRFSGSGSGT
 DFTLT⁶ISSLPEDFATYYCQQYSTFPWTFGGGT⁷KVEIKRTVAAPSVFIFPPSDEQLKSGTASVVCLLN⁸NF
 YPREAKVQWKVDNALQSGNSQESVTEQDSK⁹DSTYLSSTLTLSKADYEKHKVYACEVTHQGLSSPVTKSF
 NRGEC