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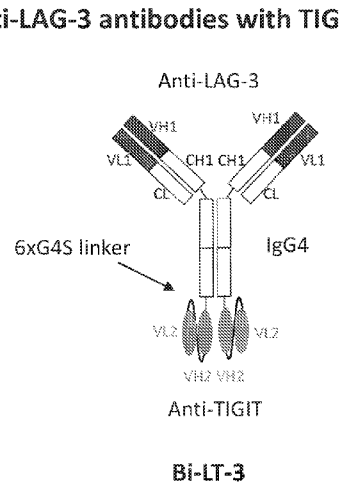

FIG. 30B

(57) Abstract: Antitumor antagonists that bind specifically to immune checkpoint regulator are disclosed. Also disclosed is a method of treating proliferative disorders with the antitumor antagonists.

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[Continued on next page]

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TITLE

ANTITUMOR IMMUNE CHECKPOINT REGULATOR ANTAGONISTS

[0001] This application claims priority to U.S. Provisional Patent Application Serial No. 62/691,658, filed June 29, 2018 and U.S. Provisional Patent Application Serial No. 62/823,989, filed March 26, 2019, the contents of which are expressly incorporated herein by reference herein.

FIELD

[0002] The present application relates generally to cancer treatment and, in particular, to bispecific inhibitors capable of modulating pathways associated with tumorigenesis and tumor immunity.

BACKGROUND

[0003] The inability of the host to eliminate cancer cells remains a major problem. Although an increasing number of therapeutic monoclonal antibodies have been approved for treatment of various cancers, emergence of resistance to these antibodies is frequently observed, given the many different molecular pathways underlying cancer growth and progression to metastasis. Although the immune system is the principal mechanism of cancer prevention, cancer cells counteract immunosurveillance. Natural control mechanisms have been identified that limit T-cell activation so as to prevent collateral damage resulting from unrestrained T-cell activity. This process has been exploited by tumor cells to evade immune responses. Restoring the capacity of immune effector cells, especially T cells, to recognize and eliminate cancer is a major objective in immunotherapy.

[0004] The need exists for improved therapeutic binding antagonists or antibodies and methods of treating cancer and chronic viral infections with such reagents.

SUMMARY

[0005] One aspect of the present application relates to bispecific antitumor antagonists that comprise a first targeting domain that specifically binds to an immune checkpoint regulator; a second targeting domain in the form of an scFv that specifically binds to TIGIT; and an immunoglobulin scaffold structurally linked to the first and second targeting domains, wherein the first targeting domain is positioned at N-terminal end of the antagonist, and wherein the second targeting domain is positioned at a C-terminal end of the antagonist and is linked to the immunoglobulin scaffold through a linker. In some embodiments, linker comprises 4 or 6 copies of the amino acid sequence G4S (4x G4S and 6xG4S, respectively).

[0006] In some embodiments, the first targeting domain specifically binds to PD-1,

PD-L1 or LAG-3.

[0007] Another aspect of the present application relates to humanized anti-LAG-3 antibodies that inhibit binding of ligands to LAG-3.

[0008] Another aspect of the present application relates to a method for treating a cell proliferative disorder. The method comprises administering to a subject in need thereof an effective amount of a bispecific antitumor antagonist or anti-LAG-3 antibody of the present application.

BRIEF DESCRIPTION OF THE DRAWINGS

[0009] **FIG. 1** shows complementarity determining region (CDR) sequences of certain anti-TIGIT mAbs. The framework region (FR) sequences flanking the anti-TIGIT CDR sequences are listed in **FIG. 39A** as **SEQ ID NOS: 216-262**.

[0010] **FIGS. 2A-2C** show several embodiments of anti-TIGIT antibody variable domain sequences.

[0011] **FIG. 3** shows CDR sequences of certain anti-PD-1 mAbs. The FR sequences flanking the anti-PD-1 CDR sequences are listed in **FIG. 39B** as **SEQ ID NOS: 263-292**.

[0012] **FIGS. 4A-4B** show several embodiments of anti-PD-1 antibody variable domain sequences.

[0013] **FIG. 5** shows CDR sequences of certain anti-PD-L1 mAbs. The FR sequences flanking the anti-PD-L1 CDR sequences are listed in **FIG. 39C** as **SEQ ID NOS: 293-315**.

[0014] **FIGS. 6A-6C** show several embodiments of anti-PD-L1 antibody variable domain sequences.

[0015] **FIGS. 7A-7C** depict three exemplary bispecific antitumor antagonists, Bi-TPM-93 (**FIG. 7A**), Bi-TPM-94A (**FIG. 7B**), and Bi-TPM-94B (**FIG. 7C**).

[0016] **FIG. 8** summarizes the arrangement of functional domains in the bispecific antagonists depicted in **FIGS. 7A-7C**.

[0017] **FIGS. 9A-9B** show the heavy chain (HC) and light chain (LC) amino acid sequences corresponding to the bispecific antagonists depicted in **FIGS. 7A-7C**.

[0018] **FIG. 10** depicts a blocking assay showing that Bi-TPM-94A blocks the interaction between PD-1 and its ligand (IC50=0.15 nM), PD-L1 better than Bi-TPM-93 (IC50=0.83 nM).

[0019] **FIG. 11** shows a non-reducing PAGE analysis of Bi-TPM-94Aand Bi-TPM-94B transiently expressed in human embryonic kidney (HEK) 293 cells.

[0020] **FIG. 12** shows a size-exclusion ultra-high performance liquid chromatography (SE-UHPLC) analysis illustrating species heterogeneity in Bi-TPM-93 and Bi-TPM-94, which is eliminated by linker modification in Bi-TPM-94B.

[0021] FIG. 13A shows that the binding affinities of Bi-TPM-94A and Bi-TPM-94B for PD-1 are stronger than the binding affinity of a benchmark anti-PD-1 antibody for PD-1. **FIG. 13B** shows that the binding affinities of Bi-TPM-94A and Bi-TPM-94B for TIGIT are stronger than the binding affinity of a benchmark anti-TIGIT antibody for TIGIT.

[0022] FIGS. 14A-14B show Bi-TPM-94A and Bi-TPM-94B potently block both TIGIT binding to its ligand, human PVR (CD155) (**FIG. 14A**) and block PD-1 binding to its ligand, PD-L1 (**FIG. 14B**).

[0023] FIG. 15 shows the results of an ELISA assay demonstrating simultaneous binding of PD-1 and TIGIT by Bi-TPM-94A and Bi-TPM-94B in which huPD-1-Fc coated 96 well plates were incubated with serially diluted samples of Bi-TPM-94A and Bi-TPM-94B, followed by His-tagged huTIGIT protein, whereby bound molecules were detected using HRP-conjugated anti-His tag Ab and TMB substrate.

[0024] FIGS. 16A-16B show increased IFN-γ secretion from human PBMCs (Donor 287, **FIG. 16A**; Donor 401, **FIG. 16B**) with Bi-TPM-94B relative to the individual or combination of parental anti-PD-1 and anti-TIGIT antibodies, as well as the negative controls.

[0025] FIGS. 17A-17B show that Bi-TPM-94B enhances proliferation of primary human T cells from Donor 287 PBMCs (**FIG. 17A**) and Donor 401 PBMCs (**FIG. 17B**) to a greater extent than the individual or combination of parental anti-PD-1 and anti-TIGIT antibodies, as well as the negative controls.

[0026] FIG. 18 is a pharmacokinetic profile showing that Bi-TPM-94A and Bi-TPM-94B have similar *in vivo* half-lives ($T_{1/2}$) following a tail vein injection into 6-10 week old female CD1 mice. The bispecific antagonists were recovered from serum taken at various times post-injection and subjected to analysis by ELISA.

[0027] FIG. 19A shows the heavy chain CDR sequences corresponding to the anti-LAG-3 mAbs 2L2A.1, 2L2A.6, 2L27B and 3L1A. **FIG. 19B** shows the light chain CDR sequences corresponding to the anti-LAG-3 mAbs 2L2A.1, 2L2A.6, 2L27B and 3L1A. The FR sequences flanking the anti-LAG-3 CDR sequences are listed in **FIG. 39C** as **SEQ ID NOS: 316-337**.

[0028] FIG. 20 shows the VH and VL sequences of anti-LAG-3 mAbs 2L2A.1, 2L2A.6, 2L27B and 3L1A.

[0029] FIGS. 21A-21B show the results of assays confirming the ability of the anti-LAG-3 mAbs to block LAG-3 binding.

[0030] FIG. 22 shows the results of a cell-based blocking assay measuring the ability of the anti-LAG-3 mAb 2L2A.1, a benchmark (BM) anti-LAG-3 mAb, and a chimeric 2L2A

antibody (**SEQ ID NOS: 203 and 204**) to block the interaction between LAG-3-muFc and its major ligand, major histocompatibility complex (MHC) antigen expressed on Raji cells. The assay data was used to calculate IC50 values (nm) depicted.

[0031] FIGS. 23A-23B show affinity analysis of anti-LAG-3 2L2A.1 mAb for binding human LAG-3-His (**FIG. 23A**) or human LAG-3-mIgG2a (**FIG. 23B**) as determined by surface plasmon resonance (SPR), along with corresponding binding affinity constants.

[0032] FIG. 24 depicts the binding of anti-LAG-3 mAb variant, 2L2A.1 or a benchmark antibody (BM) to LAG-3, including the half maximal effective concentrations (EC_{50}) producing a response halfway between the baseline and the maximum.

[0033] FIG. 25 shows a non-denaturing polyacrylamide gel (PAGE) analysis of humanized anti-LAG-3 mAb variant 2L2A.1 transiently expressed by HEK293. The positive control is HybPL1 (1PL11 CDRg-VH:1PL25 CDRg-VL).

[0034] FIG. 26 is a FACS analysis showing co-expression of LAG-3 and PD-1 in activated human $CD3^+$ T cells.

[0035] FIGS. 27A-27B show IFN-γ production, PBMCs from two donors (Donor 0105, **FIG. 27A**; Donor 0817, **FIG. 27B**) were stimulated with Staphylococcal enterotoxin B (SEB; lanes 2-4) or not stimulated with SEB (lane 1) in a 96 well plate. Following stimulation, the donor PBMCs were incubated with: no antibody (lanes 1, 2), an anti-LAG-3 benchmark (BM) antibody (lane 3), or the anti-LAG-3 mAb, 2L2A.1. The results of this assay showed that 2L2A.1 induces more IFN-γ production in both donor PBMCs than the anti-LAG-3 benchmark antibody.

[0036] FIGS. 28A-28C show increased IFN-γ production from three donor human PBMCs (Donor 223, **FIG. 28A**; Donor 224, **FIG. 28B;** Donor 225, **FIG. 28C**) stimulated with SEB (lanes 2-4) or not stimulated with SEB (lane 1). In addition, the donor PBMCs were incubated with: no antibody (lanes 1, 2), an anti-LAG-3 benchmark (BM) antibody (lane 3) or with the anti-LAG-3 mAb 2L2A.1.

[0037] FIGS. 29A-29C show that the anti-LAG-3 mAb 2L2A.1 enhances proliferation of primary human T cells from Donor 223 (**FIG. 29A**), Donor 224 (**FIG. 29B**) and Donor 225 (**FIG. 29C**) to a greater extent than the benchmark anti-LAG-3 antibody.

[0038] FIGS. 30A-30B depict two exemplary bispecific antitumor antagonists, Bi-LT-1 (**FIG. 30A**) and Bi-LT-3 (**FIG. 30B**).

[0039] FIG. 31 summarizes the arrangement of functional domains in the bispecific antagonists depicted in **FIGS. 30A-30B**.

[0040] FIG. 32 show the heavy chain (HC) and light chain (LC) amino acid sequences corresponding to the bispecific antagonists depicted in **FIGS. 30A-30B**.

[0041] FIGS. 33A-33B shows the results of a cell-based blocking assay measuring the ability of the bispecific antagonists, LT-1 and LT-3, and the bispecific antagonist, Bi-TPM-94B or an anti-LAG-3 benchmark mAb to block the interaction between LAG-3-muFc and its major ligand, major histocompatibility complex (MHC) antigen expressed on Raji cells (**FIG. 33A**) or block the interaction between TIGIT and its ligand, human PVR (CD155) (**FIG. 33B**). The assay data was used to calculate IC50 values (nm) depicted.

[0042] FIG. 34 shows the results of an ELISA assay demonstrating simultaneous binding of LAG-3 and TIGIT by Bi-LT-1, Bi-LT-3 or the parental anti-LAG-3 mAb in which LAG-3-muFc coated 96 well plates were incubated with serially diluted samples of Bi-LT-1, Bi-LT-3 or the parental anti-LAG-3 mAb, followed by His-tagged huTIGIT protein, whereby bound molecules were detected using HRP-conjugated anti-His tag HRP and TMB substrate. The assay data was used to calculate EC50 values (nm) depicted.

[0043] FIGS. 35A-35D depict pharmacokinetic profiles and *in vivo* half-lives ($T_{1/2}$) corresponding to the parental anti-LAG-3 mAb (**FIG. 35A**), an anti-LAG-3 benchmark mAb (**FIG. 35B**), Bi-LT-1 (**FIG. 35C**), or Bi-LT-3 (**FIG. 35D**) following a tail vein injection into 6-10 week old female CD1 mice. The antibodies and bispecific antagonists were recovered from serum taken at various times post-injection and subjected to analysis by ELISA. The $T_{1/2}$ for parental anti-LAG-3 mAb, Bi-LT-1 and Bi-LT-3 are five to six days, the $T_{1/2}$ for anti-LAG-3 BM mAb is either 2 days (mouse 3) or 7 days (mouse 4).

[0044] FIG. 36A shows a size exclusion chromatography (SEC) profile of Bi-LT-1 and Bi-LT-3 showing homogeneity and good stability at 4^{o} C after 7 days. **FIG. 36B** shows a size-exclusion ultra-high performance liquid chromatography (SE-UHPLC) analysis illustrating species homogeneity of protein A purified Bi-LT-1 and Bi-LT-3, as reflected in low levels of high molecular weight (HMW) species at Day 0 and Day 7 (1.3%, 1.5% respectively for Bi-LT-1 and 1.3%, 1.4%, respectively for Bi-LT-3) and low molecular weight (LMW) species at Day 0 and Day 7 (0.2%, 0.2%, respectively for Bi-LT-1 and 0.3%, 0.5%, respectively for Bi-LT-3) in comparison to dimer species at Day 0 and Day 7 (98.4%, 98.3%, respectively for Bi-LT-1 and 98.4%, 98.1%, respectively for Bi-LT-3).

[0045] FIGS. 37A-37B show IFN-γ production from human PBMCs stimulated with Staphylococcal enterotoxin B (SEB; lanes 2-8) or not stimulated with SEB (lane 1) in the presence of SHP-77 cells (**FIG. 37A**) or H358 cells (**FIG. 37B**), followed by incubation with: no antibody (lanes 1, 2); human IgG (lane 3); parental anti-TIGIT B21-35 mAb (lane 4); parental anti-LAG-3 2L2A.1 mAb (lane 5); parental mAbs, anti-TIGIT B21-35 and anti-LAG-3 2L2A.1 mAb (lane 6); Bi-LT-1 (lane 7); and Bi-LT-3 (lane 8).

[0046] FIG. 38 shows the proliferation of CD4 T cells from human PBMCs

stimulated with SEB in the presence of SHP-77 cells (lanes 2-8) and human IgG control (lane 3), anti-TIGIT mAb B21-35 (lane 4), anti-LAG-3 mAb (lane 5), the combination of anti-TIGIT mAb and anti-LAG-3 mAb (lane 6), Bi-LT-1 (lane 7) or Bi-LT-3 (lane 8).

[0047] **FIG. 39A** shows the framework regions (FRs) corresponding to the anti-TIGIT CDRs in **FIG. 1**. **FIG. 39B** shows the FRs corresponding to the anti-PD-1 CDRs in **FIG.** 3. **FIG. 39C** shows the FRs corresponding to the anti-PD-L1 CDRs in **FIG. 5** and the FRs corresponding to the anti-LAG-3 CDR in **FIGS. 19A** and **19B.**

DETAILED DESCRIPTION

Definitions

[0048] Unless defined otherwise, all technical and scientific terms used herein have the same meanings as commonly understood by one of skill in the art to which the disclosed method and compositions belong. It must be noted that as used herein and in the appended claims, the singular forms "a," "an," and "the" include plural reference unless the context clearly dictates otherwise. Thus, for example, reference to "a peptide" includes "one or more" peptides or a "plurality" of such peptides. With respect to the teachings in the present application, any issued patent or patent application publication described in this application is expressly incorporated by reference herein.

[0049] As used herein, the term "TIGIT" refers to any form of TIGIT and variants thereof that retain at least part of the activity of TIGIT. Unless indicated differently, such as by specific reference to human TIGIT, TIGIT includes all mammalian species of native sequence TIGIT, e.g., human, canine, feline, equine, and bovine.

[0050] As used herein, the term "PD-1" refers to any form of PD-1 and variants thereof that retain at least part of the activity of PD-1. Unless indicated differently, such as by specific reference to human PD-1, PD-1 includes all mammalian species of native sequence PD-1, e.g., human, canine, feline, equine, and bovine.

[0051] As used herein, the term "PD-L1" refers to any form of PD-L1 and variants thereof that retain at least part of the activity of PD-L1. Unless indicated differently, such as by specific reference to human PD-L1, PD-L1 includes all mammalian species of native sequence PD-L1, e.g., human, canine, feline, equine, and bovine.

[0052] The term "agonist" refers to a substance which promotes (i.e., induces, causes, enhances, or increases) the biological activity or effect of another molecule. The term agonist encompasses substances which bind receptor, such as an antibody, and substances which promote receptor function without binding thereto (*e.g.*, by activating an associated protein).

[0053] The term "antagonist" or "inhibitor" refers to a substance that prevents, blocks, inhibits, neutralizes, or reduces a biological activity or effect of another molecule,

such as a receptor or ligand. An antagonist can be a monospecific antibody or a bispecific antibody.

[0054] As used herein, the term "antibody" refers to a polypeptide or a polypeptide complex that specifically recognizes and binds to an antigen through one or more immunoglobulin variable regions. An antibody can be a whole antibody, an antigen binding fragment or a single chain thereof. The term "antibody" encompasses various broad classes of polypeptides that can be distinguished biochemically. Those skilled in the art will appreciate that heavy chains are classified as alpha, delta, epsilon, gamma, and mu, or α, δ, ε, γ and μ) with some subclasses among them (e.g., γ1-γ 4). It is the nature of this chain that determines the "class" of the antibody as IgG, IgM, IgA IgG, or IgE, respectively. The immunoglobulin subclasses (isotypes) e.g., IgG1, IgG2, IgG3, IgG4, etc. are well characterized and are known to confer functional specialization. Modified versions of each of these classes and isotypes are readily discernable to the skilled artisan in view of the instant disclosure and, accordingly, are within the scope of the instant disclosure. All immunoglobulin classes are within the scope of the present disclosure, the following discussion will generally be directed to the IgG class of immunoglobulin molecules.

[0055] Antibodies of the disclosure include, but are not limited to, polyclonal, monoclonal, multispecific, bispecific, human, humanized, primatized, chimeric and single chain antibodies. Antibodies disclosed herein may be from any animal origin, including birds and mammals. Preferably, the antibodies are human, murine, rat, donkey, rabbit, goat, guinea pig, camel, llama, horse, or chicken antibodies. In some embodiments, the variable region may be condricthoid in origin (e.g., from sharks).

[0056] The terms "antibody fragment" and "antigen-binding fragment" are used with reference to a portion of an antibody, such as F(ab')2, F(ab)2, Fab', Fab, Fv, single-chain Fvs (scFv), single-chain antibodies, disulfide-linked Fvs (sdFv), fragments comprising either a VL or VH domain, fragments produced by a Fab expression library, and anti-idiotypic (anti-Id) antibodies. Regardless of structure, an antibody fragment binds with the same antigen that is recognized by the intact antibody. The term "antibody fragment" includes DARTs, and diabodies. The term "antibody fragment" also includes any synthetic or genetically engineered proteins comprising immunoglobulin variable regions that act like an antibody by binding to a specific antigen to form a complex. A "single-chain fragment variable" or "scFv" refers to a fusion protein of the variable regions of the heavy (VH) and light chains (VL) of immunoglobulins. In some aspects, the regions are connected with a short linker peptide of ten to about 25 amino acids. The linker can be rich in glycine for flexibility, as well as serine or threonine for solubility, and can either connect the N-terminus of the VH

with the C-terminus of the VL, or vice versa. This protein retains the specificity of the original immunoglobulin, despite removal of the constant regions and the introduction of the linker. With regard to IgGs, a standard immunoglobulin molecule comprises two identical light chain polypeptides of molecular weight approximately 23,000 Daltons, and two identical heavy chain polypeptides of molecular weight 53,000-70,000. The four chains are typically joined by disulfide bonds in a "Y" configuration where the light chains bracket the heavy chains starting at the mouth of the "Y" and continuing through the variable region.

[0057] Both the light and heavy chains are divided into regions of structural and functional homology. The terms "constant" and "variable" are used functionally. In this regard, it will be appreciated that the variable domains of both the light (VL) and heavy (VH) chain portions determine antigen recognition and specificity. Conversely, the constant domains of the light chain (CL) and the heavy chain (CH1, CH2 or CH3) confer important biological properties such as secretion, transplacental mobility, Fc receptor binding, complement binding, and the like. By convention, the numbering of the constant region domains in conventional antibodies increases as they become more distal from the antigen-binding site or amino-terminus of the antibody. In conventional antibodies, the N-terminal portion is a variable region and at the C-terminal portion is a constant region; the CH3 and CL domains actually comprise the carboxy-terminus of the heavy and light chain, respectively.

[0058] As indicated above, the variable region allows the antibody to selectively recognize and specifically bind epitopes on antigens. That is, the VL domain and VH domain, or subset of the complementarity determining regions (CDRs), of an antibody combine to form the variable region that defines a three dimensional antigen-binding site. This quaternary antibody structure forms the antigen-binding site present at the end of each arm of the Y. More specifically, the antigen-binding site is defined by three CDRs on each of the VH and VL chains (i.e. HCDR1, HCDR2, HCDR3, LCDR1, LCDR2 and LCDR3). In some instances, e.g., certain immunoglobulin molecules are derived from camelid species or engineered based on camelid immunoglobulins. Alternatively, an immunoglobulin molecule may consist of heavy chains only with no light chains or light chains only with no heavy chains.

[0059] In naturally occurring antibodies, the six CDRs present in each antigen-binding domain are short, non-contiguous sequences of amino acids that are specifically positioned to form the antigen-binding domain as the antibody assumes its three dimensional configuration in an aqueous environment. The remainder of the amino acids in the antigen-binding domains, referred to as "framework" regions, show less inter-molecular variability.

The framework regions largely adopt a β-sheet conformation and the CDRs form loops which connect, and in some cases form part of, the β-sheet structure. Thus, framework regions act to form a scaffold that provides for positioning the CDRs in correct orientation by inter-chain, non-covalent interactions. The antigen-binding domain formed by the positioned CDRs defines a surface complementary to the epitope on the immunoreactive antigen. This complementary surface promotes the non-covalent binding of the antibody to its cognate epitope. The amino acids comprising the CDRs and the framework regions, respectively, can be readily identified for any given heavy or light chain variable region by one of ordinary skill in the art, since they have been precisely defined.

[0060] As used herein, the terms "VH1" and "VH2" refer to immunoglobulin heavy chain variable domains corresponding to two different binding specificities. Likewise, the terms "VL1" and "VL2" refer to light chain variable domains corresponding to two different binding specificities. When used together, it is to be understood that VH1 and VL1 regions define a common binding specificity and that VH2 and VL2 domains define a second binding specificity.

[0061] The term "framework region (FR)" as used herein refers to variable domain residues other than the CDR residues. Each variable domain typically has four FRs flanking the corresponding CDRs. For example, a VH domain typically has four HFRs: HFR1, HFR2, HFR3 and HFR4 flanking the three HCDRs in the configuration of HFR1-HCDR1-HFR2-HCDR2-HFR3-HCDR3-HFR4. Similarly, an LH domain typically has four LFRs flanking the three LCDRs in the configuration of: LFR1-LCDR1-LFR2-LCDR2-LFR3-LCDR3-LFR4. Exemplary FRs that may be utilized in the antagonists described herein are summarized in FIGS. 39A-39C.

[0062] Light chains are classified as either kappa or lambda (K, λ). Each heavy chain class may be bound with either a kappa or lambda light chain. In general, the light and heavy chains are covalently bonded to each other, and the "tail" portions of the two heavy chains are bonded to each other by covalent disulfide linkages or non-covalent linkages when the immunoglobulins are generated either by hybridomas, B cells or genetically engineered host cells. In the heavy chain, the amino acid sequences run from an N-terminus at the forked ends of the Y configuration to the C-terminus at the bottom of each chain.

[0063] As used herein, the terms "light chain constant region" or "CL" are used interchangeably herein with reference to amino acid sequences derived from antibody light chain. Preferably, the light chain constant region comprises at least one of a constant kappa domain or constant lambda domain.

[0064] As used herein, the term "heavy chain constant region" includes amino acid

sequences derived from an immunoglobulin heavy chain. A polypeptide comprising a heavy chain constant region comprises at least one of: a CH1 domain, a hinge (e.g., upper, middle, and/or lower hinge region) domain, a CH2 domain, a CH3 domain, or a variant or fragment thereof. For example, an antigen-binding polypeptide for use in the disclosure may comprise a polypeptide chain comprising a CH1 domain; a polypeptide chain comprising a CH1 domain, at least a portion of a hinge domain, and a CH2 domain; a polypeptide chain comprising a CH1 domain and a CH3 domain; a polypeptide chain comprising a CH1 domain, at least a portion of a hinge domain, and a CH3 domain, or a polypeptide chain comprising a CH1 domain, at least a portion of a hinge domain, a CH2 domain, and a CH3 domain. In some embodiments, a polypeptide of the disclosure comprises a polypeptide chain comprising a CH3 domain. Further, an antibody for use in the disclosure may lack at least a portion of a CH2 domain (e.g., all or part of a CH2 domain). It should be understood that the heavy chain constant region may be modified such that they vary in amino acid sequence from the naturally occurring immunoglobulin molecule. For example, the inventors of the present application have found that an Fc loop in the CH3 domain can tolerate or accommodate significant insertions (e.g., greater than 100 aa).

[0065] The heavy chain constant region of an antibody disclosed herein may be derived from different immunoglobulin molecules. For example, a heavy chain constant region of a polypeptide may comprise a CH1 domain derived from an IgG1 molecule and a hinge region derived from an IgG3 molecule. In another example, a heavy chain constant region can comprise a hinge region derived, in part, from an IgG1 molecule and, in part, from an IgG3 molecule. In another example, a heavy chain portion can comprise a chimeric hinge derived, in part, from an IgG1 molecule and, in part, from an IgG4 molecule.

[0066] A "light chain-heavy chain pair" refers to the collection of a light chain and heavy chain that can form a dimer through a disulfide bond between the CL domain of the light chain and the CH1 domain of the heavy chain.

[0067] The subunit structures and three dimensional configurations of the constant regions of the various immunoglobulin classes are well known. As used herein, the term "VH domain" includes the amino terminal variable domain of an immunoglobulin heavy chain and the term "CH1 domain" includes the first (most amino terminal) constant region domain of an immunoglobulin heavy chain. The CH1 domain is adjacent to the VH domain and is amino terminal to the hinge region of an immunoglobulin heavy chain molecule.

[0068] As used herein the term "CH2 domain" includes the portion of a heavy chain molecule that extends, e.g., from about residue 244 to residue 360 of an antibody using conventional numbering schemes (residues 244 to 360, Kabat numbering system; and

residues 231-340, EU numbering system). The CH2 domain is unique in that it is not closely paired with another domain. Rather, two N-linked branched carbohydrate chains are interposed between the two CH2 domains of an intact native IgG molecule. The CH3 domain extends from the CH2 domain to the C-terminal of the IgG molecule and comprises approximately 108 residues.

[0069] As used herein, the term "hinge region" includes the portion of a heavy chain molecule that joins the CH1 domain to the CH2 domain. This hinge region comprises approximately 25 residues and is flexible, thus allowing the two N-terminal antigen-binding regions to move independently. Hinge regions can be subdivided into three distinct domains: upper, middle, and lower hinge domains.

[0070] As used herein the term "disulfide bond" includes a covalent bond formed between two sulfur atoms. The amino acid cysteine comprises a thiol group that can form a disulfide bond or bridge with a second thiol group. In most naturally occurring IgG molecules, the CH1 and CL regions are linked by a disulfide bond and the two heavy chains are linked by two disulfide bonds at positions corresponding to 239 and 242 using the Kabat numbering system (position 226 or 229, EU numbering system).

[0071] As used herein, a "variant" of antibody, antibody fragment or antibody domain refers to antibody, antibody fragment or antibody domain that (1) shares a sequence identity of at least 80%, at least 85%, at least 90%, at least 95%, at least 96%, at least 97%, at least 98% or at least 99% identity with the original antibody, antibody fragment or antibody domain, and (2) binds specifically to the same target that the original antibody, antibody fragment or antibody domain binds specifically. It should be understood that where a measure of sequence identity is presented in the form of the phrase "at least x % identical" or "at least x % identity", such an embodiment includes any and all whole number percentages equal to or above the lower limit. Further it should be understood that where an amino acid sequence is presented in the present application, it should be construed as additionally disclosing or embracing amino acid sequences having a sequence identity of at least 80%, at least 85%, at least 90%, at least 95%, at least 96%, at least 97%, at least 98% or at least 99% identity to that amino acid sequence.

[0072] As used herein, the phrase "humanized antibody" refers to an antibody derived from a non-human antibody, typically a mouse monoclonal antibody. Alternatively, a humanized antibody may be derived from a chimeric antibody that retains or substantially retains the antigen binding properties of the parental, non-human, antibody but which exhibits diminished immunogenicity as compared to the parental antibody when administered to humans.

[0073] As used herein, the phrase "chimeric antibody," refers to an antibody where the immunoreactive region or site is obtained or derived from a first species and the constant region (which may be intact, partial or modified in accordance with the instant disclosure) is obtained from a second species. In certain embodiments the target binding region or site will be from a non-human source (e.g., mouse or primate) and the constant region is human.

[0074] Included within the scope of the multispecific antibodies of the present application are various compositions and methodologies, including asymmetric IgG-like antibodies (e.g., triomab/quadroma, Trion Pharma/Fresenius Biotech); knobs-into-holes antibodies (Genentech); Cross MAbs (Roche); electrostatically matched antibodies (AMGEN); LUZ-Y (Genentech); strand exchange engineered domain (SEED) body (EMD Serono; biolonic, Merus); Fab-exchanged antibodies (Genmab), symmetric IgG-like antibodies (e.g. dual targeting (DT)-Ig (GSK/Domantis); two-in-one antibody (Genentech); crosslinked MAbs (Karmanos Cancer Center), mAb2 (F-star); Cov X-body (Cov X/Pfizer); dual variable domain (DVD)-Ig fusions (Abbott); IgG-like bispecific antibodies (Eli Lilly); Ts2Ab (Medimmune/AZ); BsAb (ZymoGenetics); HERCULES (Biogen Idec,TvAb, Roche); scFv/Fc fusions; SCORPION (Emergent BioSolutions/Trubion, ZymoGenetics/BMS); dual affinity retargeting technology (Fc-DART), MacroGenics; dual (scFv)2-Fabs (National Research Center for Antibody Medicine); F(ab)2 fusions (Medarex/AMGEN); dual-action or Bis-Fab (Genentech); Dock-and-Lock (DNL, ImmunoMedics); Fab-Fv (UCB-Celltech); scFv- and diabody-based antibodies (e.g., bispecific T cell engagers (BiTEs, Micromet); tandem diabodies (Tandab, Affimed); DARTs (MacroGenics); single-chain diabodies; TCR-like antibodies (AIT, Receptor Logics); human serum albumin scFv fusion (Merrimack); COMBODIES (Epigen Biotech); and IgG/non-IgG fusions (e.g., immunocytokines (EMDSerono, Philogen, ImmunGene, ImmunoMedics).

[0075] By "specifically binds" or "has specificity to", it is generally meant that an antibody binds to an epitope via its antigen-binding domain, and that the binding entails some complementarity between the antigen-binding domain and the epitope. According to this definition, an antibody is said to "specifically bind" to an epitope when it binds to that epitope via its antigen-binding domain more readily than it would bind to a random, unrelated epitope. The term "specificity" is used herein to qualify the relative affinity by which a certain antibody binds to a certain epitope. For example, antibody "A" may be deemed to have a higher specificity for a given epitope than antibody "B," or antibody "A" may be said to bind to epitope "C" with a higher specificity than it has for related epitope "D." In some embodiments, an antibody or an antibody fragment "has specificity to" an antigen if the antibody or antibody fragment forms a complex with the antigen with a dissociation constant

(K_d) of 10^{-6}M or less, 10^{-7}M or less, 10^{-8}M or less, 10^{-9}M or less, or 10^{-10}M or less.

[0076] As used herein, the phrase "chimeric antibody," refers to an antibody where the immunoreactive region or site is obtained or derived from a first species and the constant region (which may be intact, partial or modified in accordance with the instant disclosure) is obtained from a second species. In certain embodiments the target binding region or site will be from a non-human source (e.g., mouse or primate) and the constant region is human.

[0077] The term "antagonist antibody" refers to an antibody that binds to a target and prevents or reduces the biological effect of that target. In some embodiments, the term can denote an antibody that prevents the target, e.g., TIGIT, to which it is bound from performing a biological function.

[0078] As used herein, an "anti-PD-1 antagonist antibody" refers to an antibody that is able to inhibit PD-1 biological activity and/or downstream events(s) mediated by PD-1. Anti-PD-1 antagonist antibodies encompass antibodies that block, antagonize, suppress or reduce (to any degree including significantly) PD-1 biological activity, including downstream events mediated by PD-1, such as PD-1 binding and downstream signaling, inhibition of T cell proliferation, inhibition of T cell activation, inhibition of IFN secretion, inhibition of IL-2 secretion, inhibition of TNF secretion, induction of IL-10, and inhibition of anti-tumor immune responses. For purposes of the present invention, it will be explicitly understood that the term "anti-PD-1 antagonist antibody" (interchangeably termed "antagonist PD-1 antibody", "antagonist anti-PD-1 antibody" or "PD-1 antagonist antibody") encompasses all the previously identified terms, titles, and functional states and characteristics whereby PD-1 itself, a PD-1 biological activity, or the consequences of the biological activity, are substantially nullified, decreased, or neutralized in any meaningful degree. In some embodiments, an anti-PD-1 antagonist antibody binds PD-1 and upregulates an anti-tumor immune response.

[0079] As used herein, an "anti-PD-L1 antagonist antibody" refers to an antibody that is able to inhibit PD-L1 biological activity and/or downstream events(s) mediated by PD-L1. Anti-PD-L1 antagonist antibodies encompass antibodies that block, antagonize, suppress or reduce (to any degree including significantly) PD-L1 biological activity, including downstream events mediated by PD-L1, such as PD-L1 binding and downstream signaling, inhibition of T cell proliferation, inhibition of T cell activation, inhibition of IFN secretion, inhibition of IL-2 secretion, inhibition of TNF secretion, induction of IL-10, and inhibition of anti-tumor immune responses. For purposes of the present invention, it will be explicitly understood that the term "anti-PD-L1 antagonist antibody" (interchangeably termed "antagonist PD-L1 antibody", "antagonist anti-PD-L1 antibody" or "PD-L1 antagonist

antibody") encompasses all the previously identified terms, titles, and functional states and characteristics whereby PD-L1 itself, a PD-L1 biological activity, or the consequences of the biological activity, are substantially nullified, decreased, or neutralized in any meaningful degree. In some embodiments, an anti-PD-L1 antagonist antibody binds PD-L1 and upregulates an anti-tumor immune response.

[0080] The phrase "immune checkpoint regulator" refers to a functional class of agents, which inhibit or stimulate signaling through an immune checkpoint. An "immune checkpoint regulator" includes receptors and their associated ligands, which together provide a means for inhibiting or stimulating signaling pathways that otherwise lead to T-cell activation. Exemplary immune checkpoint regulators include, but are not limited to, TIGIT and its CD155 ligand, PVR; PD-1 and its ligands, PD-L1 and PD-L2; CTLA-4 and its ligands, B7-1 and B7-2; TIM-3 and its ligand, Galectin-9; LAG-3 and its ligands, including liver sinusoidal endothelial cell lectin (LSECtin) and Galectin-3; CD122 and its CD122R ligand; CD70, B7H3, B and T lymphocyte attenuator (BTLA), and VISTA.

[0081] The phrases "checkpoint regulator antagonist", "immune checkpoint binding antagonist" and "immune checkpoint antagonist" are used interchangeably herein with reference to a class of agents that interfere with (or inhibit) the activity of an immune checkpoint regulator so that, as a result of the binding to the checkpoint regulator or its ligand, signaling through the checkpoint regulator receptor is blocked or inhibited. By inhibiting this signaling, immune-suppression can be reversed so that T cell immunity against cancer cells can be re-established or enhanced. Immune checkpoint regulator antagonists include antibody fragments, peptide inhibitors, dominant negative peptides and small molecule drugs, either in isolated forms or as part of a fusion protein or conjugate.

[0082] The phrases "immune checkpoint binding agonist" and "immune checkpoint agonist" are used interchangeably herein with reference to a class of agents that stimulate the activity of an immune checkpoint regulator so that, as a result of the binding to the checkpoint regulator or its ligand, signaling through the checkpoint regulator receptor is stimulated. By stimulating this signaling, T cell immunity against cancer cells can be re-established or enhanced. Exemplary immune checkpoint regulator agonists include, but are not limited to members of the tumor necrosis factor (TNF) receptor superfamily, such as CD27, CD40, OX40 (CD 134), glucocorticoid-induced TNFR family-related protein (GITR), and 4-1BB (CD137) and their ligands. Additional checkpoint regulator agonists belong to the B7-CD28 superfamily, including CD28 and ICOS.

[0083] The phrases "dominant-negative protein" or "dominant-negative peptide" refer to a protein or peptide derived from a wild type protein that has been genetically modified by

mutation and/or deletion so that the modified protein or peptide interferes with the function of the endogenous wild-type protein from which it is derived.

[0084] The phrase "small molecule drug" refers to a molecular entity, often organic or organometallic, that is not a polymer, that has medicinal activity, and that has a molecular weight less than about 2 kDa, less than about 1 kDa, less than about 900Da, less than about 800Da or less than about 700Da. The term encompasses most medicinal compounds termed "drugs" other than protein or nucleic acids, although a small peptide or nucleic acid analog can be considered a small molecule drug. Examples include chemotherapeutic anticancer drugs and enzymatic inhibitors. Small molecules drugs can be derived synthetically, semi-synthetically (i.e., from naturally occurring precursors), or biologically.

[0085] When describing polypeptide domain arrangements with hyphens between individual domains (e.g., CH2-CH3), it should be understood that the order of the listed domains is from the amino terminal end to the carboxy terminal end.

[0086] By "specifically binds" or "has specificity to", it is generally meant that an antibody binds to an epitope via its antigen-binding domain, and that the binding entails some complementarity between the antigen-binding domain and the epitope. According to this definition, an antibody is said to "specifically bind" to an epitope when it binds to that epitope via its antigen-binding domain more readily than it would bind to a random, unrelated epitope. The term "specificity" is used herein to qualify the relative affinity by which a certain antibody binds to a certain epitope. For example, antibody "A" may be deemed to have a higher specificity for a given epitope than antibody "B," or antibody "A" may be said to bind to epitope "C" with a higher specificity than it has for related epitope "D."

[0087] The term "immunoconjugate" refers to an antibody which is fused by covalent linkage to an inhibitory peptide or small molecule drug. The peptide or small molecule drug can be linked to the C-terminus of a constant heavy chain or to the N-terminus of a variable light and/or heavy chain.

[0088] A "linker" may be used to link the peptide or small molecule drug, such as a maytansinoid, to the antitumor antagonists in a stable, covalent manner. Linkers can be susceptible to or be substantially resistant to acid-induced cleavage, light-induced cleavage, peptidase-induced cleavage, esterase-induced cleavage, and disulfide bond cleavage, at conditions under which the compound or the antibody remains active. Suitable linkers are well known in the art and include, for example, disulfide groups, thioether groups, acid labile groups, photolabile groups, peptidase labile groups and esterase labile groups. Linkers also include charged linkers, and hydrophilic forms thereof as described herein and know in the art. The immunoconjugate may further include a flexible 3-15 amino acid peptide (or spacer)

between an antitumor antagonist and the peptide and/or small molecule drug.

[0089] As used herein, the term "immunoglobulin scaffold", refers to any polymer of amino acids that exhibits properties desired to support the function of an antagonist, including addition of antibody specificity, enhancement of antibody function or support of antibody structure and stability. An immunoglobulin scaffold may have one or more immunoglobulin constant regions, including CH1, CH2, and/or CH3 regions from an immunoglobulin heavy chain and/or a CL region from an immunoglobulin light chain. The immunoglobulin scaffold can be grafted with binding domains of a donor polypeptide to confer the binding specificity of the donor polypeptide onto the scaffold.

[0090] As used herein, the phrase "multispecific inhibitor" refers to a molecule comprising at least two targeting domains with different binding specificities. In some embodiments, the multispecific inhibitor is a polypeptide comprising a scaffold and two or more immunoglobulin antigen binding domains targeting different antigens or epitopes. In certain embodiments, the multispecific inhibitor is a bispecific antibody.

[0091] As used herein, the phrase "bispecific" refers to a molecule comprising at least two targeting domains with different binding specificities. Each targeting domain is capable of binding specifically to a target molecule and inhibiting a biological function of the target molecule upon binding to the target molecule. In some embodiments, the bispecific checkpoint regulator antagonist is a polymeric molecule having two or more peptides. In some embodiments, the targeting domain comprises an antigen binding domain or a CDR of an antibody. In some embodiments, the bispecific inhibitor is a bispecific antibody.

[0092] The terms "bispecific antibody," and "bispecific antagonist" are used interchangeably herein with reference to an antibody that can specifically bind two different antigens (or epitopes). In some embodiments, the bispecific antibody is a full-length antibody that binds one antigen (or epitope) on one of its two binding arms (one pair of HC/LC), and binds a different antigen (or epitope) on its second arm (a different pair of HC/LC). In these embodiments, the bispecific antibody has two distinct antigen binding arms (in both specificity and CDR sequences), and is monovalent for each antigen it binds to.

[0093] In other embodiments, the bispecific antibody is a full-length antibody that can bind two different antigens (or epitopes) in each of its two binding arms (two pairs of HC/LC) In these embodiments, the bispecific antibody has two identical antigen binding arms, with identical specificity and identical CDR sequences, and is bivalent for each antigen it binds to.

[0094] Exemplary bispecific antibodies may include asymmetric IgG-like antibodies (e.g., triomab/quadroma, Trion Pharma/Fresenius Biotech); knobs-into-holes antibodies

(Genentech); Cross MAbs (Roche); electrostatically matched antibodies (AMGEN); LUZ-Y (Genentech); strand exchange engineered domain (SEED) body (EMD Serono; biolonic, Merus); Fab-exchanged antibodies (Genmab), symmetric IgG-like antibodies (e.g. dual targeting (DT)-Ig (GSK/Domantis); two-in-one antibody (Genentech); crosslinked MAbs (Karmanos Cancer Center), mAb2 (F-star); Cov X-body (Cov X/Pfizer); dual variable domain (DVD)-Ig fusions (Abbott); IgG-like bispecific antibodies (Eli Lilly); Ts2Ab (Medimmune/AZ); BsAb (ZymoGenetics); HERCULES (Biogen Idec,TvAb, Roche); scFv/Fc fusions; SCORPION (Emergent BioSolutions/Trubion, ZymoGenetics/BMS); dual affinity retargeting technology (Fc-DART), MacroGenics; dual (scFv)2-Fabs (National Research Center for Antibody Medicine); F(ab)2 fusions (Medarex/AMGEN); dual-action or Bis-Fab (Genentech); Dock-and-Lock (DNL, ImmunoMedics); Fab-Fv (UCB-Celltech); scFv- and diabody-based antibodies (e.g., bispecific T cell engagers (BiTEs, Micromet); tandem diabodies (Tandab, Affimed); DARTs (MacroGenics); single-chain diabodies; TCR-like antibodies (AIT, Receptor Logics); human serum albumin scFv fusion (Merrimack); COMBODIES (Epigen Biotech); and IgG/non-IgG fusions (e.g., immunocytokines (EMDSerono, Philogen, ImmunGene, ImmunoMedics).

[0095] The terms “treat” and “treatment” refer to the amelioration of one or more symptoms associated with a cell proliferative disorder; prevention or delay of the onset of one or more symptoms of a cell proliferative disorder; and/or lessening of the severity or frequency of one or more symptoms of cell proliferative disorder.

[0096] The phrases “to a patient in need thereof”, “to a patient in need of treatment” or “a subject in need of treatment” includes subjects, such as mammalian subjects, that would benefit from administration of the antitumor antagonist of the present disclosure for treatment of a cell proliferative disorder.

[0097] The terms “therapeutically effective amount”, “pharmacologically effective amount”, and “physiologically effective amount” are used interchangeably to mean the amount of an antitumor antagonist that is needed to provide a threshold level of active antagonist agents in the bloodstream or in the target tissue. The precise amount will depend upon numerous factors, e.g., the particular active agent, the components and physical characteristics of the composition, intended patient population, patient considerations, and the like, and can readily be determined by one skilled in the art, based upon the information provided herein or otherwise available in the relevant literature.

[0098] The terms, “improve”, “increase” or “reduce”, as used in this context, indicate values or parameters relative to a baseline measurement, such as a measurement in the same individual prior to initiation of the treatment described herein, or a measurement in a control

individual (or multiple control individuals) in the absence of the treatment described herein.

[0099] A "control individual" is an individual afflicted with the same cell proliferative disorder as the individual being treated, who is about the same age as the individual being treated (to ensure that the stages of the disease in the treated individual and the control individual(s) are comparable). The individual (also referred to as "patient" or "subject") being treated may be a fetus, infant, child, adolescent, or adult human with a cell proliferative disorder.

[0100] The term "cell proliferative disorder" refers to a disorder characterized by abnormal proliferation of cells. A proliferative disorder does not imply any limitation with respect to the rate of cell growth, but merely indicates loss of normal controls that affect growth and cell division. Thus, in some embodiments, cells of a proliferative disorder can have the same cell division rates as normal cells but do not respond to signals that limit such growth. Within the ambit of "cell proliferative disorder" is a neoplasm, cancer or tumor.

[0101] The term "cancer" or "tumor" refers to any one of a variety of malignant neoplasms characterized by the proliferation of cells that have the capability to invade surrounding tissue and/or metastasize to new colonization sites, and includes leukemia, lymphoma, carcinoma, melanoma, sarcoma, germ cell tumor and blastoma. Exemplary cancers for treatment with the methods of the instant disclosure include cancer of the brain, bladder, breast, cervix, colon, head and neck, kidney, lung, non-small cell lung, mesothelioma, ovary, prostate, stomach and uterus, leukemia, and medulloblastoma.

[0102] The term "leukemia" refers to progressive, malignant diseases of the blood-forming organs and is generally characterized by a distorted proliferation and development of leukocytes and their precursors in the blood and bone marrow. Exemplary leukemias include, for example, acute nonlymphocytic leukemia, chronic lymphocytic leukemia, acute granulocytic leukemia, chronic granulocytic leukemia, acute promyelocytic leukemia, adult T-cell leukemia, aleukemic leukemia, a leukocythemic leukemia, basophylic leukemia, blast cell leukemia, bovine leukemia, chronic myelocytic leukemia, leukemia cutis, embryonal leukemia, eosinophilic leukemia, Gross' leukemia, hairy-cell leukemia, hemoblastic leukemia, hemocytoblastic leukemia, histiocytic leukemia, stem cell leukemia, acute monocytic leukemia, leukopenic leukemia, lymphatic leukemia, lymphoblastic leukemia, lymphocytic leukemia, lymphogenous leukemia, lymphoid leukemia, lymphosarcoma cell leukemia, mast cell leukemia, megakaryocytic leukemia, micromyeloblastic leukemia, monocytic leukemia, myeloblastic leukemia, myelocytic leukemia, myeloid granulocytic leukemia, myelomonocytic leukemia, Naegeli leukemia, plasma cell leukemia, plasmacytic leukemia, promyelocytic leukemia, Rieder

cell leukemia, Schilling's leukemia, stem cell leukemia, subleukemic leukemia, and undifferentiated cell leukemia.

[0103] The term "carcinoma" refers to the malignant growth of epithelial cells tending to infiltrate the surrounding tissues and give rise to metastases. Exemplary carcinomas include, for example, acinar carcinoma, acinous carcinoma, adenocystic carcinoma, adenoid cystic carcinoma, carcinoma adenomatosum, carcinoma of adrenal cortex, alveolar carcinoma, alveolar cell carcinoma, basal cell carcinoma, carcinoma basocellulare, basaloid carcinoma, basosquamous cell carcinoma, bronchioalveolar carcinoma, bronchiolar carcinoma, bronchogenic carcinoma, cerebriform carcinoma, cholangiocellular carcinoma, chorionic carcinoma, colloid carcinoma, comedo carcinoma, corpus carcinoma, cribriform carcinoma, carcinoma en cuirasse, carcinoma cutaneum, cylindrical carcinoma, cylindrical cell carcinoma, duct carcinoma, carcinoma durum, embryonal carcinoma, encephaloid carcinoma, epiennoid carcinoma, carcinoma epitheliale adenoides, exophytic carcinoma, carcinoma ex ulcere, carcinoma fibrosum, gelatiniform carcinoma, gelatinous carcinoma, giant cell carcinoma, carcinoma gigantocellulare, glandular carcinoma, granulosa cell carcinoma, hair-matrix carcinoma, hematoid carcinoma, hepatocellular carcinoma, Hurthle cell carcinoma, hyaline carcinoma, hypemephroid carcinoma, infantile embryonal carcinoma, carcinoma in situ, intraepidermal carcinoma, intraepithelial carcinoma, Krompecher's carcinoma, Kulchitzky-cell carcinoma, large-cell carcinoma, lenticular carcinoma, carcinoma lenticulare, lipomatous carcinoma, lymphoepithelial carcinoma, carcinoma medullare, medullary carcinoma, melanotic carcinoma, carcinoma molle, mucinous carcinoma, carcinoma muciparum, carcinoma mucocellulare, mucoepidermoid carcinoma, carcinoma mucosum, mucous carcinoma, carcinoma myxomatodes, naspharyngeal carcinoma, oat cell carcinoma, carcinoma ossificans, osteoid carcinoma, papillary carcinoma, periportal carcinoma, preinvasive carcinoma, prickle cell carcinoma, pultaceous carcinoma, renal cell carcinoma of kidney, reserve cell carcinoma, carcinoma sarcomatodes, schneiderian carcinoma, scirrhous carcinoma, carcinoma scroti, signet-ring cell carcinoma, carcinoma simplex, small-cell carcinoma, solanoid carcinoma, spheroidal cell carcinoma, spindle cell carcinoma, carcinoma spongiosum, squamous carcinoma, squamous cell carcinoma, string carcinoma, carcinoma telangiectaticum, carcinoma telangiectodes, transitional cell carcinoma, carcinoma tuberosum, tuberous carcinoma, verrucous carcinoma, and carcinoma villosum.

[0104] The term "sarcoma" refers to a tumor made up of a substance like the embryonic connective tissue and is generally composed of closely packed cells embedded in a fibrillar or homogeneous substance. Exemplary sarcomas include, for example,

chondrosarcoma, fibrosarcoma, lymphosarcoma, melanosarcoma, myxosarcoma, osteosarcoma, Abemethy's sarcoma, adipose sarcoma, liposarcoma, alveolar soft part sarcoma, ameloblastic sarcoma, botryoid sarcoma, chloroma sarcoma, chorio carcinoma, embryonal sarcoma, Wilns' tumor sarcoma, endometrial sarcoma, stromal sarcoma, Ewing's sarcoma, fascial sarcoma, fibroblastic sarcoma, giant cell sarcoma, granulocytic sarcoma, Hodgkin's sarcoma, idiopathic multiple pigmented hemorrhagic sarcoma, immunoblastic sarcoma of B cells, lymphomas (*e.g.*, Non-Hodgkin Lymphoma), immunoblastic sarcoma of T-cells, Jensen's sarcoma, Kaposi's sarcoma, Kupffer cell sarcoma, angiosarcoma, leukosarcoma, malignant mesenchymoma sarcoma, parosteal sarcoma, reticulocytic sarcoma, Rous sarcoma, serocystic sarcoma, synovial sarcoma, and telangiectaltic sarcoma.

[0105] The term "melanoma" refers to a tumor arising from the melanocytic system of the skin and other organs. Melanomas include, for example, acral-lentiginous melanoma, amelanotic melanoma, benign juvenile melanoma, Cloudman's melanoma, S91 melanoma, Harding-Passey melanoma, juvenile melanoma, lentigo maligna melanoma, malignant melanoma, nodular melanoma subungal melanoma, and superficial spreading melanoma.

[0106] Additional cancers include, for example, Hodgkin's Disease, multiple myeloma, neuroblastoma, breast cancer, ovarian cancer, lung cancer, rhabdomyosarcoma, primary thrombocytosis, primary macroglobulinemia, small-cell lung tumors, primary brain tumors, stomach cancer, colon cancer, malignant pancreatic insulanoma, malignant carcinoid, premalignant skin lesions, testicular cancer, thyroid cancer, neuroblastoma, esophageal cancer, genitourinary tract cancer, malignant hypercalcemia, cervical cancer, endometrial cancer, and adrenal cortical cancer.

I. Checkpoint Regulator Antagonists

[0107] In one aspect, the present application provides an antitumor antagonist comprising an immunoglobulin scaffold with (1) a pair of arms containing variable domain regions that specifically bind to a first immune checkpoint regulator and (2) a single chain (scFv) that specifically binds to a second immune checkpoint regulator.

[0108] In another aspect, the present application provides an antitumor antagonist comprising an immunoglobulin scaffold structurally linked to both a first immune checkpoint regulator antagonist, and a second immune checkpoint regulator antagonist in the form of an scFv.

[0109] In both aspects, the immunoglobulin scaffold may comprise one or more immunoglobulin constant regions, *e.g.,* IgG CH1, CH2, and/or CH3. In certain embodiments, the immunoglobulin scaffold is an Fc (hinge-CH2-CH3).

[0110] In some embodiments, the antitumor antagonist comprises an immunoglobulin scaffold in which the N-terminal end of the antagonist includes a first immune checkpoint regulator antagonist structurally linked thereto in which the first immune checkpoint regulator antagonist specifically binds to PD-1, PD-L1, LAG-3, TIGIT, and a second immune checkpoint regulator antagonist is positioned at the C-terminal end of the antagonist as an scFv that specifically binds to PD-1, PD-L1, LAG-3 or TIGIT.

[0111] In some embodiments, the scFv comprises a linker joining heavy chain variable regions to light chain variable regions. In one embodiment, the linker comprises an amino acid sequence comprising between 3, 4, 5, 6, 7, 8, 9, or 10 copies of the amino acid sequence G4S. In another embodiment, the linker comprises an amino acid sequence set forth in either one of **SEQ ID NOS:** 188-191. In a more particular embodiment, the linker comprises the amino acid sequence of SEQ ID NO: 191.

[0112] In one embodiment, the anti-TIGIT scFv comprises one or more heavy chain CDRs selected from **SEQ ID NOS: 1-25** and one or more light chain CDRs selected from **SEQ ID NOS:** 26-47.

[0113] In another embodiment, the anti-TIGIT scFv comprising heavy chain/light chain variable regions, wherein the scFv has a heavy chain variable region (HCVR) having at least 80%, at least 85%, at least 90%, at least 95%, or at least 99% identity to an amino acid sequence selected from the group consisting of **SEQ ID NOS: 48, 50, 52, 54, 56, 58, 60, 62, 64,** and **66** and a light chain variable region (LCVR) having at least at least 80%, at least 85%, at least 90%, at least 95%, or at least 99% to an LCVR having an amino acid sequence selected from the group consisting of **SEQ ID NOS: 49, 51, 53, 55, 57, 59, 61, 63,** and **67**.

[0114] In a more particular embodiment, the anti-TIGIT scFv comprises an HCVR having at least 80%, at least 85%, at least 90%, at least 95%, or at least 99% identity to the amino acid sequence of **SEQ ID NO:66** and an LCVR having at least 80%, at least 85%, at least 90%, at least 95%, or at least 99% to an LCVR having the amino acid sequence of **SEQ ID NO:67**.

[0115] In another embodiment, the anti-TIGIT scFv includes: an HCVR that comprises (1) an HCDR1 of **SEQ ID NO:23**, an HCDR2 of **SEQ ID NO:24** and an HCDR 3 of **SEQ ID NO:25** in combination with (2) an HFR1 having at least 80%, 85% or 90% identity to the amino acid sequence of **SEQ ID NO:260**, an HFR2 having at least 80%, 85% or 90% identity to the amino acid sequence of **SEQ ID NO:247**, an HFR3 having at least 80%, 85% or 90% identity to the amino acid sequence of **SEQ ID NO:261**, and an HFR4 having at least 80%, 85% or 90% identity to the amino acid sequence of **SEQ ID NO:236**; and further includes an immunoglobulin LCVR that comprises (1) an LCDR1 of **SEQ ID NO:45**, an LCDR2 of **SEQ**

ID NO:46 and an LCDR 3 of **SEQ ID NO:47** in combination with (2) an LFR1 having at least 80%, 85% or 90% identity to the amino acid sequence of **SEQ ID NO:220**, an LFR2 having at least 80%, 85% or 90% identity to the amino acid sequence of SEQ ID **NO:228**, an LFR3 having at least 80%, 85% or 90% identity to the amino acid sequence of **SEQ ID NO:234**, an LFR4 having at least 80%, 85% or 90% identity to the amino acid sequence of **SEQ ID NO:262.**

[0116] In another embodiment, the first targeting domain comprises one or more variable regions from an anti-PD-1 antibody, and the second targeting domain comprises an anti-TIGIT scFv as described above.

[0117] In one embodiment, the anti-PD-1 targeting domain comprises one or more heavy chain CDRs selected from **SEQ ID NOS: 68-81** and/or one or more light chain CDRs selected from **SEQ ID NOS: 82-95**.

[0118] In another embodiment, the anti-PD-1 targeting domain comprises: an HCVR having at least 80%, at least 85%, at least 90%, at least 95%, or at least 99% identity to an amino acid sequence selected from the group consisting of **SEQ ID NOS: 96, 98, 100, 102, 104,** and **106;** an LCVR having at least 80%, at least 85%, at least 90%, at least 95%, or at least 99% identity to an LCVR having an amino acid sequence selected from the group consisting of **SEQ ID NOS: 97, 99, 101, 103, 105, and 107**; or both.

[0119] In another embodiment, the anti-PD-1 targeting domain includes: an HCVR that comprises (1) an HCDR1 of **SEQ ID NO:79**, an HCDR2 of **SEQ ID NO:80** and an HCDR 3 of **SEQ ID NO:81** in combination with (2) an HFR1 having at least 80%, 85% or 90% identity to the amino acid sequence of **SEQ ID NO:283**, an HFR2 having at least 80%, 85% or 90% identity to the amino acid sequence of **SEQ ID NO:277**, an HFR3 having at least 80%, 85% or 90% identity to the amino acid sequence of **SEQ ID NO:288**, an HFR4 having at least 80%, 85% or 90% identity to the amino acid sequence of **SEQ ID NO:274**; an immunoglobulin LCVR that comprises (1) an LCDR1 of **SEQ ID NO:93**, an LCDR2 of **SEQ ID NO:94** and an LCDR 3 of **SEQ ID NO:95** in combination with (2) an LFR1 having at least 80%, 85% or 90% identity to the amino acid sequence of **SEQ ID NO:289**, an LFR2 having at least 80%, 85% or 90% identity to the amino acid sequence of SEQ ID **NO:290**, an LFR3 having at least 80%, 85% or 90% identity to the amino acid sequence of **SEQ ID NO:291**, and an LFR4 having at least 80%, 85% or 90% identity to the amino acid sequence of **SEQ ID NO:292**; or both.

[0120] In another embodiment, the anti-PD-1 targeting domain comprises an HCVR having at least 80%, at least 85%, at least 90%, at least 95%, or at least 99% identity to the amino acid sequence of **SEQ ID NO:106**; an LCVR having at least at least 80%, at least 85%, at

least 90%, at least 95%, or at least 99% identity to the amino acid sequence of **SEQ ID NO: 107**; or both.

[0121] In a particular embodiment, a bispecific anti-PD-1/anti-TIGIT antagonist includes a first targeting domain comprising an HCVR having the amino acid sequence of **SEQ ID NO:106** and/or an LCVR having the amino acid sequence of **SEQ ID NO:107** in combination with an anti-TIGIT scFv comprising an HCVR having the amino acid sequence of **SEQ ID NO:66** and an LCVR having the amino acid sequence of **SEQ ID NO:67**.

[0122] In a more particular embodiment, the scFv in the bispecific anti-PD-1/anti-TIGIT antagonist comprises a linker joining heavy chain variable regions to light chain variable regions in the second targeting domain, where the linker comprises the amino acid sequence of **SEQ ID NO:191**.

[0123] In another embodiment, the bispecific anti-PD-1/anti-TIGIT antagonist includes: an immunoglobulin heavy chain comprising the amino acid sequence of **SEQ ID NO:160;** an immunoglobulin light chain comprising the amino acid sequence of **SEQ ID NO:161**; or both.

[0124] In another embodiment, the bispecific anti-PD-1/anti-TIGIT antagonist includes: an immunoglobulin heavy chain comprising the amino acid sequence of **SEQ ID NO:162;** an immunoglobulin light chain comprising the amino acid sequence of **SEQ ID NO:161**; or both.

[0125] In another embodiment, the bispecific anti-PD-1/anti-TIGIT antagonist comprises an immunoglobulin heavy chain comprising the amino acid sequence of **SEQ ID NO: 160**, and an immunoglobulin light chain comprising the amino acid sequence of **SEQ ID NO: 161**.

[0126] In another embodiment, the first targeting domain comprises one or more variable regions from an anti-PD-L1 antibody, and the second targeting domain comprises an anti-TIGIT scFv as described above.

[0127] In one embodiment, the anti-PD-L1 targeting domain comprises one or more heavy chain CDRs selected from **SEQ ID NOS: 108-122** and/or one or more light chain CDRs selected from **SEQ ID NOS: 123-138**.

[0128] In another embodiment, the anti-PD-L1 targeting domain comprises an HCVR having at least 80%, at least 85%, at least 90%, at least 95%, or at least 99% identity to an amino acid sequence selected from the group consisting of **SEQ ID NOS: 139, 141, 143, 145, 147, 149, 151,** and **153** and/or an LCVR having at least 80%, at least 85%, at least 90%, at least 95%, or at least 99% identity to an amino acid sequence selected from the group consisting of **SEQ ID NOS: 140, 142, 144, 146, 148, 152,** and **154**.

[0129] In another embodiment, the anti-PD-L1 targeting domain comprises an HCVR having at least 80%, at least 85%, at least 90%, at least 95%, or at least 99% identity to the amino acid sequence of **SEQ ID NO:153** and/or an LCDR having at least 80%, at least 85%, at

least 90%, at least 95%, or at least 99% identity to an LCVR having the amino acid sequence of **SEQ ID NO:154**.

[0130] In another embodiment, the anti-PD-L1 targeting domain includes: an immunoglobulin HCVR that comprises (1) an HCDR1 of **SEQ ID NO:111**, an HCDR2 of **SEQ ID NO:114** and an HCDR 3 of **SEQ ID NO:115** in combination with (2) an HFR1 having at least 80%, 85% or 90% identity to the amino acid sequence of **SEQ ID NO:283**, an HFR2 having at least 80%, 85% or 90% identity to the amino acid sequence of **SEQ ID NO:277**, an HFR3 having at least 80%, 85% or 90% identity to the amino acid sequence of **SEQ ID NO:300**, and an HFR4 having at least 80%, 85% or 90% identity to the amino acid sequence of **SEQ ID NO:274**; an immunoglobulin LCVR that comprises (1) an LCDR1 of **SEQ ID NO:123**, an LCDR2 of **SEQ ID NO:124** and an LCDR 3 of **SEQ ID NO:125** in combination with (2) an LFR1 having at least 80%, 85% or 90% identity to the amino acid sequence of **SEQ ID NO:294**, an LFR2 having at least 80%, 85% or 90% identity to the amino acid sequence of **SEQ ID NO:295**, an LFR3 having at least 80%, 85% or 90% identity to the amino acid sequence of **SEQ ID NO:296**, and an LFR4 having at least 80%, 85% or 90% identity to the amino acid sequence of **SEQ ID NO:276**; or both.

[0131] In a particular embodiment, a bispecific anti-PD-L1/anti-TIGIT antagonist includes a first targeting domain comprising: an HCVR having the amino acid sequence of **SEQ ID NO: 153** and/or an LCVR having the amino acid sequence of **SEQ ID NO: 154** in combination with a second targeting domain in the form of an anti-TIGIT scFv comprising an HCVR having the amino acid sequence of **SEQ ID NO: 66** and an LCVR having the amino acid sequence of **SEQ ID NO: 67**. In a more particular embodiment, the scFv in the bispecific anti-PD-L1/anti-TIGIT antagonist comprises a linker joining anti-TIGIT HCVR to the anti-TIGIT LCVR in the second targeting domain, where the linker comprises the amino acid sequence of **SEQ ID NO: 191**.

[0132] In another embodiment, the first targeting domain comprises one or more variable regions from an anti-LAG-3 antibody, and the second targeting domain comprises an anti-TIGIT scFv as described above.

[0133] In one embodiment, the anti-LAG-3 targeting domain comprises one or more immunoglobulin heavy chain CDRs selected from **SEQ ID NOS: 163-171** and/or one or more immunoglobulin light chain CDRs selected from **SEQ ID NOS: 172-178**.

[0134] In another embodiment, the anti-LAG-3 targeting domain comprises an HCVR having at least 80%, at least 85%, at least 90%, at least 95%, or at least 99% identity to an amino acid sequence selected from the group consisting of **SEQ ID NOS: 180, 182, 184**, and **186** and/or an LCVR having at least 80%, at least 85%, at least 90%, at least 95%, or at least 99%

identity to an LCVR having an amino acid sequence selected from the group consisting of **SEQ ID NOS: 181, 183, 185, and 187**.

[0135] In another embodiment, the anti-LAG-3 targeting domain includes: an immunoglobulin HCVR comprising (1) an HCDR1 of **SEQ ID NO:163**, an HCDR2 of **SEQ ID NO:164** and an HCDR 3 of **SEQ ID NO:165** in combination with (2) an HFR1 having at least 80%, 85% or 90% identity to the amino acid sequence of **SEQ ID NO:316**, an HFR2 having at least 80%, 85% or 90% identity to the amino acid sequence of **SEQ ID NO:317**, an HFR3 having at least 80%, 85% or 90% identity to the amino acid sequence of **SEQ ID NO:318**, and an HFR4 having at least 80%, 85% or 90% identity to the amino acid sequence of **SEQ ID NO:319**; an immunoglobulin LCVR comprising (1) an LCDR1 of **SEQ ID NO:172**, an LCDR2 of **SEQ ID NO:173** and an LCDR 3 of **SEQ ID NO:174** in combination with (2) an LFR1 having at least 80%, 85% or 90% identity to the amino acid sequence of **SEQ ID NO:320**, an LFR2 having at least 80%, 85% or 90% identity to the amino acid sequence of **SEQ ID NO:321**, an LFR3 having at least 80%, 85% or 90% identity to the amino acid sequence of **SEQ ID NO:322**, and an LFR4 having at least 80%, 85% or 90% identity to the amino acid sequence of **SEQ ID NO:323**; or both**.**

[0136] In another embodiment, the anti-LAG-3 targeting domain comprises an immunoglobulin HCVR having an amino acid sequence that is 90%, 95%, 99%, or 100% identical to the amino acid sequence of **SEQ ID NO:180** and/or an immunoglobulin LCVR having an amino acid sequence that is 90%, 95%, 99%, or 100% identical to the amino acid sequence of **SEQ ID NO:181**.

[0137] In a particular embodiment, a bispecific anti-LAG-3/anti-TIGIT antagonist includes a first targeting domain comprising an HCVR having the amino acid sequence of **SEQ ID NO:180** and/or an LCVR having the amino acid sequence of **SEQ ID NO:181** in combination with an anti-TIGIT scFv comprising an HCVR having the amino acid sequence of **SEQ ID NO: 66** and an LCVR having the sequence of **SEQ ID NO:67**. In a more particular embodiment, the scFv in the bispecific anti-LAG-3/anti-TIGIT antagonist comprises a linker joining anti-TIGIT HCVR to the anti-TIGIT LCVR in the second targeting domain, where the linker comprises the amino acid sequence of **SEQ ID NO:189** or **SEQ ID NO:191**.

[0138] In one embodiment, the bispecific anti-LAG-3/anti-TIGIT antagonist includes an immunoglobulin heavy chain comprising the amino acid sequence of **SEQ ID NO:192 or 193**; an immunoglobulin light chain comprising the amino acid sequence of **SEQ ID NO:181**, or both.

Anti-LAG-3 antibodies and antigen binding fragments thereof

[0139] In another aspect, the present application provides antibodies, including antigen-binding portions thereof, which specifically bind LAG-3. **FIG. 19A** shows the heavy chain CDR1, CD2 and CDR3 sequences corresponding to the anti-LAG-3 mAbs 2L2A.1, 2L2A.6, 2L27B and 3L1A. **FIG. 19B** shows the light chain CDR1, CD2 and CDR3 sequences corresponding to the anti-LAG-3 mAbs 2L2A.1, 2L2A.6, 2L27B and 3L1A. **FIG. 20** shows the VH and VL sequences of anti-LAG-3 mAbs 2L2A.1, 2L2A.6, 2L27B and 3L1A.

[0140] In one embodiment, an anti-LAG-3 antibody or antigen-binding portion thereof includes: an immunoglobulin heavy chain CDR1 (HCDR1) having at least 80%, at least 85%, or at least 90% sequence identity to an HCDR1 amino acid sequence selected from the group consisting of **SEQ ID NOS: 163, 166,** and **169**; an immunoglobulin heavy chain CDR2 (HCDR2) sequence having at least 80%, at least 85%, or at least 90% sequence identity to an HCDR2 amino acid sequence selected from the group consisting of **SEQ ID NOs. 164, 167,** and **170**; an immunoglobulin heavy chain CDR3 (HCDR3) having at least 80%, at least 85%, or at least 90% sequence identity to an HCDR3 amino acid sequence selected from the group consisting of **SEQ ID NOs. 165, 168,** and **171**; an immunoglobulin light chain CDR1 (LCDR1) having at least 80%, at least 85%, or at least 90% sequence identity to an LCDR1 amino acid sequence selected from the group consisting of **SEQ ID NOs. 172, 175,** and **177**; an immunoglobulin light chain CDR2 (LCDR2) having at least 80%, at least 85%, or at least 90% sequence identity to an LCDR2 amino acid sequence selected from the group consisting of **SEQ ID NOs. 173** and **178**; and an immunoglobulin light chain CDR3 (LCDR3) having at least 80%, at least 85%, or at least 90% sequence identity to an LCDR3 amino acid sequence selected from the group consisting of **SEQ ID NOs. 174, 176** and **179**.

[0141] In anothe embodiment, an anti-LAG-3 antibody or antigen-binding portion thereof includes: an immunoglobulin HCDR1 amino acid sequence selected from the group consisting of **SEQ ID NOS: 163, 166,** and **169**; an immunoglobulin HCDR2 amino acid sequence selected from the group consisting of **SEQ ID NOs. 164, 167,** and **170**; an immunoglobulin HCDR3 amino acid sequence selected from the group consisting of **SEQ ID NOs. 165, 168,** and **171**; an immunoglobulin LCDR1 amino acid sequence selected from the group consisting of **SEQ ID NOs. 172, 175,** and **177**; an immunoglobulin LCDR2 amino acid sequence selected from the group consisting of **SEQ ID NOs. 173** and **178**; and an immunoglobulin LCDR3 amino acid sequence selected from the group consisting of **SEQ ID NOs. 174, 176** and **179**.

[0142] In another embodiment, the anti-LAG-3 antibody or antigen-binding portion thereof includes: an immunoglobulin HCVR having at least 80%, 85%, 90%, 95% or 99% identity to an amino acid sequence selected from the group consisting of **SEQ ID NOS: 180,**

182, 184, and **186**; an immunoglobulin LCVR having at least 80%, 85%, 90%, 95% or 99% identity to an LCVR having an amino acid sequence selected from the group consisting of **SEQ ID NOs. 181, 183, 185**, and **187**; or both.

[0143] In another embodiment, the anti-LAG-3 antibody or antigen-binding portion thereof includes: an immunoglobulin HCVR having an amino acid sequence selected from the group consisting of **SEQ ID NOS: 180, 182, 184**, and **186**; an immunoglobulin LCVR having an amino acid sequence selected from the group consisting of **SEQ ID NOs. 181, 183, 185,** and **187**; or both.

[0144] In another embodiment, the anti-LAG-3 antibody or antigen-binding portion thereof includes: an immunoglobulin heavy chain sequence that is at least 80%, at least 85%, at least 90%, at least 95%, at least 99%, or 100% identical to the amino acid sequence of **SEQ ID NO: 180**; an immunoglobulin light chain sequence that is at least 80%, at least 85%, at least 90%, at least 95%, at least 99%, or 100% identical to the amino acid sequence of **SEQ ID NO:181**; or both.

[0145] In a more particular embodiment, the anti-LAG-3 antibody or antigen-binding portion thereof includes: the immunoglobulin heavy chain sequence of **SEQ ID NO:180**; the immunoglobulin light chain sequence of **SEQ ID NO:181**; or both.

[0146] In another aspect, the present application provides one or more nucleic acids encoding any anti-LAG-3 antibody or any antigen-binding portion thereof as described herein.

[0147] In another aspect, the present application provides one or more expression vectors comprising one or more nucleic acids encoding any anti-LAG-3 antibody or any antigen-binding portion thereof as described herein.

[0148] In another aspect, the present application provides a host cell transformed with the one or more nucleic acids or the one or more expression vectors encoding any anti-LAG-3 antibody or any antigen-binding portion as described herein.

[0149] In another aspect, the present application provides a bispecific antitumor antagonist comprising a first targeting domain specifically binding LAG-3; and a second targeting domain specifically binding PD-1, PD-L1 or TIGIT, where the first targeting domain comprises any of the above-described LAG-3-binding portions. Preferably, the anti-LAG-3 bispecific antitumor antagonist includes an immunoglobulin scaffold comprising one or more IgG constant regions, e.g., CH1, CH2, CH3, and/or CL.

[0150] In some embodiments, the first targeting domain is positioned at the N-terminal end and the second targeting domain is positioned at the C-terminal end. In other embodiments, the first targeting domain is positioned at the C-terminal end and the second targeting domain is

positioned at the N-terminal end. In yet other embodiments, the second targeting domain is inserted within a loop region of *e.g.*, the CH3 domain.

[0151] In one embodiment, a bispecific antitumor antagonist comprises a first targeting domain specifically binding LAG-3, and a second targeting domain specifically binding PD-1, where the first targeting domain comprises any of the anti-LAG-3 binding fragments described above, and where the second targeting domain comprises any of the PD-1 binding fragments described below. For example, in one embodiment, the first targeting domain includes the HCVR amino acid sequence of **SEQ ID NO: 180** in combination with an LCVR having the amino acid sequence of **SEQ ID NO: 181**, and the second targeting domain includes an HCVR having the amino acid sequence of **SEQ ID NO: 106** in combination with an LCVR having the amino acid sequence of **SEQ ID NO: 107**. Alternatively, the second domain may be configured in the form of a PD-1 ECD.

[0152] In another embodiment, a bispecific antitumor antagonist includes a first targeting domain specifically binding LAG-3 and a second targeting domain specifically binding PD-L1, where the first targeting domain comprises any of the anti-LAG-3 binding fragments described above, and where the second targeting domain comprises any of the PD-L1 binding fragments described below. For example, in one embodiment, the first targeting domain includes an HCVR having the amino acid sequence of **SEQ ID NO:180** in combination with an LCVR having the amino acid sequence of **SEQ ID NO: 181**, and the second targeting domain includes an HCVR having the amino acid sequence of **SEQ ID NO: 153** in combination with an LCVR having the amino acid sequence of **SEQ ID NO: 154**.

[0153] In another embodiment, a bispecific antitumor antagonist comprises a first targeting domain specifically binding LAG-3 and a second targeting domain specifically binding TIGIT, where the first targeting domain comprises any of the anti-LAG-3 binding fragments described above, and where the second targeting domain comprises any of the TIGIT binding fragments described below. For example, in one embodiment, the first targeting domain includes an HCVR having the amino acid sequence of **SEQ ID NO:180** in combination with an LCVR having the amino acid sequence of **SEQ ID NO: 181**, and the second targeting domain includes an HCVR having the amino acid sequence of **SEQ ID NO: 66** in combination with an LCVR having the amino acid sequence of **SEQ ID NO: 67**. Alternatively, the second domain may be configured in the form of a TIGIT ECD.

[0154] Exemplary immunoglobulin scaffolds include, for example, a complete CH1-CH2-CH3 segment as set forth in **SEQ ID NOS: 155-157** and **205-215**, or an Fc (hinge-CH2-CH3) comprising the amino acid sequence set forth in any one of **SEQ ID NOS: 195-202**.

Anti-TIGIT antibody and anti-TIGIT antibody fragments

[0155] In some embodiments, the checkpoint regulator antagonist includes an anti-TIGIT antibody or antigen-binding fragment(s) thereof. **FIG. 1** shows CDR sequences of anti-TIGIT mAbs, while **FIGS. 2A-2B** show several embodiments of anti-TIGIT antibody variable domain sequences for use in the present application.

[0156] In one embodiment, the anti-TIGIT antibody or antigen-binding fragment(s) thereof includes: (1) an immunoglobulin HCVR comprising three complementarity determining regions (HCDRs): HCDR1, HCDR2 and HCDR3, where the HCDR1 has an amino acid sequence that is at least 80%, at least 85%, at least 90%, at least 95%, at least 99%, or 100% identical to an amino acid sequence selected from the group consisting of **SEQ ID NOS: 1, 6, 11, 15, 17, 20** and **23**, where the HCDR2 has an amino acid sequence that is at least 80%, at least 85%, at least 90%, at least 95%, at least 99%, or 100% identical to an amino acid sequence selected from the group consisting of **SEQ ID NOS: 2, 4, 7, 9, 12, 13, 16, 18, 21** and 24, and where the HCDR3 has an amino acid sequence that is at least 80%, at least 85%, at least 90%, at least 95%, at least 99%, or 100% identical to an amino acid sequence selected from the group consisting of **SEQ ID NOS: 3, 5, 8, 10, 14, 19, 22** and **25**; and (2) an immunoglobulin LCVR comprising LCDR1, LCDR2 and LCDR3 sequences, where the LCDR1 has an amino acid sequence that is at least 80%, at least 85%, at least 90%, at least 95%, at least 99%, or 100% identical to an amino acid sequence selected from the group consisting of **SEQ ID NOS: 26, 29, 31, 33, 35, 39, 42** and **45**, wherein the LCDR2 has an amino acid sequence that is at least 80%, at least 85%, at least 90%, at least 95%, at least 99%, or 100% identical to an amino acid sequence selected from the group consisting of **SEQ ID NOS: 27, 30, 36, 37, 40, 43** and **46**, and where the LCDR3 has an amino acid sequence that is at least 80%, at least 85%, at least 90%, at least 95%, at least 99%, or 100% identical to an amino acid sequence selected from the group consisting of **SEQ ID NOS: 28, 32, 34, 38, 41, 44** and **47**; where the antibody or the antigen-binding portion thereof binds specifically to human TIGIT.

[0157] In another embodiment, the anti-TIGIT antibody or antigen-binding fragment(s) thereof includes: (1) an immunoglobulin HCVR having an amino acid sequence that is at least 80%, at least 85%, at least 90%, at least 95%, at least 99%, or 100% identical to an amino acid sequence selected from the group consisting of **SEQ ID NOS: 48, 50, 52, 54, 56, 58, 60, 62, 64**, and **66**; and (2) an immunoglobulin LCVR having an amino acid sequence that is at least 80%, at least 85%, at least 90%, at least 95%, at least 99%, or 100% identical to an amino acid sequence selected from the group consisting of **SEQ ID NOS: 49, 51, 53, 55, 57, 59, 61, 63, 65**, and **67**; where the antibody or the antigen-binding portion thereof binds specifically to human TIGIT.

Anti-PD-1 antibodies and antigen-binding fragments thereof

[0158] In some embodiments, the checkpoint regulator antagonist includes an anti-PD-1 antibody or antigen-binding fragment(s) thereof. **FIG. 3** shows CDR sequences of anti-PD-1 mAbs and **FIGS. 4A-4C** show several embodiments of anti-PD-1 antibody variable domain sequences for use in the present application.

[0159] In one embodiment, the anti-PD-1 antibody or antigen-binding fragment(s) thereof includes: (1) an immunoglobulin HCVR comprising HCDR1, HCDR2 and HCDR3 sequences, where the HCDR1 has an amino acid sequence that is at least 80%, at least 85%, at least 90%, at least 95%, at least 99%, or 100% identical to an amino acid sequence selected from the group consisting of **SEQ ID NOS: 68, 71, 74, 76**, and **79**, where the HCDR2 has an amino acid sequence that is at least 80%, at least 85%, at least 90%, at least 95%, at least 99%, or 100% identical to an amino acid sequence selected from the group consisting of **SEQ ID NOS: 69, 72, 77**, and **80**, and where the HCDR3 has an amino acid sequence that is at least 80%, at least 85%, at least 90%, at least 95%, at least 99%, or 100% identical to an amino acid sequence selected from the group consisting of **SEQ ID NOS: 70, 73, 75, 78**, and **81**; and (2) an immunoglobulin LCVR comprising LCDR1, LCDR2 and LCDR3 sequences, where the LCDR1 has an amino acid sequence that is at least 80%, at least 85%, at least 90%, at least 95%, at least 99%, or 100% identical to an amino acid sequence selected from the group consisting of **SEQ ID NOS: 82, 85, 88, 89, 90**, and **93**, where the LCDR2 has an amino acid sequence that is at least 80%, at least 85%, at least 90%, at least 95%, at least 99%, or 100% identical to an amino acid sequence selected from the group consisting of **SEQ ID NOS: 83, 86, 91**, and **94**, and where the LCDR3 has an amino acid sequence that is at least 80%, 85%, 90%, 95%, 99%, or 100% identical to an amino acid sequence selected from the group consisting of **SEQ ID NOS: 84, 87, 92**, and **95**, where the antibody or the antigen-binding portion thereof binds specifically to human PD-1.

[0160] In some embodiments, the anti-PD-1 antibody or antigen-binding fragment(s) thereof include: (1) an immunoglobulin HCVR having an amino acid sequence that is at least 80%, at least 85%, at least 90%, at least 95%, at least 99%, or 100% identical to an amino acid sequence selected from the group consisting of **SEQ ID NOS: 96, 98, 100, 102, 104**, and **106**; and (2) an immunoglobulin LCVR having an amino acid sequence that is at least 80%, at least 85%, at least 90%, at least 95%, at least 99%, or 100% identical to an amino acid sequence selected from the group consisting of **SEQ ID NOS: 97, 99, 101, 103, 105**, and **107**, where the antibody, or the antigen-binding portion thereof, binds specifically to human PD-1.

Anti-PD-L1 antibodies and antigen-binding fragment thereof

[0161] In some embodiments, the checkpoint regulator antagonist includes an anti-PD-L1 antibody or antigen-binding fragment(s) thereof. **FIG. 5** shows CDR sequences of anti-PD-L1 mAbs and **FIGS. 6A-6C** show several embodiments of anti-PD-L1 antibody variable domain sequences for use in the present application.

[0162] In one embodiment, the PD-L1 antibody or antigen-binding fragment(s) thereof includes: (1) an immunoglobulin HCVR comprising HCDR1, HCDR2 and HCDR3 sequences, where the HCDR1 has an amino acid sequence that is at least 80%, at least 85%, at least 90%, at least 95%, at least 99%, or 100% identical to an amino acid sequence selected from the group consisting of **SEQ ID NOS: 108, 111, 117**, and **120**, where the HCDR2 has an amino acid sequence that is at least 80%, at least 85%, at least 90%, at least 95%, at least 99%, or 100% identical to an amino acid sequence selected from the group consisting of **SEQ ID NOS: 109, 112, 114, 116, 118**, and **121**, where the HCDR3 has an amino acid sequence that is at least 80%, at least 85%, at least 90%, at least 95%, at least 99%, or 100% identical to an amino acid sequence selected from the group consisting of **SEQ ID NOS: 110, 113, 115, 119**, and **122**; and (2) an immunoglobulin LCVR, wherein the light chain variable region comprises three complementarity determining regions (LCDRs): LCDR1, LCDR2 and LCDR3, wherein the LCDR1 has an amino acid sequence that is at least 80%, at least 85%, at least 90%, at least 95%, at least 99%, or 100% identical to an amino acid sequence selected from the group consisting of **SEQ ID NOS: 123, 126, 130, 133**, and **136**, wherein the LCDR2 has an amino acid sequence that is at least 80%, at least 85%, at least 90%, at least 95%, at least 99%, or 100% identical to an amino acid sequence selected from the group consisting of **SEQ ID NOS: 124, 127, 131, 134**, and **137**, and wherein the LCDR3 has an amino acid sequence that is at least 80%, at least 85%, at least 90%, at least 95%, at least 99%, or 100% identical to an amino acid sequence selected from the group consisting of **SEQ ID NOS: 125, 128, 129, 132, 135**, and **138**, wherein the antibody, or the antigen-binding portion thereof, binds specifically to human PD-L1.

[0163] In some embodiments, the PD-L1 antibody or antigen-binding fragment(s) thereof include: (1) an immunoglobulin HCVR having an amino acid sequence that is at least 80%, at least 85%, at least 90%, at least 95%, at least 99%, or 100% identical to an amino acid sequence selected from the group consisting of **SEQ ID NOS: 139, 141, 143, 145, 147, 149, 151,** and **153**; and (2) an immunoglobulin LCVR having an amino acid sequence that is at least 80%, at least 85%, at least 90%, at least 95%, at least 99%, or 100% identical to an amino acid sequence selected from the group consisting of **SEQ ID NOS: 140, 142, 144, 146, 148, 150, 152** and **154**, where the antibody, or the antigen-binding portion thereof, binds specifically to human PD-L1.

II. Miscellaneous embodiments

[0164] The HCVRs and LCVRs described herein may be linked to an immunoglobulin scaffold. In some embodiments, the immunological scaffold is configured as an IgG1, IgG2 or IgG4. The immunoglobulin scaffold may include CH1-CH2-CH3 regions or it may include a naturally-occurring Fc region or a non-naturally occurring or mutated Fc region, *e.g.*, an effectorless or mostly effectorless Fc (*e.g.*, human IgG2 or IgG4) or, alternatively, an Fc with enhanced binding to one or more activating Fc receptors (FcγRI, FcγRIIa or FcγRIIIa) so as to enhance T_{reg} depletion in the tumor environment. Accordingly, in certain embodiments the anti-TIGIT, anti-PD-1, anti-PD-L1, anti-LAG-3, HCVRs and LCVRs described herein may be linked to an Fc comprising one or more modifications, typically to alter one or more functional properties of the antibody, such as serum half-life, complement fixation, Fc receptor binding, and/or antigen-dependent cellular cytotoxicity.

[0165] In one embodiment, the immunoglobulin scaffold for use in the present application includes a CH1-CH2-CH3 region having an amino acid sequence set forth in **SEQ ID NOS: 155-157** and **205-215**. In another embodiment, the immunoglobulin scaffold includes or substantially consists of an Fc receptor, such as one having the amino acid sequence set forth in any one of **SEQ ID NOS: 195-202**.

[0166] Furthermore, an antibody described herein may be chemically modified (*e.g.*, one or more chemical moieties can be attached to the antibody) or it may be modified to alter its glycosylation, to alter one or more functional properties of the antibody. More specifically, in certain embodiments, the antibodies in the present application may include modifications in the Fc region in order to generate an Fc variant with (a) increased or decreased antibody-dependent cell-mediated cytotoxicity (ADCC), (b) increased or decreased complement mediated cytotoxicity (CDC), (c) increased or decreased affinity for C1q and/or (d) increased or decreased affinity for a Fc receptor relative to the parent Fc. Such Fc region variants will generally comprise at least one amino acid modification in the Fc region. Combining amino acid modifications is thought to be particularly desirable. For example, the variant Fc region may include two, three, four, five, etc. substitutions therein, *e.g.*, of the specific Fc region positions identified herein.

[0167] For uses where effector function is to be avoided altogether, *e.g.*, when antigen binding alone is sufficient to generate the desired therapeutic benefit, and effector function only leads to (or increases the risk of) undesired side effects, IgG4 antibodies may be used, or antibodies or fragments lacking the Fc region or a substantial portion thereof can be devised, or the Fc may be mutated to eliminate glycosylation altogether (*e.g.*, N297A). Alternatively, a hybrid construct of human IgG2 (CH1 domain and hinge region) and human IgG4 (CH2

and CH3 domains) may be generated that is devoid of effector function, lacking the ability to bind FcγRs (like IgG2) and activate complement (like IgG4). When using an IgG4 constant domain, it is usually preferable to include the substitution S228P, which mimics the hinge sequence in IgG1 and thereby stabilizes IgG4 molecules, reducing Fab-arm exchange between the therapeutic antibody and endogenous IgG4 in the patient being treated.

[0168] In preferred embodiments, the the first and second targeting domains are presented in a humanized immunoglobulin scaffold. Additionally, the IgG scaffold may have a N297A or K447A amino acid substitution.

[0169] In certain embodiments, the anti-TIGIT, anti-PD-1, anti-PD-L1, anti-LAG-3, or fragments thereof may be modified to increase its biological half-life. Various approaches may be employed, including *e.g.,* that increase the binding affinity of the Fc region for FcRn. In one embodiment, the antibody is altered within the CH1 or CL region to contain a salvage receptor binding epitope taken from two loops of a CH2 domain of an Fc region of an IgG, as described in U.S. Pat. Nos. 5,869,046 and 6,121,022. The numbering of residues in the Fc region is that of the EU index. Sequence variants disclosed herein are provided with reference to the residue number followed by the amino acid that is substituted in place of the naturally occurring amino acid, optionally preceded by the naturally occurring residue at that position. Where multiple amino acids may be present at a given position, *e.g.*, if sequences differ between naturally occurring isotypes, or if multiple mutations may be substituted at the position, they are separated by slashes (*e.g.*, "X/Y/Z").

[0170] Exemplary Fc variants that increase binding to FcRn and/or improve pharmacokinetic properties include substitutions at positions 259, 308, and 434, including for example 259I, 308F, 428L, 428M, 434S, 434H, 434F, 434Y, and 434M. Other variants that increase Fc binding to FcRn include: 250E, 250Q, 428L, 428F, 250Q/428L (Hinton et al., 2004, J. Biol. Chem. 279(8): 6213-6216, Hinton et al. 2006 Journal of Immunology 176:346-356), 256A, 272A, 305A, 307A, 31 IA, 312A, 378Q, 380A, 382A, 434A (Shields et al. (2001) J. Biol. Chem., 276(9):6591-6604), 252F, 252Y, 252W, 254T, 256Q, 256E, 256D, 433R, 434F, 434Y, 252Y/254T/256E, 433K/434F/436H (Dall'Acqua et al. (2002) J. Immunol., 169:5171-5180, Dall'Acqua et al. (2006) J. Biol. Chem., 281:23514-23524, and U.S. Pat. No. 8,367,805.

[0171] Modification of certain conserved residues in IgG Fc (1253, H310, Q311, H433, N434), such as the N434A variant (Yeung et al. (2009) J. Immunol. 182:7663), have been proposed as a way to increase FcRn affinity, thus increasing the half-life of the antibody in circulation (WO 98/023289). The combination Fc variant comprising M428L and N434S has been shown to increase FcRn binding and increase serum half-life up to five-fold

(Zalevsky et al. (2010) Nat. Biotechnol. 28:157). The combination Fc variant comprising T307A, E380A and N434A modifications also extends half-life of IgG1 antibodies (Petkova et al. (2006) Int. Immunol. 18:1759). In addition, combination Fc variants comprising M252Y-M428L, M428L-N434H, M428L-N434F, M428L-N434Y, M428L-N434A, M428L-N434M, and M428L-N434S variants have also been shown to extend half-life (U.S. 2006/173170). Further, a combination Fc variant comprising M252Y, S254T and T256E was reported to increase half-life-nearly 4-fold. Dall'Acqua et al. (2006) J. Biol. Chem. 281:23514.

[0172] The bispecific antitumor antagonists of the present application may be constructed with an IgG backbone. More specifically, any of the bispecific antagonists of the present application may be constructed with an IgG1 or IgG4 backbone. Use of an IgG1 backbone is preferable for cancer treatment where a target is present on antigen presenting cells that can mediate antibody-dependent cell-mediated cytotoxicity (ADCC). Use of an IgG4 backbone allows targeting of antigen where antigen binding alone is sufficient to generate the desired therapeutic benefits. IgG4-based antagonists preclude undesirable effector functions associated with *e.g.,* IgG1 antibodies, including FcγR binding and complement activation.

Homodimers and heterodimers

[0173] One of the challenges for efficiently producing bispecific antibody preparations concerns mispairing of heavy and light chains, when co-expressing chains of different binding specificities. **Table 1** lists several amino acid substitution options for overcoming mispairing between heavy chains of different binding specificities, which "enforce" or preferentially promote correct association between desired heavy chains. Any approach to prevent or reduce mispairing between heavy chains may be used to make the bispecific antitumor antagonists according to the present disclosure.

[0174] The "knobs-into-hole" (KiH) approach relies on modifications of the interface between the two CH3 domains where most interactions occur. Typically, a bulky residue is introduced into the CH3 domain of one antibody heavy chain and acts similarly to a key. In the other heavy chain, a "hole" is formed that is able to accommodate this bulky residue, mimicking a lock. The resulting heterodimeric Fc-part can be further stabilized by artificial disulfide bridges.

[0175] An alternative approach is based on charged residues with ionic interactions or steric complementarity. This includes altering the charge polarity in the CH3 interface so that co-expression of electrostatically matched Fc domains support favorable attractive interactions and heterodimer formation while retaining the hydrophobic core, whereas

unfavorable repulsive charge interactions suppress homodimerization. See **Table 1**. The amino acid numbering in Table 1 follows the Kabat numbering scheme and can be applied to heavy chain amino acid sequences of the antibodies described herein.

[0176] In some embodiments, an immunological scaffold may be substituted with another dimer structure containing, for example, leucine zipper (LZ) domains. A leucine zipper is a common three-dimensional structural motif in proteins, typically as part of a DNA-binding domain in various transcription factors. A single LZ typically contains 4-5 leucine residues at approximately 7-residue intervals, which forms an amphipathic alpha helix with a hydrophobic region running along one side. In a particular embodiment, a heterodimeric protein scaffold comprises a LZ from the c-jun transcription factor associated with a LZ from the c-fos transcription factor. Although c-jun is known to form jun-jun homodimers and c-fos does not form homodimers, the formation of jun-fos heterodimers is greatly favored over jun-jun homodimers.

[0177] A leucine zipper domain may be incorporated in place of CH2-CH3 sequences in the protein scaffold or it may be placed at the carboxy terminal end of the two heavy chains in the bispecific antitumor antagonist. In the case of the latter, a furin cleavage site may be introduced between the carboxy terminal end of CH3 and the amino terminal end of the leucine zipper. This can facilitate furin-mediated cleavage of the leucine zipper following the heterodimerization step when co-expressing the heavy and light chains of the bispecific antitumor antagonist in an appropriate mammalian cell expression system (see Wranik et al., J. Biol. Chem., 287(5):43331-43339, 2012).

Table 1

Type	HC1	HC2
Knobs-into-holes	Y349C, T366S, L368A, Y407V	S354C, T366W
Ionic, electrostatic	S183E, E356K, E357K, D399K	S183K, K370E, K409D, K439E
Ionic, electrostatic	K392D, K409D	E356K, D399K
HA-TF substitutions	S364H, F405A	Y349T, T394F
HF-TA substitutions	S364H, T394F	Y349T, F405A
Leucine zipper heterodimer	human c-Jun leucine zipper	human c-fos leucine zipper

[0178] The amino acid numbering in **Table 1** follows the Kabat numbering scheme and can be applied to heavy chain amino acid sequences of the antibodies described herein. The mutations described in Table 1 may be applied to the sequence (published or otherwise)

of any immunoglobulin IgG1 heavy chain, as well as other immunoglobulin classes, and subclasses (or isotypes) therein.

[0179] When co-expressing heavy and light chains of monospecific, bispecific antibodies, a light chain of one binding specificity can also mispair with a heavy chain of a different binding specificity. Therefore, in certain embodiments, portions of the heavy chain, light chain or both may be modified relative to the "wild-type" antibody chains from which they are derived to prevent or reduce mispairing of both heavy chain constant regions to one another, as well mispairing of light chain constant regions to their heavy chain counterparts.

[0180] The light chain mispairing problem can be addressed in several ways. In some embodiments, sterically complementary mutations and/or disulfide bridges may be incorporated into the two VL/VH interfaces. In other embodiments, mutations can be incorporated based on ionic or electrostatic interactions. In some embodiments, light chain mispairing may be prevented or reduced by employing a first arm with an S183E mutation in the CH1 domain of the heavy chain and an S176K mutation in the CL domain of the light chain. A second arm may include an S183K mutation in the in the CH1 domain of the heavy chain and an S176E mutation in the CL domain of the light chain. In other embodiments, a "CrossMab" approach is employed, where one arm in the bispecific antitumor antagonist (e.g., Fab) is left untouched, but in the other arm containing the other binding specificity, one or more domains in the light chain are swapped with one or more domains in the heavy chain at the heavy chain:light chain interface.

[0181] Methods, immunoglobulin domain sequences, including specific mutations for preventing mispairing of heavy and light chains as disclosed above are further described in U.S. Patent Application Publication Nos. 2014/0243505, 2013/0022601.

Conjugates

[0182] In certain embodiments, the antitumor antagonists of the present application are chemically conjugated to one or more peptides and/or small molecule drugs. The peptides or small molecule drug can be the same or different. The peptides or small molecule drugs can be attached, for example to reduced SH groups and/or to carbohydrate side chains. Methods for making covalent or non-covalent conjugates of peptides or small molecule drugs with antibodies are known in the art and any such known method may be utilized.

[0183] In some embodiments the peptide or small molecule drug is attached to the hinge region of a reduced antibody component via disulfide bond formation. Alternatively, such agents can be attached using a heterobifunctional cross-linkers, such as N-succinyl 3-(2-pyridyldithio)propionate (SPDP). General techniques for such conjugation are well-known in the art. In some embodiments, the peptide or small molecule drug is conjugated via a

carbohydrate moiety in the Fc region of the antibody. The carbohydrate group can be used to increase the loading of the same agent that is bound to a thiol group, or the carbohydrate moiety can be used to bind a different therapeutic or diagnostic agent. Methods for conjugating peptide inhibitors or small molecule drugs to antibodies via antibody carbohydrate moieties are well-known to those of skill in the art. For example, in one embodiment, the method involves reacting an antibody component having an oxidized carbohydrate portion with a carrier polymer that has at least one free amine function. This reaction results in an initial Schiff base (imine) linkage, which can be stabilized by reduction to a secondary amine to form the final conjugate. Exemplary methods for conjugating small molecule drugs and peptides to antibodies are described in U.S. Patent Application Publication No. 2014/0356385.

[0184] Preferably, the antitumor antagonists in the present disclosure retain certain desirable characteristics and pharmacokinetic properties of antibodies, including a desirable *in vitro* and *in vivo* stability (*e.g.*, lone half-life and shelf-life stability), efficient delivery into desired target cells, increased affinity for binding partners, desirable antibody-dependent cell-mediated cytotoxicity and complement-dependent cytotoxicity, and reduced renal clearance or excretion. Accordingly, careful attention to size and need for particular constant region effector functions may be considered in the design of the antitumor antagonists.

[0185] The anti-TIGIT, anti-PD-1 and anti-PD-L1 inhibitors, including monospecific, bispecific antitumor antagonists therefrom, may range in size from 50 kD to 300 kD, from 50 kD to 250 kD, from 60 kD to 250 kD, from 80 kDa to 250 kD, from 100 kD to 250 kD, from 125 kD to 250 kD, from 150 kD to 250 kD, from 60 kD to 225 kD, from 75 kD to 225 kD, from 100 kD to 225 kD, from 125 kD to 225 kD, from 150 kD to 225 kD, from 60 kD to 200 kD, from 75 kD to 200 kD, from 100 kD to 125 kD to 200 kD, from 150 kD to 200 kD, from 60 kD to 150 kD, from 75 kD to 150 kD, from 100 kD to 150 kD, from 60 kD to 125 kD, from 75 kD to 125 kD, from 75 kD to 100 kD, or any range encompassed by any combination of whole numbers listed in the above cited ranges or any ranges specified by any combination of whole numbers between any of the above cited ranges.

Kits

[0186] The present application further provides a kit comprising any one or more of the checkpoint regulator antagonist or antitumor antagonist of the present application. In some embodiments, the kit further contains additional components, including syringes and needles for administration, as well as reagents, including secondary antibodies for detection, and additional human antibodies described herein for use in combination therapies therewith. A kit typically includes a label and/or instructions indicating the intended use of the contents

of the kit. The label or instruction may include any writing, or recorded material supplied on or with the kit, or which otherwise accompanies the kit.

III. Methods of Using the Antitumor Antagonists

[0187] The antitumor antagonists of the present application have numerous *in vitro* and *in vivo* utilities including, for example, enhancement of immune responses and treatment of cancers, infectious diseases or autoimmune diseases.

[0188] In certain embodiments, the present application provides a method for treating: a cell proliferative disorder; a method of reducing or depleting regulatory T cells in a tumor; a method for treating a microbial infection; or a method for treating an immunological disorder, where the method comprises administering to a subject in need thereof an effective amount of an antitumor antagonist according to the present application.

[0189] In some embodiments, the antitumor antagonists of the present application are administered to cells in culture, *in vitro* or *ex vivo*, or to human subjects, *e.g.*, *in vivo*, to enhance immunity in a variety of diseases. Accordingly, provided herein are methods of modifying an immune response in a subject comprising administering to the subject an antibody or antigen-binding fragment thereof as described herein such that the immune response in the subject is enhanced, stimulated or up-regulated. Preferred subjects include human patients in whom enhancement of an immune response would be desirable. The methods are particularly suitable for treating human patients having a disorder that can be treated by augmenting an immune response (*e.g.*, the T-cell mediated immune response). The methods are particularly suitable for treatment of cancer or chronic infections *in vivo*. For example, the anti-TIGIT, anti-PD-1, anti-PD-L1 or anti-LAG-3 compositions may be administered together with an antigen of interest or the antigen may already be present in the subject to be treated (*e.g.*, a tumor-bearing or virus-bearing subject) to enhance antigen-specific immunity. When anti-TIGIT antibodies are administered together with another agent, the two can be administered separately or simultaneously.

[0190] In some embodiments, the checkpoint regulator antagonist used in the above-described method is an anti-TIGIT, anti-PD-1, anti-PD-L1 antibody, anti-LAG-3 antibody, a fragment thereof, or combination thereof. In some embodiments, the checkpoint regulator antagonist is a monospecific or bispecific antibody.

[0191] In some embodiments, the checkpoint regulator antagonist or antitumor antagonist is in the form of an antibody or antibody fragment. In some embodiments, the antibodies described herein are human or humanized antibodies.

[0192] Also encompassed are methods for detecting and/or measuring the presence of human TIGIT, human PD-1, human PD-L1 or human LAG3 in a sample comprising

contacting the sample, and a control sample, with a human monoclonal antibody thereof, or an antigen binding fragment thereof, which specifically binds to human TIGIT, human PD-1 or human PD-L1 under conditions that allow for formation of a complex between the antibody or fragment thereof and human TIGIT, human PD-1 or human PD-L1. The formation of a complex is then detected, wherein a difference in complex formation between the sample compared to the control sample is indicative the presence of human TIGIT antigen in the sample.

[0193] Given the ability of anti-TIGIT, anti-PD-1, anti-PD-L1 and anti-LAG-3 antibodies to block inhibition or co-inhibition of T cell responses, *e.g.*, antigen-specific T cell responses, provided herein are *in vitro* and *in vivo* methods of using the antibodies described herein to stimulate, enhance or upregulate antigen-specific T cell responses, *e.g.*, anti-tumor T cell responses. In certain embodiments, CD3 stimulation is also provided (*e.g.*, by co-incubation with a cell expressing membrane CD3), which stimulation can be provided at the same time, before, or after treatment with an anti-TIGIT, anti-PD-1, anti-PD-L1 or anti-LAG-3 antibody. For example, the present application provides a method of enhancing an antigen-specific T cell response comprising contacting a T cell with an anti-TIGIT, anti-PD-1, anti-PD-L1 or anti-LAG-3 antibody described herein, and optionally with CD3, such that an antigen-specific T cell response is enhanced, *e.g.*, by removal of a TIGIT, PD-1, PD-L1 or LAG-3 mediated inhibitory effect. Any suitable indicator of an antigen-specific T cell response can be used to measure the antigen-specific T cell response. Non-limiting examples of such suitable indicators include increased T cell proliferation in the presence of the antibody and/or increase cytokine production in the presence of the antibody. In a preferred embodiment, interleukin-2 and/or interferon-.gamma. production by the antigen-specific T cell is enhanced.

[0194] Further encompassed are methods for enhancing an immune response (*e.g.*, an antigen-specific T cell response) in a subject comprising administering an anti-TIGIT antibody, an anti-PD-1 antibody, an anti-PD-L1 antibody, an anti-LAG-3 antibody, or a bispecific antitumor antagonist described herein to the subject such that an immune response (*e.g.*, an antigen-specific T cell response) in the subject is enhanced. In a preferred embodiment, the subject is a tumor-bearing subject and an immune response against the tumor is enhanced. A tumor may be a solid tumor or a liquid tumor, *e.g.*, a hematological malignancy. In certain embodiments, a tumor is an immunogenic tumor. In other embodiments, a tumor is non-immunogenic. In certain embodiments, a tumor is PD-L1 positive. In other embodiments a tumor is PD-L1 negative. A subject may also be a virus-bearing subject in which an immune response against the virus is enhanced as a consequence

of administering an anti-TIGIT antibody, anti-PD-1 antibody, anti-PD-L1 antibody, anti-LAG-3 antibody, monospecific antitumor antagonist or bispecific antitumor antagonist, as described herein.

[0195] In one embodiment, a method for inhibiting the growth of tumor cells in a subject comprises administering to the subject an anti-TIGIT antibody, anti-PD-1 antibody, anti-PD-L1 antibody, anti-LAG-3 antibody or bispecific antitumor antagonist described herein such that growth of the tumor is inhibited in the subject. Also provided are methods of treating chronic viral infection in a subject comprising administering to the subject an anti-TIGIT antibody, anti-PD-1 antibody, anti-PD-L1 antibody, anti-LAG-3 antibody or bispecific antitumor antagonist as described herein such that the chronic viral infection is treated in the subject.

[0196] Also encompassed herein are methods for depleting T_{reg} cells from the tumor microenvironment of a subject with a tumor, *e.g.*, cancerous tumor, comprising administering to the subject a therapeutically effective amount of an anti-TIGIT antibody, anti-PD-1 antibody, anti-PD-L1 antibody, anti-LAG-3 antibody, or bispecific antitumor antagonist described herein that comprises an Fc that stimulates depletion of T_{reg} cells in the tumor microenvironment. An Fc may, *e.g.*, be an Fc with effector function or enhanced effector function, such as binding or having enhanced binding to one or more activating Fc receptors.

[0197] In a preferred embodiment, T_{reg} depletion occurs without significant depletion or inhibition of T_{eff} in the tumor microenvironment, and without significant depletion or inhibition of T_{eff} cells and T_{reg} cells outside of the tumor microenvironment. In certain embodiments, the subject has higher levels of TIGIT on T_{reg} cells than on T_{eff} cells, *e.g.*, in the tumor microenvironment. In certain embodiments, anti-TIGIT antibodies or antagonists may deplete T_{regs} in tumors and/or T_{regs} in tumor infiltrating lymphocytes (TILs). For example, in the CT26 tumor model, an anti-mouse TIGIT antibody formatted as a mouse IgG2a (which exhibits effector function) partially depleted both Treg and $CD8^+$ T cells, but did not deplete $CD4^+$ T cells. An effectorless counterpart anti-TIGIT antibody, formatted as a mouse IgG1 D265A, did not deplete T cells.

[0198] When considering whether or not to employ Fc effector function or an effectorless anti-TIGIT antibody, due consideration must be given to the tradeoff between depletion of T_{regs}, which may enhance anti-tumor immune response, and depletion of $CD8^+$ T cells, which would eliminate some of the cells needed to actually kill tumor cells. Although depletion of T_{regs} might be expected to enhance anti-tumor activity, recent studies have demonstrated that ligation of TIGIT on $TIGIT^+$ T_{regs} promotes T_{reg} cell-mediated suppression of T_{eff} cell proliferation (Joller et al. (2014) Immunity 40:569), suggesting that blocking of

TIGIT signaling (*e.g.*, using an antagonist anti-TIGIT antibody of the present invention) might also enhance anti-tumor activity. Accordingly, it may be most efficacious to use an antagonist anti-TIGIT antibody lacking effector function, which: i) blocks TIGIT signaling in T_{regs} thus reducing their immunosuppressive activity; ii) activates anti-tumor $CD8^+$ T cells by blocking TIGIT's inhibitory effects, while at the same time avoiding their effector-function-mediated depletion; and iii) enhances DNAM-mediated activation by allowing DNAM to bind to PVR (CD155, the TIGIT ligand) that would otherwise have been bound by TIGIT (and by reducing direct TIGIT-DNAM interactions) (Johnston et al. (2014) Cancer Cell 26:923). The same is applicable to use of anti-PD-1 antibodies, anti-PD-L1 antibodies or bispecific antitumor antagonists.

[0199] In certain embodiments, an anti-TIGIT antibody, anti-PD-1 antibody, anti-PD-L1 antibody, anti-LAG-3 antibody or bispecific antitumor antagonist described herein is given to a subject as an adjunctive therapy. Treatment of cancer patient with an anti-TIGIT antibody, anti-PD-1 antibody, anti-PD-L1 antibody, anti-LAG-3 antibody or bispecific antitumor antagonist according to the present application may lead to a long-term durable response relative to the current standard of care; long term survival of at least 1, 2, 3, 4, 5, 10 or more years, recurrence free survival of at least 1, 2, 3, 4, 5, or 10 or more years. In certain embodiments, treatment of a cancer patient with an anti-TIGIT antibody, anti-PD-1 antibody, anti-PD-L1 antibody, anti-LAG-3 antibody or bispecific antitumor antagonist prevents recurrence of cancer or delays recurrence of cancer by, *e.g.*, 1, 2, 3, 4, 5, or 10 or more years. An anti-TIGIT, anti-PD-1, anti-PD-L1 and/or anti-LAG-3 treatment can be used as a primary or secondary line of treatment.

[0200] In certain preferred embodiments, the subject has a cell proliferative disease or cancer. Blocking of PVR/Nectin-2 signaling through TIGIT by anti-TIGIT antibodies can enhance the immune response to cancerous cells in the patient. Similarly, blocking of Provided herein are methods for treating a subject having cancer, comprising administering to the subject an anti-TIGIT, anti-PD-1, anti-PD-L1, anti-LAG-3 or bispecific antitumor antagonist thereof as described herein, such that the subject is treated, *e.g.,* such that growth of cancerous tumors is inhibited or reduced and/or that the tumors regress. An anti-TIGIT anti-PD-1, anti-PD-L1, anti-LAG-3 or bispecific antitumor antagonist thereof as described herein can be used alone to inhibit the growth of cancerous tumors. Alternatively, any of these antitumor antagonists can be used in conjunction with another agent, *e.g.*, other anti-cancer targets, immunogenic agents, standard cancer treatments, or other antibodies, as described below.

[0201] Accordingly, provided herein are methods of treating cancer, *e.g.,* by inhibiting growth of tumor cells, in a subject, comprising administering to the subject a therapeutically effective amount of an anti-TIGIT, anti-PD-1, anti-PD-L1 or anti-LAG-3 antagonist, or a bispecific antitumor antagonist, as described herein. Preferably, the antibody is a human anti-TIGIT, anti-PD-1, anti-PD-L1 or anti-LAG-3 antibody comprising the anti-TIGIT, anti-PD-1, anti-PD-L1 or anti-LAG-3 HCVRs and LCVR described herein, or it may be a chimeric, humanized, or non-human anti-hu TIGIT, anti-hu PD-1, anti-PD-L1 antibody or anti-LAG-3 antibody, *e.g.,* a chimeric, humanized, or non-human anti-TIGIT, anti-PD-1, anti-PD-L1 or anti-LAG-3 antibody that competes for binding with, or binds to the same epitope as, at least one of the anti-TIGIT, anti-PD-1, anti-PD-L1 or anti-LAG-3 antibodies described herein.

[0202] Cancers whose growth may be inhibited using the antibodies of the invention include cancers typically responsive to immunotherapy. Non-limiting examples of cancers for treatment include squamous cell carcinoma, small-cell lung cancer, non-small cell lung cancer, squamous non-small cell lung cancer (NSCLC), non NSCLC, glioma, gastrointestinal cancer, renal cancer (*e.g.* clear cell carcinoma), ovarian cancer, liver cancer, colorectal cancer, endometrial cancer, kidney cancer (*e.g.*, renal cell carcinoma (RCC)), prostate cancer (*e.g.* hormone refractory prostate adenocarcinoma), thyroid cancer, neuroblastoma, pancreatic cancer, glioblastoma (glioblastoma multiforme), cervical cancer, stomach cancer, bladder cancer, hepatoma, breast cancer, colon carcinoma, and head and neck cancer (or carcinoma), gastric cancer, germ cell tumor, pediatric sarcoma, sinonasal natural killer, melanoma (*e.g.*, metastatic malignant melanoma, such as cutaneous or intraocular malignant melanoma), bone cancer, skin cancer, uterine cancer, cancer of the anal region, testicular cancer, carcinoma of the fallopian tubes, carcinoma of the endometrium, carcinoma of the cervix, carcinoma of the vagina, carcinoma of the vulva, cancer of the esophagus, cancer of the small intestine, cancer of the endocrine system, cancer of the parathyroid gland, cancer of the adrenal gland, sarcoma of soft tissue, cancer of the urethra, cancer of the penis, solid tumors of childhood, cancer of the ureter, carcinoma of the renal pelvis, neoplasm of the central nervous system (CNS), primary CNS lymphoma, tumor angiogenesis, spinal axis tumor, brain stem glioma, pituitary adenoma, Kaposi's sarcoma, epidermoid cancer, squamous cell cancer, T-cell lymphoma, environmentally-induced cancers including those induced by asbestos, virus-related cancers (*e.g.,* human papilloma virus (HPV)-related tumor), and hematologic malignancies derived from either of the two major blood cell lineages, i.e., the myeloid cell line (which produces granulocytes, erythrocytes, thrombocytes, macrophages and mast cells) or lymphoid cell line (which produces B, T, NK and plasma cells), such as all types of

leukemias, lymphomas, and myelomas, *e.g.*, acute, chronic, lymphocytic and/or myelogenous leukemias, such as acute leukemia (ALL), acute myelogenous leukemia (AML), chronic lymphocytic leukemia (CLL), and chronic myelogenous leukemia (CML), undifferentiated AML (MO), myeloblastic leukemia (M1), myeloblastic leukemia (M2; with cell maturation), promyelocytic leukemia (M3 or M3 variant [M3V]), myelomonocytic leukemia (M4 or M4 variant with eosinophilia [M4E]), monocytic leukemia (M5), erythroleukemia (M6), megakaryoblastic leukemia (M7), isolated granulocytic sarcoma, and chloroma; lymphomas, such as Hodgkin's lymphoma (HL), non-Hodgkin's lymphoma (NEIL), B-cell lymphomas, T-cell lymphomas, lymphoplasmacytoid lymphoma, monocytoid B-cell lymphoma, mucosa-associated lymphoid tissue (MALT) lymphoma, anaplastic (*e.g.*, Ki 1+) large-cell lymphoma, adult T-cell lymphoma/leukemia, mantle cell lymphoma, angio immunoblastic T-cell lymphoma, angiocentric lymphoma, intestinal T-cell lymphoma, primary mediastinal B-cell lymphoma, precursor T-lymphoblastic lymphoma, T-lymphoblastic; and lymphoma/leukemia (T-Lbly/T-ALL), peripheral T-cell lymphoma, lymphoblastic lymphoma, post-transplantation lymphoproliferative disorder, true histiocytic lymphoma, primary central nervous system lymphoma, primary effusion lymphoma, lymphoblastic lymphoma (LBL), hematopoietic tumors of lymphoid lineage, acute lymphoblastic leukemia, diffuse large B-cell lymphoma, Burkitt's lymphoma, follicular lymphoma, diffuse histiocytic lymphoma (DHL), immunoblastic large cell lymphoma, precursor B-lymphoblastic lymphoma, cutaneous T-cell lymphoma (CTLC) (also called mycosis fungoides or Sezary syndrome), and lymphoplasmacytoid lymphoma (LPL) with Waldenstrom's macroglobulinemia; myelomas, such as IgG myeloma, light chain myeloma, nonsecretory myeloma, smoldering myeloma (also called indolent myeloma), solitary plasmocytoma, and multiple myelomas, chronic lymphocytic leukemia (CLL), hairy cell lymphoma; hematopoietic tumors of myeloid lineage, tumors of mesenchymal origin, including fibrosarcoma and rhabdomyoscarcoma; seminoma, teratocarcinoma, tumors of the central and peripheral nervous, including astrocytoma, schwannomas; tumors of mesenchymal origin, including fibrosarcoma, rhabdomyoscaroma, and osteosarcoma; and other tumors, including melanoma, xeroderma pigmentosum, keratoacanthoma, seminoma, thyroid follicular cancer and teratocarcinoma, hematopoietic tumors of lymphoid lineage, for example T-cell and B-cell tumors, including but not limited to T-cell disorders such as T-prolymphocytic leukemia (T-PLL), including of the small cell and cerebriform cell type; large granular lymphocyte leukemia (LGL) preferably of the T-cell type; a/d T-NHL hepatosplenic lymphoma; peripheral/post-thymic T cell lymphoma (pleomorphic and immunoblastic subtypes); angiocentric (nasal) T-cell lymphoma; cancer of the head or neck, renal cancer, rectal cancer, cancer of the thyroid

gland; acute myeloid lymphoma, as well as any combinations of said cancers. The methods described herein may also be used for treatment of metastatic cancers, refractory cancers (*e.g.*, cancers refractory to previous immunotherapy, *e.g.*, with a blocking CTLA-4 or PD-1 antibody), and recurrent cancers.

[0203] An anti-TIGIT, anti-PD-1 antibody, anti-PD-L1 antibody, anti-LAG-3 antibody or bispecific antitumor antagonist can be administered alone, in combination with another antitumor antagonist, or concurrently with another antitumor antagonist. An anti-TIGIT, anti-PD-1 antibody, anti-LAG-3 antibody or bispecific antitumor antagonist can also be administered in combination, or concurrently with, an immunogenic agent, such as cancerous cells, tumor vaccines, purified tumor antigens (including recombinant proteins, peptides, and carbohydrate molecules), cells transfected with genes encoding immune stimulating cytokines, in a cancer vaccine strategy (He et al. (2004) J. Immunol. 173:4919-28), or an oncolytic virus.

[0204] Many experimental strategies for vaccination against tumors have been devised. In one of these strategies, a vaccine is prepared using autologous or allogeneic tumor cells. Some of these cellular vaccines have been shown to be most effective when the tumor cells are transduced to express GM-CSF. GM-CSF has been shown to be a potent activator of antigen presentation for tumor vaccination (Dranoff et al. (1993) Proc. Natl. Acad. Sci. U.S.A. 90: 3539-43). Cancer vaccines have been shown to enhance effector T-cell infiltration into the tumors in preclinical models. The major types of cancer vaccines include peptide vaccines, vector-based antigen specific vaccines, whole-cell vaccines, and dendritic cell vaccines. All vaccine-based therapies are designed to deliver either single or multiple antigenic epitopes or antigens from the whole cells to the patients and induce tumor-specific effector T cells. Thus, a vaccine-based therapy may be the most efficient way to induce T-cell infiltration into the tumor.

[0205] The study of gene expression and large scale gene expression patterns in various tumors has led to the definition of so called tumor specific antigens (Rosenberg, S A (1999) Immunity 10: 281-7). In many cases, these tumor specific antigens are differentiation antigens expressed in the tumors and in the cell from which the tumor arose, for example melanocyte antigens gp100, MAGE antigens, and Trp-2. More importantly, many of these antigens can be shown to be the targets of tumor specific T cells found in the host.

[0206] TIGIT, PD-1, PD-L1 and/or LAG-3 inhibition may be used in conjunction with a collection of recombinant proteins and/or peptides expressed in a tumor in order to generate an immune response to these proteins. Such proteins may be viewed by the immune system as self-antigens and are therefore tolerant to them. The tumor antigen can include the

protein telomerase, which is required for the synthesis of telomeres of chromosomes and which is expressed in more than 85% of human cancers and in only a limited number of somatic tissues (Kim et al. (1994) Science 266: 2011-2013). Tumor antigens can also be "neo-antigens" expressed in cancer cells because of somatic mutations that alter protein sequence or create fusion proteins between two unrelated sequences (i.e., bcr-abl in the Philadelphia chromosome), or idiotype from B cell tumors.

[0207] Non-limiting examples of tumor vaccines include sipuleucel-T (Provenge®), an FDA-approved tumor vaccine for metastatic prostate cancer; tumor cells transfected to express the cytokine granulocyte macrophage colony-stimulating factor (GM-CSF), such as the whole cell GM-CSF-secreting irradiated, allogeneic pancreatic cancer vaccine (GVAX; Johns Hopkins); a multi-peptide vaccine consisting of immunogenic peptides derived from breast cancer antigens, neu, legumain, and β-catenin, which prolonged the vaccine-induced progression-free survival of breast tumor-bearing mice when administered in combination with anti-PD-1 antibody (Karyampudi L. et al. (2014) Cancer Res 74:2974-2985); peptides of melanoma antigens, such as peptides of gp100, MAGE antigens, Trp-2, MARTI and/or tyrosinase, or. Other tumor vaccines include proteins from viruses implicated in human cancers such as human papilloma viruses (HPV)(*e.g.*, Gardasil®, Gardasil 9®, and Cervarix®; hepatitis B virus (*e.g.*, Engerix-B and Recombivax HB); hepatitis C virus (HCV), Kaposi's sarcoma associated herpes sarcoma virus (KSHV). Another form of tumor specific antigen that can be used in conjunction with TIGIT inhibition is purified heat shock proteins (HSP) isolated from the tumor tissue itself. These heat shock proteins contain fragments of proteins from the tumor cells and these HSPs are highly efficient at delivery to antigen presenting cells for eliciting tumor immunity. Talimogene laherparepvec (T-VEC, or Imlygic®) is an FDA-approved oncolytic virus for the treatment of some patients with metastatic melanoma that cannot be surgically removed.

[0208] Dendritic cells (DC) are potent antigen presenting cells that can be used to prime antigen-specific responses. DC's can be produced *ex vivo* and loaded with various protein and peptide antigens, as well as tumor cell extracts (Nestle et al. (1998) Nature Medicine 4: 328-332). DCs can also be transduced by genetic means to express these tumor antigens as well. DCs have also been fused directly to tumor cells for the purposes of immunization (Kugler et al. (2000) Nature Medicine 6:332-336). As a method of vaccination, DC immunization may be effectively combined with TIGIT blocking to activate (unleash) more potent anti-tumor responses.

[0209] TIGIT, PD-1, PD-L1 and/or LAG-3 inhibition can also be combined with standard cancer treatments (*e.g.*, surgery, radiation, and chemotherapy). In particular, TIGIT,

PD-1, PD-L1 and/or LAG-3 inhibition can be effectively combined with chemotherapeutic regimes. In these instances, it may be possible to reduce the dose of chemotherapeutic reagent administered (Mokyr et al. (1998) Cancer Research 58: 5301-5304). An example of such a combination is an antitumor antagonist in combination with decarbazine for the treatment of melanoma. Another example of such a combination is a checkpoint regulator antagonist or antitumor antagonist in combination with interleukin-2 (IL-2) for the treatment of melanoma. For example, the scientific rationale behind the combined use of TIGIT, PD-1, PD-L1 and/or LAG-3 inhibition and chemotherapy to promote cell death is a consequence of the cytotoxic action of most chemotherapeutic compounds, should result in increased levels of tumor antigen in the antigen presentation pathway. Other combination therapies that may result in synergy with TIGIT, PD-1, PD-L1 and/or LAG-3 inhibition through cell death are radiation, surgery, and hormone deprivation. Each of these protocols creates a source of tumor antigen in the host. Angiogenesis inhibitors can also be combined with TIGIT, PD-1, PD-L1 and/or LAG-3 inhibition. Inhibition of angiogenesis leads to tumor cell death, which may feed tumor antigen into host antigen presentation pathways.

[0210] The anti-TIGIT antibodies, anti-PD-1 antibodies, anti-PD-L1 antibodies, anti-LAG-3 antibodies, and bispecific antitumor antagonists described herein may also be used in combination with bispecific antibodies that target Fcα or Fcγ receptor-expressing effectors cells to tumor cells (*see, e.g.*, U.S. Pat. Nos. 5,922,845 and 5,837,243). Bispecific antibodies can be used to target two separate antigens. For example anti-Fc receptor/antitumor antigen (*e.g.*, Her-2/neu) bispecific antibodies have been used to target macrophages to sites of tumor. This targeting may more effectively activate tumor specific responses. The T cell arm of these responses would be augmented by the inhibition of TIGIT, PD-1, PD-L1 and/or LAG-3. Alternatively, antigen may be delivered directly to DCs by the use of bispecific antibodies that bind to tumor antigen and a dendritic cell specific cell surface marker.

[0211] Tumors evade host immune surveillance by a large variety of mechanisms. Many of these mechanisms may be overcome by the inactivation of immunosuppressive proteins expressed by the tumors. These include among others TGF-β, IL-10, and Fas ligand. Antibodies to each of these entities can be used in combination with the antitumor antagonists described herein to counteract the effects of the immunosuppressive agent and favor tumor immune responses by the host.

[0212] Other antibodies that activate host immune responsiveness can be used in combination with the antitumor antagonists described herein. These include molecules on the surface of dendritic cells that activate DC function and antigen presentation. Anti-CD40 antibodies are able to substitute effectively for T cell helper activity (Ridge et al. (1998)

Nature 393: 474-478) and can be used in conjunction with anti-TIGIT antibodies. Activating antibodies to T cell costimulatory molecules, such as OX-40 (Weinberg et al. (2000) Immunol 164: 2160-2169), CD137/4-1BB (Melero et al. (1997) Nature Medicine 3: 682-685 (1997), and ICOS (Hutloff et al. (1999) Nature 397: 262-266) may also provide for increased levels of T cell activation. In addition, inhibitors of other immune checkpoint regulators may also be used in conjunction with other antitumor antagonists described herein, as further described below.

[0213] Bone marrow transplantation is currently being used to treat a variety of tumors of hematopoietic origin. While graft versus host disease is a consequence of this treatment, TIGIT inhibition may be used to increase the effectiveness of the donor engrafted tumor specific T cells by reducing graft vs. tumor responses.

[0214] *Ex vivo* activation and expansion of antigen specific T cells and adoptive transfer of these cells into recipients in order to stimulate antigen-specific T cells against cancers or viral infections in the presence of anti-TIGIT antibodies can increase the frequency and activity of the adoptively transferred T cells.

[0215] There are also several experimental treatment protocols that involve *ex vivo* activation and expansion of antigen specific T cells and adoptive transfer of these cells into recipients in order to stimulate antigen-specific T cells against tumor (Greenberg & Riddell (1999) Science 285: 546-51). These methods can also be used to activate T cell responses to infectious agents such as CMV. *Ex vivo* activation in the presence of anti-TIGIT antibodies can increase the frequency and activity of the adoptively transferred T cells.

[0216] In certain embodiments, an antitumor antagonist described herein may be administered to a subject with an infectious disease, especially chronic infections. In this case, similar to its application to cancer, antibody-mediated TIGIT, PD-1, PD-L1 and/or LAG-3 inhibition can be used alone, or as an adjuvant, in combination with vaccines, to enhance immune responsiveness to pathogens, toxins, and self-antigens. Exemplary pathogens for which this therapeutic approach can be applied include, but are not limited to, HIV, Hepatitis (A, B, & C), Influenza, Herpes, Giardia, Malaria, Leishmania, Staphylococcus aureus, and Pseudomonas aeruginosa. TIGIT, PD-1, PD-L1 and/or LAG-3 inhibition is particularly useful against established infections by agents such as HIV that present novel or altered antigens over the course of the infections. Administration of the anti-TIGIT antibodies, anti-PD-1 antibodies, anti-PD-L1 antibodies or bispecific antitumor antagonists can allow for recognition of these antigens as foreign so as to provoke an appropriate T cell response.

[0217] Other pathogenic viruses causing infections treatable by the methods described herein include HIV, hepatitis (A, B, or C), herpesvirus infections (*e.g.*, VZV, HSV-1, HAV-6, HSV-II, and CMV, Epstein Barr virus), and infections caused by an adenovirus, influenza virus, flavivirus, echoviruses, rhinoviruses, coxsackie viruses, coronaviruses, respiratory syncytial viruses, mumps viruses, rotavirus, measles virus, rubella virus, parvovirus, vaccinia virus, HTLV virus, dengue virus, papillomavirus, molluscum virus, poliovirus, rabies virus, JC virus, arboviral encephalitis virus, or combination thereof.

[0218] Exemplary pathogenic bacteria or diseases caused therefrom which may be treatable by the methods described herein include *Chlamydia, Rickettsia, Mycobacteria, Staphylococci, Streptococci, Pneumonococci, Meningococci* and *Gonococci, Klebsiella, Proteus, Serratia, Pseudomonas, Legionella, Diphtheria, Salmonella, Bacilli, Cholera, Leptospirosis* tetanus, botulism, anthrax, plague, and Lyme disease.

[0219] Exemplary pathogenic fungi causing infections treatable by the methods described herein include *Candida* (*e.g.*, *albicans, krusei, glabrata, tropicalis*, etc.), *Cryptococcus neoformans*, *Aspergillus* (*e.g.*, *fumigatus, niger*, etc.), *Mucorales* (*e.g.*, *mucor, absidia, rhizopus*), *Sporothrix schenkii*, *Blastomyces dermatitidis*, *Paracoccidioides brasiliensis*, *Coccidioides immitis* and *Histoplasma capsulatum*.

[0220] Exemplary pathogenic parasites causing infections treatable by the methods described herein include *Entamoeba histolytica*, *Balantidium coli*, *Naegleriafowleri*, *Acanthamoeba* sp., *Giardia Zambia*, *Cryptosporidium* sp., Pneumocystis carinii, *Plasmodium vivax*, *Babesia microti*, *Trypanosoma brucei*, *Trypanosoma cruzi*, *Leishmania donovani*, *Toxoplasma gondii*, *Nippostrongylus brasiliensis*.

[0221] In all of the above methods, TIGIT, PD-1, PD-L1 and/or LAG-3 inhibition can be combined with other forms of immunotherapy, such as cytokine treatment (*e.g.*, interferons, GM-CSF, G-CSF, IL-2), or bispecific antibody therapy using two different binding specificities to provide enhanced presentation of tumor antigens.

[0222] Anti-TIGIT antibodies, anti-PD-1 antibodies, anti-PD-L1 antibodies, anti-LAG-3 antibodies and bispecific antitumor antagonists described herein can be used to enhance antigen-specific immune responses by co-administration of one or more of any of these antibodies with an antigen of interest (*e.g.*, a vaccine). Accordingly, provided herein are methods of enhancing an immune response to an antigen in a subject, comprising administering to the subject: (i) the antigen; and (ii) an anti-TIGIT antibody, anti-PD-1 antibody, anti-PD-L1 antibody, anti-LAG-3 antibody or bispecific antitumor antagonist, or combination thereof, such that an immune response to the antigen in the subject is enhanced. The antigen can be, for example, a tumor antigen, a viral antigen, a bacterial antigen or an

antigen from a pathogen. Non-limiting examples of such antigens include those discussed in the sections above, such as the tumor antigens (or tumor vaccines) discussed above, or antigens from the viruses, bacteria or other pathogens described above.

[0223] In certain embodiments, a peptide or fusion protein comprising the epitope to which an anti-TIGIT antibody, anti-PD-1 antibody, anti-PD-L1 antibody, LAG-3 antibody or bispecific antitumor antagonist binds may be used as a vaccine instead of, or in addition to, the antitumor antagonist(s).

[0224] Suitable routes of administering the antibody compositions (*e.g.*, human monoclonal antibodies, multi-specific antibodies or antagonists and immunoconjugates) described herein *in vivo* and *in vitro* are well known in the art and can be selected by those of ordinary skill. For example, the antibody compositions can be administered by injection (*e.g.*, intravenous or subcutaneous). Suitable dosages of the molecules used will depend on the age and weight of the subject and the concentration and/or formulation of the antibody composition.

Combination therapies

[0225] In another aspect, the present application provides combination therapies for enhancing an antigen-specific T cell response in a subject. In one embodiment, the method includes contacting a T cell with an anti-TIGIT antibody, anti-PD-1 antibody, anti-PD-L1 antibody, anti-LAG-3 antibody, antibody fragment thereof or bispecific antitumor antagonist in combination with a second antibody, antibody fragment, antagonist or drug such that an antigen-specific T cell response or apoptotic pathway is enhanced. For example, in some embodiments, the first antibody or antibody fragment specifically binds TIGIT and the second antibody or antibody fragment specifically binds to PD-1, PD-L1 or LAG-3.

[0226] In a related aspect, a method of reducing or depleting regulatory T cells in a tumor of a subject in need thereof includes administering an effective amount of an antibody or antibody fragment in combination with a second antibody, antibody fragment, antagonist or drug such that the number of regulatory T cells in the subject is reduced.

[0227] In some embodiments, the subject has a cell proliferative disease or cancer as described herein.

[0228] In other embodiments, the subject has a chronic viral infection, inflammatory disease or autoimmune disease as described herein.

[0229] The provision of two distinct signals to T-cells is a widely accepted model for lymphocyte activation of resting T lymphocytes by antigen-presenting cells (APCs). This model further provides for the discrimination of self from non-self and immune tolerance. The primary signal, or antigen specific signal, is transduced through the T-cell receptor

(TCR) following recognition of foreign antigen peptide presented in the context of the major histocompatibility-complex (MHC). The second or co-stimulatory signal is delivered to T-cells by co-stimulatory molecules expressed on antigen-presenting cells (APCs). This induces T-cells to promote clonal expansion, cytokine secretion and effector function. In the absence of co-stimulation, T-cells can become refractory to antigen stimulation, which results in a tolerogenic response to either foreign or endogenous antigens.

[0230] In the two-signal model, T-cells receive both positive co-stimulatory and negative co-inhibitory signals. The regulation of such positive and negative signals is critical to maximize the host's protective immune responses, while maintaining immune tolerance and preventing autoimmunity. Negative signals seem necessary for induction of T-cell tolerance, while positive signals promote T-cell activation. Both co-stimulatory and co-inhibitory signals are provided to antigen-exposed T cells, and the interplay between co-stimulatory and co-inhibitory signals is essential to controlling the magnitude of an immune response. Further, the signals provided to the T cells change as an infection or immune provocation is cleared, worsens, or persists, and these changes powerfully affect the responding T cells and re-shape the immune response.

[0231] The mechanism of co-stimulation is of therapeutic interest because the manipulation of co-stimulatory signals has shown to provide a means to either enhance or terminate cell-based immune response. Recently, it has been discovered that T cell dysfunction or anergy can occur concurrently with an induced and sustained expression of immune checkpoint regulators, such as programmed death 1 polypeptide (PD-1) and its ligands, PD-L1 and PD-L2. PD-L1 is overexpressed in many cancers and is often associated with poor prognosis (Thompson R H et al., Cancer Res 2006, 66(7):3381). Further, the majority of tumor infiltrating T lymphocytes predominantly express PD-1, in contrast to T lymphocytes in normal tissues and peripheral blood T lymphocytes indicating that up-regulation of PD-1 on tumor-reactive T cells can contribute to impaired antitumor immune responses (Blood 2009 114(8):1537). This may be due to exploitation of PD-L1 signaling mediated by PD-L1 expressing tumor cells interacting with PD-1 expressing T cells to result in attenuation of T cell activation and evasion of immune surveillance. Inhibition of the PD-L1/PD-1 interaction provides a means to enhance T cell immunity, including CD8+ T cell-mediated killing of cancer cells and tumors. Similar enhancements to T cell immunity have been observed by inhibiting the binding of PD-L1 to the binding partner B7-1. Consequently, therapeutic targeting of PD-1 and other immune checkpoint regulators are an area of intense interest.

[0232] Combining inhibition of TIGIT, PD-1, PD-L1 and/or LAG-3 signaling with other signaling pathways deregulated in tumor cells can provide a means for enhance treatment efficacy. In recent years, a number of immune checkpoint regulators in the form of receptors and their ligands have been identified. One important family of membrane-bound ligands that bind to co-stimulatory or co-inhibitory receptors is the B7 family, which includes CTLA-4 and its ligands, B7-1 and B7-2; PD-1 and its ligands, PD-L1 (B7-H1) and PD-L2 (B7-DC); B7-H2 (ICOS-L), B7-H3, B7-H4, B7-H5 (VISTA), and B7-H6. Additional immune checkpoint antagonists include, but are not limited to TIM-3 and its ligand, Galectin-9; LAG-3 and its ligands, including liver sinusoidal endothelial cell lectin (LSECtin) and Galectin-3; CD122 and its CD122R ligand; CD70, B7H3, B and T lymphocyte attenuator (BTLA), and VISTA (Le Mercier et al. (2015) Front. Immunol., (6), Article 418). In addition, a number of checkpoint regulator antagonists have been identified and tested in various clinical and pre-clinical models and/or approved by the FDA (Kyi et al., FEBS Letters, 588:368-376 (2014). The concept of inhibitory receptor blockade, also known as immune checkpoint blockade, has been validated by virtue of *e.g.*, the FDA approval of the PD-1 inhibitors, nivolumab and pembrolizumab, as well as the anti-CTLA-4 antibody, ipilimumab for metastatic melanoma.

[0233] An immune checkpoint antagonist modulates or interferes with the activity of the immune checkpoint regulator so that, as a result of the binding to the checkpoint regulator or its ligand, signaling through the checkpoint regulator receptor is blocked or inhibited. By inhibiting this signaling, immune-suppression can be reversed so that T cell immunity against cancer cells can be re-established or enhanced. In contrast, an immune checkpoint agonist (of *e.g.*, a costimulatory molecule) stimulates the activity of an immune checkpoint regulator so that, as a result of the binding to the checkpoint regulator or its ligand, signaling through the checkpoint regulator receptor is stimulated. By stimulating this signaling, T cell immunity against cancer cells can be re-established or enhanced.

[0234] Accordingly, in one embodiment, a method for stimulating an immune response in a subject comprises administering to the subject an anti-TIGIT antibody, anti-PD-1 antibody, anti-PD-L1 antibody, anti-LAG-3 antibody, antibody fragment(s) thereof (*e.g.*, anti-TIGIT HCVR and/LCVRs) or bispecific antitumor antagonist described herein in combination with another immune checkpoint regulator described herein above, such that an immune response is stimulated in the subject, for example to inhibit tumor growth or to stimulate an anti-viral response.

[0235] In one embodiment, an anti-TIGIT antibody, anti-PD-1 antibody, anti-PD-L1 antibody, anti-LAG-3 antibody, antibody fragment(s) thereof, or bispecific antitumor

antagonist, according to the present application is administered in combination with another immune checkpoint regulator, either as separate antibodies or in multi-specific antibody comprising binding specificities to multiple products. Generally, an anti-TIGIT antibody, anti-PD-1 antibody, anti-PD-L1 antibody, anti-LAG-3 antibody, or bispecific antitumor antagonist, described herein can be combined to stimulate an immune response with (i) an antagonist of the IgSF family protein, B7 family or TNF family that inhibit T cell activation, or antagonist of a cytokine that inhibits T cell activation (*e.g.*, IL-6, IL-10, TGF-β, VEGF, or other immunosuppressive cytokines) and/or (ii) an agonist of a stimulatory receptors of the IgSF family, B7 family or TNF family or of cytokines to stimulate T cell activation, for stimulating an immune response.

[0236] In one embodiment, the subject is administered an anti-TIGIT antibody or HCVR and/or LCVR fragments thereof in combination with an anti-PD-1 antibody or PD-1 antagonist. In another embodiment, the subject is administered is administered an anti-TIGIT antibody or HCVR and/or LCVR fragments thereof in combination with an anti-PD-L1 antibody or PD-L1 antagonist. In another embodiment, the subject is administered an anti-TIGIT antibody or HCVR and/or LCVR fragments thereof in combination with an anti-CTLA-4 antibody or CTLA-4 antagonist.

[0237] In certain embodiments, only subjects with a cancer exhibiting high expression of a ligand for an immune checkpoint regulator are selected for combination treatment with the anti-TIGIT, anti-PD-1, anti-PD-L1 and/or anti-LAG-3 antibody, fragment thereof, or any of the bispecific antagonists of the present application. By way of example, in one embodiment, a subject with a cancer exhibiting high expression of PVR (CD155) and/or Nectin-2 (CD112) and/or low expression PD-L1 may be selected for monotherapy with anti-TIGIT antibodies, fragments thereof, or TIGIT antagonists of the present application, or combination therapy with a PD-1 antagonist or other immune checkpoint regulator.

[0238] The anti-TIGIT antibody, anti-PD-1 antibody, anti-PD-L1 antibody may be administered separately from the second antibody, antibody fragment or antagonist, or a multispecific antibody or antagonist may be administered comprising at least one binding specificity for TIGIT and a second binding specificity for the other targeted product. Further, the anti-TIGIT, anti-PD-1 antibody, anti-PD-L1 antibody, anti-LAG-3 antibody or bispecific antagonist in accordance with the present application may be co-administered with one or more additional agents, *e.g.,* antibodies, antagonists, or drugs in amount(s) effective in stimulating an immune response and/or apoptosis so as to further enhance, stimulate or upregulate an immune response and/or apoptosis in a subject.

[0239] In some embodiments, the anti-TIGIT, anti-PD-1, anti-PD-L1 or anti-LAG-3 antibody or fragment(s) thereof is administered subsequent to treatment with a different antitumor antagonist. For example, in one embodiment, anti-TIGIT, anti-PD-1, anti-PD-L1 or anti-LAG-3 antibodies may be administered only after treatment with a PD-1/PD-L1 antagonist has failed, has led to incomplete therapeutic response, or there has been recurrence of the tumor or relapse (or "PD-1 failure"). In some embodiments, cancers exhibiting such failures may be screened for expression of *e.g.*, PVR and/or Nectin-2 and only those having high level expression are treated with an anti-TIGIT, anti-PD-1, anti-PD-L1 or anti-LAG-3 antibody, fragment or antagonist of the present application.

[0240] In one embodiment, the anti-TIGIT, anti-PD-1, anti-PD-L1 or anti-LAG-3 antibody or fragment(s) thereof are administered in combination with a PD-1, PD-L1, PD-L2, TIGIT, or LAG-3 antagonist.

[0241] Other anti-PD-1 antibodies include, but are not limited to, nivolumab (BMS-936558, MDX-1106, OPDIVO™), a humanized immunoglobulin G4 (IgG4) mAb (Bristol-Myers Squibb); pembrolizumab (MK-3475, lambrolizumab, KEYTRUDA™)(Merck); pidilizumab (CT-011)(Medivation); and AMP-224 (Merck). Anti-PD-1 antibodies are commercially available, for example from ABCAM (AB137132), BIOLEGEND™ (EH12.2H7, RMP1-14) and AFFYMETRIX EBIOSCIENCE (J105, J116, MIH4).

[0242] Other anti-PD-L1 antibodies include atezolizumab (MPDL3280A, RG7446), a fully human IgG4 mAb Genentech/Roche); BMS-936559 (MDX-1105), a fully humanized IgG4 mAb (Bristol-Myers Squibb); MEDI4736, a humanized IgG antibody (Medimmune/AstraZeneca); and MSB0010718C, a fully human IgG4 monoclonal antibody (Merck, EMD Serono).

[0243] Exemplary anti-CTLA-4 antibodies for use in accordance with the present methods include ipilimumab, trevilizumab and tremelimumab.

[0244] In certain embodiments, the antitumor antagonist is a dominant negative protein of the immune checkpoint regulator. In particular embodiments, the dominant negative protein comprises an extracellular domain derived from a member selected from the group consisting of PD-L1, PD-L2, PD-1, B7-1, B7-2, B7H3, CTLA-4, LAG-3, TIM-3, TIGIT, BTLA, VISTA, CD70, and combinations thereof. In certain particular embodiments, these extracellular domains are fused to an immunoglobulin constant region or Fc receptor in the presently described antibodies. Such mutants can bind to the endogenous receptor so as to form a complex that is deficient in signaling. In certain embodiments, the extracellular domain is fused to an immunoglobulin constant region or Fc fragment or to a monomer in the oligomeric protein complex.

[0245] In certain embodiments, a dominant negative PD-L1 antagonist comprises an extracellular domain of PD-1. An exemplary dominant negative protein is AMP-224 (co-developed by Glaxo Smith Kline and Amplimmune), a recombinant fusion protein comprising the extracellular domain of PD-L2 and the Fc region of human IgG. In another embodiment, a dominant-negative PD-L1 antagonist includes one or more mutation(s) in PD-1 preventing its ability to bind PD-L1.

[0246] Exemplary immune checkpoint regulator agonists include, but are not limited to members of the tumor necrosis factor (TNF) receptor superfamily, such as CD27, CD40, OX40, GITR and 4-1BB (CD137) and their ligands, or members of the B7-CD28 superfamily, including CD28 and ICOS (CD278). Additional checkpoint regulator agonists include CD2, CDS, ICAM-1, LFA-1 (CD11a/CD18), CD30, BAFFR, HVEM, CD7, LIGHT, NKG2C, SLAMF7, NKp80, CD160, B7-H3, CD83 ligand. Immune checkpoint agonists can include antibodies or soluble fusion protein agonists comprising one or more costimulatory domains. Agonist antibodies include, but are not limited to anti-CD40 mAbs, such as CP-870,893, lucatumumab, and dacetuzumab; anti-CD137 mAbs, such as BMS-663513 urelumab, and PF-05082566; anti-OX40 mAbs; anti-GITR mAbs, such as TRX518; anti-CD27 mAbs, such as CDX-1127; and anti-ICOS mAbs.

[0247] Exemplary GITR agonists include, *e.g.*, GITR fusion proteins and anti-GITR antibodies (*e.g.*, bivalent anti-GITR antibodies) such as, *e.g.*, a GITR fusion protein described in U.S. Pat. Nos. 6,111,090 and 8,586,023; European Patent No.: 090505B1, U.S. Pat. No. PCT Publication Nos.: WO 2010/003118 and 2011/090754. Anti-GITR antibodies are described in, *e.g.*, in U.S. Pat. Nos. 7,025,962, 7,618,632, 7,812,135, 8,388,967, and 8,591,886; European Patent Nos.: 1947183B1 and 1866339; PCT Publication Nos.: WO 2011/028683, WO 2013/039954, WO2005/007190, WO 2007/133822, WO2005/055808, WO 99/40196, WO 2001/03720, WO99/20758, WO2006/083289, WO 2005/115451, WO 2011/051726. An exemplary anti-GITR antibody is TRX518.

[0248] Another family of membrane bound ligands that bind to co-stimulatory or co-inhibitory receptors is the TNF family of molecules that bind to cognate TNF receptor family members, which include CD40 and CD40L, OX-40, OX-40L, CD70, CD27L, CD30, CD30L, 4-1BBL, CD137/4-1BB, TRAIL/Apo2-L, TRAILR1/DR4, TRAILR2/DR5, TRAILR3, TRAILR4, OPG, RANK, RANKL, TWEAKR/Fn14, TWEAK, BAFFR, EDAR, XEDAR, TACI, APRIL, BCMA, LTβR, LIGHT, DcR3, HVEM, VEGI/TL1A, TRAMP/DR3, EDAR, EDA1, XEDAR, EDA2, TNFR1, Lymphotoxin α/TNF γ, TNFR2, TNFα, LTβR, Lymphotoxin α 1(32, FAS, FASL, RELT, DR6, TROY, NGFR (*see, e.g.*, Tansey, M.G. et al. (2009) Drug Discovery Today, 14(23-24):1082-1088).

[0249] Immune checkpoint agonists or costimulatory molecules include cell surface molecules other than antigen receptors or their ligands that are required for an efficient immune response, and include, but are not limited to MHC class I molecules, MHC class II molecules, TNF receptor proteins, immunoglobulin-like proteins, cytokine receptors, integrins, signaling lymphocytic activation molecules (SLAM proteins), activating NK cell receptors, BTLA, Toll ligand receptor, OX40, CD2, CD7, CD27, CD28, CD30, CD40, CDS, ICAM-1, LFA-1 (CD11a/CD18), 4-1BB (CD137), B7-H3, CDS, ICAM-1, ICOS (CD278), GITR, BAFFR, LIGHT, HVEM (LIGHTR), KIRDS2, SLAMF7, NKp80 (KLRF1), NKp44, NKp30, NKp46, CD19, CD4, CD8alpha, CD8beta, IL2R beta, IL2R gamma, IL7R alpha, ITGA4, VLA1, CD49a, ITGA4, IA4, CD49D, ITGA6, VLA-6, CD49f, ITGAD, CD11d, ITGAE, CD103, ITGAL, CD11a, LFA-1, ITGAM, CD11b, ITGAX, CD11c, ITGB1, CD29, ITGB2, CD18, LFA-1, ITGB7, NKG2D, NKG2C, TNFR2, TRANCE/RANKL, DNAM1 (CD226), SLAMF4 (CD244, 2B4), CD84, CD96 (Tactile), CEACAM1, CRTAM, Ly9 (CD229), CD160 (BY55), PSGL1, CD100 (SEMA4D), CD69, SLAMF6 (NTB-A, Ly108), SLAM (SLAMF1, CD150, IPO-3), BLAME (SLAMF8), SELPLG (CD162), LTBR, LAT, GADS, SLP-76, PAG/Cbp, CD19a, and a ligand that specifically binds with CD83.

[0250] In one aspect, T cell responses can be stimulated by a combination of the anti-TIGIT, anti-PD-1, anti-PD-L1 or anti-LAG-3 mAbs of the present invention and one or more of (i) an antagonist of a protein that inhibits T cell activation (*e.g.*, immune checkpoint inhibitors), such as CTLA-4, PD-1, PD-L1, PD-L2, LAG-3, TIM-3, Galectin 9, CEACAM-1, BTLA, CD69, Galectin-1, CD113, GPR56, VISTA, 2B4, CD48, GARP, PD-1H, LAIR1, TIM-1, CD96 and TIM-4, and (ii) an agonist of a protein that stimulates T cell activation such as B7-1, B7-2, CD28, 4-1BB (CD137), 4-1BBL, ICOS, CD40, ICOS-L, OX40, OX40L, GITR, GITRL, CD70, CD27, CD40, DR3 and CD28H.

[0251] Exemplary agents that modulate one of the above proteins and may be combined with the anti-TIGIT antibodies, anti-PD-1 antibodies, anti-PD-L1 antibodies, and/or anti-LAG-3 antibodies of the present application for treating cancer, include: YERVOY™/ipilimumab or tremelimumab (to CTLA-4), galiximab (to B7.1), OPDIVO™/nivolumab/BMS-936558 (to PD-1), pidilizumab/CT-011 (to PD-1), KEYTRUDA™/pembrolizumab/MK-3475 (to PD-1), AMP224 (to B7-DC/PD-L2), BMS-936559 (to B7-H1), MPDL3280A (to B7-H1), MEDI-570 (to ICOS), AMG557 (to B7H2), MGA271 (to B7H3), IMP321 (to LAG-3), urelumab/BMS-663513 and PF-05082566 (to CD137/4-1BB), CDX-1127 (to CD27), MEDI-6383 and MEDI-6469 (to OX40), RG-7888 (to OX40L), Atacicept (to TACI), CP-870893 (to CD40), lucatumumab (to CD40), dacetuzumab (to CD40), and muromonab-CD3 (to CD3).

[0252] Other molecules that can be combined with the antitumor antagonists described herein for the treatment of cancer include antagonists of inhibitory receptors on NK cells or agonists of activating receptors on NK cells. For example, antagonist anti-TIGIT, anti-PD-1, and/or anti-PD-L1 antibodies can be combined with antagonists of KIR (*e.g.*, lirilumab), CSF-1R antagonists, such as RG7155.

[0253] Tumors evade host immune surveillance by a large variety of mechanisms. Many of these mechanisms may be overcome by the inactivation of immunosuppressive proteins expressed by the tumors. These include among others TGF-β, IL-10, and Fas ligand. Antibodies to each of these entities can be used in combination with the antitumor antagonists described herein to counteract the effects of the immunosuppressive agent and favor tumor immune responses by the host.

[0254] Other antibodies that activate host immune responsiveness can be used in combination with the antitumor antagonists described herein. These include molecules on the surface of dendritic cells that activate DC function and antigen presentation. Anti-CD40 antibodies are able to substitute effectively for T cell helper activity and can be used in conjunction with the antitumor antagonists described herein. Activating antibodies to T cell costimulatory molecules such as OX-40, CD137/4-1BB, and ICOS may also provide for increased levels of T cell activation.

[0255] In certain embodiments, the antitumor antagonists described herein can be co-administered with one or other more therapeutic agents, *e.g.*, anti-cancer agents, radiotoxic agents or an immunosuppressive agent. Such co-administration can solve problems due to development of resistance to drugs, changes in the antigenicity of the tumor cells that would render them unreactive to the antibody, and toxicities (by administering lower doses of one or more agents).

[0256] The antitumor antagonists described herein can be linked to the agent (as an immuno-complex) or can be administered separate from the agent. In the latter case (separate administration), the antibody can be administered before, after or concurrently with the agent or can be co-administered with other known therapies, *e.g.*, an anti-cancer therapy, *e.g.*, radiation. The antitumor antagonists described herein may be co-administered with one or more anti-cancer agents so as to provide two anti-cancer agents operating synergistically via different mechanisms to yield a cytotoxic effect in human cancer cells.

[0257] The antitumor antagonists described herein may be combined with an anti-cancer agent, such an alkylating agent; an anthracycline antibiotic; an anti-metabolite; a detoxifying agent; an interferon; a polyclonal or monoclonal antibody; an EGFR inhibitor; a HER2 inhibitor; a histone deacetylase inhibitor; a hormone; a mitotic inhibitor; a

phosphatidylinositol-3-kinase (PI3K) inhibitor; an Akt inhibitor; a mammalian target of rapamycin (mTOR) inhibitor; a proteasomal inhibitor; a poly(ADP-ribose) polymerase (PARP) inhibitor; a Ras/MAPK pathway inhibitor; a centrosome declustering agent; a multi-kinase inhibitor; a serine/threonine kinase inhibitor; a tyrosine kinase inhibitor; a VEGF/VEGFR inhibitor; a taxane or taxane derivative, an aromatase inhibitor, an anthracycline, a microtubule targeting drug, a topoisomerase poison drug, an inhibitor of a molecular target or enzyme (*e.g.*, a kinase or a protein methyltransferase), a cytidine analogue or combination thereof.

[0258] Exemplary alkylating agents include, but are not limited to, cyclophosphamide (Cytoxan; Neosar); chlorambucil (Leukeran); melphalan (Alkeran); carmustine (BiCNU); busulfan (Busulfex); lomustine (CeeNU); dacarbazine (DTIC-Dome); oxaliplatin (Eloxatin); carmustine (Gliadel); ifosfamide (Ifex); mechlorethamine (Mustargen); busulfan (Myleran); carboplatin (Paraplatin); cisplatin (CDDP; Platinol); temozolomide (Temodar); thiotepa (Thioplex); bendamustine (Treanda); or streptozocin (Zanosar).

[0259] Exemplary anthracycline antibiotics include, but are not limited to, doxorubicin (Adriamycin); doxorubicin liposomal (Doxil); mitoxantrone (Novantrone); bleomycin (Blenoxane); daunorubicin (Cerubidine); daunorubicin liposomal (DaunoXome); dactinomycin (Cosmegen); epirubicin (Ellence); idarubicin (Idamycin); plicamycin (Mithracin); mitomycin (Mutamycin); pentostatin (Nipent); or valrubicin (Valstar).

[0260] Exemplary anti-metabolites include, but are not limited to, fluorouracil (Adrucil); capecitabine (Xeloda); hydroxyurea (Hydrea); mercaptopurine (Purinethol); pemetrexed (Alimta); fludarabine (Fludara); nelarabine (Arranon); cladribine (Cladribine Novaplus); clofarabine (Clolar); cytarabine (Cytosar-U); decitabine (Dacogen); cytarabine liposomal (DepoCyt); hydroxyurea (Droxia); pralatrexate (Folotyn); floxuridine (FUDR); gemcitabine (Gemzar); cladribine (Leustatin); fludarabine (Oforta); methotrexate (MTX; Rheumatrex); methotrexate (Trexall); thioguanine (Tabloid); TS-1 or cytarabine (Tarabine PFS).

[0261] Exemplary detoxifying agents include, but are not limited to, amifostine (Ethyol) or mesna (Mesnex).

[0262] Exemplary interferons include, but are not limited to, interferon alfa-2b (Intron A) or interferon alfa-2a (Roferon-A).

[0263] Exemplary polyclonal or monoclonal antibodies include, but are not limited to, trastuzumab (Herceptin); ofatumumab (Arzerra); bevacizumab (Avastin); rituximab (Rituxan); cetuximab (Erbitux); panitumumab (Vectibix); tositumomab/iodine131

tositumomab (Bexxar); alemtuzumab (Campath); ibritumomab (Zevalin; In-111; Y-90 Zevalin); gemtuzumab (Mylotarg); eculizumab (Soliris) ordenosumab.

[0264] Exemplary EGFR inhibitors include, but are not limited to, gefitinib (Iressa); lapatinib (Tykerb); cetuximab (Erbitux); erlotinib (Tarceva); panitumumab (Vectibix); PKI-166; canertinib (CI-1033); matuzumab (Emd7200) or EKB-569.

[0265] Exemplary HER2 inhibitors include, but are not limited to, trastuzumab (Herceptin); lapatinib (Tykerb) or AC-480.

[0266] Exemplary histone deacetylase inhibitors include, but are not limited to, vorinostat (Zolinza), valproic acid, romidepsin, entinostat abexinostat, givinostat, and mocetinostat.

[0267] Exemplary hormones include, but are not limited to, tamoxifen (Soltamox; Nolvadex); raloxifene (Evista); megestrol (Megace); leuprolide (Lupron; Lupron Depot; Eligard; Viadur); fulvestrant (Faslodex); letrozole (Femara); triptorelin (Trelstar LA; Trelstar Depot); exemestane (Aromasin); goserelin (Zoladex); bicalutamide (Casodex); anastrozole (Arimidex); fluoxymesterone (Androxy; Halotestin); medroxyprogesterone (Provera; Depo-Provera); estramustine (Emcyt); flutamide (Eulexin); toremifene (Fareston); degarelix (Firmagon); nilutamide (Nilandron); abarelix (Plenaxis); or testolactone (Teslac).

[0268] Exemplary mitotic inhibitors include, but are not limited to, paclitaxel (Taxol; Onxol; Abraxane); docetaxel (Taxotere); vincristine (Oncovin; Vincasar PFS); vinblastine (Velban); etoposide (Toposar; Etopophos; VePesid); teniposide (Vumon); ixabepilone (Ixempra); nocodazole; epothilone; vinorelbine (Navelbine); camptothecin (CPT); irinotecan (Camptosar); topotecan (Hycamtin); amsacrine or lamellarin D (LAM-D).

[0269] Exemplary phosphatidyl-inositol-3 kinase (PI3K) inhibitors include wortmannin an irreversible inhibitor of PI3K, demethoxyviridin a derivative of wortmannin, LY294002, a reversible inhibitor of PI3K; BKM120 (Buparlisib); Idelalisib (a PI3K Delta inhibitor); duvelisib (IPI-145, an inhibitor of PI3K delta and gamma); alpelisib (BYL719), an alpha-specific PI3K inhibitor; TGR 1202 (previously known as RP5264), an oral PI3K delta inhibitor; and copanlisib (BAY 80-6946), an inhibitor PI3Kα,δ isoforms predominantly.

[0270] Exemplary Akt inhibitors include, but are not limited to miltefosine, AZD5363, GDC-0068, MK2206, Perifosine, RX-0201, PBI-05204, GSK2141795, and SR13668.

[0271] Exemplary MTOR inhibitors include, but are not limited to, everolimus (Afinitor) or temsirolimus (Torisel); rapamune, ridaforolimus; deforolimus (AP23573), AZD8055 (AstraZeneca), OSI-027 (OSI), INK-128, BEZ235, PI-103, Torin1, PP242, PP30, Ku-0063794, WAY-600, WYE-687, WYE-354, and CC-223.

[0272] Exemplary proteasomal inhibitors include, but are not limited to, bortezomib (PS-341), ixazomib (MLN 2238), MLN 9708, delanzomib (CEP-18770), carfilzomib (PR-171), YU101, oprozomib (ONX-0912), marizomib (NPI-0052), and disufiram.

[0273] Exemplary PARP inhibitors include, but are not limited to, olaparib, iniparib, velaparib, BMN-673, BSI-201, AG014699, ABT-888, GPI21016, MK4827, INO-1001, CEP-9722, PJ-34, Tiq-A, Phen, PF-01367338 and combinations thereof.

[0274] Exemplary Ras/MAPK pathway inhibitors include, but are not limited to, trametinib, selumetinib, cobimetinib, CI-1040, PD0325901, AS703026, RO4987655, RO5068760, AZD6244, GSK1120212, TAK-733, U0126, MEK162, and GDC-0973.

[0275] Exemplary centrosome declustering agents include, but are not limited to, griseofulvin; noscapine, noscapine derivatives, such as brominated noscapine (*e.g.*, 9-bromonoscapine), reduced bromonoscapine (RBN), N-(3-brormobenzyl) noscapine, aminonoscapine and water-soluble derivatives thereof; CW069; the phenanthridene-derived poly(ADP-ribose) polymerase inhibitor, PJ-34; N2-(3-pyridylmethyl)-5-nitro-2-furamide, N2-(2-thienylmethyl)-5-nitro-2-furamide, and N2-benzyl-5-nitro-2-furamide.

[0276] Exemplary multi-kinase inhibitors include, but are not limited to, regorafenib; sorafenib (Nexavar); sunitinib (Sutent); BIBW 2992; E7080; Zd6474; PKC-412; motesanib; or AP24534.

[0277] Exemplary serine/threonine kinase inhibitors include, but are not limited to, ruboxistaurin; eril/easudil hydrochloride; flavopiridol; seliciclib (CYC202; Roscovitrine); SNS-032 (BMS-387032); Pkc412; bryostatin; KAI-9803; SF1126; VX-680; Azd1152; Arry-142886 (AZD-6244); SCIO-469; GW681323; CC-401; CEP-1347 or PD 332991.

[0278] Exemplary tyrosine kinase inhibitors include, but are not limited to, erlotinib (Tarceva); gefitinib (Iressa); imatinib (Gleevec); sorafenib (Nexavar); sunitinib (Sutent); trastuzumab (Herceptin); bevacizumab (Avastin); rituximab (Rituxan); lapatinib (Tykerb); cetuximab (Erbitux); panitumumab (Vectibix); everolimus (Afinitor); alemtuzumab (Campath); gemtuzumab (Mylotarg); temsirolimus (Torisel); pazopanib (Votrient); dasatinib (Sprycel); nilotinib (Tasigna); vatalanib (Ptk787; ZK222584); CEP-701; SU5614; MLN518; XL999; VX-322; Azd0530; BMS-354825; SKI-606 CP-690; AG-490; WHI-P154; WHI-P131; AC-220; or AMG888.

[0279] Exemplary VEGF/VEGFR inhibitors include, but are not limited to, bevacizumab (Avastin); sorafenib (Nexavar); sunitinib (Sutent); ranibizumab; pegaptanib; or vandetinib.

[0280] Exemplary microtubule targeting drugs include, but are not limited to, paclitaxel, docetaxel, vincristin, vinblastin, nocodazole, epothilones and navelbine.

[0281] Exemplary topoisomerase poison drugs include, but are not limited to, teniposide, etoposide, adriamycin, camptothecin, daunorubicin, dactinomycin, mitoxantrone, amsacrine, epirubicin and idarubicin.

[0282] Exemplary taxanes or taxane derivatives include, but are not limited to, paclitaxel and docetaxol.

[0283] Exemplary general chemotherapeutic, anti-neoplastic, anti-proliferative agents include, but are not limited to, altretamine (Hexalen); isotretinoin (Accutane; Amnesteem; Claravis; Sotret); tretinoin (Vesanoid); azacitidine (Vidaza); bortezomib (Velcade) asparaginase (Elspar); levamisole (Ergamisol); mitotane (Lysodren); procarbazine (Matulane); pegaspargase (Oncaspar); denileukin diftitox (Ontak); porfimer (Photofrin); aldesleukin (Proleukin); lenalidomide (Revlimid); bexarotene (Targretin); thalidomide (Thalomid); temsirolimus (Torisel); arsenic trioxide (Trisenox); verteporfin (Visudyne); mimosine (Leucenol); (1M tegafur-0.4 M 5-chloro-2,4-dihydroxypyrimidine-1 M potassium oxonate) or lovastatin.

[0284] In certain embodiments, TIGIT, PD-1, PD-L1 and/or LAG-3 inhibition is combined with standard cancer treatments (*e.g.*, surgery, radiation, and chemotherapy). TIGIT, PD-1, PD-L1 and/or LAG-3 inhibition can be effectively combined with chemotherapeutic regimes. In these instances, it may be possible to reduce the dose of chemotherapeutic reagent administered. An example of such a combination is an anti-TIGIT, anti-PD-1, anti-PD-L1 or anti-LAG-3 antibody in combination with decarbazine for the treatment of melanoma. Another example of such a combination is an anti-TIGIT, anti-PD-1, anti-PD-L1 or anti-LAG-3 antibody in combination with interleukin-2 (IL-2) for the treatment of melanoma. It is believed that the combined use of TIGIT, PD-1, PD-L1 and/or LAG-3 inhibition and chemotherapy can enhance apoptosis and increase tumor antigen presentation for cytotoxic immunity. Other synergistic combination therapies include TIGIT, PD-1, PD-L1 and/or LAG-3 inhibition through cell death when used in combination with radiation, surgery or hormone deprivation. Each of these protocols creates a source of tumor antigen in the host.

[0285] In certain embodiments, the checkpoint regulator antagonists described herein can be used in multi-specific antagonists or in combination with bispecific antibodies targeting Fcα or Fcγ receptor-expressing effector cells to tumor cells (*see, e.g.*, U.S. Pat. Nos. 5,922,845 and 5,837,243). Bispecific antibodies can be used to target two separate antigens. For example anti-Fc receptor/anti-tumor antigen (*e.g.*, Her-2/neu) bispecific antibodies have been used to target macrophages to cancer cells or tumors. This targeting may more effectively activate tumor specific responses. The T cell arm of these responses would be

augmented by the inhibition of TIGIT, PD-1, PD-L1 and/or LAG-3. Alternatively, antigen may be delivered directly to DCs by the use of bispecific antibodies that bind to tumor antigen and a dendritic cell specific cell surface marker.

IV. Nucleic Acids and Host Cells for Expressing Checkpoint Regulator

[0286] In another aspect, the present application provides nucleic acids encoding the antitumor antagonists of the present application, including the heavy and light chains, as well as expression vectors comprising such nucleic acids. In particular, the nucleic acids encode one or more HCDRs, LCDRs, HCVRs and/or LCVRs corresponding to any of the antibodies, antagonists or fragments described herein.

[0287] Thus, in one aspect, the present application provides one or more nucleic acids encoding any of the antitumor antagonists, antibodies or antigen-binding portions thereof as described herein.

[0288] In another aspect, the present application provides one or more expression vectors comprising the one or more nucleic acids encoding any of the antitumor antagonists, antibodies or antigen-binding portions thereof as described herein.

[0289] In another aspect, the present application provides a host cell transformed with the one or more expression vectors comprising the one or more nucleic acids encoding any of the antitumor antagonists, antibodies or antigen-binding portions thereof as described herein.

[0290] DNA(s) encoding antigen binding sites can be isolated and sequenced from a monoclonal antibody produced in hybridoma cells 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 monoclonal antibodies). Alternatively, amino acid sequences from immunoglobulins of interest may be determined by direct protein sequencing, and suitable encoding nucleotide sequences can be designed according to a universal codon table. In other cases, nucleotide and amino acid sequences of antigen binding sites or other immunoglobulin sequences, including constant regions, hinge regions and the like may be obtained from published sources well known in the art.

[0291] Expression vectors encoding a particular monospecific or bispecific antitumor antagonist may be used to synthesize the antitumor antagonists of the present disclosure in cultured cells *in vitro* or they may be directly administered to a patient to express the antitumor antagonist *in vivo* or *ex vivo*. As used herein, an "expression vector" refers to a viral or non-viral vector comprising a polynucleotide encoding one or more polypeptide chains corresponding to the monospecific or bispecific antitumor antagonists of the present disclosure in a form suitable for expression from the polynucleotide(s) in a host cell for antibody preparation purposes or for direct administration as a therapeutic agent.

[0292] A nucleic acid sequence is "operably linked" to another nucleic acid sequence when the former is placed into a functional relationship with the latter. For example, a DNA for a presequence or signal peptide 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 signal peptide, 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, synthetic oligonucleotide adaptors or linkers may be used in accordance with conventional practice.

[0293] Nucleic acid sequences for expressing the antitumor antagonists typically include an amino terminal signal peptide sequence, which is removed from the mature protein. Since the signal peptide sequences can affect the levels of expression, the polynucleotides may encode any one of a variety of different N-terminal signal peptide sequences. It will be appreciated by those skilled in the art that the design of the expression vector can depend on such factors as the choice of the host cell to be transformed, the level of expression of protein desired, and the like.

[0294] The above described "regulatory sequences" refer to DNA sequences necessary for the expression of an operably linked coding sequence in one or more host organisms. The term "regulatory sequences" is intended to include promoters, enhancers and other expression control elements (*e.g.*, polyadenylation signals). Regulatory sequences include those which direct constitutive expression of a nucleotide sequence in many types of host cells or those which direct expression of the nucleotide sequence only in certain host cells (*e.g.*, tissue-specific regulatory sequences). Expression vectors generally contain sequences for transcriptional termination, and may additionally contain one or more elements positively affecting mRNA stability.

[0295] The expression vector contains one or more transcriptional regulatory elements, including promoters and/or enhancers, for directing the expression of antitumor antagonists. A promoter comprises a DNA sequence that functions to initiate transcription from a relatively fixed location in regard to the transcription start site. A promoter contains core elements required for basic interaction of RNA polymerase and transcription factors, and may operate in conjunction with other upstream elements and response elements.

[0296] As used herein, the term "promoter" is to be taken in its broadest context and includes transcriptional regulatory elements (TREs) from genomic genes or chimeric TREs

therefrom, including the TATA box or initiator element for accurate transcription initiation, with or without additional TREs (*i.e.*, upstream activating sequences, transcription factor binding sites, enhancers, and silencers) which regulate activation or repression of genes operably linked thereto in response to developmental and/or external stimuli, and trans-acting regulatory proteins or nucleic acids. A promoter may contain a genomic fragment or it may contain a chimera of one or more TREs combined together.

[0297] Preferred promoters are those capable of directing high-level expression in a target cell of interest. The promoters may include constitutive promoters (*e.g.*, HCMV, SV40, elongation factor-1α (EF-1α)) or those exhibiting preferential expression in a particular cell type of interest. Enhancers generally refer to DNA sequences that function away from the transcription start site and can be either 5' or 3' to the transcription unit. Furthermore, enhancers can be within an intron as well as within the coding sequence. They are usually between 10 and 300 bp in length, and they function in cis. Enhancers function to increase and/or regulate transcription from nearby promoters. Preferred enhancers are those directing high-level expression in the antibody producing cell. Cell or tissue-specific transcriptional regulatory elements (TREs) can be incorporated into expression vectors to restrict expression to desired cell types. Pol III promoters (H1 or U6) are particularly useful for expressing shRNAs from which siRNAs are expressed. An expression vector may be designed to facilitate expression of the antitumor antagonist in one or more cell types.

[0298] In certain embodiments, one or more expression vectors may be engineered to express both the antitumor antagonist and one or more siRNA targeting the Tie2 pathway, the VEGF pathway or an immune checkpoint regulator.

[0299] An siRNA is a double-stranded RNA that can be engineered to induce sequence-specific post-transcriptional gene silencing of mRNAs. Synthetically produced siRNAs structurally mimic the types of siRNAs normally processed in cells by the enzyme Dicer. When expressed from an expression vector, the expression vector is engineered to transcribe a short double-stranded hairpin-like RNA (shRNA) that is processed into a targeted siRNA inside the cell. Synthetic siRNAs and shRNAs may be designed using well known algorithms and synthesized using a conventional DNA/RNA synthesizer.

[0300] To co-express the individual chains of the antitumor antagonist, a suitable splice donor and splice acceptor sequences may be incorporated for expressing both products. Alternatively, an internal ribosome binding sequence (IRES) or a 2A peptide sequence, may be employed for expressing multiple products from one promoter. An IRES provides a structure to which the ribosome can bind that does not need to be at the 5' end of the mRNA. It can therefore direct a ribosome to initiate translation at a second initiation codon within a

mRNA, allowing more than one polypeptide to be produced from a single mRNA. A 2A peptide contains short sequences mediating co-translational self-cleavage of the peptides upstream and downstream from the 2A site, allowing production of two different proteins from a single transcript in equimolar amounts. CHYSEL is a non-limiting example of a 2A peptide, which causes a translating eukaryotic ribosome to release the growing polypeptide chain that it is synthesizing without dissociating from the mRNA. The ribosome continues translating, thereby producing a second polypeptide.

[0301] An expression vector may comprise a viral vector or a non-viral vector. A viral vectors may be derived from an adeno-associated virus (AAV), adenovirus, herpesvirus, vaccinia virus, poliovirus, poxvirus, a retrovirus (including a lentivirus, such as HIV-1 and HIV-2), Sindbis and other RNA viruses, alphavirus, astrovirus, coronavirus, orthomyxovirus, papovavirus, paramyxovirus, parvovirus, picornavirus, togaviruses and the like. A non-viral vector is simply a "naked" expression vector that is not packaged with virally derived components (*e.g.*, capsids and/or envelopes).

[0302] In certain cases, these vectors may be engineered to target certain diseases or cell populations by using the targeting characteristics inherent to the virus vector or engineered into the virus vector. Specific cells may be "targeted" for delivery of polynucleotides, as well as expression. Thus, the term "targeting", in this case, may be based on the use of endogenous or heterologous binding agents in the form of capsids, envelope proteins, antibodies for delivery to specific cells, the use of tissue-specific regulatory elements for restricting expression to specific subset(s) of cells, or both.

[0303] In some embodiments, expression of the antibody chains is under the control of the regulatory element such as a tissue specific or ubiquitous promoter. In some embodiments, a ubiquitous promoter such as a CMV promoter, CMV-chicken beta-actin hybrid (CAG) promoter, a tissue specific or tumor-specific promoter to control the expression of a particular antibody heavy or light chain or single-chain derivative therefrom.

[0304] Non-viral expression vectors can be utilized for non-viral gene transfer, either by direct injection of naked DNA or by encapsulating the antitumor antagonist-encoding polynucleotides in liposomes, microparticles, microcapsules, virus-like particles, or erythrocyte ghosts. Such compositions can be further linked by chemical conjugation to targeting domains to facilitate targeted delivery and/or entry of nucleic acids into desired cells of interest. In addition, plasmid vectors may be incubated with synthetic gene transfer molecules such as polymeric DNA-binding cations like polylysine, protamine, and albumin, and linked to cell targeting ligands such as asialoorosomucoid, insulin, galactose, lactose or transferrin.

[0305] Alternatively, naked DNA may be employed. Uptake efficiency of naked DNA may be improved by compaction or by using biodegradable latex beads. Such delivery may be improved further by treating the beads to increase hydrophobicity and thereby facilitate disruption of the endosome and release of the DNA into the cytoplasm.

V. Methods for Producing Monospecific or Multispecific Antibodies

[0306] In another aspect, the present application provides host cells transformed with the anti-TIGIT, anti-PD-1, anti-PD-L1 and/or anti-LAG-3 HCVRs and/or LCVRs, encoding nucleic acids or expression vectors, or nucleic acids/expression vectors encoding the monospecific or bi-specific antitumor antagonist of the present application. The host cells can be any bacterial or eukaryotic cell capable of expressing the anti-TIGIT, anti-PD-1, anti-PD-L1 and/or anti-LAG-3 HCVRs and/or LCVRs encoding nucleic acids or expression vectors or any of the other co-administered antibodies or antagonists described herein.

[0307] In another aspect, a method of producing an antitumor antagonist comprises culturing a host cell transformed with one or more anti-TIGIT, anti-PD-1, anti-PD-L1 and/or anti-LAG-3 HCVRs and/or LCVRs encoding nucleic acids or expression vectors under conditions that allows production of the antibody or fragment, and purifying the antibody from the cell.

[0308] In a further aspect, the present application provides a method for producing an antibody comprising culturing a cell transiently or stably expressing one or more constructs encoding one or more polypeptide chains in the antibody; and purifying the antibody from the cultured cells. Any cell capable of producing a functional antibody may be used. In preferred embodiments, the antibody-expressing cell is of eukaryotic or mammalian origin, preferably a human cell. Cells from various tissue cell types may be used to express the antibodies. In other embodiments, the cell is a yeast cell, an insect cell or a bacterial cell. Preferably, the antibody-producing cell is stably transformed with a vector expressing the antibody.

[0309] One or more expression vectors encoding the antibody heavy or light chains can be introduced into a cell by any conventional method, such as by naked DNA technique, cationic lipid-mediated transfection, polymer-mediated transfection, peptide-mediated transfection, virus-mediated infection, physical or chemical agents or treatments, electroporation, *etc.* In addition, cells may be transfected with one or more expression vectors for expressing the antibody along with a selectable marker facilitating selection of stably transformed clones expressing the antibody. The antibodies produced by such cells may be collected and/or purified according to techniques known in the art, such as by centrifugation, chromatography, etc.

[0310] Examples of suitable selectable markers for mammalian cells include dihydrofolate reductase (DHFR), thymidine kinase, neomycin, neomycin analog G418, hydromycin, and puromycin. When such selectable markers are successfully transferred into a mammalian host cell, the transformed mammalian host cell can survive if placed under selective pressure. There are two widely used distinct categories of selective regimes. The first category is based on a cell's metabolism and the use of a mutant cell line which lacks the ability to grow independent of a supplemented media. Two examples are CHO DHFR$^-$ cells and mouse LTK$^-$ cells. These cells lack the ability to grow without the addition of such nutrients as thymidine or hypoxanthine. Because these cells lack certain genes necessary for a complete nucleotide synthesis pathway, they cannot survive unless the missing nucleotides are provided in a supplemented media. An alternative to supplementing the media is to introduce an intact DHFR or TK gene into cells lacking the respective genes, thus altering their growth requirements. Individual cells which were not transformed with the DHFR or TK gene will not be capable of survival in non-supplemented media.

[0311] The second category is dominant selection which refers to a selection scheme used in any cell type and does not require the use of a mutant cell line. These schemes typically use a drug to arrest growth of a host cell. Those cells which have a novel gene would express a protein conveying drug resistance and would survive the selection. Examples of such dominant selection use the drugs neomycin, mycophenolic acid, or hygromycin. The three examples employ bacterial genes under eukaryotic control to convey resistance to the appropriate drug G418 or neomycin (geneticin), xgpt (mycophenolic acid) or hygromycin, respectively. Others include the neomycin analog G418 and puromycin.

[0312] Exemplary antibody-expressing cells include human Jurkat, human embryonic kidney (HEK) 293, Chinese hamster ovary (CHO) cells, mouse WEHI fibrosarcoma cells, as well as unicellular protozoan species, such as *Leishmania tarentolae*. In addition, stably transformed, antibody producing cell lines may be produced using primary cells immortalized with c-myc or other immortalizing agents.

[0313] In one embodiment, the cell line comprises a stably transformed *Leishmania* cell line, such as *Leishmania tarentolae*. *Leishmania* are known to provide a robust, fast-growing unicellular host for high level expression of eukaryotic proteins exhibiting mammalian-type glycosylation patterns. A commercially available *Leishmania* eukaryotic expression kit is available (Jena Bioscience GmbH, Jena, Germany).

[0314] In some embodiments, the cell line expresses at least 1 mg, at least 2 mg, at least 5 mg, at least 10 mg, at least 20 mg, at least 50 mg, at least 100 mg, at least 200 mg, at least 300 mg, at least 400 mg, or at least 500 mg of the antibody/liter of culture.

[0315] The antibodies in the present application may be isolated from antibody expressing cells following culture and maintenance in any appropriate culture medium, such as RPMI, DMEM, and AIM V®. The antibodies can be purified using conventional protein purification methodologies (*e.g.*, affinity purification, chromatography, *etc.*), including the use of Protein-A or Protein-G immunoaffinity purification. In some embodiments, antibodies are engineered for secretion into culture supernatants for isolation therefrom.

VI. Pharmaceutical Compositions and Methods of Treatment

[0316] Another aspect of the present application relates to pharmaceutical compositions and methods for treating a cell proliferative disorder, such as cancer, chronic infections, or immunologically compromised disease states. In one embodiment, the pharmaceutical composition comprises one or more antitumor antagonists of the present application. In some embodiments, the antitumor antagonist(s) comprise one or more checkpoint regulator antagonists, such as anti-T cell Ig and ITIM domain (TIGIT) inhibitors, PD-1 inhibitors, PD-L1 inhibitors and LAG-3 inhibitors. The antagonist(s) are formulated together with a pharmaceutically acceptable carrier. Pharmaceutical composition of the present application may include one or more different antibodies, one or more multispecific antibodies, one or more immunoconjugates, or a combination thereof as described herein.

[0317] As described above, methods for using the pharmaceutical compositions described herein comprise administering to a subject in need thereof an effective amount of the pharmaceutical composition according to the present disclosure.

[0318] Any suitable route or mode of administration can be employed for providing the patient with a therapeutically or prophylactically effective dose of the antibody or antagonist. Exemplary routes or modes of administration include parenteral (*e.g.*, intravenous, intraarterial, intramuscular, subcutaneous, intratumoral), oral, topical (nasal, transdermal, intradermal or intraocular), mucosal (*e.g.*, nasal, sublingual, buccal, rectal, vaginal), inhalation, intralymphatic, intraspinal, intracranial, intraperitoneal, intratracheal, intravesical, intrathecal, enteral, intrapulmonary, intralymphatic, intracavital, intraorbital, intracapsular and transurethral, as well as local delivery by catheter or stent.

[0319] A pharmaceutical composition comprising an antibody or antagonist in accordance with the present disclosure may be formulated in any pharmaceutically acceptable carrier(s) or excipient(s). As used herein, the term "pharmaceutically acceptable carrier" includes any and all solvents, dispersion media, coatings, antibacterial and antifungal agents, isotonic and absorption delaying agents, and the like that are physiologically compatible. Pharmaceutical compositions may comprise suitable solid or gel phase carriers or excipients. Exemplary carriers or excipients include but are not limited to, calcium carbonate, calcium

phosphate, various sugars, starches, cellulose derivatives, gelatin, and polymers such as polyethylene glycols. Exemplary pharmaceutically acceptable carriers include one or more of water, saline, phosphate buffered saline, dextrose, glycerol, ethanol and the like, as well as combinations thereof. In many cases, it will be preferable to include isotonic agents, for example, sugars, polyalcohols such as mannitol, sorbitol, or sodium chloride in the composition. Pharmaceutically acceptable carriers may further comprise minor amounts of auxiliary substances such as wetting or emulsifying agents, preservatives or buffers, which enhance the shelf life or effectiveness of the therapeutic agents.

[0320] The antitumor antagonist can be incorporated into a pharmaceutical composition suitable for parenteral administration. Suitable buffers include but are not limited to, sodium succinate, sodium citrate, sodium phosphate or potassium phosphate. Sodium chloride can be used to modify the toxicity of the solution at a concentration of 0-300 mM (optimally 150 mM for a liquid dosage form). Cryoprotectants can be included for a lyophilized dosage form, principally 0-10% sucrose (optimally 0.5-1.0%). Other suitable cryoprotectants include trehalose and lactose. Bulking agents can be included for a lyophilized dosage form, principally 1-10% mannitol (optimally 2-4%). Stabilizers can be used in both liquid and lyophilized dosage forms, principally 1-50 mM L-Methionine (optimally 5-10 mM). Other suitable bulking agents include glycine, arginine, can be included as 0-0.05% polysorbate-80 (optimally 0.005-0.01%). Additional surfactants include but are not limited to polysorbate 20 and BRIJ surfactants.

[0321] Therapeutic antitumor antagonist preparations can be lyophilized and stored as sterile powders, preferably under vacuum, and then reconstituted in bacteriostatic water (containing, for example, benzyl alcohol preservative) or in sterile water prior to injection. Pharmaceutical composition may be formulated for parenteral administration by injection *e.g.*, by bolus injection or continuous infusion.

[0322] The therapeutic agents in the pharmaceutical compositions may be formulated in a "therapeutically effective amount" or a "prophylactically effective amount". A "therapeutically effective amount" refers to an amount effective, at dosages and for periods of time necessary, to achieve the desired therapeutic result. A therapeutically effective amount of the recombinant vector may vary depending on the condition to be treated, the severity and course of the condition, the mode of administration, whether the antibody or agent is administered for preventive or therapeutic purposes, the bioavailability of the particular agent(s), the ability of the antitumor antagonist to elicit a desired response in the individual, previous therapy, the age, weight and sex of the patient, the patient's clinical history and response to the antibody, the type of the antitumor antagonist used, discretion of the attending

physician, etc. A therapeutically effective amount is also one in which any toxic or detrimental effects of the recombinant vector is outweighed by the therapeutically beneficial effects. A "prophylactically effective amount" refers to an amount effective, at dosages and for periods of time necessary, to achieve the desired prophylactic result.

[0323] Preferably, the polypeptide domains in the antitumor antagonist are derived from the same host in which they are to be administered in order to reduce inflammatory responses against the administered therapeutic agents.

[0324] The antitumor antagonist is suitably administered to the patent at one time or over a series of treatments and may be administered to the patient at any time from diagnosis onwards. The antitumor antagonist may be administered as the sole treatment or in conjunction with other drugs or therapies useful in treating the condition in question.

[0325] As a general proposition, a therapeutically effective amount or prophylactically effective amount of the antitumor antagonist will be administered in a range from about 1 ng/kg body weight/day to about 100 mg/kg body weight/day whether by one or more administrations. In a particular embodiment, each antitumor antagonist is administered in the range of from about 1 ng/kg body weight/day to about 10 mg/kg body weight/day, about 1 ng/kg body weight/day to about 1 mg/kg body weight/day, about 1 ng/kg body weight/day to about 100 μg/kg body weight/day, about 1 ng/kg body weight/day to about 10 μg/kg body weight/day, about 1 ng/kg body weight/day to about 1 μg/kg body weight/day, about 1 ng/kg body weight/day to about 100 ng/kg body weight/day, about 1 ng/kg body weight/day to about 10 ng/kg body weight/day, about 10 ng/kg body weight/day to about 100 mg/kg body weight/day, about 10 ng/kg body weight/day to about 10 mg/kg body weight/day, about 10 ng/kg body weight/day to about 1 mg/kg body weight/day, about 10 ng/kg body weight/day to about 100 μg/kg body weight/day, about 10 ng/kg body weight/day to about 10 μg/kg body weight/day, about 10 ng/kg body weight/day to about 1 μg/kg body weight/day, 10 ng/kg body weight/day to about 100 ng/kg body weight/day, about 100 ng/kg body weight/day to about 100 mg/kg body weight/day, about 100 ng/kg body weight/day to about 10 mg/kg body weight/day, about 100 ng/kg body weight/day to about 1 mg/kg body weight/day, about 100 ng/kg body weight/day to about 100 μg/kg body weight/day, about 100 ng/kg body weight/day to about 10 μg/kg body weight/day, about 100 ng/kg body weight/day to about 1 μg/kg body weight/day, about 1 μg/kg body weight/day to about 100 mg/kg body weight/day, about 1 μg /kg body weight/day to about 10 mg/kg body weight/day, about 1 μg /kg body weight/day to about 1 mg/kg body weight/day, about 1 μg /kg body weight/day to about 100 μg/kg body weight/day, about 1 μg /kg body weight/day to about 10 μg/kg body weight/day, about 10 μg/kg body weight/day to about 100 mg/kg body

weight/day, about 10 μg /kg body weight/day to about 10 mg/kg body weight/day, about 10 μg /kg body weight/day to about 1 mg/kg body weight/day, about 10 μg /kg body weight/day to about 100 μg/kg body weight/day, about 100 μg/kg body weight/day to about 100 mg/kg body weight/day, about 100 μg /kg body weight/day to about 10 mg/kg body weight/day, about 100 μg /kg body weight/day to about 1 mg/kg body weight/day, about 1 mg/kg body weight/day to about 100 mg/kg body weight/day, about 1 mg/kg body weight/day to about 10 mg/kg body weight/day, about 10 mg/kg body weight/day to about 100 mg/kg body weight/day.

[0326] In other embodiments, the antitumor antagonist is administered at a dose of 500 μg to 20 g every three days, or 25 mg/kg body weight every three days.

[0327] In other embodiments, each antitumor antagonist is administered in the range of about 10 ng to about 100 ng per individual administration, about 10 ng to about 1 μg per individual administration, about 10 ng to about 10 μg per individual administration, about 10 ng to about 100 μg per individual administration, about 10 ng to about 1 mg per individual administration, about 10 ng to about 10 mg per individual administration, about 10 ng to about 100 mg per individual administration, about 10 ng to about 1000 mg per injection, about 10 ng to about 10,000 mg per individual administration, about 100 ng to about 1 μg per individual administration, about 100 ng to about 10 μg per individual administration, about 100 ng to about 100 μg per individual administration, about 100 ng to about 1 mg per individual administration, about 100 ng to about 10 mg per individual administration, about 100 ng to about 100 mg per individual administration, about 100 ng to about 1000 mg per injection, about 100 ng to about 10,000 mg per individual administration, about 1 μg to about 10 μg per individual administration, about 1 μg to about 100 μg per individual administration, about 1 μg to about 1 mg per individual administration, about 1 μg to about 10 mg per individual administration, about 1 μg to about 100 mg per individual administration, about 1 μg to about 1000 mg per injection, about 1 μg to about 10,000 mg per individual administration, about 10 μg to about 100 μg per individual administration, about 10 μg to about 1 mg per individual administration, about 10 μg to about 10 mg per individual administration, about 10 μg to about 100 mg per individual administration, about 10 μg to about 1000 mg per injection, about 10 μg to about 10,000 mg per individual administration, about 100 μg to about 1 mg per individual administration, about 100 μg to about 10 mg per individual administration, about 100 μg to about 100 mg per individual administration, about 100 μg to about 1000 mg per injection, about 100 μg to about 10,000 mg per individual administration, about 1 mg to about 10 mg per individual administration, about 1 mg to about 100 mg per individual administration, about 1 mg to about 1000 mg per injection, about 1 mg

to about 10,000 mg per individual administration, about 10 mg to about 100 mg per individual administration, about 10 mg to about 1000 mg per injection, about 10 mg to about 10,000 mg per individual administration, about 100 mg to about 1000 mg per injection, about 100 mg to about 10,000 mg per individual administration and about 1000 mg to about 10,000 mg per individual administration. The antitumor antagonist may be administered daily, every 2, 3, 4, 5, 6 or 7 days, or every 1, 2, 3 or 4 weeks.

[0328] In other particular embodiments, the amount of the antitumor antagonist may be administered at a dose of about 0.0006 mg/day, 0.001 mg/day, 0.003 mg/day, 0.006 mg/day, 0.01 mg/day, 0.03 mg/day, 0.06 mg/day, 0.1 mg/day, 0.3 mg/day, 0.6 mg/day, 1 mg/day, 3 mg/day, 6 mg/day, 10 mg/day, 30 mg/day, 60 mg/day, 100 mg/day, 300 mg/day, 600 mg/day, 1000 mg/day, 2000 mg/day, 5000 mg/day or 10,000 mg/day. As expected, the dosage will be dependent on the condition, size, age and condition of the patient.

[0329] In certain embodiments, the coding sequences for a antitumor antagonist are incorporated into a suitable expression vector (*e.g.*, viral or non-viral vector) for expressing an effective amount of the antitumor antagonist in patient with a cell proliferative disorder. In certain embodiments comprising administration of *e.g.*, one or more recombinant AAV (rAAV) viruses, the pharmaceutical composition may comprise the rAAVs in an amount comprising at least 10^{10}, at least 10^{11}, at least 10^{12}, at least 10^{13}, or at least 10^{14} genome copies (GC) or recombinant viral particles per kg, or any range thereof. In certain embodiments, the pharmaceutical composition comprises an effective amount of the recombinant virus, such as rAAV, in an amount comprising at least 10^{10}, at least 10^{11}, at least 10^{12}, at least 10^{13}, at least 10^{14}, at least 10^{15} genome copies or recombinant viral particles genome copies per subject, or any range thereof.

[0330] Dosages can be tested in several art-accepted animal models suitable for any particular cell proliferative disorder.

[0331] Delivery methodologies may also include the use of polycationic condensed DNA linked or unlinked to killed viruses, ligand linked DNA, liposomes, eukaryotic cell delivery vehicles cells, deposition of photopolymerized hydrogel materials, use of a handheld gene transfer particle gun, ionizing radiation, nucleic charge neutralization or fusion with cell membranes, particle mediated gene transfer and the like.

[0332] The present invention is further illustrated by the following examples which should not be construed as limiting. The contents of all references, patents and published patent applications cited throughout this application, as well as the Figures and Tables are incorporated herein by reference.

EXAMPLES

Example 1: Generation of Monoclonal Antibodies

[0333] Monoclonal antibodies (mAbs) of the present application are generated and screened using techniques well known in the art, see, *e.g.*, Harlow and Lane (1988) *Antibodies, A Laboratory Manual*, Cold Spring Harbor Publications, New York. The antigen specific hybridoma mAbs are cloned, sequenced and engineered using techniques well known in the art, see *e.g.*, Lo. B.K.C Methods in Molecular Biology™. Volume 248 2004. Antibody Engineering.

[0334] **FIG. 1** shows CDR sequences of anti-TIGIT mAbs. **FIGS. 2A-2B** show several embodiments of anti-TIGIT antibody variable domain sequences. **FIG. 3** shows CDR sequences of anti-PD-1 mAbs. **FIGS. 4A-4C** show several embodiments of anti-PD-1 antibody variable domain sequences. **FIG. 5** shows CDR sequences of anti-PD-L1 mAbs. **FIGS. 6A-6C** show several embodiments of anti-PD-L1 antibody variable domain sequences.

Example 2: Design of bispecific anti-PD-1 antibodies with anti-TIGIT scFv

[0335] **FIGS. 7A-7C** show three exemplary bispecific antitumor antagonists, Bi-TPM-93 (**FIG. 7A**), Bi-TPM-94A (**FIG. 7B**), and Bi-TPM-94B (**FIG. 7C**). These antagonists contain an anti-PD-1 (PD-01) antibody backbone (**FIG. 7A**) or an anti-PD-1 (PD-06/2P17) antibody backbone (**FIGS. 7A, 7B**) along with an anti-TIGIT scFv with heavy chain and light chain variable regions from the anti-TIGIT mAb T-10/B21 separated by a 3xG4S linker (**FIGS. 7A, 7B**) or a 6xG4S linker (**FIG. 7C**).

[0336] **FIG. 8** shows functional domain sequences present in the bispecific antibodies depicted in **FIGS. 7A-7C**.

[0337] **FIGS. 9A-9B** show exemplary heavy chain (HC) and light chain (LC) sequences corresponding to the bispecific antibodies depicted in **FIGS. 7A-7C**.

Example 3: Expression and functional characterization of bispecific anti-PD-1/anti-TIGIT antibodies

[0338] To evaluate the PD-1 blocking ability of Bi-TPM-93 and Bi-TPM-94A, a PD-1 IC50 assay was conducted in which serial dilutions of the bispecific mAbs were incubated at 4^{o} C for 30 mins with human PD-1 transfected CHOK1 cells and 7μg/ml FITC labeled human PD-L1-Fc protein, followed by washing and fixation of the cells prior to analysis with an iQue intellicyt system. The results of this assay in **FIG. 10** show that Bi-TPM-94 containing VH and VL sequences from PD-06/2P17 blocks the interaction between PD-1 and its ligand, PD-L1 better (IC50=0.15 nM) than Bi-TPM-93 containing VH and VL sequences from PD-01 (IC50=0.83 nM).

[0339] **FIG. 11** shows a non reducing PAGE analysis demonstrating robust transient expression of both Bi-TPM-94A and Bi-TPM-94B in human embryonic kidney (HEK) 293 cells.

[0340] To evaluate the degree of homogeneity of the antagonist species corresponding to Bi-TPM93, Bi-TPM-94A, and Bi-TPM-94B, the samples were purified and subjected to a size-exclusion ultra-high performance liquid chromatography (SE-UHPLC) analysis. Purification of the samples was carried out as follows. First, harvested cell culture fluids (HCCFs) were subjected to 0.2 μm filtration following by affinity purification using Hitrap Protein A HP chromatography (GE Healthcare) at 1 mL/min. Following affinity purification, the material was subjected to cation exchange (CEX) chromatography using Sepax Proteomix® SCX-NP5 columns with gradient elution at 0.8 mL/min. The amounts of aggregate (high molecular weight, HMW), dimer, and low molecular weight (LMW) fragments were determined by SE-UPLC using Tosoh TSKgel UP-G3000SWXL columns.

[0341] The results of this analysis (**FIG. 12**) unexpectedly revealed a level of species heterogeneity in Bi-TPM 93 and Bi-TPM-94A that was eliminated by linker modification in Bi-TPM-94B, i.e., increasing the length of linker from 3xG4S to 6xG4S.

[0342] To evaluate the binding affinities and kinetics of binding to His tagged human PD-1 by Bi-TPM-94A, Bi-TPM-94B, and a parental anti-PD-1 benchmark (BM) mAb, bio-layer interferometry was carried out using the Octet RED96 system (ForteBio). Briefly, 20 nM of the bispecific antagonist were loaded onto anti-human IgG capture biosensors. Association of analyte (His tagged human PD-1 protein or His tagged human TIGIT protein) was observed by placing the biosensors in wells containing serial dilution of His tagged PD-1 or His tagged TIGIT for 5 mins. Dissociation was measured after transfer of the biosensors into kinetic buffer alone and monitoring of the interferometry signal for 10 minutes. The observed on and off rates (K_a and K_d) were fit using a 1:1 binding global fit model comprising at least 5 concentrations tested, followed by calculation of the equilibrium binding constant K_D.

[0343] The results of this analysis in **FIG. 13A** show that the binding affinities of Bi-TPM-94A and Bi-TPM-94B to PD-1 are stronger than the binding affinity of a benchmark anti-PD-1 antibody to PD-1. Likewise, **FIG. 13B** shows that the binding affinities of Bi-TPM-94A and Bi-TPM-94A to TIGIT are stronger than the binding affinity of a benchmark anti-TIGIT antibody to TIGIT.

[0344] A blocking assay using CHO cells stabley expressing TIGIT and 1ug/ml biotinylated PVR-muFc was used to compare Bi-TPM-94A and Bi-TPM-94B ability to block the binding of TIGIT to its PVR ligand TIGIT. Briefly, cells were incubated with biotinylated PVR-Fc and the Bi-TPM molecules, washed and bound PVR-muFc was detected with PE

streptavidin using in the iQue Intellicyt system. **FIG. 14A** shows, both molecules similarly block the binding of TIGIT and PVR. Similarly, both molecules can block the binding of PD-1 to its PD-L1 ligand (**FIG. 14B**). The results of these assay further revealed that Bi-TPM-94A and Bi-TPM-94B exhibited IC50 values slightly better than corresponding anti-TIGIT and anti-PD-1 benchmark (BM) antibodies.

[0345] To determine whether Bi-TPM-94A and Bi-TPM-94B can bind both PD-1 and TIGIT simultaneously, huPD-1-Fc-coated (5μg/ml) 96 well ELISA plates were blocked with 1% BSA in PBS and incubated for 2 hours with serial dilutions of the anti-PD-1/anti-TIGIT bispecific antibodies, followed by addition and incubation for 2 hours with His-tagged huTIGIT protein. After washing, HRP conjugated anti-His tag Ab and TMB substrate were added as detection agents and quantified with a Perkin Elmer multimode plate reader. The results of this assay in **FIG. 15** show simultaneous binding of PD-1 and TIGIT by Bi-TPM-94A and Bi-TPM-94B.

[0346] To evaluate the ability of Bi-TPM-94B to induce IFN-γ production, 250,000 human PBMCs from donors screened for CMV antigen reactivity, i.e., Donor 287 (**FIG. 16A**) and Donor 401 (**FIG. 16B**) were stimulated with 0.1 μg/ml of CMV-infected cell lysates (lanes 2-7) to stimulate CMV reactive T cells or not stimulated with CMV-infected cell lysates (lane 1). Shp-77 cells were co-cultured with the PBMCs to provide an immune function inhibitory environment and further incubated with human IgG (lane 3), parental anti-TIGIT mAb B21-35 (lane 4), parental anti-PD-1 mAb 2P17 (lane 5), parental anti-TIGIT mAb B21-35 in combination with parental anti-PD-1 mAb 2P17 (lane 6), or Bi-TPM-94B (lane 7). 5 days later, cell culture supernatants were examined for IFN-γ production by ELISA.

[0347] The results of this analysis in **FIGS. 16A-16B** show increased IFN-γ secretion from human PBMCs (Donor 287, **FIG. 16A**; Donor 401, **FIG. 16B**) with Bi-TPM-94B relative to the monospecific parental mAbs or the combination of monospecific parental anti-PD-1 and anti-TIGIT antibodies, as well as the negative controls.

[0348] To evaluate the ability of Bi-TPM-94B to induce T cell proliferation, 250,000 human PBMCs from the Donor 287 (**FIG. 17A**) and Donor 401 (**FIG. 17B**) were stimulated with 0.1 μg/ml of CMV-infected cell lysates for 2 days to stimulate CMV reactive T cells and then labeled with carboxyfluorescein succinimidyl ester (CFSE). The CFSE-labeled PBMCs were then co-cultured with Shp-77 cells to provide an immune function inhibitory environment and further incubated with human IgG (lane 1), parental anti-TIGIT mAb B21-35 (lane 2), parental anti-PD-1 mAb 2P17 (lane 3), parental anti-TIGIT mAb B21-35 in combination with parental anti-PD-1 mAb 2P17 (lane 4), or Bi-TPM-94B (lane 5). 5 days later, CSFE signal on

CD3+ T cells were analyzed with an iQue intellicyt system and a proliferation index was calculated, based on the loss of CFSE signal.

[0349] The results of this analysis in **FIGS. 17A-17B** show that Bi-TPM-94B enhances proliferation of primary human T cells from Donor 287 PBMCs (**FIG. 17A**) and Donor 401 PBMCs (**FIG. 17B**) to a greater extent than the individual or combination of parental anti-PD-1 and anti-TIGIT antibodies, as well as the negative controls.

[0350] To evaluate the pharmacokinetic properties of Bi-TPM-94A and Bi-TPM-94B *in vivo*, a pharmacokinetic profile was generated. Briefly, 10 mg/kg of Bi-TMP-94A or Bi-TPM-94B was intravenously injected into the tail vein of 6-10 week old female CD1 mice (n=2 mice). Serum was harvested at 3 minutes, 3 hours, 1 day, 3 days, 7 days and 10 days post injection. To detect the antibodies in the serum, 96 well ELISA plates were coated with 5 μg/ml goat anti-human IgG (Fc specific) F(ab')2 fragment (Sigma, #SAB3701274) and then blocked with 5% milk in PBS. Following the blocking step, both the mouse serum and purified Bi-TPM-94A or Bi-TPM-94B molecules (as a standards) were serially diluted in 5% milk, and were then added to the plate and incubated for 2 hr. After the incubation, the wells were washed and then incubated with Peroxidase AffiniPure Mouse Anti-Human IgG Fcγ Fragment Specific (Jackson ImmunoResearch #209-035-098) and TMB-ELISA Substrate Solution (Thermo Scientific #34029) and quantified by OD650 signal with a Perkin Elmer multimode plate reader.

[0351] The results of this analysis in **FIG. 18** showed that the half-life ($T_{1/2}$) of the bispecific antagonist Bi-TPM-94A and Bi-TMP-94B are 7-10 days. Thus, the Bi-TPM-94A and B molecules have the improved property of higher affinity binding as compared to Bi-TPM-93, and Bi-TPM-94B, with the extended G4S linker in the anti-TIGIT scfv, is further improved as compared to Bi-TPM-93 and Bi-TPM-94A with regards to homogeneity.

Example 4: Identification and functional characterization of anti-LAG-3 monoclonal antibodies

[0352] In another aspect, the present application relates to the screening and characterization of monoclonal antibodies or the antigen-binding portions thereof that specifically bind to the human immune checkpoint regulator, LAG-3. **FIG. 19A** shows the heavy chain CDR1, CD2 and CDR3 sequences corresponding to the anti-LAG-3 mAbs 2L2A.1, 2L2A.6, 2L27B and 3L1A that were isolated. **FIG. 19B** shows the light chain CDR1, CD2 and CDR3 sequences corresponding to the anti-LAG-3 mAbs 2L2A.1, 2L2A.6, 2L27B and 3L1A. **FIG. 20** shows the VH and VL sequences of anti-LAG-3 mAbs 2L2A.1, 2L2A.6, 2L27B and 3L1A.

[0353] A blocking assay was carried out to show that the mAbs 2L2A and 2L27B (**FIG. 21A**) and 2L37A and 3L1A (**FIG. 21B**) to block the interaction between human LAG-

3 and its major ligand, major histocompatibility complex II (MHC II) antigen expressed on Raji cells. Briefly, 2-fold serial dilutions of the mAbs 2L2A and 2L27B (**FIG. 21A**) and 2L37A and 3L1A (**FIG. 21B**) were prepared. The serial dilutions and human LAG-3-huFc were incubated for 30 mins at room temperature and then added to Raji cells and further incubated for 30 minutes on ice. Then, Raji cells were washed and LAG-3-huFc binding on Raji cells was detected with anti-human IgG PE. Cells were fixed prior to analysis with an iQue Intellicyt system. The results of this analysis in **FIGS. 21A-21B** confirm the ability of the mouse anti-LAG-3 mAbs to block the interaction between LAG-3 and its major ligand, major histocompatibility complex II (MHC II) antigen to a degree comparable to that of an anti-LAG-3 benchmark (BM) mAb.

[0354] The above-described blocking assay was further used to calculate IC50 values reflecting inhibition of binding of LAG-3 to MHC II by anti-LAG-3 antibodies, specifically, a humanized anti-LAG-3 variant, 2L2A.1, an anti-LAG-3 benchmark (BM) antibody (BMS-986016, Bristol-Myers Squibb), and a chimeric 2L2A antibody comprising mouse 2L2A CDRs in a human Ig.

[0355] The results of these assay in **FIG. 22** show that the humanized anti-LAG-3 mAb 2L2A.1 is a better blocker than the the BM mAb and the chimeric 2L2A antibody comprising mouse 2L2A CDRs in a human Ig as reflected in the lower IC50 value obtained.

[0356] To evaluate the binding affinities and kinetics of binding to His tagged human LAG-3 by the anti-LAG-3 mAb 2L2A.1, bio-layer interferometry was carried out using the Octet RED96 system (ForteBio), essentially as described above in Example 3 above with reference to **FIGS. 13A-13B** as determined by surface plasmon resonance (SPR), along with corresponding binding affinity constants. **FIG. 23A** shows the binding affinities of the humanized 2L2A.1 antibody to His-tagged human LAG-3. **FIG. 23B** similarly shows the binding affinity constants for binding of the humanized 2L2A.1 antibody to human LAG-3 fused to mouse IgG2a (huLAG-3-mIgG2a).

[0357] To further evaluate the ability of 2L2A.1 to bind human LAG-3, serial dilutions of 2L2A.1 or a LAG-3 benchmark (BM) antibody were added to CHO-K1 cells (20,000 cells/well) expressing human LAG-3. The mixtures were incubated at 4° C for 20 min, washed 3 times, and stained with the secondary antibody, PE labeled F(ab')2-Goat anti-human IgG Fc (Thermo Scientific #H10104) by incubation at 4°C for 20 min. Cells were washed and resuspended in 7AAD solution and fixed in 10% neutral buffered formalin solution for 15 minutes before analysis with the iQue Intellicyt system. Corresponding EC50 values reflecting the half maximal effective concentrations (EC_{50}) producing a response halfway between the baseline and maximum response with respect to binding human LAG-3 were also determined.

As shown in **FIG. 24**, the results show that 2L2A.1 has a higher affinity of binding for human LAG-3 than the BM antibody.

[0358] **FIG. 25** shows a non-denaturing polyacrylamide gel (PAGE) analysis demonstrating robust expression of humanized anti-LAG-3 mAb variant 2L2A.1 in transiently expressed human embryonic kidney (HEK) 293 cells as compared to a control (ctrl) antibody.

[0359] To evaluate whether LAG-3 and PD-1 are coexpressed in activated human CD3+ T cells, FACS analysis was carried out on donor PBMCs activated with anti-human CD3/CD28. The results of this analysis in **FIG. 26** confirmed the co-expression of LAG-3 and PD-1 by human PBMCs activated with Staphylococcal enterotoxin B (SEB).

[0360] To evaluate the ability of 2L2A.1 to induce IFN-γ production, 100,000 human PBMCs from two donors, i.e., Donor 0105 (**FIG. 27A**) and Donor 0817 (**FIG. 27B**) were plated on a 96-well ELISA plate. PBMCs were either not stimulated (lane 1) or stimulated with 0.5 μg/ml of Staphylococcal enterotoxin B (SEB; lanes 2-4). To the stimulated cells, Benchmark (BM) anti-LAG-3 mAb (lane 3), the 2L2A.1 mAb (lane 4), or an isotype-matched control antibody (lane 2), were added and incubated at 37°C for 5 days. Cell culture supernatants were then examined for IFN-γ production by ELISA. The results in **FIGS. 27A-27B** show increased IFN-γ secretion from both human donor PBMCs with 2L2A.1 relative to the anti-LAG-3 BM.

[0361] A similar analysis was done, but also assessed proliferation of the PBMCs with 3 additional donor PBMCs, Donor 223 (**FIG. 28A**), Donor 224 (**FIG. 28B**), and Donor 225 (**FIG. 28C**). The results in **FIGS. 28A-28B** further establish increased IFN-γ secretion from human donor PBMCs with 2L2A.1 relative to the anti-LAG-3 BM. To assess proliferation, the 3 donor PBMCs were labeled with CSFE, stimulated with 100ng/ml SEB, and Benchmark (BM) anti-LAG-3 mAb (lane 3), the 2L2A.1 mAb (lane 4), or an isotype-matched control antibody (lane 2), were added. The PBMC mixtures were incubated at 37°C for 5 days, and loss of CFSE signal on CD4 T cells were quantified by FACS to generate a Proliferation Index. The results shown in **FIGS. 29A and 29B** establish that 2L2A.1 can induce more primary T cell proliferation than a benchmark (BM) anti-LAG-3 mAb.

Example 5: Design and functional characterization of bispecific anti-LAG-3/anti-TIGIT scFv antagonist

[0362] Based on the design and characterization of the bispecific anti-PD-1/anti-TIGIT scFv described in Example 1, it was of interest to evaluate whether the benefits in manufacturability and functionality of this design can be extended to an analogous bispecific anti-LAG-3/anti-TIGIT scFv design. **FIGS. 30A-30B** depict two exemplary bispecific antitumor antagonists, Bi-LT-1, with the extended scfv linker 4xG4S (**FIG. 30A**) and Bi-LT-3, with the extended scfv linker 6xG4S (**FIG. 30B**). **FIG. 31** summarizes the arrangement of

functional domains in the bispecific antagonists depicted in **FIGS. 30A-30B**. **FIG. 32** show the heavy chain (HC) and light chain (LC) amino acid sequences corresponding to the bispecific antagonists depicted in **FIGS. 30A-30B**.

[0363] To evaluate the ability of the bispecific antitumor antagonists, Bi-LT-1 and Bi-LT-3 to block the interaction between TIGIT and its ligand, human PVR (CD155), and additionally block the interaction between LAG-3 and its major ligand, major histocompatibility complex II (MHC II) antigen, cell-based blocking assays were carried as follows.

[0364] Briefly, to show that Bi-LT-1 and Bi-LT-3 can block the interaction between TIGIT and its ligand, human PVR (CD155), a cell-based blocking assay was carried out in which serial dilutions of Bi-LT-1, Bi-LT-3, or Bi-TPM-94B (described in Example 1 above) were incubated with human TIGIT-transfected CHOK1 cells and CD155/PVR-mouse IgG2a for 30 minutes on ice. CD155/PVR-mouse IgG2a binding on CHOK1 cells was detected with anti-mouse IgG PE. Cells were fixed prior to analysis with an iQue intellicyt system. The results from this assay are shown in **FIG. 33A** and reveal that Bi-LT-1 and Bi-LT-3, with the extended scfv linkers retain their bioactivity for TIGIT.

[0365] To show that the bispecific antitumor antagonists, Bi-LT-1 and Bi-LT-3 can also block the interaction between LAG-3 and its major ligand, major histocompatibility complex II (MHC II) antigen a cell-based blocking assay was carried out in which serial dilutions of Bi-LT-1, Bi-LT-3, or an anti-LAG-3 benchmark (BM) antibody were incubated with LAG-3-mouse IgG2a for 30 mins at room temperature, added to Raji cells, and further incubated for 30 minutes on ice. The Raji cells were then washed, and LAG-3-mouse IgG2a binding on Raji cells was detected with anti-mouse IgG PE. Cells were fixed prior to analysis with an iQue intellicyt system. The results of this assay are shown in **FIG. 33B** indicate that Bi-LT-1 and Bi-LT-1 retain their bioactivity for LAG-3 similar to the benchmark anti-LAG3 antibody. The assay data was further used to calculate IC50 values (nm) depicted, which are comparable to the IC50 value for the anti-LAG-3 benchmark (BM) antibody. Taken together, the results in **FIGS. 33A** and **33B** establish that Bi-LT-1 and Bi-LT-3 retain their bioactivity for both TIGIT and LAG-3.

[0366] To see whether Bi-LT-1 and Bi-LT-3 can simultaneously bind both LAG-3 and TIGIT, 5 μg/ml LAG-3-mIgG was coated on an ELISA plate at 4° C overnight and then blocked before adding serial dilutions of LT-1, LT-3 or a parental LAG-3 mAb. Following a 1 hr incubation at room temperature, the plate was washed and 500ng/ml His tagged HuTIGIT was added and incubated for 1 hr at room temperature. Plate bound His tagged HuTIGIT was then detected using anti-His tag HRP and a TMB substrate. This results of this assay in **FIG. 34** demonstrate that Bi-LT-1 and Bi-LT-3 can simultaneously bind both LAG-3 and TIGIT.

[0367] **FIGS. 35A-35D** depict pharmacokinetic profiles and in vivo half-lives ($T_{1/2}$) corresponding to the parental anti-LAG-3 mAb (FIG. 35A), an anti-LAG-3 benchmark mAb (**FIG. 35B**), Bi-LT-1 (**FIG. 35C**), or Bi-LT-3 (**FIG. 35D**) following a tail vein injection into 6-10 week old female CD1 mice. The antibodies and bispecific antagonists were recovered from serum taken at various times post-injection and subjected to analysis by ELISA. The results indicate both Bi-LT-1 and Bi-LT-3 have similar pharmacokinetics as compared to the anti-LAG3 parental antibody and to the benchmark antibody, with half-lives (T1/2) of 5-6 days.

[0368] To evaluate species homogeneity and stability of protein A purified Bi-LT-1 and Bi-LT-3, a size exclusion chromatography (SEC) profile of Bi-LT-1 and Bi-LT-3 was generated as described above. The results of this analysis in **FIG. 36A** are consistent with both of the extended scfv linkers creating species homogeneity and good stability after 7 days at 4° C. **FIG. 36B** shows a size-exclusion ultra-high performance liquid chromatography (SE-UHPLC) analysis carried out as described above. The results from this analysis are consistent with the species homogeneity observed in FIG. 36A, as reflected in low levels of high molecular weight (HMW) species at Day 0 and Day 7 (1.3%, 1.5%, respectively for Bi-LT-1 and 1.3%, 1.4%, respectively for Bi-LT-3) and low molecular weight (LMW) species at Day 0 and Day 7 (0.2%, 0.2%, respectively, for Bi-LT-1 and 0.3%, 0.5%, respectively for Bi-LT-3) in comparison to dimer species at Day 0 and Day 7 (98.4%, 98.3%, respectively for Bi-LT-1 and 98.4%, 98.1%, respectively for Bi-LT-3).

[0369] To evaluate the ability of Bi-LT-1 and Bi-LT-3 to induce IFN-γ production, human PBMCs were stimulated with 100 ng/ml SEB in the presence of either SHP-77 cells (**FIG. 37A**) or H358 cells (**FIG. 37B**) to provide an immune suppressive signal. Human IgG control (lane 3), anti-TIGIT mAb (B21-35) alone (lane 4), anti-LAG-3 mAb alone (lane 5), a combination of anti-TIGIT mAb and anti-LAG-3 mAb (lane 6), Bi-LT-1 (lane 7), or Bi-LT-3 (lane 8) were then added to rescue T cell function (**FIGS. 37A, 37B**). 4 days later, cell culture supernatants were collected and examined for IFN-γ level by ELISA. The results of these analyses in **FIGS. 37A** and **37B** show that LT-1 and LT-3 are more potent than the combination of parental TIGIT and LAG-3 antibodies.

[0370] **FIG. 38** shows an evaluation of the ability of Bi-LT-1 and Bi-LT-3 to induce proliferation of CD4 T cells. Human PMBC were labeled with CSFE and then stimulated with 100ng/ml SEB, in the presence of SHP-77 cells to provide immune suppressive signal. 128nM human IgG control (lane 3), anti-TIGIT mAb B21-35 (lane 4), anti-LAG-3 mAb (lane 5), the combination of anti-TIGIT mAb and anti-LAG-3 mAb (lane 6), Bi-LT-1 (lane 7) or Bi-LT-3 (lane 8) were used to rescue T cell function. 5 days later, loss of CFSE signal on CD4 T cells was quantified by FACS to determine the Proliferation Index. Similar to the increased

stimulation of IFNγ over the parental antibodies and the combination of the 2 parental antibodies, both Bi-LT-1 and Bi-LT-3 have increased ability to stimulate proliferation of human CD4 T cells.

[0371] The above description is for the purpose of teaching a person of ordinary skill in the art how to practice the present invention and is not intended to detail all those obvious modifications and variations which will become apparent to the skilled worker upon reading the description. It is intended, however, that all such obvious modifications and variations be included within the scope of the present invention, which is defined by the following claims. The claims are intended to cover the claimed components and steps in any sequence which is effective to meet the objectives there intended, unless the context specifically indicates the contrary.

WHAT IS CLAIMED IS:

1. An antitumor antagonist comprising:

a first targeting domain that specifically binds to PD-1, PD-L1 or LAG-3; and

a second targeting domain comprising an scFv that specifically binds to TIGIT;

2. The antitumor antagonist of Claim 1, wherein the scFv comprises:

an immunoglobulin heavy chain variable region (HCVR) having at at least 90% identity to an amino acid sequence selected from the group consisting of **SEQ ID NOS:** 48, 50, 52, 54, 56, 58, 60, 62, 64, and 66; and/or

immunoglobulin light chain variable region (LCVR) having at at least 90% identity comprising an amino acid sequence selected from the group consisting of **SEQ ID NOS:** 49, 51, 53, 55, 57, 59, 61, 63, 65, and 67, or both.

3. The antitumor antagonist of Claim 1 or Claim 2, wherein the scFv comprises:

an immunoglobulin HCVR having at least 90% identity to the amino acid sequence of SEQ ID NO:66; and/or

an immunoglobulin LCVR comprising at least 90% identity to the amino acid sequence of SEQ ID NO:67.

4. The antitumor antagonist of any one of Claims 1-3, wherein the scFv comprises:

an immunoglobulin HCVR comprising the amino acid sequence of SEQ ID NO:66; and/or

an immunoglobulin LCVR comprising the amino acid sequence of SEQ ID NO:67.

5. The antitumor antagonist of any one of Claims 1-4, comprising an immunoglobulin scaffold having an amino terminus and a carboxy terminus.

6. The antitumor antagonist of Claim 5, wherein the first targeting domain is linked to the amino terminus of the immunoglobulin scaffold and the second targeting domain is linked to the carboxy terminus of immunoglobulin scaffold via a peptide linker.

7. The antitumor antagonist of Claim 6, wherein the peptide linker comprises an amino acid sequence selected from the group consisting of **SEQ ID NOS:** 188-191.

8. The antitumor antagonist of Claim 6 or Claim 7, wherein the peptide linker comprises the amino acid sequence of SEQ ID NO:191.

9. The antitumor antagonist of any one of Claims 1-8, wherein the first targeting domain specifically binds to PD-1.

10. The antitumor antagonist of Claim 9, wherein the first targeting domain comprises:

(1) an immunoglobulin HCVR comprising three complementarity determining regions (HCDRs): HCDR1, HCDR2 and HCDR3,

wherein the HCDR1 comprises an amino acid sequence selected from the group consisting of **SEQ ID NOS:** 68, 71, 74, 76 and 79,

wherein the HCDR2 comprises an amino acid sequence selected from the group consisting of **SEQ ID NOS:** 69, 72, 77 and 80,

wherein the HCDR3 comprises an amino acid sequence selected from the group consisting of **SEQ ID NOS:** 70, 73, 75, 78 and 81; and

(2) an immunoglobulin LCVR comprising three complementarity determining regions (LCDRs): LCDR1, LCDR2 and LCDR3,

wherein the LCDR1 comprises an amino acid sequence selected from the group consisting of **SEQ ID NOS:** 82, 85, 88, 89, 90 and 93,

wherein the LCDR2 comprises an amino acid sequence selected from the group consisting of **SEQ ID NOS:** 83, 86, 91 and 94, and

wherein the LCDR3 comprises an amino acid sequence selected from the group consisting of **SEQ ID NOS:** 84, 87, 92 and 95.

11. The antitumor antagonist of Claim 9 or Claim 10, wherein the first targeting domain comprises:

an immunoglobulin HCVR having at least 90% identity to an amino acid sequence selected from the group consisting of **SEQ ID NOS:** 96, 98, 100, 102, 104, and 106; and/or

an immunoglobulin LCVR having at least 90% identity to an amino acid sequence selected from the group consisting of **SEQ ID NOS:** 97, 99, 101, 103, 105, and 107.

12. The antitumor antagonist of any one of Claims 9-11, wherein the first targeting domain comprises:

an immunoglobulin HCVR comprising the amino acid sequence of SEQ ID NO:106; and/or

an immunoglobulin LCVR comprising the amino acid sequence of SEQ ID NO:107.

13. The antitumor antagonist of any one of Claims 9-12, comprising:

an immunoglobulin heavy chain having the amino acid sequence of SEQ ID NO: 160 or SEQ ID NO: 162, and/or an immunoglobulin light chain having the amino acid sequence of SEQ ID NO: 161.

14. The antitumor antagonist of any one of Claims 1-8, wherein the first targeting domain specifically binds to PD-L1.

15. The antitumor antagonist of Claim 14, wherein the first targeting domain comprises:

(1) an immunoglobulin HCVR comprising three complementarity determining regions (HCDRs): HCDR1, HCDR2 and HCDR3,

wherein the HCDR1 comprises an amino acid sequence selected from the group consisting of **SEQ ID NOS:** 108, 111, 117 and 120,

wherein the HCDR2 comprises an amino acid sequence selected from the group consisting of **SEQ ID NOS:** 109, 112, 114, 116, 118, 121 and 125,

wherein the HCDR3 comprises an amino acid sequence selected from the group consisting of **SEQ ID NOS:** 110, 113, 115, 119 and 122; and

(2) an immunoglobulin LCVR comprising three complementarity determining regions (LCDRs): LCDR1, LCDR2 and LCDR3,

wherein the LCDR1 comprises an amino acid sequence selected from the group consisting of **SEQ ID NOS:** 123, 126, 130, 133 and 136,

wherein the LCDR2 comprises an amino acid sequence selected from the group consisting of **SEQ ID NOS:** 126, 127, 131, 134 and 137, and

wherein the LCDR3 comprises an amino acid sequence selected from the group consisting of **SEQ ID NOS:** 125, 128, 129, 132, 135 and 138.

16. The antitumor antagonist of Claim 14 or Claim 15, wherein the first targeting domain comprises:

an immunoglobulin HCVR having at least 90% identity to an amino acid sequence selected from the group consisting of **SEQ ID NOS:** 139, 141, 143, 145, 147, 149, 151 and 153;

an immunoglobulin LCVR having at least 90% identify to an amino acid sequence selected from the group consisting of **SEQ ID NOS:** 140, 142, 144, 146, 148, 150, 152 and 154; or both.

17. The antitumor antagonist of any one of Claims 14-16, wherein the first targeting domain comprises:

an immunoglobulin HCVR having the amino acid sequence of SEQ ID NO:153;

an immunoglobulin LCVR having the amino acid sequence of SEQ ID NO:154, or both.

18. An antitumor antagonist of any one of Claims 14-17, comprising:

an immunoglobulin heavy chain having an amino acid sequence selected from the group consisting of SEQ ID NO: 339-341; and/or

an immunoglobulin light chain having the amino acid sequence of SEQ ID NO: 338.

19. The antitumor antagonist of any one of Claims 1-8, wherein the first targeting domain specifically binds LAG-3.

20. The antitumor antagonist of Claim 19, wherein the first targeting domain comprises:

(1) an immunoglobulin HCVR comprising three complementarity determining regions (HCDRs): HCDR1, HCDR2 and HCDR3,

wherein the HCDR1 comprises an amino acid sequence selected from the group consisting of **SEQ ID NOS:** 163, 166 and 169,

wherein the HCDR2 comprises an amino acid sequence selected from the group consisting of **SEQ ID NOS:** 164, 167 and 170, and

wherein the HCDR3 comprises an amino acid sequence selected from the group consisting of **SEQ ID NOS:** 165, 168 and 171; and

(2) an immunoglobulin LCVR comprising three complementarity determining regions (LCDRs): LCDR1, LCDR2 and LCDR3,

wherein the LCDR1 comprises an amino acid sequence selected from the group consisting of **SEQ ID NOS:** 172, 175 and 177,

wherein the LCDR2 comprises an amino acid sequence selected from the group consisting of **SEQ ID NOS:** 173 and 178, and

wherein the LCDR3 comprises an amino acid sequence selected from the group consisting of **SEQ ID NOS:** 174, 176 and 179.

21. The antitumor antagonist of Claim 19 or Claim 20, wherein the first targeting domain comprises:

an immunoglobulin HCVR having at least 90% identity to an amino acid sequence selected from the group consisting of **SEQ ID NOS:** 180, 182, 184, and 186; and/or

an immunoglobulin LCVR having at least 90% identity to an amino acid sequence selected from the group consisting of **SEQ ID NOS:** 181, 183, 185, and 187.

22. The antitumor antagonist of any one of Claims 19-21, wherein the first targeting domain comprises:

an immunoglobulin HCVR having the amino acid sequence of SEQ ID NO:180; and/or

an immunoglobulin LCVR having the amino acid sequence of SEQ ID NO:181.

23. An antitumor antagonist of any one of Claims 19-22, wherein the first targeting domain comprises:

an immunoglobulin heavy chain comprising the amino acid sequence of SEQ ID NO:192 or SEQ ID NO:193; and/or

an immunoglobulin light chain comprising the amino sequence of SEQ ID NO: 194.

24. An antibody or antigen-binding portion thereof, comprising:

(1) an immunoglobulin HCVR comprising three complementarity determining regions (HCDRs): HCDR1, HCDR2 and HCDR3,

wherein the HCDR1 comprises an amino acid sequence selected from the group consisting of **SEQ ID NOS:** 163, 166, and 169,

wherein the HCDR2 comprises an amino acid sequence selected from the group consisting of **SEQ ID NOS:** 164, 167, and 170,

wherein the HCDR3 comprises an amino acid sequence selected from the group consisting of SEQ ID NOs. 165, 168, and 171;

(2) an immunoglobulin LCVR comprising three complementarity determining regions (LCDRs): LCDR1, LCDR2 and LCDR3,

wherein the LCDR1 comprises an amino acid sequence selected from the group consisting of SEQ ID NOs. 172, 175, and 177,

wherein the LCDR2 comprises an amino acid sequence selected from the group consisting of SEQ ID NOs. 173 and 178,

wherein the LCDR13 comprises an amino acid sequence selected from the group consisting of SEQ ID NOs. 174, 176 and 179, and

wherein the antibody, or the antigen-binding portion thereof, binds specifically to human LAG-3.

25. The antibody or an antigen-binding portion thereof of Claim 24, comprising:

an immunoglobulin HCVR having at least 90% identity to an amino acid sequence selected from the group consisting of **SEQ ID NOS:** 180, 182, 184, and 186; and/or

an immunoglobulin LCVR having at least 90% identity to an amino acid sequence selected from the group consisting of **SEQ ID NOS:** 181, 183, 185, and 187.

26. The antibody or an antigen-binding portion thereof of Claim 24 or Claim 25, comprising:

an immunoglobulin HCVR comprising the amino acid sequence of SEQ ID NO: 180; and/or

an immunoglobulin LCVR comprising the amino acid sequence of SEQ ID NO:181.

27. One or more nucleic acids encoding the antitumor antagonist, antibody or antigen-binding portion thereof of any one of Claims 1-26.

28. One or more expression vectors comprising the one or more nucleic acids of Claim 27.

29. A host cell transformed with the one or more expression vectors of Claim 28.

30. A method of treating a cell proliferative disorder in a subject, comprising:

administering to a subject in need thereof an effective amount of the antitumor antagonist of any one of Claims 1-26.

Anti-TIGIT mab sequences

Mab	Heavy Chain CDR1		Heavy Chain CDR2		Heavy Chain CDR3	
T-01	SDYAWN	(SEQ ID:1)	YISYSGSTGYNPSLKS	(SEQ ID:2)	RMIGYAMDY	(SEQ ID:3)
T-02	SDYAWN	(1)	YITYSGGTSYNPSLKS	(SEQ ID:4)	RQIGLGFTY	(SEQ ID:5)
T-03	DHTIH	(SEQ ID:6)	YFYPRDGSTKYNEKFKG	(SEQ ID:7)	GMLRWFAD	(SEQ ID:8)
T-04	DHTIH	(6)	YIYPRDGSSKYNVKFKG	(SEQ ID:9)	GMLRWFAY	(SEQ ID:10)
T-05	DQAIH	(SEQ ID:11)	YIYPRDGSTKYNETFKG	(SEQ ID:12)	GMLRWFAY	(10)
T-06	SDYAWN	(1)	YITYSGSTSYNPSLKS	(SEQ ID:13)	RQVGLGFAY	(SEQ ID:14)
T-07	SDSAWN	(SEQ ID:15)	YITYSGSTNYNPSLRS	(SEQ ID:16)	RQVGLGFAY	(14)
T-08	NYGMN	(SEQ ID:17)	WINTYTGEPTYADDFKG	(SEQ ID:18)	APPYGYDVRFAY	(SEQ ID:19)
T-09	TFAMGVG	(SEQ ID:20)	HIWWDDDKYYNPALKS	(SEQ ID:21)	MDYSYFAWFAY	(SEQ ID:22)
T-10 (B21-35)	SYYMH	(SEQ ID:23)	INPSGGRTSYAQMFQG	(SEQ ID:24)	DREEQWPVGGFDY	(SEQ ID:25)

Mab	Light Chain CDR1		Light Chain CDR2		Light Chain CDR3	
T-01	KASQDVSTVVA	(SEQ ID:26)	SASYRYT	(SEQ ID:27)	QQHYSTPWT	(SEQ ID:28)
T-02	KASQDLSTAVA	(SEQ ID:29)	SSSYRYT	(SEQ ID:30)	QQHYSTPWT	(28)
T-03	KASQDVSTTVA	(SEQ ID:31)	SASYRYT	(27)	QQHYSTPLT	(SEQ ID:32)
T-04	KASQDVFTAVA	(SEQ ID:33)	SASYRYT	(27)	QQHYSIPLT	(SEQ ID:34)
T-05	KASQDVSTAVA	(SEQ ID:35)	SASYRYT	(27)	QQHYSTPLT	(32)
T-06	KASQDVSTAVA	(35)	SASYHYT	(SEQ ID:36)	QQHYSTPWT	(28)
T-07	KASQDVSTAVA	(35)	SASYRFT	(SEQ ID:37)	QHHYSTPWT	(SEQ ID:38)
T-08	RSSQSIVHSNGNTYLE	(SEQ ID:39)	KVSDRFS	(SEQ ID:40)	FQGSHVPWT	(SEQ ID:41)
T-09	RSSTGAVTTSNYAN	(SEQ ID:42)	GTNNRAP	(SEQ ID:43)	ALWYSNHWV	(SEQ ID:44)
T-10	RASQSIRRYLN	(SEQ ID:45)	SASNLQS	(SEQ ID:46)	QQSYIPPT	(SEQ ID:47)

FIG. 1

Anti-TIGIT Antibody Variable Domain Sequences

T-01
VH (SEQ ID NO: 48)
QVQLQESGPGLVKPSQTLSLTCTVSGYSITSDYAWNWIRQPPGKGLEWIGYISYSGSTGY
NPSLKSRVTISVDTSKNQFSLKLSSVTAADTAVYYCARRMIGYAMDYWGQGTSVTVSS

VL (SEQ ID NO: 49)
DIQMTQSPSSLSASVGDRVTITCKASQDVSTVVAWHQQKPGKAPKLLIYSASYRYTGVP
SRFSGSGSGTDFTLTISSLQPEDFATYYCQQSYSTPQQHYSTPWTFGGGTKLEIKR

T-02
VH (SEQ ID NO: 50)
QVKLQESGPGLVKPSQTLSLTCTVTGYSITSDYAWNWIRQPPGKGLEWIGYITYSGGTSY
NPSLKSRVTISVDTSKNQFSLKLSSVTAADTAVYSCARRQIGLGFTYWGQGTLVTVSA

VL (SEQ ID NO: 51)
DIQMTQSPSSLSASVGDRVTIPCKASQDLSTAVAWYQQKPGKAPKLLIYSSSYRYTGVPS
RFSGSGSGTDFTLTISSLQPEDFATYYCQQSYSTPQQHYSTPWTFGEGTKLEIK

T-03
VH (SEQ ID NO: 52)
EVQLVQSGAEVKKPGATVKISCKVSGYTFTDHTIHWVQQAPGKGLEWMGYFYPRDGST
KYNEKFKGRVTITADTSTDTAYMELSSLRSEDTAVYYCATGMLRWFADWGQGTLITVS
VA

VH (SEQ ID NO: 53)
DIQMTQSPSSLSASVGDRVTITCKASQDVSTTVAWYQQKPGKAPKLLIYSASYRYTGVP
SRFSGSGSGTDFTLTISSLQPEDFATYYCQQHYSTPLTFGAGTKLELK

FIG. 2A

T-04
VH (SEQ ID NO: 54):
EVQLVQSGAEVKKPGATVKISCKVSGYTFTDHTIHWVQQAPGKGLEWMGYIYPRDGSS
KYNVKFKGRVTITADTSTDTAYMELSSLRSEDTAVYYCATGMLRWFAYWGQGTLVTV
SS

VL (SEQ ID NO: 55):
DIQMTQSPSSLSASVGDRVTITCKASQDVFTAVAWYQQKPGKAPKLLIYSASYRYTGVP
SRFSGSGSGTDFTFTISSLQPEDIATYYCQQHYSIPLTFGAGTKLEIK

T-05
VH (SEQ ID NO: 56):
EVQLKQSGAEVKKPGATVKISCKVSGYTFTDQAIHWVQQAPGKGLEWMGYIYPRDGST
KYNETFKGRVTITADTSTDTAYMELSSLRSEDTAVYFCARGMLRWFAYWGQGTLVTVS
S

VL (SEQ ID NO: 57):
DIQMTQSPSSLSASVGDRVTITCKASQDVSTAVAWYQQKPGKAPKLLIYSASYRYTGVP
SRFSGSGSGTDFTLTISSLQPEDFATYYCQQHYSTPLTFGAGTKLELK

T-06
VH (SEQ ID NO: 58):
QVQLQESGPGLVKPSQTLSLTCTVSGGSVSSDYAWNWIRQPPGKGLEWIGYITYSGSTS
YNPSLKSRVTISVDTSKNQFSLKLSSVTAADTAVYYCARRQVGLGFAYWGQGTLVTVS
A

VL (SEQ ID NO: 59):
DIQMTQSPSSLSASVGDRVTITCKASQDVSTAVAWYQQKPGKAPKLLIYSASYHYTGVP
SRFSGSGSGTDFTLTISSLQPEDFATYYCQQHYSTPWTFGGGTKLEIK

T-07
VH (SEQ ID NO: 60):
EVQLQESGPGLVKPSDTLSLTCAVSGYSITSDSAWNWIRQPPGKGLEWIGYITYSGSTNY
NPSLRSRVTMSVDTSKNQFSLKLSSVTAVDTAVYYCTRRQVGLGFAYWGQGTLVTVSA

VL (SEQ ID NO: 61):
DIQMTQSPSSLSASVGDRVTITCKASQDVSTAVAWYQQKPGKAPKLLIYSASYRFTGAP
SRFSGSGSGTDFTLTISSLQPEDFGIYYCQHHYSTPWTFGGGTKLEFK

FIG. 2B

T-08
VH (SEQ ID NO: 62):
QVQLVQSGSELKKPGASVKVSCKASGYTFTNYGMNWVRQAPGQGLEWMGWINTYTG
EPTYADDFKGRFVFSLDTSVSTAYLQISSLKAEDTAVYYCARAPPYGYDVRFAYWGQG
TLVTVSS

VL (SEQ ID NO: 63):
DVVMTQSPLSLPVTLGQPASISCRSSQSIVHSNGNTYLEWFQQRPGQSPRVLIYKVSDRF
SGVPDRFSGSGSGTDFTLKISRVEAEDVGVYYCFQGSHVPWTFGRGTKLEIK

T-09
VH (SEQ ID NO: 64):
QVTLKESGPTLVKPTQTLTLTCTFSGFSLSTFAMGVGWIRQPPGKALEWLAHIWWDDD
KYYNPALKSRLTITKDTSKNQVVLTMTNMDPVDTATYYCARMDYSYFAWFAYWGQG
TLVTVSS

VL (SEQ ID NO: 65):
QAVVTQEPSLTVSPGGTVTLTCRSSTGAVTTSNYANWVQQKPGQLFRGLIGGTNNRAP
WVPARFSGSLIGDKAALTLSGVQPEDEAEYFCALWYSNHWVFGGGTKLTVL

T-10 (B21-35)
VH (SEQ ID NO: 66):
QVQLVQSGAEVKKPGASVKVSCKASGYTFTSYYMHWVRQAPGQGLEWMGIINPSGGR
TSYAQMFQGRVTMTRDTSTSTVYMELSSLRSEDTAVYYCARDREEQWPVGGFDYWGQ
GTLVTVSS

VL (SEQ ID NO: 67):
DIQMTQSPSSLSASVGDRVTITCRASQSIRRYLNWYQQKPGKAPKLLIYSASNLQSGVPS
RFSGSGSGTDFTLTISSLQPEDFATYYCQQSYIIPPTFGQGTKVEIK

FIG. 2C

Anti-PD1 mab sequences

Mab	Heavy Chain CDR1		Heavy Chain CDR2		Heavy Chain CDR3	
D-01	NFLMS	(SEQ ID:68)	TISGGGRDTYYVDSVKG	(SEQ ID:69)	RTTYSMDY	(SEQ ID:70)
D-02	NSYLY	(SEQ ID:71)	GINPSNGGTNFNEKFKT	(SEQ ID:72)	RDYNYDGGFDS	(SEQ ID:73)
D-03	NSYIY	(SEQ ID:74)	GINPSNGGTNFNEKFKT	(72)	RRDYRYDGGFDS	(SEQ ID:75)
D-04	NSYIY	(74)	GINPSNGGTNFNEKFKT	(72)	RDYNYDGGFDS	(53)
D-05 (2P16)	TYYIY	(SEQ ID:76)	GINPGNGGTNFNEKFKI	(SEQ ID:77)	RYHGYDGGLDY	(SEQ ID:78)
D-06 (2P17)	SYYIH	(SEQ ID:79)	WIFPGSGNSKYNENFKG	(SEQ ID:80)	SETYDYGDY	(SEQ ID:81)
Iab	**Light Chain CDR1**		**Light Chain CDR2**		**Light Chain CDR3**	
D-01	LASQTIGTWLA	(SEQ ID:82)	AATSLAD	(SEQ ID:83)	QQFYSIPWT	(SEQ ID:84)
D-02	RASSTLYSNYLH	(SEQ ID:85)	RASFLAS	(SEQ ID:86)	QQGSSIPLT	(SEQ ID:87)
D-03	SASSSLYSSYLH	(SEQ ID:88)	RASFLAS	(86)	QQGSSIPLT	(87)
D-04	RASSSLYSNYLH	(SEQ ID:89)	RASFLAS	(86)	QQGSSIPLT	(87)
D-05	RASKSVSTSGFSYIH	(SEQ ID:90)	LASNLES	(SEQ ID:91)	QHTWELPNT	(SEQ ID:92)
D-06	KASQNVGTNVA	(SEQ ID:93)	SASYRYS	(SEQ ID:94)	QQYYSYPYT	(SEQ ID:95)

FIG. 3

anti-PD-1 Antibody Variable Domain Sequences

PD-01
VH (SEQ ID NO: 96):
EVQLVESGGGLVQPGGSLRLSCAASGFTFSNFLMSWVRQAPGKGLEWVSTISGGGR
DTYYVDSVKGRFTISRDNSKNTLYLQMNSLRAEDTAVYYCAKRTTYSMDYWGQGT
SVTVSS

VL (SEQ ID NO: 97):
DIQMTQSPSSVSASVGDRVTITCLASQTIGTWLAWYQQKPGKAPKLLIYAATSLADG
VPSRFSGSGSGTDFTLTISSLQPEDFATYYCQQFYSIPWTFGGGTKLEIK

PD-02
VH (SEQ ID NO: 98):
QVQLVQSGAEVKKPGASVKVSCKASDYTFTNSYLYWLRQAPGQGLEWMGGINPSN
GGTNFNEKFKTRTTSTRDTSISTAYMELSRLRSDDTVVYYCTRRDYNYDGGFDSWG
QGTLVTVSS

VL (SEQ ID NO: 99):
DIQMTQSPSSLSASVGDRVTFTCRASSTLYSNYLHWYQQKPGKAPKLLIYRASFLAS
GVPSRFSGSGSGTDFTLTISSLQPEDFATYYCQQGSSIPLTFGGGTKVEIK

PD-03
VH (SEQ ID NO: 100)
QVQLVQSGAEVKKPGASVKVSCKASDYTFTNSYIYWVRQAPGQGLEWMGGINPSN
GGTNFNEKFKTRVTSTRDTSISTAYMELSRLRSDDTVVYYCARRDYRYDGGFDSWG
QGTTLTVSS

VL (SEQ ID NO: 101)
DIQMTQSPSSLSASVGDRVTITCSASSSLYSSYLHWYQQKPGKAPKLLIYRASFLASG
VPSRFSGSGSGTDFTLTISSLQPEDFATYYCQQGSSIPLTFGAGTKLDLK

FIG. 4A

PD-04
VH (SEQ ID NO: 102):
QVQLVQSGAEVKKPGASVKVSCKASDYTFTNSYIYWVRQAPGQGLEWMGGINPSN
GGTNFNEKFKTRVTSTRDTSISTAYMELSRLRSDDTVVYYCARRDYNYDGGFDSWG
QGTLVTVSS

VL (SEQ ID NO: 103):
DIQMTQSPSSLSASVGDRVTFTCRASSSLYSNYLHWYQQKPGKAPKLLIYRASFLAS
GVPSRFSGSGSGTDFTLTISSLQPEDFATYYCQQGSSIPLTFGGGTKVEIK

PD-05
VH (SEQ ID NO: 104):
QVQLVQSGAEVKKPGASVKVSCKASGYTFTTYYIYWVRQAPGQGLEWMGGINPGN
GGTNFNEKFKIRVTMTRDTSISTAYMELSSLRSEDTAVYYCARRYHGYDGGLDYWG
QGTLVTVSS

VL (SEQ ID NO: 105):
DIVLTQSPASLAVSPGQRATITCRASKSVSTSGFSYIHWYQQKPGQPPKLLIYLASNLE
SGVPARFSGSGSGTDFTLTINPVEANDTANYYCQHTWELPNTFGGGTKVEIK

PD-06
VH (SEQ ID NO: 106):
QVQLVQSGAEVKKPGASVKVSCKASGYTFTSYYIHWVRQAPGQGLEWMGWIFPGS
GNSKYNENFKGRVTLTADTSTSTVYMELSSLRSEDTAVYYCASETYDYGDYWGQGT
LVTVSS

VL (SEQ ID NO: 107):
DIQMTQSPSFLSASVGDRVTITCKASQNVGTNVAWYQQKPGKAPKALIYSASYRYSG
VPSRFSGSGSGTEFTLTISSLQPEDFATYYCQQYYSYPYTFGQGTKLEIK

FIG. 4B

Anti-PD-L1 mab sequences

Mab	Heavy Chain CDR1		Heavy Chain CDR2			Heavy Chain CDR3	
PL-01	NYWMH	(SEQ ID:108)	MIHPNTINNYNYNEKFKS	(SEQ ID:109)		SDYGSSPYYFDY	(SEQ ID:110)
PL-02	SYWMH	(SEQ ID:111)	MIHPNVGSTNYNEKFKS	(SEQ ID:112)		SRYGSSPYYFDY	(SEQ ID:113)
PL-03	SYWMH	(111)	MIHPNSGGNNYNEKFKS	(SEQ ID:114)		SWYGSSPYYFDY	(SEQ ID:115)
PL-04	SYWMH	(111)	MIHPTGVSTDYNEKFKS	(SEQ ID:116)		SDYGSSPYYFDY	(110)
PL-05	SDYAWN	(SEQ ID:117)	YISDSGSTSYNPSLKS	(SEQ ID:118)		SFLRLRSYFDH	(SEQ ID:119)
PL-06	SYGIN	(SEQ ID:120)	CIYIGNDYTNYNEKFKG	(SEQ ID:121)		AYYGSRVDY	(SEQ ID:122)
PL-07	SYGIN	(120)	CIYIGNDYTNYNEKFKG	(121)		AYYGSRVDY	(122)
PL-08	SYWMH	(111)	MIHPNSGGNNYNEKFKS	(114)		SWYGSSPYYFDY	(115)
Mab	Light Chain CDR1		Light Chain CDR2			Light Chain CDR3	
PL-01	RASQDIDNYLN	(SEQ ID:123)	YTSRLHS	(SEQ ID:124)		QQGYTLPWT	(SEQ ID:125)
PL-02	RASQDISNYLN	(SEQ ID:126)	YTSRLQS	(SEQ ID:127)		QQGNTLPWT	(SEQ ID:128)
PL-03	RASQDISNYLN	(126)	YTSRLHS	(124)		QQGNTLPWT	(128)
PL-04	RASQDISNYLN	(126)	YTSRLHS	(124)		QQGDTLPWT	(SEQ ID:129)
PL-05	KASQDVNVAVA	(SEQ ID:130)	WASTRHI	(SEQ ID:131)		QQHYSTPYT	(SEQ ID:132)
PL-06	KASQDINKYIA	(SEQ ID:133)	YTSTLQP	(SEQ ID:134)		LQYDNLYT	(SEQ ID:135)
PL-07	QSISDYLH	(SEQ ID:136)	CASQSISG	(SEQ ID:137)		QNGHSFPYT	(SEQ ID:138)
PL-08	RASQDIDNYLN	(123)	YTSRLHS	(124)		QQGYTLPWT	(125)

FIG. 5

anti-PD-L1 Antibody Variable Domain Sequences

PL-01
VH (SEQ ID NO: 139):
QVQLVQSGAEVKKPGASVKVSCKASGYTFTNYWMHWMKQAPGQGLEWMGMIHP
NTNNYNYNEKFKSRVTSTRDTSISTAYMELSRLRSDDTVVYYCARSDYGSSPYYFDY
WGQGTLVTVSS

VL (SEQ ID NO: 140):
DIQMTQSPSSLSASVGDRVTISCRASQDIDNYLNWYQQKPGKAPKLLIKYTSRLHSG
VPSRFSGSGSGTDFTLTISSLQPEDFATYFCQQGYTLPWTFGGGTKVEIK

PL-02
VH (SEQ ID NO: 141):
QVQLVQSGAEVKKPGASVKVSCKASGYTFTSYWMHWVRQAPGQGLEWMGMIHPN
VGSTNYNEKFKSKATMTRDKSSSTVYMELSSLRSEDTAVYYCARSRYGSSPYYFDY
WGQGTLVTVSS

VL (SEQ ID NO: 142):
DIQMTQSPSSLSASVGDRVTISCRASQDISNYLNWYQQKPGKAPKLLIYYTSRLQSGV
PSRFSGSGSGTDFTFTISSLQPEDIATYFCQQGNTLPWTFGQGTKVEIK

PL-03
VH (SEQ ID NO: 143):
QVQLVQSGAEVKKPGASVKVSCKASGYTFTSYWMHWVRQAPGQGLEWMGMIHPN
SGGNNYNEKFKSRVTMTRDTSISTAYMELSRLRSDDTAVYYCARSWYGSSPYYFDY
WGQGTLVTVSS

VL (SEQ ID NO: 144):
DIQMTQSPSSLSASVGDRVTISCRASQDISNYLNWYQQKPGKAPKLLIYYTSRLHSGV
PSRFSGSGSGTDFTFTISSLQPEDIATYFCQQGNTLPWTFGQGTKVEIK

FIG. 6A

PL-04
VH (SEQ ID NO: 145):
QVQLVQSGAEVKKPGASVKVSCKASGYTFTSYWMHWVRQAPGQGLEWMGMIHPT
GVSTDYNEKFKSRVTMTRDTSTSTVYMELSSLRSEDTAVYYCARSDYGSSPYYFDY
WGQGTLVTVSS

VL (SEQ ID NO: 146):
DIQMTQSPSSLSASVGDRVTISCRASQDISNYLNWYQQKPGKAPKLLIKYTSRLHSGV
PSRFSGSGSGTDFTLTISSLQPEDFATYFCQQGDTLPWTFGGGTKVEIK

PL-05
VH (SEQ ID NO: 147):
DVQLQESGPGLVKPSQSLSLTCTVTGYSITSDYAWNWIRQFPGNKLEWMGYISDSGS
TSYNPSLKSRISITRDTSKNQFFLQLNSVTTEDTATYYCANSFLRLRSYFDHWGQGTT
LTVSS

VL (SEQ ID NO: 148):
DIVMTQSHKFMSTSVGDRVSITCKASQDVNVAVAWYQQKPGQSPKLLIFWASTRHI
GVPDRFTGSGSGTDYTLTISSVQAEDLALYYCQQHYSTPYTFGGGTKLEIK

PL-06
VH (SEQ ID NO: 149):
QVQLVQSGAEVKKPGASVKVSCKASGYTFTSYGINWVRQAPGQRLEWMGWCIYIG
NDYTNYNEKFKGRVTITRDTSASTAYMELSSLRSEDTAVYYCARAYYGSRVDYWG
QGTLVTVSS

VL (SEQ ID NO: 150):
DIQMTQSPSSLSAFVGDRVTITCKASQDINKYIAWYQQKPGKAPKLLIHYTSTLQPGV
PSRFSGSGSGRDFTFTISSLQPEDIATYYCLQYDNLYTFGGGTKVEIK

PL-07
VH (SEQ ID NO: 151):
QVQLVQSGAEVKKPGASVKVSCKASGYTFTSYGINWVRQAPGQRLEWMGWCIYIG
NDYTNYNEKFKGRVTITRDTSASTAYMELSSLRSEDTAVYYCARAYYGSRVDYWG
QGTLVTVSS

VL (SEQ ID NO: 152):
EIVLTQSPVTLSLSPGERATLSCQSISDYLHWYLQKPGQAPRLLIKCASQSISGIPARFS
GSGSGSDFTLTISSLEPEDFAVYYCQNGHSFPYTFGGGTKVEIK

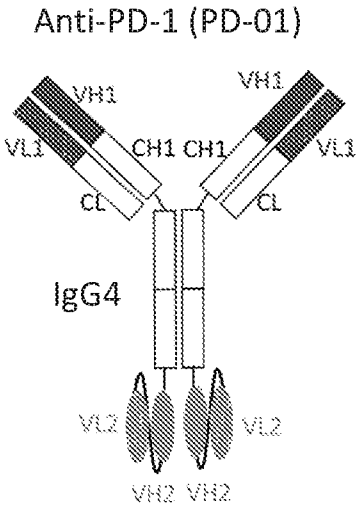
FIG. 6B

PL-08
VH (SEQ ID NO: 153):
QVQLVQSGAEVKKPGASVKVSCKASGYTFTSYWMHWVRQAPGQGLEWMGMIHPN
SGGNNYNEKFKSRVTMTRDTSISTAYMELSRLRSDDTAVYYCARSWYGSSPYYFDY
WGQGTLVTVSS

VL (SEQ ID NO: 154):
DIQMTQSPSSLSASVGDRVTISCRASQDIDNYLNWYQQKPGKAPKLLIKYTSRLHSG
VPSRFSGSGSGTDFTLTISSLQPEDFATYFCQQGYTLPWTFGGGTKVEIK

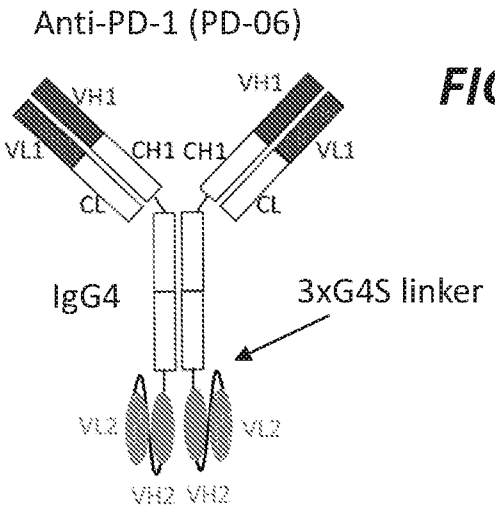
FIG. 6C

Anti-PD-1 antibodies with TIGIT scfv



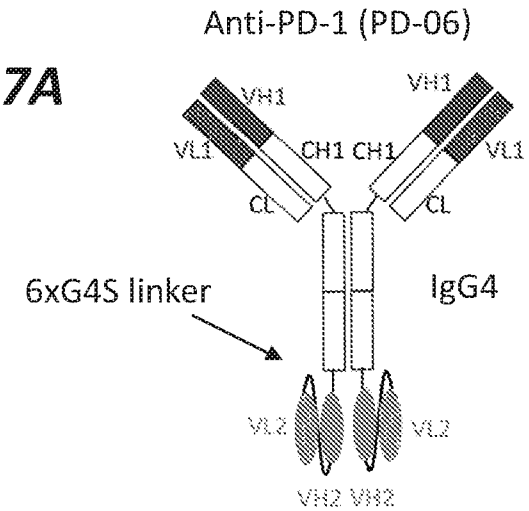
Bi-TPM-93

FIG. 7A



Bi-TPM-94A

FIG. 7B



Bi-TPM-94B

FIG. 7C

Functional Domain Sequences in FIGs. 7A-7C

VH/VL or Fusion Protein Domain	Amino Acid Sequences of Functional Domains
Anti-PD-1 HCVR (PD-01)	EVQLVESGGGLVQPGGSLRLSCAASGFTFSNFLMSWVRQAPGKGLEWVSTISGGGRDTYYVDSVK GRFTISRDNSKNTLYLQMNSLRAEDTAVYYCAKRTTYSMDYWGQGTSVTVSS (SEQ ID NO:96)
Anti-PD-1 LCVR (PD-01)	DIQMTQSPSSVSASVGDRVTITCLASQTIGTWLAWYQQKPGKAPKLLIYAATSLADGVPSRFSGSGS GTDFTLTISSLQPEDFATYYCQQFYSIPWTFGGGTKLEIK (SEQ ID NO:97)
Anti-PD-1 HCVR (PD-06, 2P17)	QVQLVQSGAEVKKPGASVKVSCKASGYTFTSYYIHWVRQAPGQGLEWMGWIFPGSGNSKYNENF KGRVTLTADTSTSTVYMELSSLRSEDTAVYYCASETYDYGDYWGQGTLVTVSS (SEQ ID NO:106)
Anti-PD-1 LCVR (PD-06, 2P17)	DIQMTQSPSFLSASVGDRVTITCKASQNVGTNVAWYQQKPGKAPKALIYSASYRYSGVPSRFSGSGS GTEFTLTISSLQPEDFATYYCQQYYSYPYTFGQGTKLEIK (SEQ ID NO:107)
Anti-TIGIT HCVR (B21-35)	QVQLVQSGAEVKKPGASVKVSCKASGYTFTSYYMHWVRQAPGQGLEWMGIINPSGGRTSYAQMF QGRVTMTRDTSTSTVYMELSSLRSEDTAVYYCARDREEQWPVGGFDYWGQGTLVTVSS (SEQ ID NO:66)
Anti-TIGIT LCVR (B21-35)	DIQMTQSPSSLSASVGDRVTITCRASQSIRRYLNWYQQKPGKAPKLLIYSASNLQSGVPSRFSGSGSG TDFTLTISSLQPEDFATYYCQQSYIIPPTFGQGTKVEIK (SEQ ID NO:67)
IgG4 CH1-CH2-CH3 (hinged stabilized S231P)	ASTKGPSVFPLAPCSRSTSESTAALGCLVKDYFPEPVTVSWNSGALTSGVHTFPAVLQSSGLYSLSSVV TVPSSSLGTKTYTCNVDHKPSNTKVDKRVESKYGPPCPPCPAPEFLGGPSVFLFPPKPKDTLMISRTPE VTCVVVDVSQEDPEVQFNWYVDGVEVHNAKTKPREEQFNSTYRVVSVLTVLHQDWLNGKEYKCK VSNKGLPSSIEKTISKAKGQPREPQVYTLPPSQEEMTKNQVSLTCLVKGFYPSDIAVEWESNGQPEN NYKTTPPVLDSDGSFFLYSRLTVDKSRWQEGNVFSCSVMHEALHNHYTQKSLSLSLGK (SEQ ID NO: 155)
3xG4S linker	GGGGSGGGGSGGGGS (SEQ ID NO:188)
6xG4S linker	GGGGSGGGGSGGGGSGGGGSGGGGSGGGGS (SEQ ID NO:191)

FIG. 8

HC and LC Sequences of Antagonists in FIGs. 7A-7C

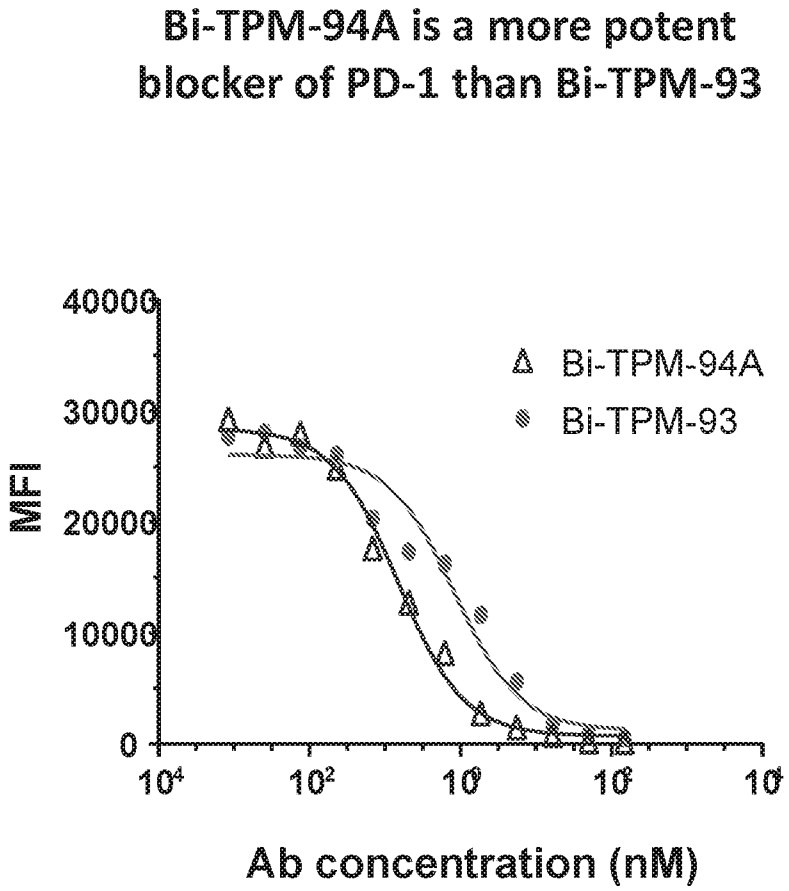
Antagonist (HC/LC)	Amino Acid Sequence
Bi-TPM-93 HC	EVQLVESGGGLVQPGGSLRLSCAASGFTFSNFLMSWVRQAPGKGLEWVSTISGGG RDTYYVDSVKGRFTISRDNSKNTLYLQMNSLRAEDTAVYYCAKRTTYSMDYWGQGT SVTVSSASTKGPSVFPLAPCSRSTSESTAALGCLVKDYFPEPVTVSWNSGALTSGVHT FPAVLQSSGLYSLSSVVTVPSSSLGTKTYTCNVDHKPSNTKVDKRVESKYGPPCPPCP APEFLGGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSQEDPEVQFNWYVDGVEVHN AKTKPREEQFNSTYRVVSVLTVLHQDWLNGKEYKCKVSNKGLPSSIEKTISKAKGQPR EPQVYTLPPSQEEMTKNQVSLTCLVKGFYPSDIAVEWESNGQPENNYKTTPPVLDSD GSFFLYSRLTVDKSRWQEGNVFSCSVMHEALHNHYTQKSLSLSLGKGGGGSGGGGS QVQLVQSGAEVKKPGASVKVSCKASGYTFTSYYMHWVRQAPGQGLEWMGIINPS GGRTSYAQMFQGRVTMTRDTSTSTVYMELSSLRSEDTAVYYCARDREEQWPVGGF DYWGQGTLVTVSSGGGGSGGGGSGGGGSDIQMTQSPSSLSASVGDRVTITCRASQ SIRRYLNWYQQKPGKAPKLLIYSASNLQSGVPSRFSGSGSGTDFTLTISSLQPEDFATY YCQQSYIIPPTFGQGTKVEIK (SEQ ID NO: 158) (PD-01)
TPM-93 LC	DIQMTQSPSSVSASVGDRVTITCLASQTIGTWLAWYQQKPGKAPKLLIDAATSLADG VPSRFSGSGSGTDFTLTISSLQPEDFATYYCQQFYSIPWTFGGGTKLEIKRTVAAPSVFI FPPSDEQLKSGTASVVCLLNNFYPREAKVQWKVDNALQSGNSQESVTEQDSKDSTY SLSSTLTLSKADYEKHKVYACEVTHQGLSSPVTKSFNRGEC (SEQ ID NO: 159) (PD-01)
Bi-TPM-94A HC	QVQLVQSGAEVKKPGASVKVSCKASGYTFTSYYIHWVRQAPGQGLEWMGWIFPG SGNSKYNENFKGRVTLTADTSTSTVYMELSSLRSEDTAVYYCASETYDYGDYWGQGT LVTVSSASTKGPSVFPLAPCSRSTSESTAALGCLVKDYFPEPVTVSWNSGALTSGVHTF PAVLQSSGLYSLSSVVTVPSSSLGTKTYTCNVDHKPSNTKVDKRVESKYGPPCPPCPA PEFLGGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSQEDPEVQFNWYVDGVEVHNA KTKPREEQFNSTYRVVSVLTVLHQDWLNGKEYKCKVSNKGLPSSIEKTISKAKGQPRE PQVYTLPPSQEEMTKNQVSLTCLVKGFYPSDIAVEWESNGQPENNYKTTPPVLDSD GSFFLYSRLTVDKSRWQEGNVFSCSVMHEALHNHYTQKSLSLSLGKGGGGSGGGGS QVQLVQSGAEVKKPGASVKVSCKASGYTFTSYYMHWVRQAPGQGLEWMGIINPS GGRTSYAQMFQGRVTMTRDTSTSTVYMELSSLRSEDTAVYYCARDREEQWPVGGF DYWGQGTLVTVSSGGGGSGGGGSGGGGSDIQMTQSPSSLSASVGDRVTITCRASQ SIRRYLNWYQQKPGKAPKLLIYSASNLQSGVPSRFSGSGSGTDFTLTISSLQPEDFATY YCQQSYIIPPTFGQGTKVEIK (SEQ ID NO: 160)
Bi-TPM-94A LC	DIQMTQSPSFLSASVGDRVTITCKASQNVGTNVAWYQQKPGKAPKALIYSASYRYSG VPSRFSGSGSGTEFTLTISSLQPEDFATYYCQQYYSYPYTFGQGTKLEIKRTVAAPSVFI FPPSDEQLKSGTASVVCLLNNFYPREAKVQWKVDNALQSGNSQESVTEQDSKDSTY SLSSTLTLSKADYEKHKVYACEVTHQGLSSPVTKSFNRGEC (SEQ ID NO: 161)

FIG. 9A

HC and LC Sequences of Antagonists in FIGs. 7A-7C

Antagonist (HC/LC)	Amino Acid Sequence
Bi-TPM-94B HC	QVQLVQSGAEVKKPGASVKVSCKASGYTFTSYYIHWVRQAPGQGLEWMGWIFPGSG NSKYNENFKGRVTLTADTSTSTVYMELSSLRSEDTAVYYCASETYDYGDYWGQGTLVTV SSASTKGPSVFPLAPCSRSTSESTAALGCLVKDYFPEPVTVSWNSGALTSGVHTFPAVLQS SGLYSLSSVVTVPSSSLGTKTYTCNVDHKPSNTKVDKRVESKYGPPCPPCPAPEFLGGPSV FLFPPKPKDTLMISRTPEVTCVVVDVSQEDPEVQFNWYVDGVEVHNAKTKPREEQFNS TYRVVSVLTVLHQDWLNGKEYKCKVSNKGLPSSIEKTISKAKGQPREPQVYTLPPSQEEM TKNQVSLTCLVKGFYPSDIAVEWESNGQPENNYKTTPPVLDSDGSFFLYSRLTVDKSRW QEGNVFSCSVMHEALHNHYTQKSLSLSLGKGGGGSGGGGSQVQLVQSGAEVKKPGAS VKVSCKASGYTFTSYYMHWVRQAPGQGLEWMGIINPSGGRTSYAQMFQGRVTMTR DTSTSTVYMELSSLRSEDTAVYYCARDREEQWPVGGFDYWGQGTLVTVSSGGGGSGG GGSGGGGSGGGGSGGGGSGGGGSDIQMTQSPSSLSASVGDRVTITCRASQSIRRYLN WYQQKPGKAPKLLIYSASNLQSGVPSRFSGSGSGTDFTLTISSLQPEDFATYYCQQSYIIPP TFGQGTKVEIK (SEQ ID NO: 162)
Bi-TPM-94B LC	DIQMTQSPSFLSASVGDRVTITCKASQNVGTNVAWYQQKPGKAPKALIYSASYRYSGVP SRFSGSGSGTEFTLTISSLQPEDFATYYCQQYYSYPYTFGQGTKLEIKRTVAAPSVFIFPPSD EQLKSGTASVVCLLNNFYPREAKVQWKVDNALQSGNSQESVTEQDSKDSTYSLSSTLTL SKADYEKHKVYACEVTHQGLSSPVTKSFNRGEC (SEQ ID NO: 161)

FIG. 9B



Molecule	IC50 (nM)
Bi-TPM-93	0.83
Bi-TPM-94A	0.15

FIG. 10

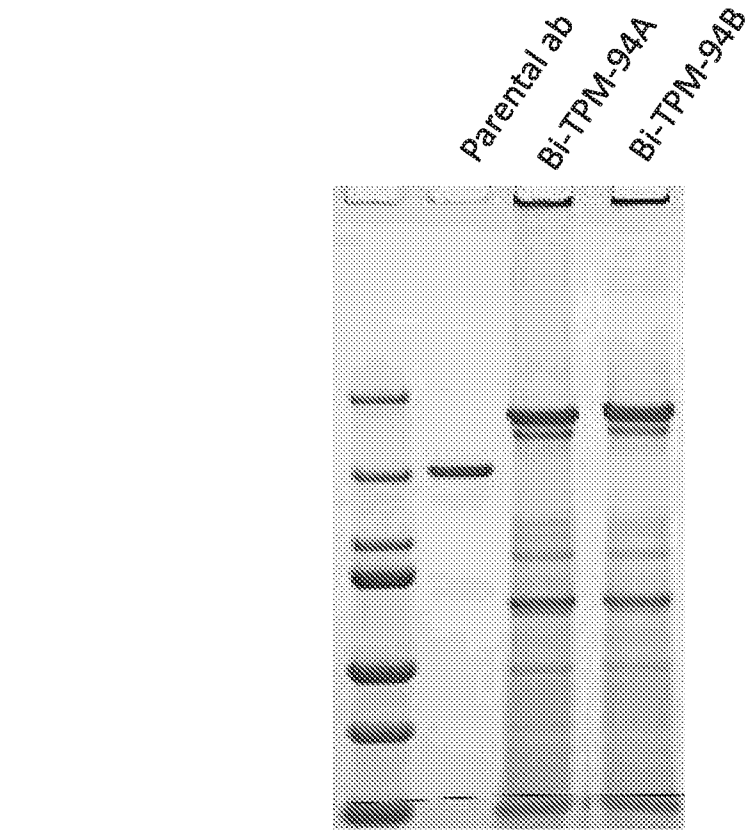

Robust transient expression levels for Bi-TPM-94B
Parental ab
Bi-TPM-94A
Bi-TPM-94B

FIG. 11

Size-exclusion ultra-high performance liquid chromatography revealed species heterogeneity in Bi-TPM-93 and Bi-TPB-94A, which was eliminated by linker modification in Bi-TPM-94B

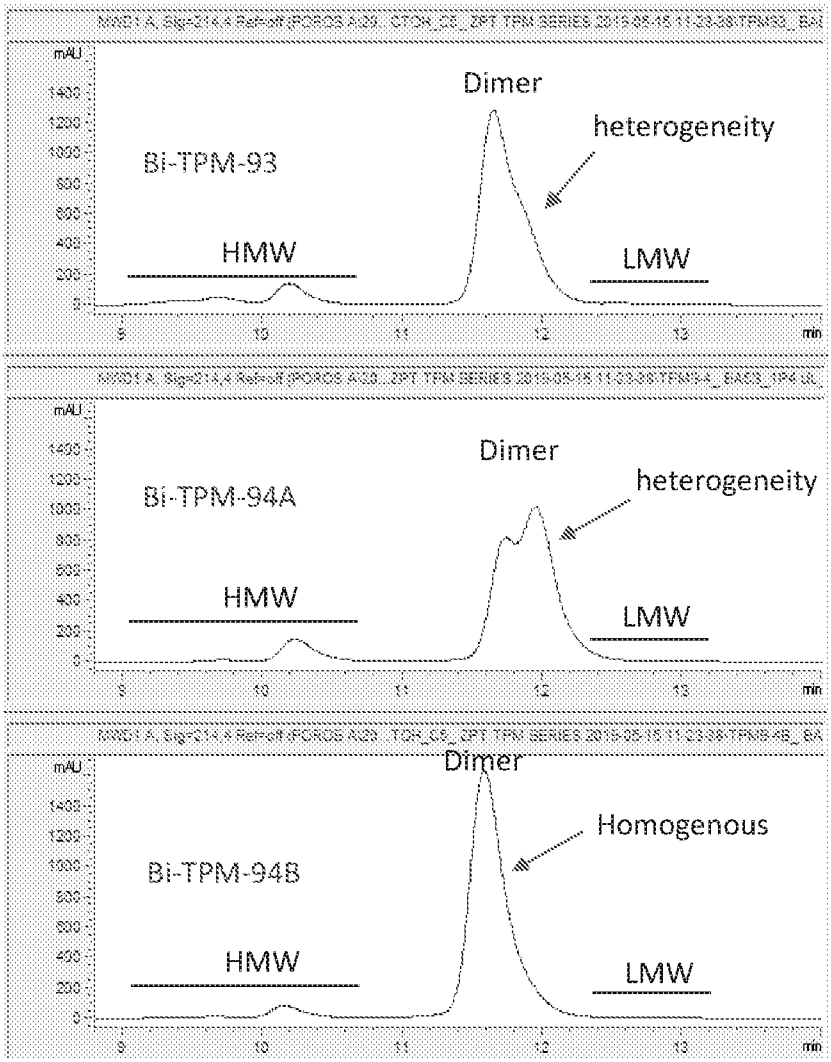
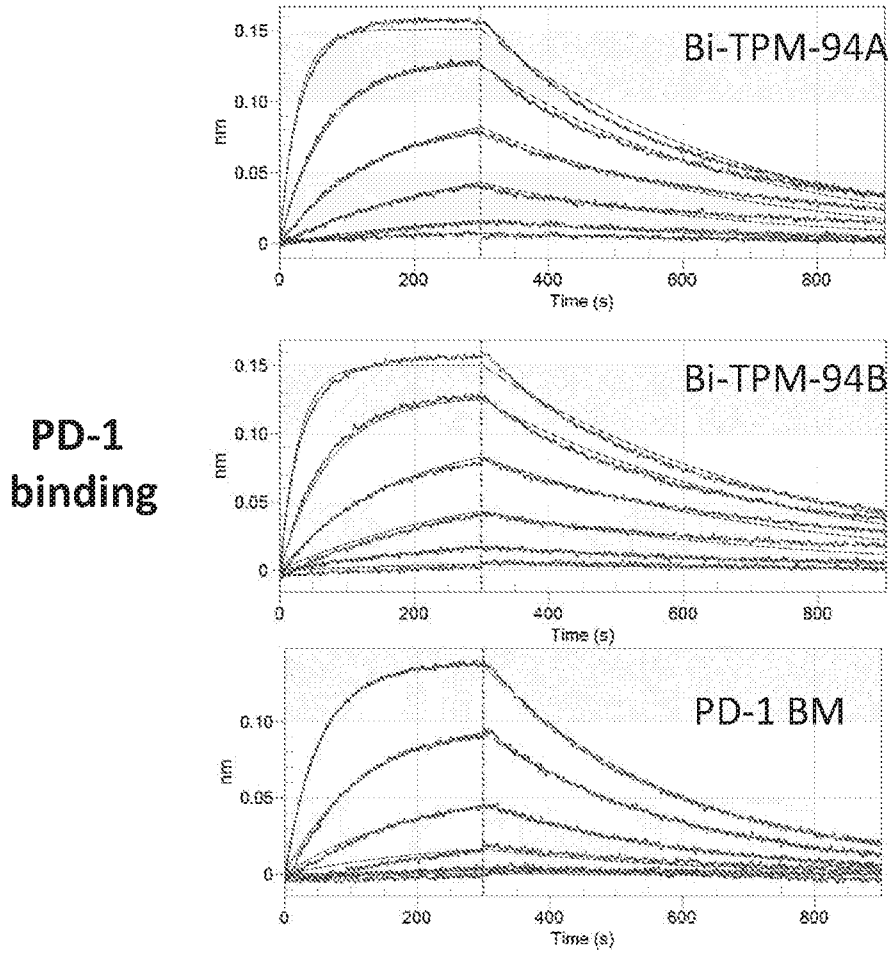


FIG. 12

Bi-TPM-94A and -94B bind PD-1 better than the benchmark Ab

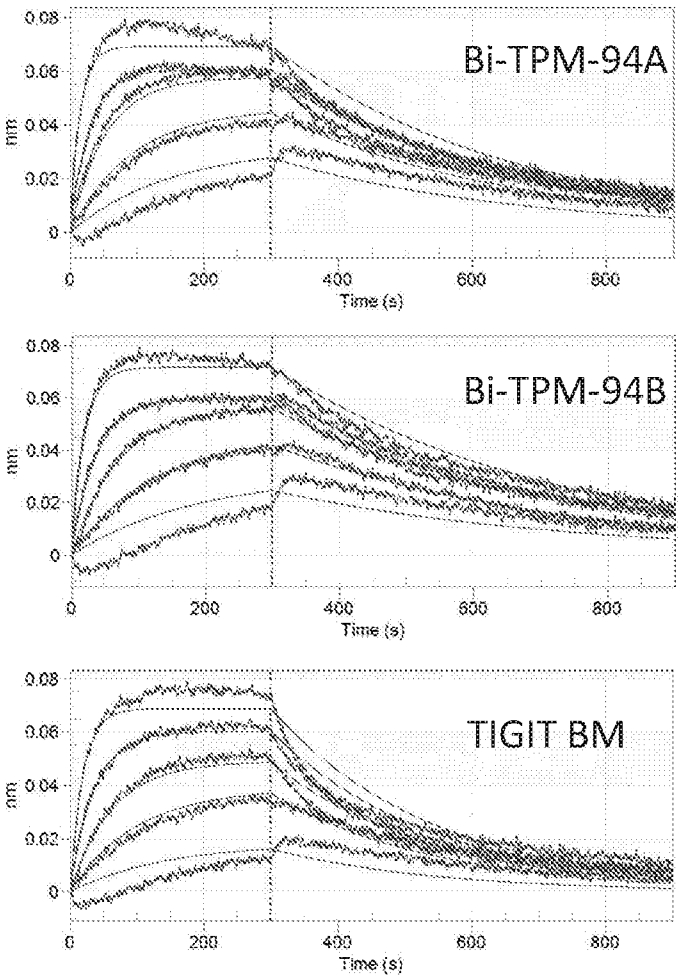


	Soluble Human PD-1		
Antibody	K_D (nM)	$K_a(M^{-1}s^{-1})$	$K_d(s^{-1})$
Bi-TPM-94A	2.9	4.37E+05	1.27E-03
Bi-TPM-94B	2.7	4.11E+05	1.10E-03
PD-1 benchmark	8.8	1.88E+05	1.66E-03

FIG. 13A

Bi-TPM-94A and -94B bind TIGIT better than the benchmark Ab

TIGIT binding



	Soluble Human TIGIT		
Antagonist	K_D (nM)	K_a ($M^{-1}s^{-1}$)	K_d (s^{-1})
Bi-TPM-94A	1.5	1.80E+06	2.71E-03
Bi-TPM-94B	1.7	1.35E+06	2.33E-03
TIGIT Benchmark	3.1	1.34E+06	4.11E-03

FIG. 13B

Bi-TPM-94A and Bi-TPM-94B potently block both PD1 and TIGIT binding to their ligands

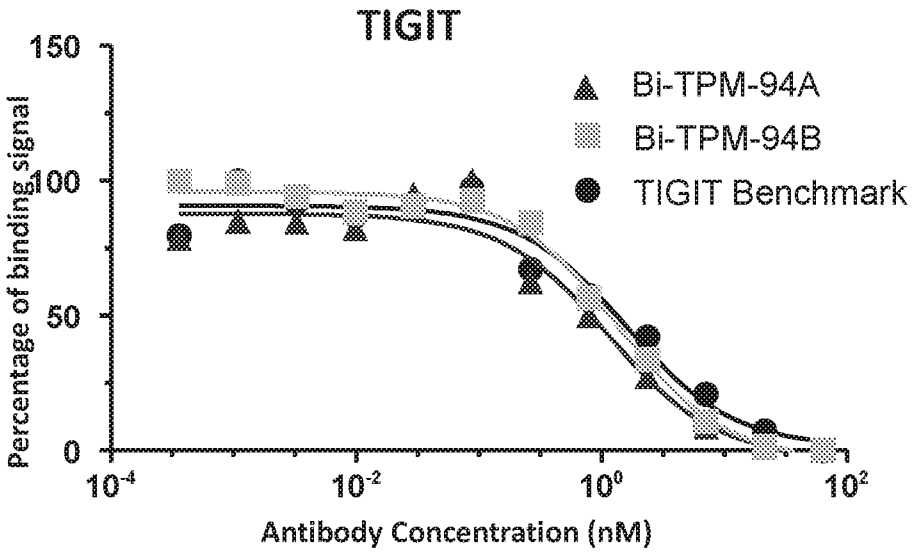


FIG. 14A

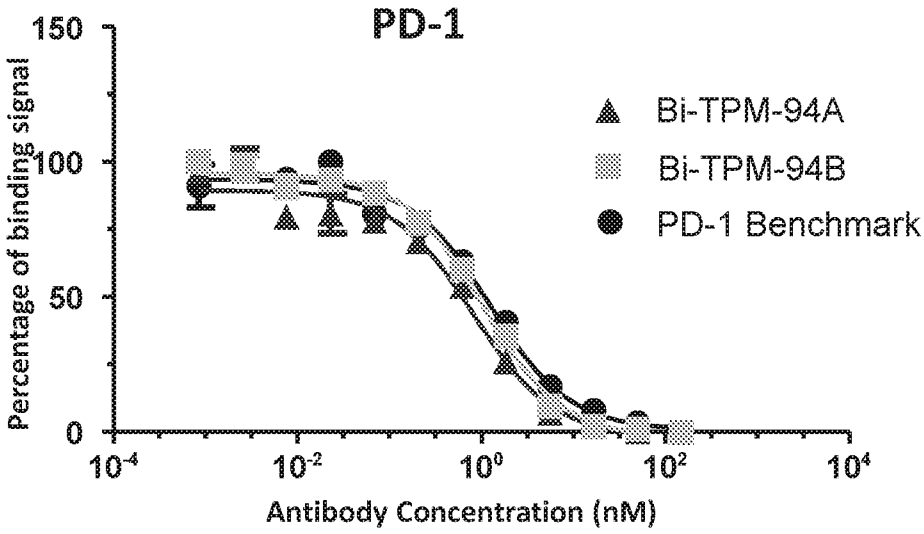


FIG. 14B

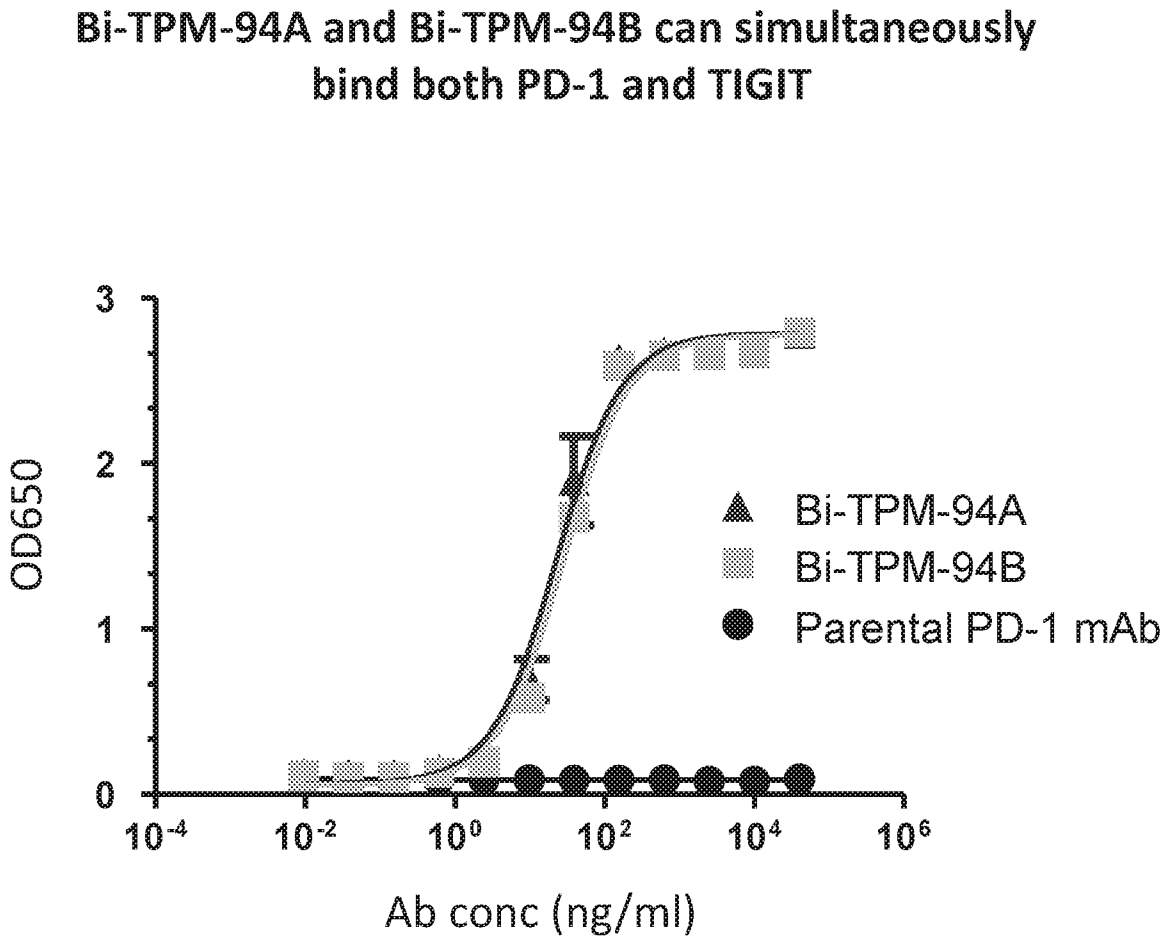

Bi-TPM-94A and Bi-TPM-94B can simultaneously bind both PD-1 and TIGIT
OD650
3
2
1
0
10^{-4}
10^{-2}
10^{0}
10^{2}
10^{4}
10^{6}
Ab conc (ng/ml)
Bi-TPM-94A
Bi-TPM-94B
Parental PD-1 mAb

FIG. 15

Bi-TPM-94B induces more IFN-γ production than the individual combination of antibodies during CMV recall response of human PBMCs

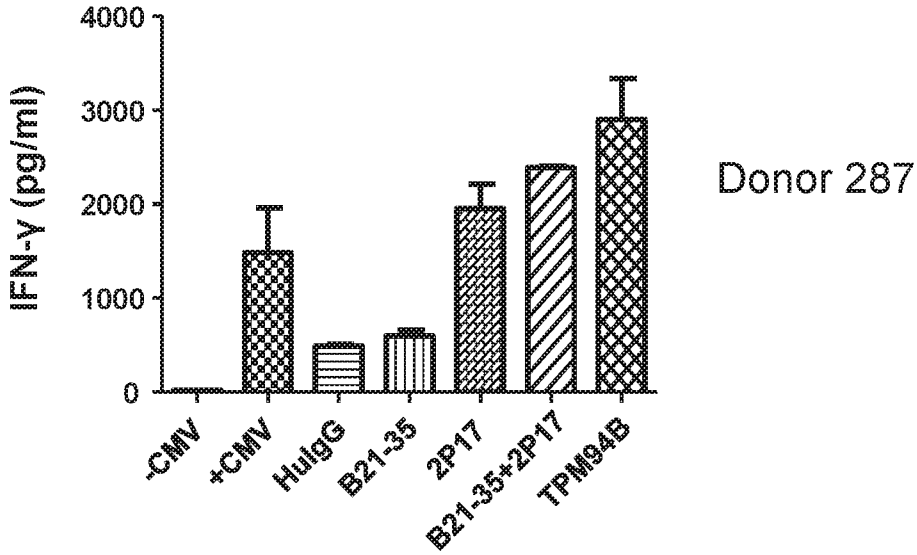


FIG. 16A

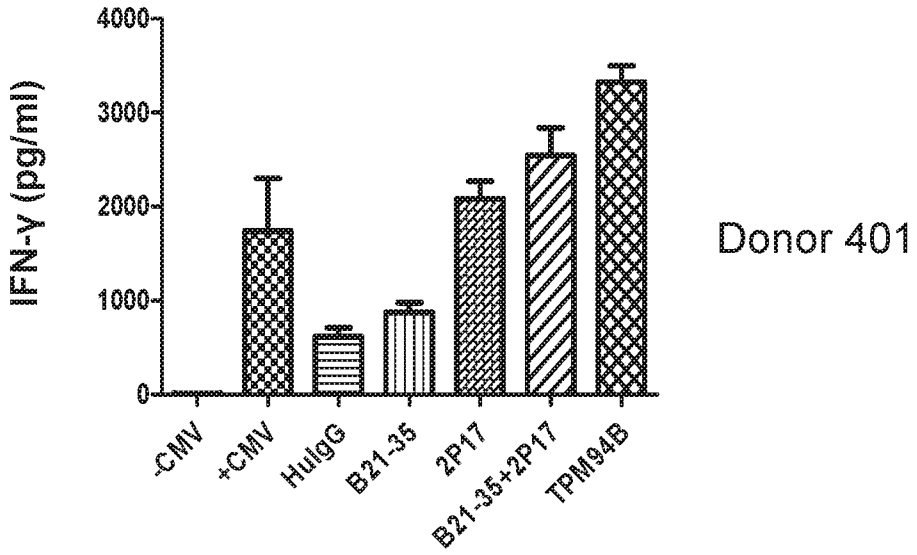
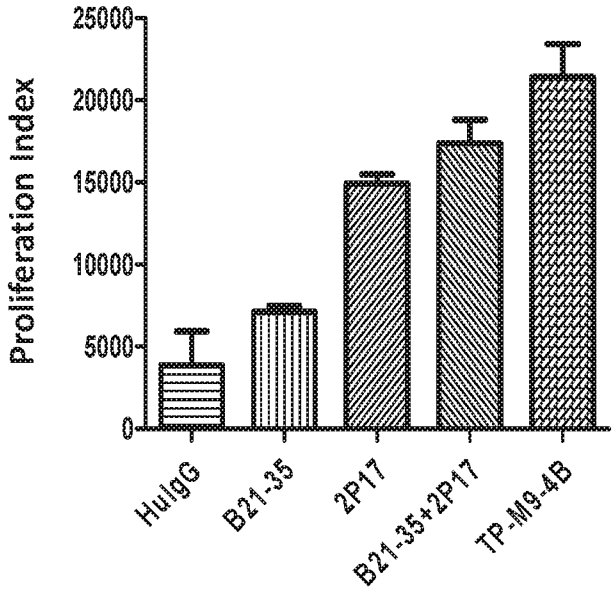


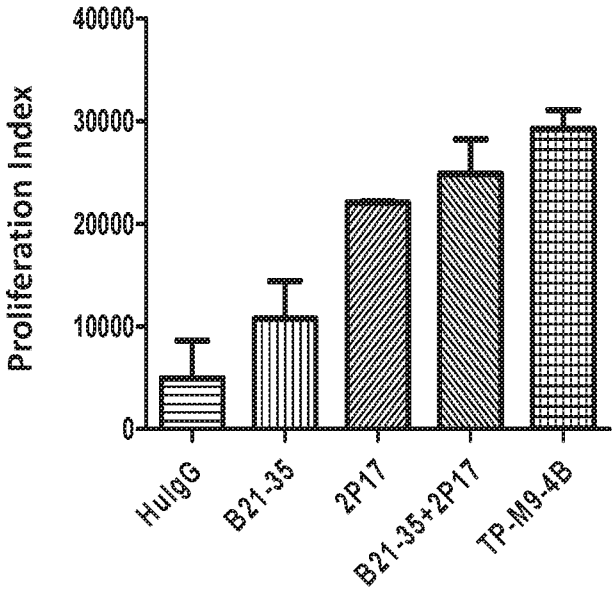
FIG. 16B

Bi-TPM94B induces more T cell proliferation than the combination of antibodies during CMV recall response of human PBMCs



Donor 287

FIG. 17A



Donor 401

FIG. 17B

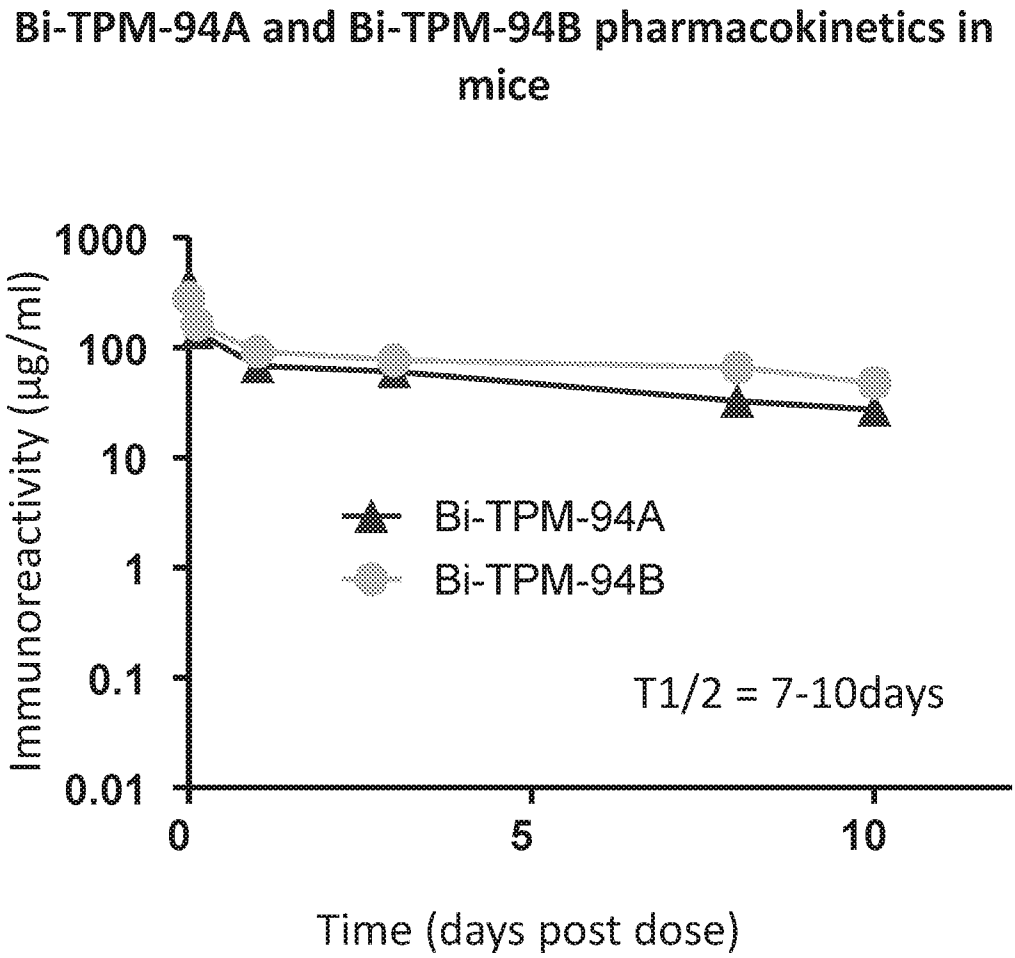

Bi-TPM-94A and Bi-TPM-94B pharmacokinetics in mice
Immunoreactivity (µg/ml)
1000
100
10
1
0.1
0.01
0
5
10
Time (days post dose)
Bi-TPM-94A
Bi-TPM-94B
T1/2 = 7-10days

FIG. 18

Anti-LAG-3 mAb CDR1, CDR2 and CDR3 Amino Acid Sequences

mAb	HCDR1	HCDR2	HCDR3
2L2A.1	DYYMN (SEQ ID:163)	VINPYNGDTSYNQKFKG (SEQ ID:164)	DDGYYVHYFDY (SEQ ID:165)
2L2A.6	DYYMN (SEQ ID:163)	VINPYNGDTSYNQKFKG (SEQ ID:164)	DDGYYVHYFDY (SEQ ID:165)
2L27B	HYYMN (SEQ ID:166)	LINPYNGDTAYNQKFKD (SEQ ID:167)	TRDDGYYVEH (SEQ ID:168)
3L1A	TAYTIH (SEQ ID:169)	WLYPGNDNIMYNENFKD (SEQ ID:170)	HEDWGPLDY (SEQ ID:171)

FIG. 19A

mAb	LCDR1	LCDR2	LCDR3
2L2A.1	RASQDISSRLT (SEQ ID:172)	ATSSLDS (SEQ ID:173)	LQYASSPLT (SEQ ID:174)
2L2A.6	RASQDISSRLT (SEQ ID:172)	ATSSLDS (SEQ ID:173)	LQYASSPLT (SEQ ID:174)
2L27B	RASQDIGSRLN (SEQ ID:175)	ATSSLDS (SEQ ID:173)	LQYASSPPT (SEQ ID:176)
3L1A	RASQSISS (SEQ ID:177)	RASNLES (SEQ ID:178)	QQSNGLPYT (SEQ ID:179)

FIG. 19B

Anti-LAG-3 mAb Variable Domain Sequences

2L2A.1
VH (SEQ ID NO: 180)
QVQLVQSGAEVKKPGASVKVSCKASGYTLTDYYMNWMRQAPGQGLEWMGVINPYNGDTSYNQKFKGRVTMTR
DTSTSTVYMELSSLRSEDTAVYYCVRDDGYYVHYFDYWGQGTLVTVSS

VL (SEQ ID NO: 181)
DIQMTQSPSSLSASVGDRVTITCRASQDISSRLTWLQQEPEKAPKRLIYATSSLDSGVPKRFSGSGSGTDFTLTISSLQP
EDFATYYCLQYASSPLTFGGGTKVEIK

2L2A.6
VH (SEQ ID NO: 182)

QVQLVQSGAEVKKPGASVKVSCKASGYTFTDYYMNWVRQAPGQGLEWMGVINPYNGDTSYNQKFKGRVTMTR
DTSTSTVYMELSSLRSEDTAVYYCARDDGYYVHYFDYWGQGTLVTVSS

VL (SEQ ID NO: 183)
DIQMTQSPSSLSASVGDRVTITCRASQDISSRLTWLQQKPGKAPKRLIYATSSLDSGVPSRFSGSGSGTDFTLTISSLQP
EDFATYYCLQYASSPLTFGGGTKVEIK

2L27B
VH (SEQ ID NO: 184)
QVQLVQSGAEVKKPGASVKVSCKASGFTFSHYYMNWVRQAPGQGLEWMGLINPYNGDTAYNQKFKDRVTMTR
DTSTSTVYMELSSLRSEDTAVYFCTRDDGYYVEHFDYWDDGYYVEHFDYWGQGTLVTVSS

VL (SEQ ID NO: 185)
DIQMTQSPSSLSASVGDRVTITCRASQDIGSRLNWYQQKPGKAPKRLIYATSSLDSGVPSRFSGSGSGTDFTLTISSLQ
PEDFATYYCLQYASSPPTFGGGTKVEIK

3L1A
VH (SEQ ID NO: 186)

QVQLVQSGAEVKKPGASVKVSCKASGYTFTAYTIHWVRQAPGQGLEWMGWLYPGNDNIMYNENFKDRVTMTR
DTSTSTVYMELSSLRSEDTAVYYCARHEDWGPLDYWGQGTLVTVSS

VL (SED ID NO: 187)
DIQMTQSPSTLSASVGDRVTITCRASQSISSWLAWYQQKPGKAPKLLIYRASNLESGVPSRFSGSGSGTEFTLTISSLQP
DDFATYYCQQSNGLPYTFGQGTKLEIK

FIG. 20

Mouse anti-LAG-3 mAbs block the interaction between LAG-3 and its MHC II ligand

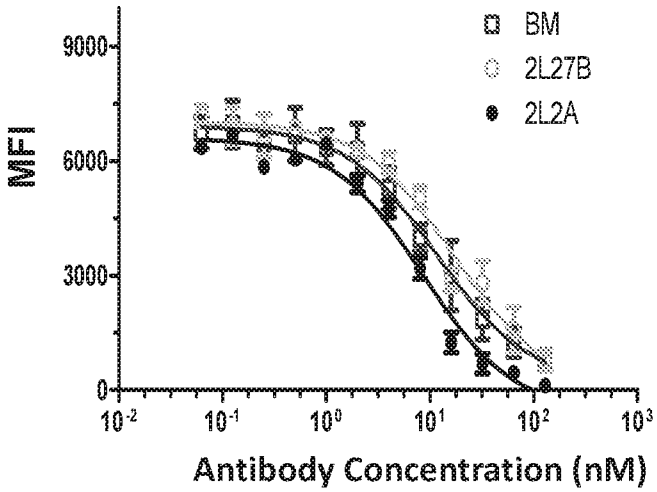


FIG. 21A

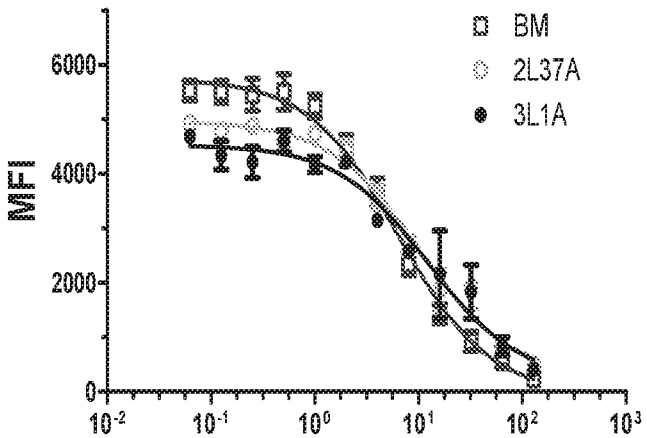
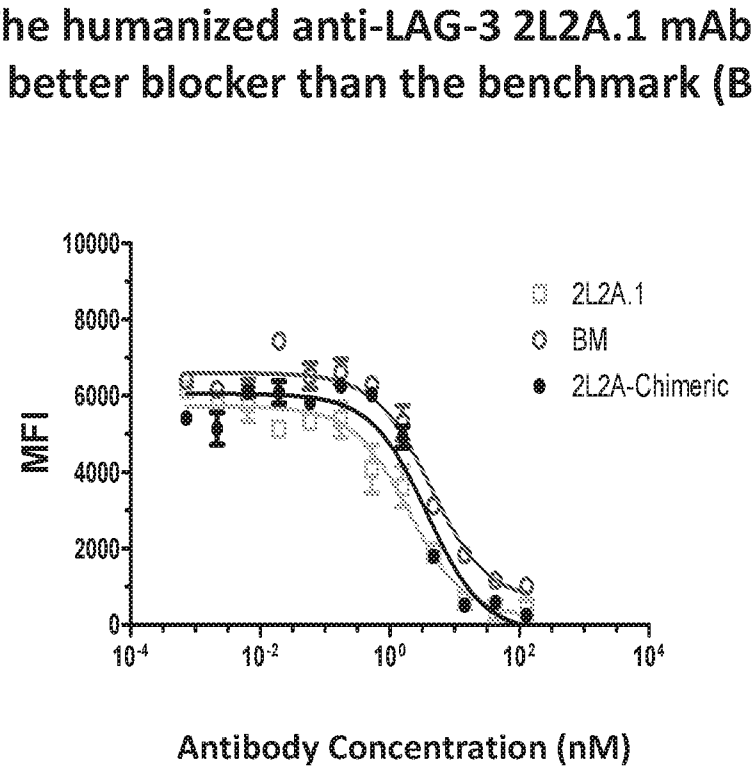


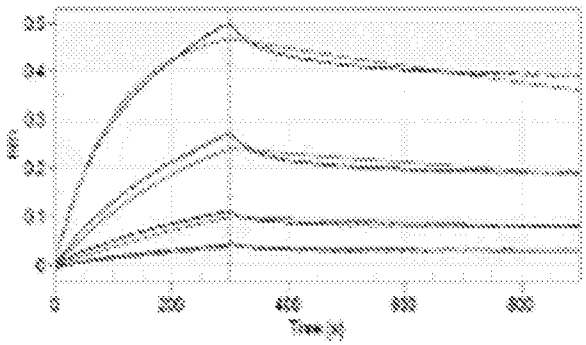
FIG. 21B



Antibody	IC50 (nM)
2L2A.1	1.97
Benchmark	4.14
2L2A- Chimeric	3.74

FIG. 22

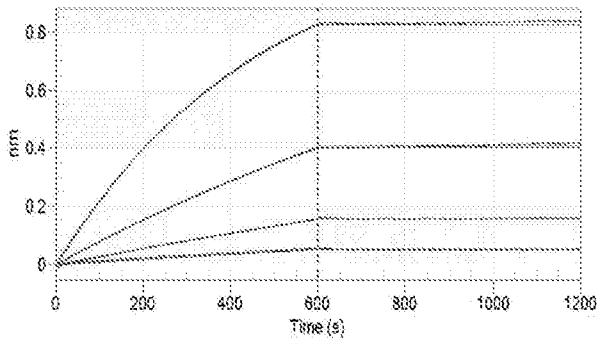
2L2A.1 affinity analysis by surface plasmon resonance (SPR)



2L2A.1

	huLAG-3-His		
Antibody	K_D (pM)	$K_a(M^{-1}s^{-1})$	$K_d(s^{-1})$
2L2A.1	1420 (±13)	3.93E+05	5.56E-04

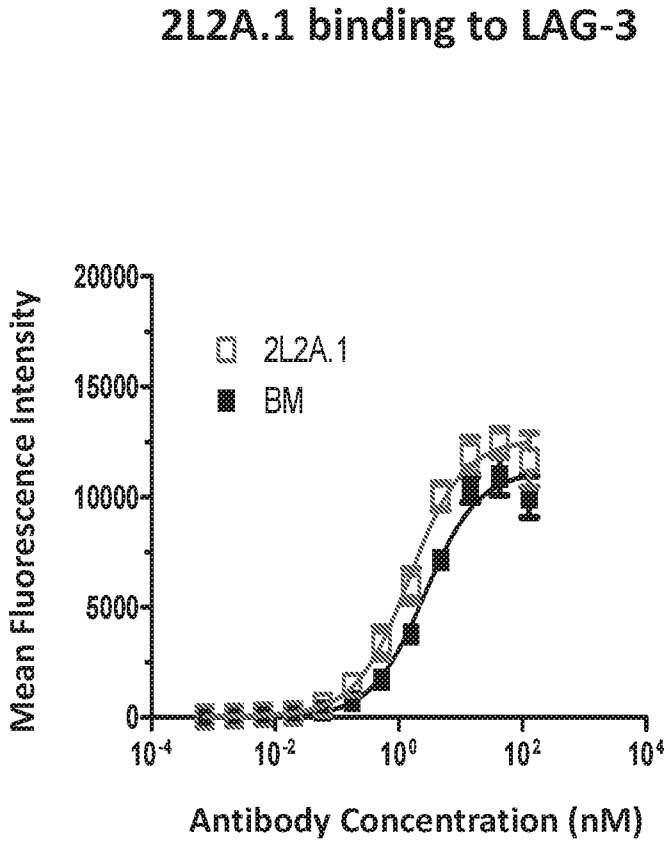
FIG. 23A



2L2A.1

	huLAG-3-mIgG2a		
Antibody	K_D (pM)	$K_a(M^{-1}s^{-1})$	$K_d(s^{-1})$
2L2A.1	1 (±11)	8.49E+04	<1.0E-07

FIG. 23B



Antibody	EC50 (nM)
Benchmark Ab	2.64
2L2A.1	1.48

FIG. 24


2L2A.1 expression
3rd day (65h)
mAb standard
+ctrl
2L2A.1
1ug
2ug

FIG. 25

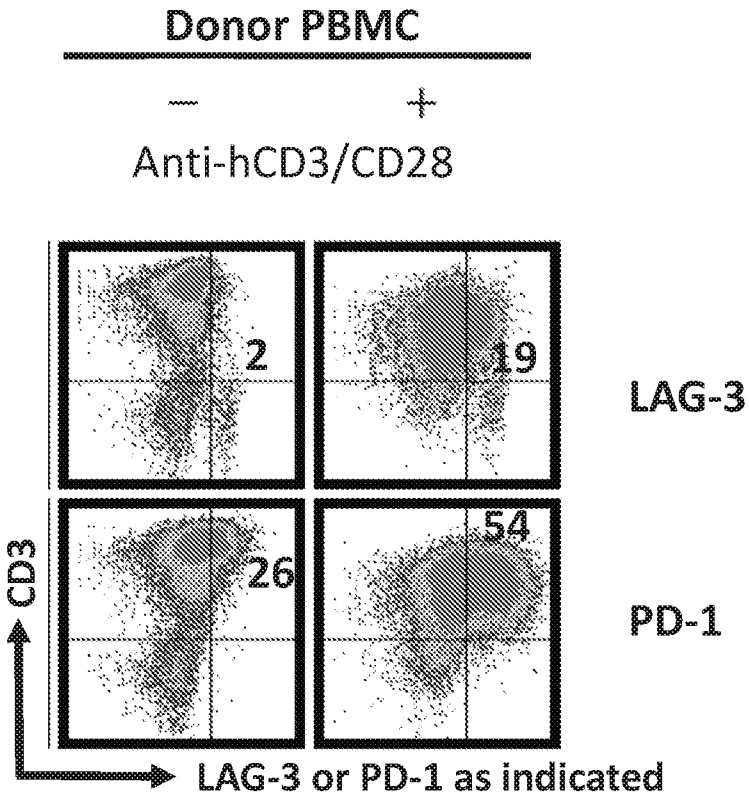

Donor PBMC
–
+
Anti-hCD3/CD28
2
19
26
54
LAG-3
PD-1
CD3
LAG-3 or PD-1 as indicated

FIG. 26

Anti-LAG-3 mAb 2L2A.1 induces more IFN-γ production than a benchmark (BM) anti-LAG-3 mAb

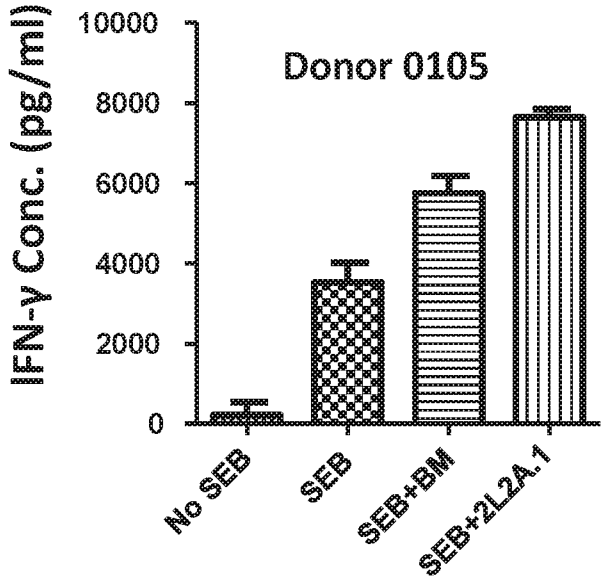


FIG. 27A

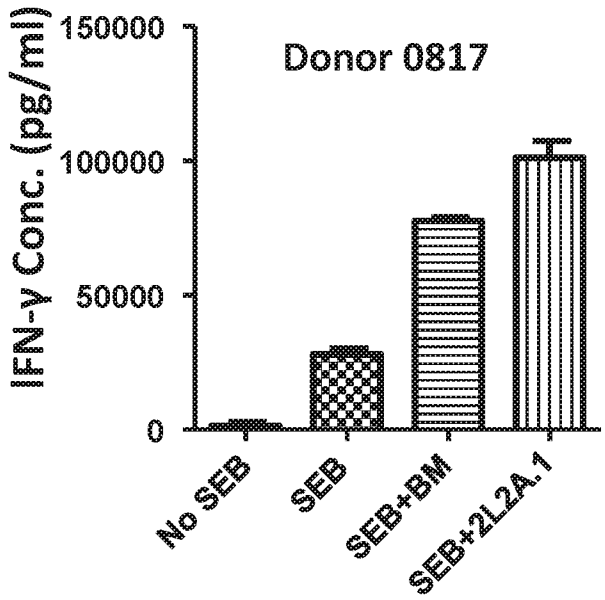


FIG. 27B

Anti-LAG-3 mAb 2L2A.1 can induce more IFN-γ production than a benchmark (BM) anti-LAG-3 mAb

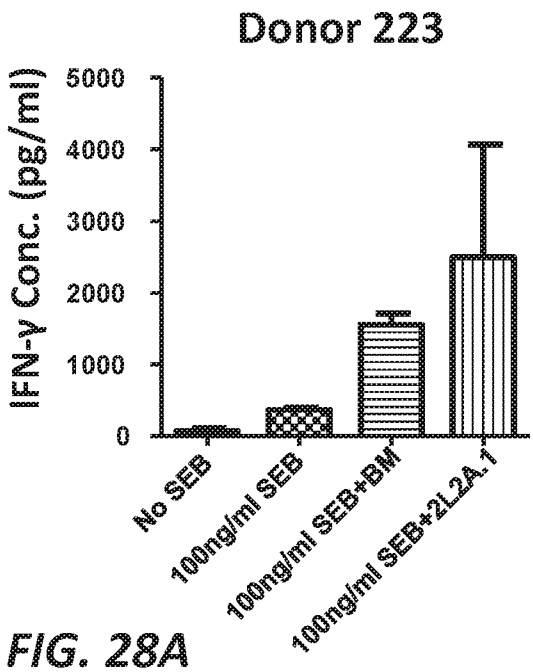


FIG. 28A

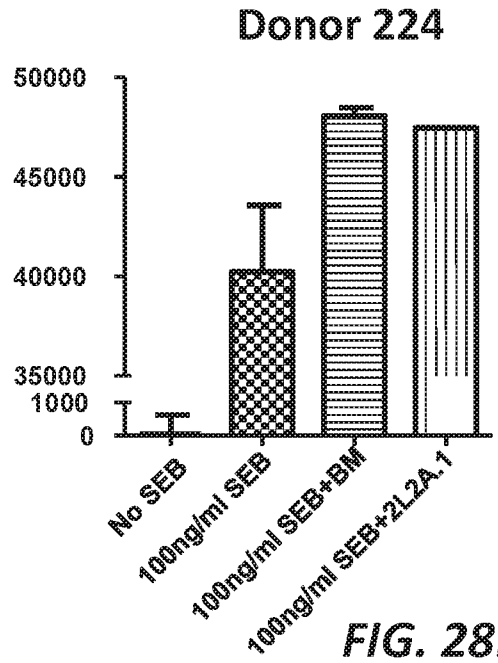


FIG. 28B

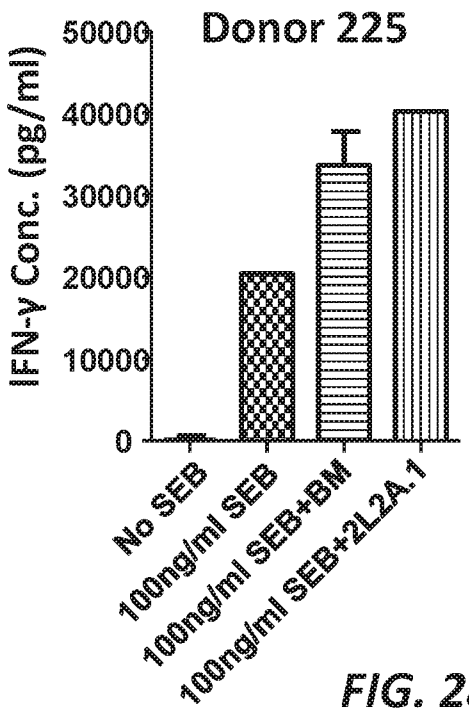


FIG. 28C

Anti-LAG-3 mAb 2L2A.1 can induce more primary T cell proliferation than a benchmark (BM) anti-LAG-3 mAb

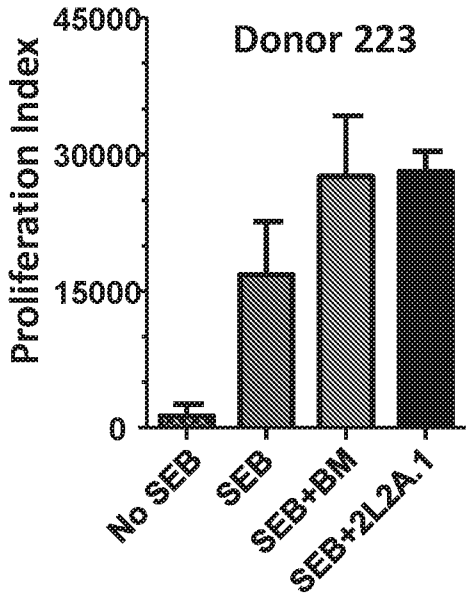


FIG. 29A

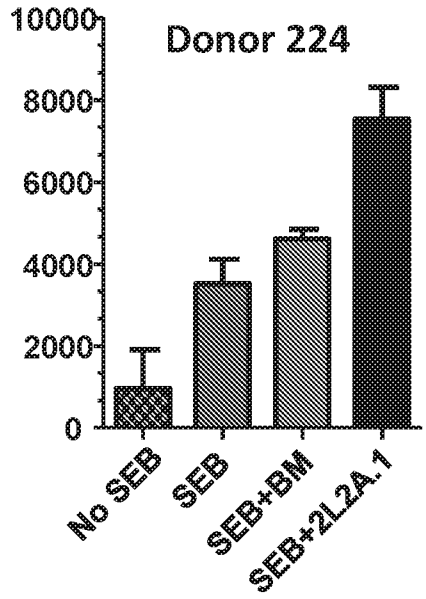


FIG. 29B

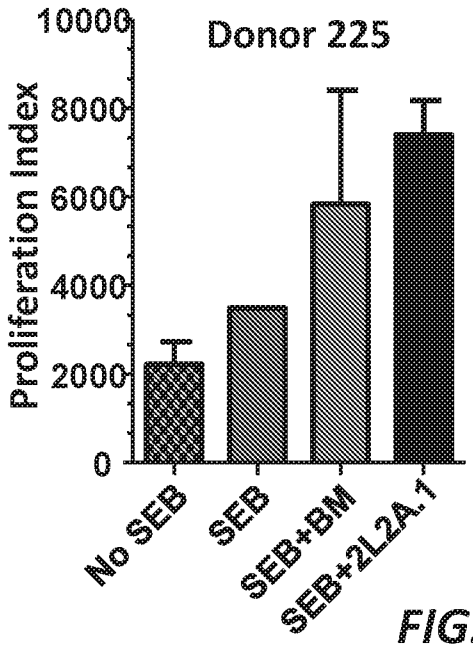
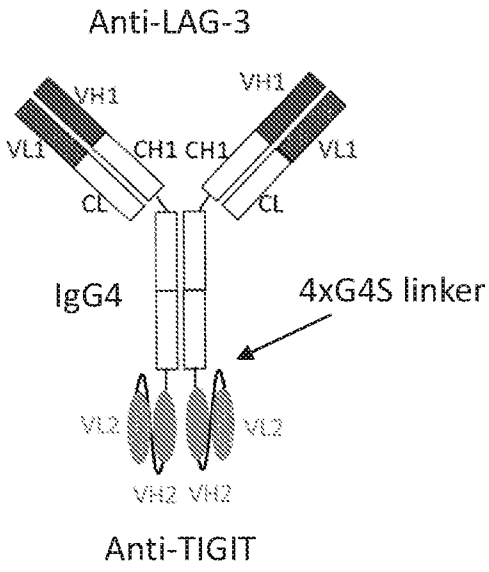


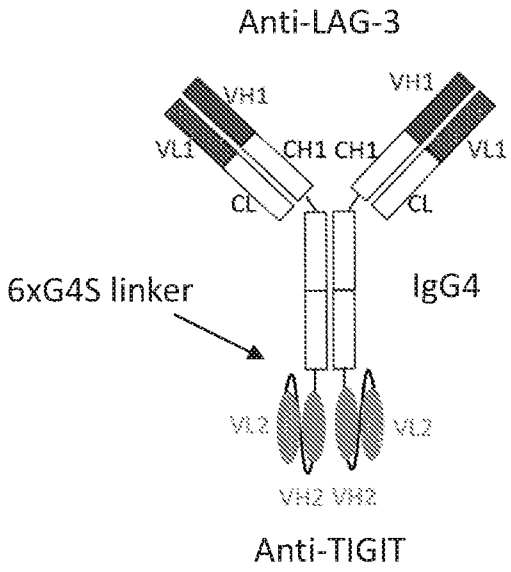
FIG. 29C

Anti-LAG-3 antibodies with TIGIT scfv



Bi-LT-1

FIG. 30A



Bi-LT-3

FIG. 30B

Functional Domain Sequences in FIGs. 30A-30B

VH/VL or Fusion Protein Domain	Amino Acid Sequences of Functional Domains
Anti-LAG-3 HCVR (2L2A.1)	QVQLVQSGAEVKKPGASVKVSCKASGYTLTDYYMNWMRQAPGQGLEWMGVINPYNGDTSYNQK FKGRVTMTRDTSTSTVYMELSSLRSEDTAVYYCVRDDGYYVHYFDYWGQGTLVTVSS(SEQ ID NO: 180)
Anti-LAG-3 LCVR (2L2A.1)	DIQMTQSPSSLSASVGDRVTITCRASQDISSRLTWLQQEPEKAPKRLIYATSSLDSGVPKRFSGSGSGT DFTLTISSLQPEDFATYYCLQYASSPLTFGGGTKVEIK (SEQ ID NO: 181)
Anti-TIGIT HCVR (B21-35)	QVQLVQSGAEVKKPGASVKVSCKASGYTFTSYYMHWVRQAPGQGLEWMGIINPSGGRTSYAQMF QGRVTMTRDTSTSTVYMELSSLRSEDTAVYYCARDREEQWPVGGFDYWGQGTLVTVSS (SEQ ID NO: 66)
Anti-TIGIT LCVR (B21-35)	DIQMTQSPSSLSASVGDRVTITCRASQSIRRYLNWYQQKPGKAPKLLIYSASNLQSGVPSRFSGSGSGT DFTLTISSLQPEDFATYYCQQSYIIPPTFGQGTKVEIK (SEQ ID NO: 67)
IgG4 CH1-CH2-CH3 (hinge stabilized S231P)	ASTKGPSVFPLAPCSRSTSESTAALGCLVKDYFPEPVTVSWNSGALTSGVHTFPAVLQSSGLYSLSSVVT VPSSSLGTKTYTCNVDHKPSNTKVDKRVESKYGPPCPPCPAPEFLGGPSVFLFPPKPKDTLMISRTPEV TCVVVDVSQEDPEVQFNWYVDGVEVHNAKTKPREEQFNSTYRVVSVLTVLHQDWLNGKEYKCKVS NKGLPSSIEKTISKAKGQPREPQVYTLPPSQEEMTKNQVSLTCLVKGFYPSDIAVEWESNGQPENNYK TTPPVLDSDGSFFLYSRLTVDKSRWQEGNVFSCSVMHEALHNHYTQKSLSLSLGK (SEQ ID NO: 155)
4xG4S linker	GGGGSGGGGSGGGGSGGGGS (SEQ ID NO: 189)
6xG4S linker	GGGGSGGGGSGGGGSGGGGSGGGGSGGGGS (SEQ ID NO: 191)

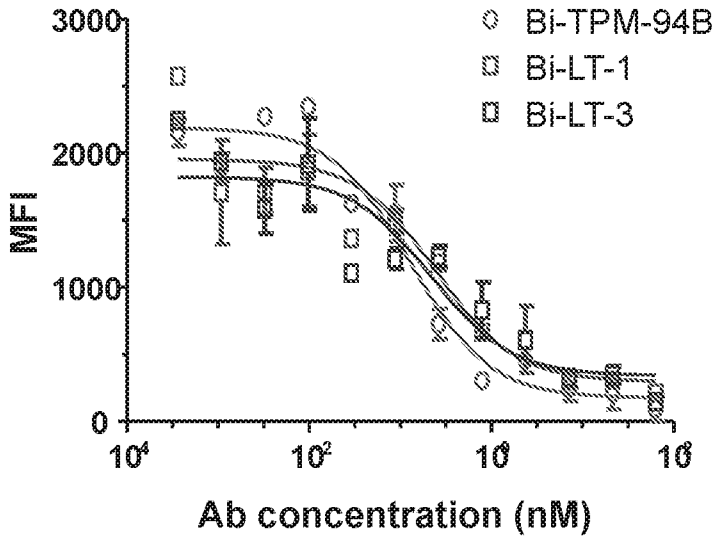
FIG. 31

HC and LC Sequences of Antagonists of Bi-LT-1 and Bi-LT-3 in FIGs. 30A-30B

Antagonist (HC/LC)	Amino Acid Sequence
Bi-LT-1 HC	QVQLVQSGAEVKKPGASVKVSCKASGYTLTDYYMNWMRQAPGQGLEWMGVINPYNGDT SYNQKFKGRVTMTRDTSTSTVYMELSSLRSEDTAVYYCVRDDGYYVHYFDYWGQGTLVTVSS ASTKGPSVFPLAPCSRSTSESTAALGCLVKDYFPEPVTVSWNSGALTSGVHTFPAVLQSSGLYS LSSVVTVPSSSLGTKTYTCNVDHKPSNTKVDKRVESKYGPPCPPCPAPEFLGGPSVFLFPPKPK DTLMISRTPEVTCVVVDVSQEDPEVQFNWYVDGVEVHNAKTKPREEQFNSTYRVVSVLTVLH QDWLNGKEYKCKVSNKGLPSSIEKTISKAKGQPREPQVYTLPPSQEEMTKNQVSLTCLVKGFY PSDIAVEWESNGQPENNYKTTPPVLDSDGSFFLYSRLTVDKSRWQEGNVFSCSVMHEALHN HYTQKSLSLSLGKGGGGSGGGGSQVQLVQSGAEVKKPGASVKVSCKASGYTFTSYYMHWVR QAPGQGLEWMGIINPSGGRTSYAQMFQGRVTMTRDTSTSTVYMELSSLRSEDTAVYYCARD REEQWPVGGFDYWGQGTLVTVSSGGGGSGGGGSGGGGSGGGGSDIQMTQSPSSLSASVG DRVTITCRASQSIRRYLNWYQQKPGKAPKLLIYSASNLQSGVPSRFSGSGSGTDFTLTISSLQPE DFATYYCQQSYIIPPTFGQGTKVEIK (SEQ ID NO:192)
Bi-LT-1 LC	DIQMTQSPSSLSASVGDRVTITCRASQDISSRLTWLQQKPEKAPKRLIYATSSLDSGVPSRFSGS GSGTDFTLTISSLQPEDFATYYCLQYASSPLTFGGGTKVEIKRTVAAPSVFIFPPSDEQLKSGTAS VVCLLNNFYPREAKVQWKVDNALQSGNSQESVTEQDSKDSTYSLSSTLTLSKADYEKHKVYA CEVTHQGLSSPVTKSFNRGEC (SEQ ID NO:194)
Bi-LT-3 HC	QVQLVQSGAEVKKPGASVKVSCKASGYTLTDYYMNWMRQAPGQGLEWMGVINPYNGDT SYNQKFKGRVTMTRDTSTSTVYMELSSLRSEDTAVYYCVRDDGYYVHYFDYWGQGTLVTVSS ASTKGPSVFPLAPCSRSTSESTAALGCLVKDYFPEPVTVSWNSGALTSGVHTFPAVLQSSGLYS LSSVVTVPSSSLGTKTYTCNVDHKPSNTKVDKRVESKYGPPCPPCPAPEFLGGPSVFLFPPKPK DTLMISRTPEVTCVVVDVSQEDPEVQFNWYVDGVEVHNAKTKPREEQFNSTYRVVSVLTVLH QDWLNGKEYKCKVSNKGLPSSIEKTISKAKGQPREPQVYTLPPSQEEMTKNQVSLTCLVKGFY PSDIAVEWESNGQPENNYKTTPPVLDSDGSFFLYSRLTVDKSRWQEGNVFSCSVMHEALHN HYTQKSLSLSLGKGGGGSGGGGSQVQLVQSGAEVKKPGASVKVSCKASGYTFTSYYMHWVR QAPGQGLEWMGIINPSGGRTSYAQMFQGRVTMTRDTSTSTVYMELSSLRSEDTAVYYCARD REEQWPVGGFDYWGQGTLVTVSSGGGGSGGGGSGGGGSGGGGSGGGGSGGGGSDIQM TQSPSSLSASVGDRVTITCRASQSIRRYLNWYQQKPGKAPKLLIYSASNLQSGVPSRFSGSGSG TDFTLTISSLQPEDFATYYCQQSYIIPPTFGQGTKVEIK (SEQ ID NO:193)
Bi-LT-3 LC	DIQMTQSPSSLSASVGDRVTITCRASQDISSRLTWLQQKPEKAPKRLIYATSSLDSGVPSRFSGS GSGTDFTLTISSLQPEDFATYYCLQYASSPLTFGGGTKVEIKRTVAAPSVFIFPPSDEQLKSGTAS VVCLLNNFYPREAKVQWKVDNALQSGNSQESVTEQDSKDSTYSLSSTLTLSKADYEKHKVYA CEVTHQGLSSPVTKSFNRGEC (SEQ ID NO:194)

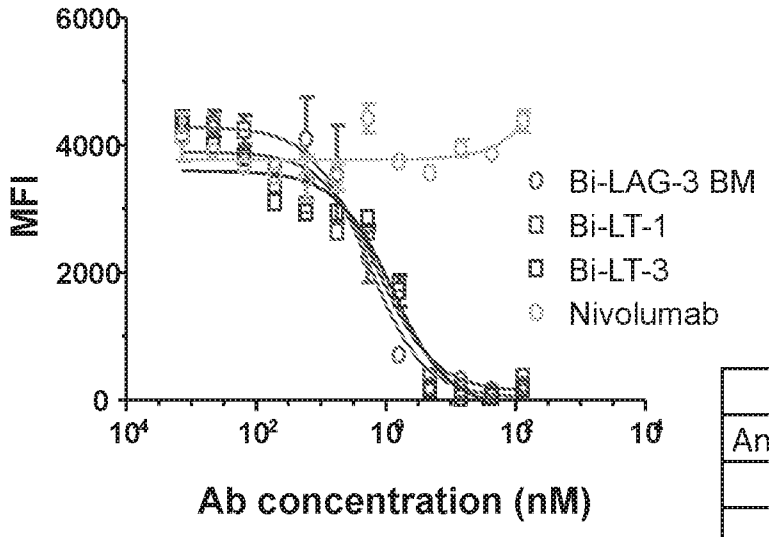
FIG. 32

Improved TIGIT scFvs with the extended 4xG4S or 6xG4S linkers fused to anti-LAG3 antibody retain bioactivity for both TIGIT and LAG3



TIGIT	IC50 (nM)
Bi-TPM-94B	0.13
Bi-LT-1	0.24
Bi-LT-3	0.22

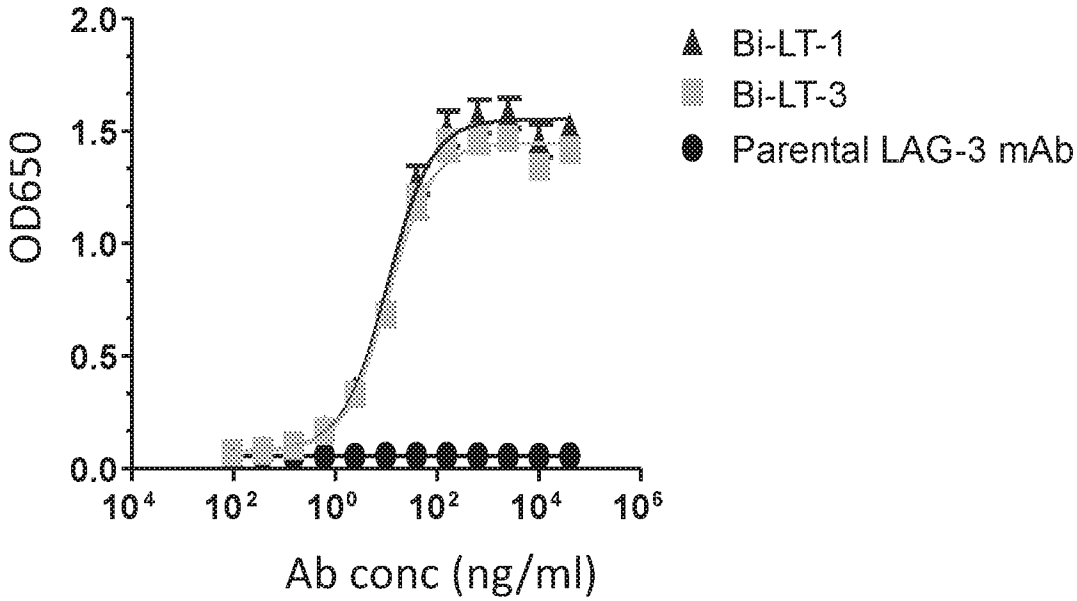
FIG. 33A



LAG-3	IC50 (nM)
Anti-LAG-3 BM	0.6
Bi-LT-1	0.7
Bi-LT-3	0.9

FIG. 33B

Bi-LT-1 and Bi-LT-3 can simultaneously bind LAG-3 and TIGIT



Antibody	Bottom	Top	EC50 (ng/ml)
LAG-3 mAb	0.06	0.06	NA
Bi-LT-1	0.08	1.55	10.67
Bi-LT-3	0.08	1.45	10.75

FIG. 34

Improved TIGIT scfvs fused to LAG-3 antibody have good pharmacokinetic properties, similar to unfused LAG3 antibody

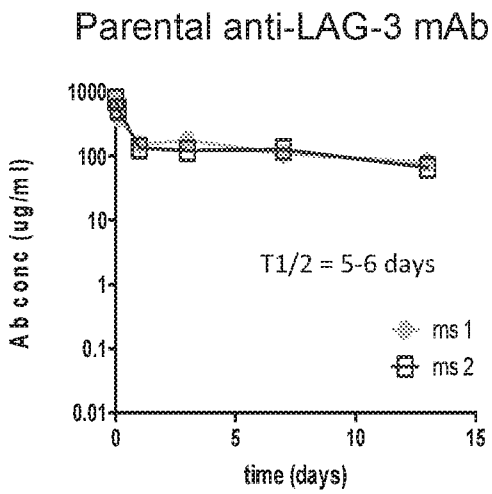


FIG. 35A

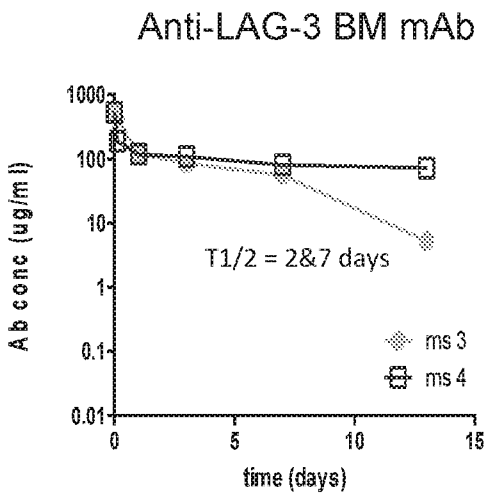


FIG. 35B

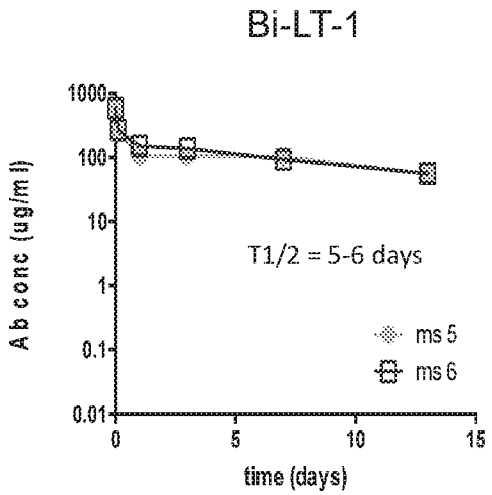


FIG. 35C

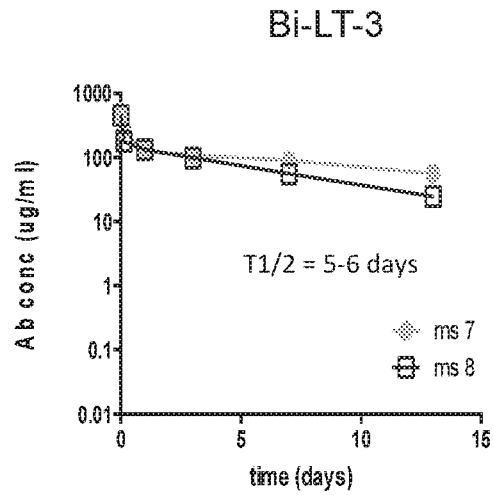


FIG. 35D

Bi-LT-1 and Bi-LT-3 with the improved TIGIT scFvs are homogenous and has good stability at 4° C for 7 days

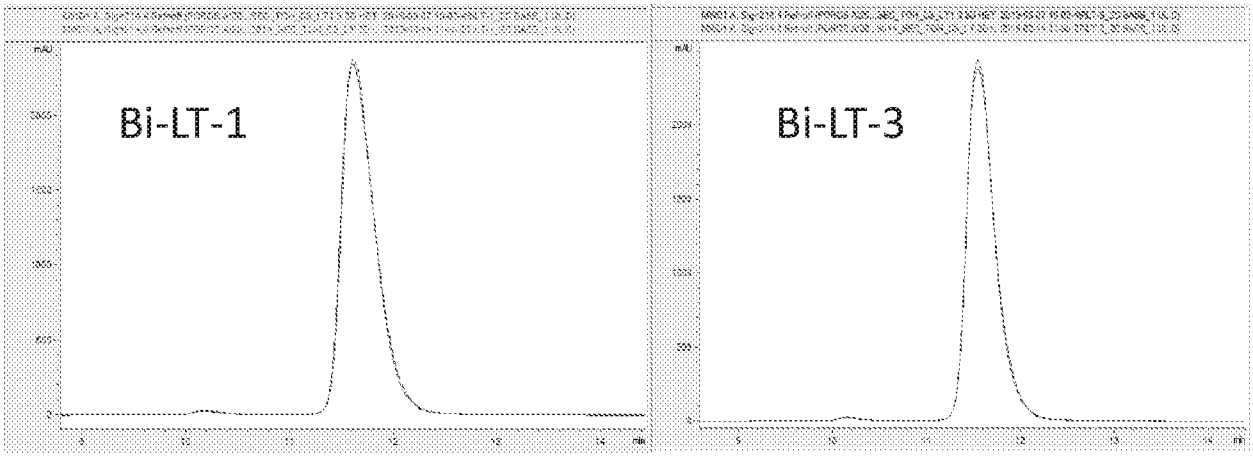


FIG. 36A

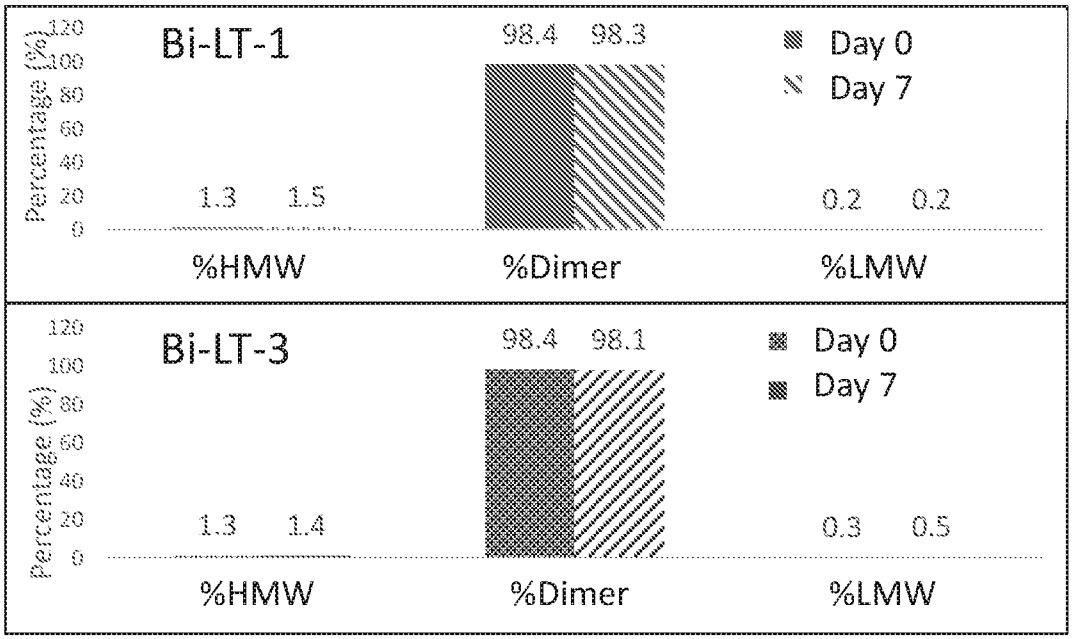


FIG. 36B

Bi-LT-1 and Bi-LT-3 are more potent than the combination of parental TIGIT and LAG-3 antibodies, since they induce more IFN-γ in a SEB stimulated PBMC assay

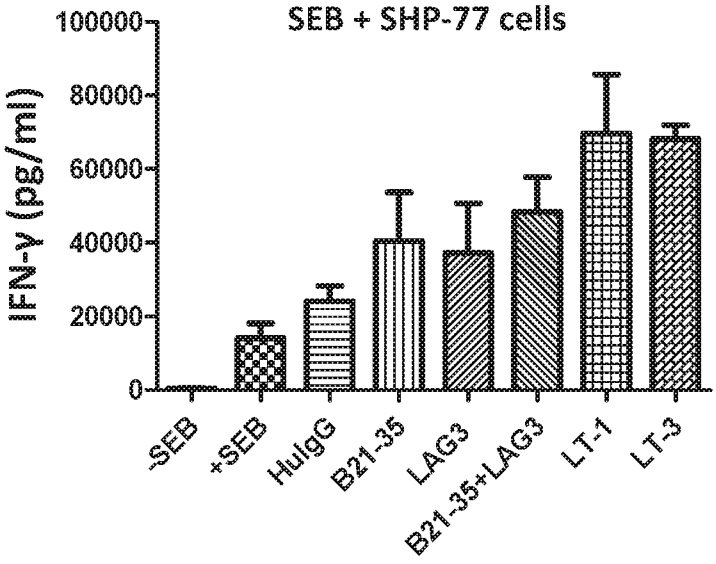


FIG. 37A

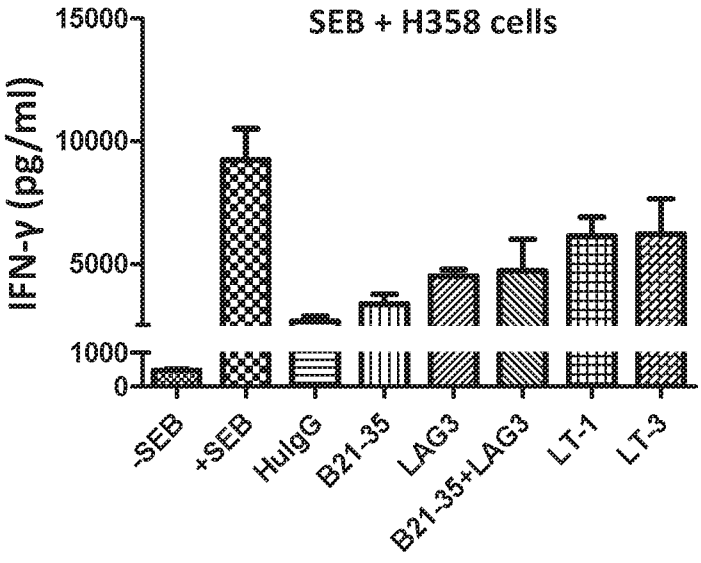


FIG. 37B

Bi-LT-1 and Bi-LT-3 are more potent than the combination of parental TIGIT and LAG-3 antibodies for the induction of T cell proliferation of SEB stimulated PBMCs

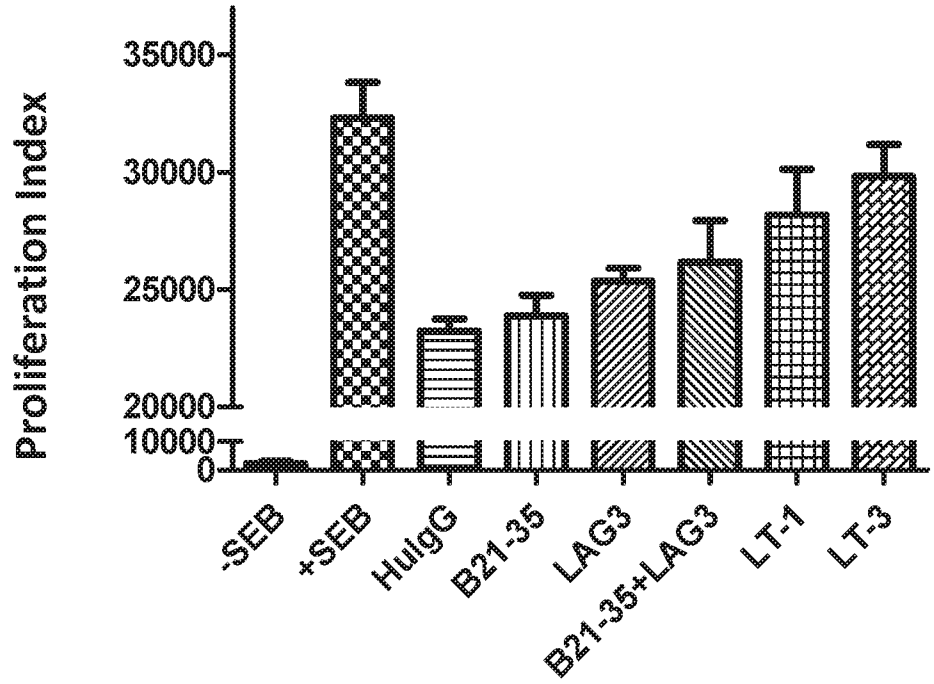


FIG. 38

SEQ ID NO	Sequence	Description/Notes
216	QVQLQESGPGLVKPSQTLSLTCTVSGYSIT	T-01 HFR1
217	WIRQPPGKGLEWIG	T-01, 02, 06, 07 HFR2
218	RVTISVDTSKNQFSLKLSSVTAADTAVYYCAR	T-01, 06 HFR3
219	WGQGTSVTVSS	T-01 HFR4
220	DIQMTQSPSSLSASVGDRVTITC	T-01, 03, 04, 05, 06, 07, 10 LFR1
221	WHQQKPGKAPKLLIY	T-01 LFR2
222	GVPSRFSGSGSGTDFTLTISSLQPEDFATYYCQQSYSTP	T-01, 02 LFR3
223	FGGGTKLEIKR	T-01, 06 LFR4
224	QVKLQESGPGLVKPSQTLSLTCTVTGYSIT	T-02 HFR1
225	RVTISVDTSKNQFSLKLSSVTAADTAVYSCAR	T-02 HFR3
226	WGQGTLVTVSA	T-02, 06, 07 HFR4
227	DIQMTQSPSSLSASVGDRVTIPC	T-02 LFR1
228	WYQQKPGKAPKLLIY	T-02, 03, 04, 05, 06, 07, 10 LFR2
229	FGEGTKLEIK	T-02 LFR4
230	EVQLVQSGAEVKKPGATVKISCKVSGYTFT	T-03, 04 HFR1
231	WVQQAPGKGLEWMG	T-03, 04, 05 HFR2
232	RVTITADTSTDTAYMELSSLRSEDTAVYYCAT	T-03, 04 HFR3
233	WGQGTLITVSVA	T-03 HFR4
234	GVPSRFSGSGSGTDFTLTISSLQPEDFATYYC	T-03, 05, 06, 10 LFR3
235	FGAGTKLELK	T-03, 05 LFR4
236	WGQGTLVTVSS	T-04, 05, 08, 09, 10 HFR4
237	GVPSRFSGSGSGTDFTFTISSLQPEDIATYYC	T-04 LFR3
238	FGAGTKLEIK	T-04 LFR4
239	EVQLKQSGAEVKKPGATVKISCKVSGYTFT	T-05 HFR1
240	RVTITADTSTDTAYMELSSLRSEDTAVYFCAR	T-05 HFR3
241	QVQLQESGPGLVKPSQTLSLTCTVSGGSVS	T-06 HFR1
242	EVQLQESGPGLVKPSDTLSLTCAVSGYSIT	T-07 HFR1
243	RVTMSVDTSKNQFSLKLSSVTAVDTAVYYCTR	T-07 HFR3
244	GAPSRFSGSGSGTDFTLTISSLQPEDFGIYYC	T-07 LFR3
245	FGGGTKLEFK	T-07 LFR4
246	QVQLVQSGSELKKPGASVKVSCKASGYTFT	T-08 HFR1
247	WVRQAPGQGLEWMG	T-08, 10 HFR2
248	RFVFSLDTSVSTAYLQISSLKAEDTAVYYCAR	T-08 HFR3
249	DVVMTQSPLSLPVTLGQPASISC	T-08 LFR1
250	WFQQRPGQSPRVLIY	T-08 LFR2
251	GVPDRFSGSGSGTDFTLKISRVEAEDVGVYYC	T-08 LFR3
252	FGRGTKLEIK	T-08 LFR4
253	QVTLKESGPTLVKPTQTLTLTCTFSGFSLS	T-09 HFR1
254	WIRQPPGKALEWLA	T-09 HFR2
255	RLTITKDTSKNQVVLTMTNMDPVDTATYYCAR	T-09 HFR3
256	QAVVTQEPSLTVSPGGTVTLTC	T-09 LFR1
257	WVQQKPGQLFRGLIG	T-09 LFR2
258	WVPARFSGSLIGDKAALTLSGVQPEDEAEYFC	T-09 LFR3
259	FGGGTKLTVL	T-09 LFR4
260	QVQLVQSGAEVKKPGASVKVSCKASGYTFT	T-10 HFR1
261	RVTMTRDTSTSTVYMELSSLRSEDTAVYYCAR	T-10 HFR3
262	FGQGTKVEIK	T-10 LFR4

FIG. 39A

SEQ ID NO	Sequence	Description/Notes
263	EVQLVESGGGLVQPGGSLRLSCAASGFTFS	PD-01 HFR1
264	WVRQAPGKGLEWVS	PD-01 HFR2
265	RFTISRDNSKNTLYLQMNSLRAEDTAVYYCAK	PD-01 HFR3
266	WGQGTSVTVSS	PD-01 HFR4
267	DIQMTQSPSSVSASVGDRVTITC	PD-01 LFR1
268	WYQQKPGKAPKLLIY	PD-01, 02, 03, 04; PL-02, 03 LFR2
269	GVPSRFSGSGSGTDFTLTISSLQPEDFATYYC	PD-01, 02, 03, 04 LFR3
270	FGGGTKLEIK	PD-01; PL-05 LFR4
271	QVQLVQSGAEVKKPGASVKVSCKASDYTFT	PD-02, 03, 04 HFR1
272	WLRQAPGQGLEWMG	PD-02 HFR2
273	RTTSTRDTSISTAYMELSRLRSDDTVVYYCTR	PD-02 HFR3
274	WGQGTLVTVSS	PD-02, 04, 05, 06; PL-01, 02, 03, 04, 06, 07, 08 HFR4
275	DIQMTQSPSSLSASVGDRVTFTC	PD-02, 04 LFR1
276	FGGGTKVEIK	PD-02, 04, 05; PL-01, 04, 06, 07, 08 LFR4
277	WVRQAPGQGLEWMG	PD-03, 04, 05, 06; PL-02, 03, 04, 08 HFR2
278	RVTSTRDTSISTAYMELSRLRSDDTVVYYCA	PD-03, 04; PL-01 HFR3
279	WGQGTTLTVSS	PD-03; PL-05 HFR4
280	DIQMTQSPSSLSASVGDRVTITC	PD-03 LFR1
281	FGAGTKLDLK	PD-03 LFR4
282	FGGGTKVEIK	PD-04, 05; PL-01, 04, 06, 07, 08 LFR4
283	QVQLVQSGAEVKKPGASVKVSCKASGYTFT	PD-05, 06; PL-01, 02, 03, 04, 06, 07, 08 HFR1
284	RVTMTRDTSISTAYMELSSLRSEDTAVYYCAR	PD-05 HFR3
285	DIVLTQSPASLAVSPGQRATITC	PD-05 LFR1
286	WYQQKPGQPPKLLIY	PD-05 LFR2
287	GVPARFSGSGSGTDFTLTINPVEANDTANYYC	PD-05 LFR3
288	RVTLTADTSTSTVYMELSSLRSEDTAVYYCA	PD-06 HFR3
289	DIQMTQSPSFLSASVGDRVTITC	PD-06 LFR1
290	WYQQKPGKAPKALIY	PD-06 LFR2
291	GVPSRFSGSGSGTEFTLTISSLQPEDFATYYC	PD-06 LFR3
292	FGQGTKLEIK	PD-06 LFR4

FIG. 39B

SEQ ID NO	Sequence	Description/Notes
293	WMKQAPGQGLEWMG	PL-01 HFR2
294	DIQMTQSPSSLSASVGDRVTISC	PL-01, 02, 03, 04, 08 LFR1
295	WYQQKPGKAPKLLIK	PL-01, 04, 08 LFR2
296	GVPSRFSGSGSGTDFTLTISSLQPEDFATYFC	PL-01, 04, 08 LFR3
297	KATMTRDKSSSTVYMELSSLRSEDTAVYYCAR	PL-02 HFR3
298	GVPSRFSGSGSGTDFTFTISSLQPEDIATYFC	PL-02, 03 LFR3
299	FGQGTKVEIK	PL-02, 03 LFR4
300	RVTMTRDTSISTAYMELSRLRSDDTAVYYCAR	PL-03, 08 HFR3
301	RVTMTRDTSTSTVYMELSSLRSEDTAVYYCAR	PL-04 HFR3
302	DVQLQESGPGLVKPSQSLSLTCTVTGYSIT	PL-05 HFR1
303	WIRQFPGNKLEWMG	PL-05 HFR2
304	RISITRDTSKNQFFLQLNSVTTEDTATYYCAN	PL-05 HFR3
305	DIVMTQSHKFMSTSVGDRVSITC	PL-05 LFR1
306	WYQQKPGQSPKLLIF	PL-05 LFR2
307	GVPDRFTGSGSGTDYTLTISSVQAEDLALYYC	PL-05 LFR3
308	WVRQAPGQRLEWMGW	PL-06, 07 HFR2
309	RVTITRDTSASTAYMELSSLRSEDTAVYYCAR	PL-06, 07 HFR3
310	DIQMTQSPSSLSAFVGDRVTITC	PL-06 LFR1
311	WYQQKPGKAPKLLIH	PL-06 LFR2
312	GVPSRFSGSGSGRDFTFTISSLQPEDIATYYC	PL-06 LFR3
313	EIVLTQSPVTLSLSPGERATLSC	PL-07 LFR1
314	WYLQKPGQAPRLLIK	PL-07 LFR2
315	IPARFSGSGSGSDFTLTISSLEPEDFAVYYC	PL-07 LFR3
316	QVQLVQSGAEVKKPGASVKVSCKASGYTLT	2L2A.1 HFR1
317	WMRQAPGQGLEWMG	2L2A.1 HFR2
318	RVTMTRDTSTSTVYMELSSLRSEDTAVYYCVR	2L2A.1 HFR3
319	WGQGTLVTVSS	2L2A.1, 2L2A.6, 3L1A HFR4
320	DIQMTQSPSSLSASVGDRVTITC	2L2A.1, 2L2A.6, 2L27B LFR1
321	WLQQKPEKAPKRLIY	2L2A.1 LFR2
322	GVPSRFSGSGSGTDFTLTISSLQPEDFATYYC	2L2A.1, 2L2A.6, 2L27B LFR3
323	FGGGTKVEIK	2L2A.1, 2L2A.6, 2L27B LFR4
324	QVQLVQSGAEVKKPGASVKVSCKASGYTFT	2L2A.6 HFR1
325	WVRQAPGQGLEWMG	2L2A.6 HFR2
326	RVTMTRDTSTSTVYMELSSLRSEDTAVYYCAR	2L2A.6 3L1A HFR3
327	WLQQKPGKAPKRLIY	2L2A.6, 3L1A LFR2
328	QVQLVQSGAEVKKPGASVKVSCKASGFTFS	2L27B HFR1
329	WVRQAPGQGLEWMGL	2L27B HFR2
330	RVTMTRDTSTSTVYMELSSLRSEDTAVYFC	2L27B HFR3
331	FDYWDDGYYVEHFDYWGQGTLVTVSS	2L27B HFR4
332	WYQQKPGKAPKRLIY	2L27B LFR2
333	QVQLVQSGAEVKKPGASVKVSCKASGYTF	3L1A HFR1
334	DIQMTQSPSTLSASVGDRVTITC	3L1A LFR1
335	WLAWYQQKPGKAPKLLIY	3L1A LFR2
336	GVPSRFSGSGSGTEFTLTISSLQPDDFATYYC	3L1A LFR3
337	FGQGTKLEIK	3L1A LFR4

FIG. 39C

INTERNATIONAL SEARCH REPORT

International application No.
PCT/US19/39994

A. CLASSIFICATION OF SUBJECT MATTER

IPC - A61K 39/395, 45/06; C07K 16/28 (2019.01)

CPC - A61K 38/1764, 38/1774, 39/395, 39/39558, 45/06; A61P 35/00; C07K 16/28, 16/2803, 16/2818

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

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)
See Search History document

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

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

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X --- Y	US 2017/0044256 A1 (GENENTECH, INC.) 16 February 2017; paragraphs [0118], [0177]; claims 11, 14, 24	1 --- 2
Y	US 2015/0337033 A1 (SAMSUNG ELECTRONICS CO LTD.) 26 November 2015; paragraph [0227]	2
P, X	WO 2018/128939 A1 (GENSON BIOPHARMA INC.) 12 July 2018; entire document	1-2

☐ Further documents are listed in the continuation of Box C. ☐ See patent family annex.

* Special categories of cited documents:
"A" document defining the general state of the art which is not considered to be of particular relevance
"D" document cited by the applicant in the international application
"E" earlier application or patent but published on or after the international filing date
"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)
"O" document referring to an oral disclosure, use, exhibition or other means
"P" document published prior to the international filing date but later than the priority date claimed
"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention
"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone
"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art
"&" document member of the same patent family

Date of the actual completion of the international search	Date of mailing of the international search report
30 October 2019 (30.10.2019)	21 NOV 2019
Name and mailing address of the ISA/US Mail Stop PCT, Attn: ISA/US, Commissioner for Patents P.O. Box 1450, Alexandria, Virginia 22313-1450 Facsimile No. 571-273-8300	Authorized officer Shane Thomas Telephone No. PCT Helpdesk: 571-272-4300

INTERNATIONAL SEARCH REPORT

International application No.
PCT/US19/39994

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

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

1. ☐ Claims Nos.:
because they relate to subject matter not required to be searched by this Authority, namely:

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

3. ☒ Claims Nos.: 4-23, 27-30
because they are dependent claims and are not drafted in accordance with the second and third sentences of Rule 6.4(a).

Box No. III Observations where unity of invention is lacking (Continuation of item 3 of first sheet)

This International Searching Authority found multiple inventions in this international application, as follows:

-***-Please See Supplemental Page-***-

1. ☐ As all required additional search fees were timely paid by the applicant, this international search report covers all searchable claims.

2. ☐ As all searchable claims could be searched without effort justifying additional fees, this Authority did not invite payment of additional fees.

3. ☐ As only some of the required additional search fees were timely paid by the applicant, this international search report covers only those claims for which fees were paid, specifically claims Nos.:

4. ☒ No required additional search fees were timely paid by the applicant. Consequently, this international search report is restricted to the invention first mentioned in the claims; it is covered by claims Nos.:
1-3, 24-26; SEQ ID NOs.: 48-49

Remark on Protest

☐ The additional search fees were accompanied by the applicant's protest and, where applicable, the payment of a protest fee.

☐ The additional search fees were accompanied by the applicant's protest but the applicable protest fee was not paid within the time limit specified in the invitation.

☐ No protest accompanied the payment of additional search fees.

INTERNATIONAL SEARCH REPORT
Information on patent family members

International application No.
PCT/US19/39994

-***-Continued from Box No. III Observations where unity of invention is lacking: -***-

This application contains the following inventions or groups of inventions which are not so linked as to form a single general inventive concept under PCT Rule 13.1. In order for all inventions to be examined, the appropriate additional examination fees must be paid.

Groups I+, Claims 1-3, 24-26; a targeting domain that binds to PD-1 (targeting domain); SEQ ID NO: 48 (TIGIT-binding HCVR) and SEQ ID NO: 49 (TIGIT-binding LCVR) are directed toward ant antitumor antagonist; and an antibody associated therewith.

The antitumor antagonist and antibody will be searched to the extent they encompass a targeting domain that binds to PD-1 (first exemplary targeting domain); SEQ ID NO: 48 (first exemplary TIGIT-binding HCVR) and SEQ ID NO: 49 (first exemplary TIGIT-binding LCVR). Applicant is invited to elect additional targeting domain(s), with specified set(s) of associated variable region(s) and/or CDR(s), with specified SEQ ID NO: for each, or with specified substitution(s) at specified site(s) of a SEQ ID NO:, such that the sequence of each elected species is fully specified (i.e. no optional or variable residues or substituents), and available as an option within at least one searchable claim, to be searched. Additional targeting domain(s) and associated set(s) of sequences will be searched upon the payment of additional fees. It is believed that claims 1 (in-part), and 2 (in-part) encompass this first named invention and thus these claims will be searched without fee to the extent that they encompass a targeting domain that binds to PD-1 (targeting domain); SEQ ID NO: 48 (TIGIT-binding HCVR) and SEQ ID NO: 49 (TIGIT-binding LCVR). Applicants must specify the searchable claims that encompass any additionally elected targeting domain(s) and associated set(s) of sequences. Applicants must further indicate, if applicable, the claims which encompass the first named invention, if different than what was indicated above for this group. Failure to clearly identify how any paid additional invention fees are to be applied to the "+" group(s) will result in only the first claimed invention to be searched/examined. An exemplary election would be a targeting domain that binds to PD-L1 (targeting domain).

No technical features are shared between the targeting domains and/or CDR sequences of Groups I+ and, accordingly, these groups lack unity a priori.

Groups I+ share the technical features including: an antitumor antagonist comprising: a first targeting domain that specifically binds to PD-1, PD-L1 or LAG-3; and a second targeting domain comprising an scFv that specifically binds to TIGIT; and an antibody or antigen-binding portion thereof, comprising: (1) an immunoglobulin HCVR comprising three complementarity determining regions (HCDRs): HCDR1, HCDR2 and HCDR3; and (2) an immunoglobulin LCVR comprising three complementarity determining regions (LCDRs): LCDR1, LCDR2 and LCDR3, and wherein the antibody, or the antigen-binding portion thereof, binds specifically to human LAG-3.

However, these shared technical features are previously disclosed by WO 2018/128939 A1 to Gensun Biopharma Inc. (hereinafter 'Gensun').

Gensun discloses an antitumor antagonist (a checkpoint regulator antagonist that upregulates an anti-tumor immune response (an antitumor antagonist); an antitumor antagonist); abstract, paragraphs [0013], [0085]) comprising: a first targeting domain that specifically binds to PD-1, PD-L1 or LAG-3 (comprising: a first targeting domain that specifically binds to PD-1 or PD-L1; paragraph [0013]); and a second targeting domain comprising an scFv that specifically binds to TIGIT (a second targeting domain comprising an scFv that specifically binds to TIGIT; paragraphs [0013], [0061], [0124]); and an antibody or antigen-binding portion thereof, comprising: (1) an immunoglobulin HCVR comprising three complementarity determining regions (HCDRs): HCDR1, HCDR2 and HCDR3 (an antibody or antigen-binding portion thereof, comprising: (1) an immunoglobulin HCVR comprising three complementarity determining regions (HCDRs): HCDR1, HCDR2 and HCDR3; paragraph [0007]); and (2) an immunoglobulin LCVR comprising three complementarity determining regions (LCDRs): LCDR1, LCDR2 and LCDR3 (an immunoglobulin LCVR comprising three complementarity determining regions (LCDRs): LCDR1, LCDR2 and LCDR3; paragraph [0007]), and an antibody, or antigen-binding portion thereof, that binds specifically to human LAG-3 (an antibody, or antigen-binding portion thereof, that binds specifically to human LAG-3; paragraph [0087], Claim 14). Gensun does not explicitly disclose wherein an antibody that specifically binds to human LAG-3 comprises an immunoglobulin HCVR comprising three complementarity determining regions (HCDRs): HCDR1, HCDR2 and HCDR3, and an immunoglobulin LCVR comprising three complementarity determining regions (LCDRs): LCDR1, LCDR2 and LCDR3. However, it would have been obvious to a person of ordinary skill in the art at the time the invention was made to have modified the disclosure of Gensun to have disclosed wherein an antibody to a particular target, such as an anti-LAG-3 antibody, as disclosed by Gensun, comprises known domains of an antibody, such as a HCVR comprising three complementarity determining regions (HCDRs): HCDR1, HCDR2 and HCDR3, and a LCVR comprising three complementarity determining regions (LCDRs): LCDR1, LCDR2 and LCDR3, in order to enable a practitioner to produce such an antibody for use as disclosed by Gensun.

Since none of the special technical features of the Groups I+ inventions is found in more than one of the inventions, and since all of the shared technical features are previously disclosed by the Gensun reference, unity of invention is lacking.