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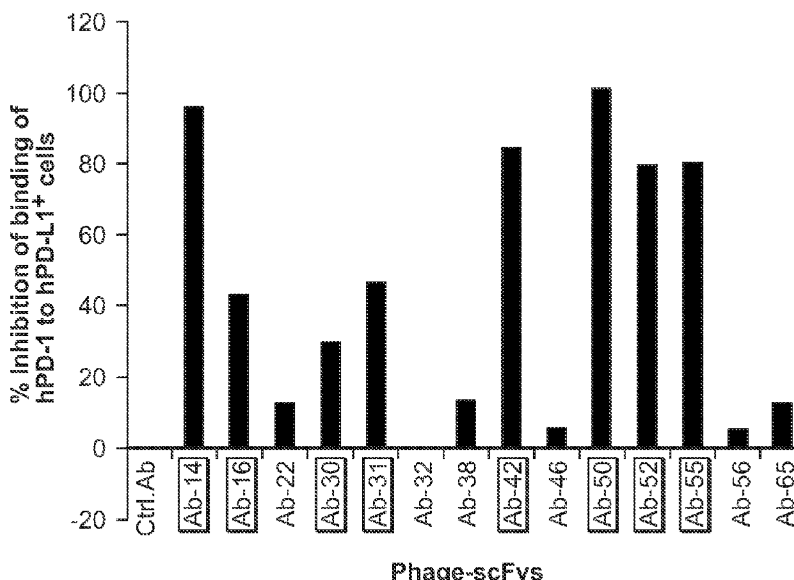
FIG. 3



(57) Abstract: The present invention comprises human monoclonal antibodies that bind to PD-L1 (also known as programmed death ligand 1 or B7H1). Binding of the invented antibody to PD-L1 inhibits binding to its receptor, PD1 (programmed death 1), and ligand-mediated activities and can be used to treat cancer and chronic viral infections.

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HUMAN MONOCLONAL ANTI-PD-L1 ANTIBODIES AND METHODS OF USE

RELATED APPLICATIONS

[0001] This application claims priority to and benefit of U.S. Provisional Application No. 61/709,731, filed on October 4, 2012, and U.S. Provisional Application No. 61/779,969 filed on March 13, 2013; the contents of which are each hereby incorporated by reference in their entireties.

FIELD OF THE INVENTION

[0002] This invention relates generally to anti-PD-L1 (also known as programmed cell death 1 ligand 1 or B7H1) antibodies as well as to methods for use thereof.

BACKGROUND OF THE INVENTION

[0003] The immune system must achieve a balance between effective responses to eliminate pathogenic entities and maintaining tolerance to prevent autoimmune disease. T cells are central to preserving this balance, and their proper regulation is primarily coordinated by the B7-CD28 family of molecules. Interactions between B7 family members, which function as ligands, and CD28 family members, which function as receptors, provide critical positive signals that not only initiate, augment and sustain T cell responses, but also contribute key negative signals that limit, terminate and/or attenuate T cell responses when appropriate. A member of the CD28 family, called PD-1 (also known as programmed cell death-1) is upregulated on activated T cells, B cells, and monocytes. PD-1 has two identified ligands in the B7 family, PD-L1 (also known as BH71 or programmed cell death-1 ligand 1) and PD-L2. While PD-L2 expression tends to be more restricted, found primarily on activated antigen-presenting cells (APCs), PD-L1 expression is more widespread, including cells of hematopoietic lineage (including activated T cells, B cells, monocytes, dendritic cells and macrophages) and peripheral nonlymphoid tissues (including heart, skeletal, muscle, placenta, lung, kidney and liver tissues). The widespread expression of PD-L1 suggests its significant role in regulating PD-1/PD-L1-mediated peripheral tolerance.

[0004] Binding between PD-L1 and PD-1 has a profound effect on the regulation of T cell responses. Specifically, PD-L1/PD-1 interaction inhibits T cell proliferation and production of effector cytokines that mediate T cell activity and immune response, such as

IL-2 and IFN-γ. This negative regulatory function is important for preventing T cell-mediated autoimmunity and immunopathology. However, the PD-1/PD-L1 axis has also been shown to play a role in T cell exhaustion, whereby the negative regulatory function inhibits T cell response to the detriment of the host. Prolonged or chronic antigenic stimulation of T cells can induce negative immunological feedback mechanisms which inhibit antigen-specific responses and results in immune evasion of pathogens. T cell exhaustion can also result in progressive physical deletion of the antigen-specific T cells themselves. T cell expression of PD-1 is up-regulated during chronic antigen stimulation, and its binding to PD-L1 results in a blockade of effector function in both CD4+ (T helper cells) and CD8+ (cytotoxic T lymphocytes or CTL) T cells, thus implicating the PD-1/PD-L1 interaction in the induction of T cell exhaustion.

[0005] More recently, it has been shown that some chronic viral infections and cancers have developed immune evasion tactics that specifically exploit the PD-1/PD-L1 axis by causing PD-1/PD-L1-mediated T cell exhaustion. Many human tumor cells and tumor-associated antigen presenting cells express high levels of PD-L1, which suggests that the tumors induce T cell exhaustion to evade anti-tumor immune responses. During chronic HIV infection, HIV-specific CD8+ T cells are functionally impaired, showing a reduced capacity to produce cytokines and effector molecules as well as a diminished ability to proliferate. Studies have shown that PD-1 is highly expressed on HIV-specific CD8+ T cells of HIV infected individuals, indicating that blocking the PD-1/PD-L1 pathway may have therapeutic potential for treatment of HIV infection and AIDS patients. Taken together, agents that block the PD-1/PD-L1 pathway will provide a new therapeutic approach for a variety of cancers, HIV infection, and/or other diseases and conditions that are associated with T-cell exhaustion. Therefore, there exists an urgent need for agents that can block or prevent PD-1/PD-L1 interaction.

SUMMARY OF THE INVENTION

[0006] The invention is based upon the discovery of monoclonal antibodies which bind PD-L1. The monoclonal antibody is fully human. The antibodies bind PD-L1. The antibodies are referred to herein as huPD-L1 antibodies.

[0007] PD-L1 is also known as programmed cell death 1 ligand 1, programmed death ligand 1, PDCD1 ligand 1, PDCD1L1, PDL1, B7 homolog 1, B7H1, B7-H, CD274 and CD274 antigen.

[0008] The present invention provides an isolated humanized monoclonal antibody having a heavy chain with three CDRs comprising the amino acid sequences SYGIS (SEQ ID NO:57), WISAYNGNTNYAQKLED (SEQ ID NO:70), and ALPSGTILVGGWFDP (SEQ ID NO:86) respectively and a light chain with three CDRs comprising the amino acid sequences TRSSGNIASNYVQ (SEQ ID NO:101), EDNQRPS (SEQ ID NO:115), and QSYDSSNLWV (SEQ ID NO:127) respectively; a heavy chain with three CDRs comprising the amino acid sequences SYALS (SEQ ID NO:58), AISGGGGSTYYADSVKD (SEQ ID NO:71), and DVFPETFSMNYGMDV (SEQ ID NO:87) respectively and a light chain with three CDRs comprising the amino acid sequences QGDSLRSYYAS (SEQ ID NO:102), GKNNRPS (SEQ ID NO:116), and NSRDSSGNHYV (SEQ ID NO:128) respectively; a heavy chain with three CDRs comprising the amino acid sequences DYAMH (SEQ ID NO:60), LISGDGGSTYYADSVKD (SEQ ID NO:73), and VLLPCSSTSCYGSVGAFDI (SEQ ID NO:88) respectively and a light chain with three CDRs comprising the amino acid sequences GGSDIGRKSVH (SEQ ID NO:103), SDRDRPS (SEQ ID NO:117), and QVWDNNSDHYV (SEQ ID NO:129) respectively; a heavy chain with three CDRs comprising the amino acid sequences NYDMS (SEQ ID NO:61), RVNWNGGSTTYADAVKD (SEQ ID NO:74), and EFVGAYDL (SEQ ID NO:89) respectively and a light chain with three CDRs comprising the amino acid sequences TGTSSDVGGYNYVS (SEQ ID NO:104), DVSNRPS (SEQ ID NO:118), and SSYTSSTLP (SEQ ID NO:130) respectively; a heavy chain with three CDRs comprising the amino acid sequences GLYIH (SEQ ID NO:62), WIIPIFGTANYAQKFED (SEQ ID NO:75), and GLRWGIWGWFDP (SEQ ID NO:90) respectively and a light chain with three CDRs comprising the amino acid sequences RASQSIGNSLA (SEQ ID NO:105), GASSRAT (SEQ ID NO:119), and QQHTIPTFS (SEQ ID NO:131) respectively; a heavy chain with three CDRs comprising the amino acid sequences DNAIS (SEQ ID NO:63), WIIPIFGKPNYAQKFED (SEQ ID NO:76), and TMVRGFLGVMDV (SEQ ID NO:91) respectively and a light chain with three CDRs comprising the amino acid sequences RASQGIGSYLA (SEQ ID NO:106), AASTLQS (SEQ ID NO:120), and QQLNNYPIT (SEQ ID NO:132) respectively; a heavy chain with three CDRs comprising the amino acid sequences SYAMS (SEQ ID NO:64), AISGSGGSTYYADSVKD (SEQ ID NO:77), and DQFVTIFGVPRYGMDV (SEQ ID NO:92) respectively and a light chain with three CDRs comprising the amino acid sequences SGDKLGNKYAY (SEQ ID NO:107), QDIKRPS (SEQ ID NO:121), and QTWDNSVV (SEQ ID NO:133) respectively; a heavy chain with

three CDRs comprising the amino acid sequences SYAIS (SEQ ID NO:57), WIIPIFGTANYAQKFED (SEQ ID NO:78), and GRQMFGAGIDF (SEQ ID NO:93) respectively and a light chain with three CDRs comprising the amino acid sequences TRSSGSIDSNYVQ (SEQ ID NO:108), EDNQRPS (SEQ ID NO:115), and QSYDSNNRHVI (SEQ ID NO:134) respectively; a heavy chain with three CDRs comprising the amino acid sequences TYALN (SEQ ID NO:65), RIVPLIGLVNYAHNFED (SEQ ID NO:79), and EVYGGNSDY (SEQ ID NO:94) respectively and a light chain with three CDRs comprising the amino acid sequences TRSSGNIGTNYVQ (SEQ ID NO:109), EDYRRPS (SEQ ID NO:122), and QSYHSSGWE (SEQ ID NO:135) respectively; a heavy chain with three CDRs comprising the amino acid sequences SHGIT (SEQ ID NO:66), WISAHNGHASNAQKVED (SEQ ID NO:80), and VHAALYYGMDV (SEQ ID NO:95) respectively and a light chain with three CDRs comprising the amino acid sequences GGNNIGSKGVH (SEQ ID NO:110), DDSDRPS (SEQ ID NO:123), and QVWDSSSDHWV (SEQ ID NO:136) respectively; a heavy chain with three CDRs comprising the amino acid sequences RHGMH (SEQ ID NO:67), VISHDGSVKYYADSMKD (SEQ ID NO:81), and GLSYQVSGWFDP (SEQ ID NO:96) respectively and a light chain with three CDRs comprising the amino acid sequences TRSSGSIASNYVQ (SEQ ID NO:111), EDNQRPS (SEQ ID NO:115), and QSYDSTTPSV (SEQ ID NO:137) respectively; a heavy chain with three CDRs comprising the amino acid sequences SYGIS (SEQ ID NO:58), WTSPHNGLTAFAQILED (SEQ ID NO:82), and VHPVFSYALDV (SEQ ID NO:97) respectively and a light chain with three CDRs comprising the amino acid sequences TRSSGSIASNYVQ (SEQ ID NO:112), EDNQRPS (SEQ ID NO:115), and QSYDGITVI (SEQ ID NO:138) respectively; a heavy chain with three CDRs comprising the amino acid sequences TYAFS (SEQ ID NO:68), RIIPILGIANYAQKFED (SEQ ID NO:83), and DGYGSDPVL (SEQ ID NO:98) respectively and a light chain with three CDRs comprising the amino acid sequences TRSSGSIASHYVQ (SEQ ID NO:113), EDNKRPS (SEQ ID NO:124), and QSYDSSNRWV (SEQ ID NO:139) respectively; or a heavy chain with three CDRs comprising the amino acid sequences NYGIS (SEQ ID NO:69), WISAYNGNTNYAQKVED (SEQ ID NO:84), and GDFRKPFDY (SEQ ID NO:99) respectively and a light chain with three CDRs comprising the amino acid sequences TLRSGLNVGSYRIY (SEQ ID NO:114), YKSDSNKQQAS (SEQ ID NO:125), and MIWYSSAVV (SEQ ID NO:140) respectively; wherein said antibody binds human PD-L1.

[0009] In one aspect, the antibody is monovalent or bivalent. In another aspect, the antibody is a single chain antibody.

[0010] The present invention provides a single chain antibody comprising a V_H nucleotide sequence comprising SEQ ID NO: 1 and a V_L nucleotide sequence comprising SEQ ID NO: 3; a V_H nucleotide sequence comprising SEQ ID NO: 5 and a V_L nucleotide sequence comprising SEQ ID NO:7; a V_H nucleotide sequence comprising SEQ ID NO: 9 and a V_L nucleotide sequence comprising SEQ ID NO: 11; a V_H nucleotide sequence comprising SEQ ID NO: 13 and a V_L nucleotide sequence comprising SEQ ID NO: 15; a V_H nucleotide sequence comprising SEQ ID NO: 17 and a V_L nucleotide sequence comprising SEQ ID NO:19; a V_H nucleotide sequence comprising SEQ ID NO: 21 and a V_L nucleotide sequence comprising SEQ ID NO:23; a V_H nucleotide sequence comprising SEQ ID NO: 25 and a V_L nucleotide sequence comprising SEQ ID NO:27; a V_H nucleotide sequence comprising SEQ ID NO: 29 and a V_L nucleotide sequence comprising SEQ ID NO:31; a V_H nucleotide sequence comprising SEQ ID NO: 33 and a V_L nucleotide sequence comprising SEQ ID NO:35; a V_H nucleotide sequence comprising SEQ ID NO: 37 and a V_L nucleotide sequence comprising SEQ ID NO:39; a V_H nucleotide sequence comprising SEQ ID NO: 41 and a V_L nucleotide sequence comprising SEQ ID NO:43; a V_H nucleotide sequence comprising SEQ ID NO: 45 and a V_L nucleotide sequence comprising SEQ ID NO:47; a V_H nucleotide sequence comprising SEQ ID NO: 49 and a V_L nucleotide sequence comprising SEQ ID NO:51; or a V_H nucleotide sequence comprising SEQ ID NO: 53 and a V_L nucleotide sequence comprising SEQ ID NO:55.

[0011] In another aspect, the present invention provides a single chain antibody comprising a V_H amino acid sequence comprising SEQ ID NO: 2 and a V_L amino acid sequence comprising SEQ ID NO: 4; a V_H amino acid sequence comprising SEQ ID NO: 6 and a V_L amino acid sequence comprising SEQ ID NO: 8; a V_H amino acid sequence comprising SEQ ID NO: 10 and a V_L amino acid sequence comprising SEQ ID NO: 12; a V_H amino acid sequence comprising SEQ ID NO: 14 and a V_L amino acid sequence comprising SEQ ID NO: 16; a V_H amino acid sequence comprising SEQ ID NO: 18 and a V_L amino acid sequence comprising SEQ ID NO: 20; a V_H amino acid sequence comprising SEQ ID NO: 22 and a V_L amino acid sequence comprising SEQ ID NO: 24; a V_H amino acid sequence comprising SEQ ID NO: 26 and a V_L amino acid sequence comprising SEQ ID NO: 28; a V_H amino acid sequence comprising SEQ ID NO: 30 and a V_L amino acid sequence comprising SEQ ID NO: 32; a V_H amino acid sequence comprising SEQ ID NO: 34 and a V_L amino acid

sequence comprising SEQ ID NO: 36; a V_H amino acid sequence comprising SEQ ID NO: 38 and a V_L amino acid sequence comprising SEQ ID NO: 40; a V_H amino acid sequence comprising SEQ ID NO: 42 and a V_L amino acid sequence comprising SEQ ID NO: 44; a V_H amino acid sequence comprising SEQ ID NO: 46 and a V_L amino acid sequence comprising SEQ ID NO: 48; a V_H amino acid sequence comprising SEQ ID NO: 50 and a V_L amino acid sequence comprising SEQ ID NO: 52; or a V_H amino acid sequence comprising SEQ ID NO: 54 and a V_L amino acid sequence comprising SEQ ID NO: 56.

[0012] In some aspects, the antibody has a binding affinity within the range of 10^{-5} M to 10^{-12} M.

[0013] In another aspect, the antibody is a bi-specific antibody that also binds to a tumor-associated antigen, a cytokine or a cell surface receptor. For example, the tumor-associated antigen is CAIX. For example, the cytokine is IL-10. For example, the cell surface receptor is CCR4, IL21R, BTLA, HVEM or TIM3.

[0014] The present invention provides an antibody linked to a therapeutic agent. For example, the therapeutic agent is a toxin, a radiolabel, a siRNA, a small molecule, or a cytokine.

[0015] The present invention provides a cell producing any of the foregoing antibodies.

[0016] The present invention also provides methods of selectively killing a tumor cell comprising contacting said cell with any of the foregoing antibodies. In one aspect, the selective killing occurs by antibody-dependent cellular toxicity (ADCC), complement-dependent cytotoxicity (CDC), antibody dependent cellular phagocytosis (ADCP). In another aspect, the tumor cell expresses PD-L1.

[0017] The present invention also provides methods of preventing or reversing T cell exhaustion comprising administering to a subject in need thereof a composition comprising any of the foregoing antibodies.

[0018] The present invention also provides methods of augmenting an immune response to an antigen comprising administering to a subject in need thereof a composition comprising any of the foregoing antibodies. In one aspect, the antigen is a viral antigen, a bacterial antigen or a tumor associated antigen. In another aspect, the viral antigen is HIV. In a further aspect, the tumor associated antigen is CAIX. In another aspect, the antibody is administered prior to or after exposure to the antigen. In another aspect, the administration of

said antibody causes an increase in antigen specific T cell activity. In another aspect, the T-cell is an effector T cell.

[0019] The present invention also provides methods of treating or alleviating a symptom of cancer, comprising administering to a subject in need thereof a composition comprising any of the foregoing antibodies. For example, the cancer is renal cell carcinoma or breast cancer. For example, the cancer is a cancer in which PD-L1 is overexpressed. In another example, the cancer is a cancer that induces T cell exhaustion.

[0020] The present invention also provides methods of treating or alleviating a symptom of a chronic viral infection, comprising administering to a subject in need thereof a composition comprising any of the foregoing antibodies. For example, the chronic viral infection is an HIV infection. For example, the chronic viral infection is a viral infection that induces T cell exhaustion.

[0021] The present invention provides a nucleic acid sequence comprising the nucleic acid sequence of SEQ ID NO: 1, 3, 5, 7, 9, 11, 13. 15. 17, 19, 21, 23, 25, 27, 29, 31, 33, 35, 37, 39, 41, 43, 45, 47, 49, 51, 53 or 55.

[0022] In another aspect, the present invention provides a nucleic acid sequence encoding the polypeptide of SEQ ID NO: 2, 4, 6, 8, 10, 12, 14, 16, 18, 20, 22, 24, 26, 28, 30, 32, 34, 36, 38, 40, 42, 44, 46, 48, 50, 52, 54 or 56.

[0023] In another aspect, the present invention provides a polypeptide comprising the amino acid sequence of SEQ ID NO: 2, 4, 6, 8, 10, 12, 14, 16, 18, 20, 22, 24, 26, 28, 30, 32, 34, 36, 38, 40, 42, 44, 46, 48, 50, 52, 54 or 56.

[0024] In another aspect, the present invention provides a vector comprising a nucleic acid sequence of SEQ ID NO: 1, 3, 5, 7, 9, 11, 13. 15. 17, 19, 21, 23, 25, 27, 29, 31, 33, 35, 37, 39, 41, 43, 45, 47, 49, 51, 53 or 55. The present invention provides a vector comprising a nucleic acid sequence of SEQ ID NO: 2, 4, 6, 8, 10, 12, 14, 16, 18, 20, 22, 24, 26, 28, 30, 32, 34, 36, 38, 40, 42, 44, 46, 48, 50, 52, 54 or 56. The present invention further provides a cell comprising any one of the foregoing vectors.

[0025] The administration routes, in any methods of this disclosure, include, but are not limited to parenteral, (*e.g.*, intravenous), intradermal, subcutaneous, oral (*e.g.*, inhalation), transdermal (*i.e.,* topical), transmucosal, and rectal administration.

[0026] The subject in any methods of this disclosure is, for example, a mammal. The mammal is, for example, a human.

[0027] Other features and advantages of the invention will be apparent from and are encompassed by the following detailed description and claims.

BRIEF DESCRIPTION OF THE DRAWINGS

[0028] **Figure 1.** Amino acid sequences of anti-PD-L1 scFv-phage clones (14 clones). Framework regions 1-4 (FW1-4), Complementarily determining regions 1-3 (CDR1-3) and family designations for both the IGHV and IGLV/IGKV are shown. Kabat number is used. Key: "." AA matches to consensus, "X" no consensus AA, and "-" is a space (i.e. no AA).

[0029] **Figure 2.** Binding analysis of huPD-L1 antibodies with human PD-L1 (hPD-L1) expressing cells by FACS. Four types of cells were tested, including parental cell line 300.9 and hPD-L1, hPD-L2 or human C-type lectin domain family 2 member (hCLEC2D) transfected 300.9 cells. GF1538 is a humanized Ab against hPD-L1. GF1757 is a humanized Ab against hPD-L2. Secondary antibody is PE-goat anti-human IgG.

[0030] **Figure 3.** Inhibition of hPD-1 binding to hPD-L1 by anti-PD-L1 phage-antibodies in a competitive FACS analysis. All anti-hPD-L1 Abs in phage-scFv form were tested for inhibition of the binding of hPD1-hFc fusion protein with hPD-L1 expressing 293T cells. 10^{12} pfu of phage-scFvs were mixed with ~0.25 μg/mL of soluble hPD1-hFc and added to hPD-L1 expressing-plasmid transfected 293T cells. After washing, the cells were incubated with FITC-anti-human IgG antibody to measure the binding of hPD1-hFc to hPD-L1 on cell surface.

[0031] **Figure 4.** Inhibition of hPD-1 binding to hPD-L1 by anti-PD-L1 soluble antibodies in a competitive FACS analysis. All anti-hPD-L1 Abs were pre-incubated with hPD-L1 expressing-plasmid transfected 300.9 cells at indicated concentrations for 30 mins, 0.125 μg of hPD-1-mouse IgG2a was then added to each reaction and incubated for another 30 mins. After washing, PE-goat-anti-mouse IgG2a Ab was added and followed by washing and FACS analysis. GF1538 is a humanized Ab against hPD-L1. GF1757 is a humanized Ab against hPD-L2.

[0032] **Figure 5.** Design and formation of bi-specific antibodies.

[0033] **Figure 6.** Bi-specific antibody (bsAb) construct determination. A) Schematic representation of the bi-specific antibody that recognizes CAIX and PD-L1, and the "knob into hole" approach of linking the CH3 domains. B) Schematic representation of the three types of bsAb constructs with different mutations in the CH2 domain to alter ADCC activity.

[0034] **Figure 7.** Generation of bi-specific antibody and its function. A) Protein gel showing the dissociation of engineered (G37 KIHA) antibody under reducing conditions compared to conjugated (non-reduced) control IgG, parental G37 (WT), and bi-specific (G37 KIHA + PD-L1 KIHB) antibodies. B) Protein gel showing the dissociation of engineered (PD-L1 KIHB) antibody under reducing conditions compared to control IgG, parental PDL-1 (WT), and bi-specific (G37 KIHA + PD-L1 KIHB) antibodies. C) Analysis of bi-specific antibody binding to $CAIX^{+}PDL\text{-}1^{-}$ SKRC-52 cells by flow cytometry.

[0035] **Figure 8.** Functional characterization of PD-L1 specific mAb42. PBMCs from four healthy donors (D1-D4) were cultured in the presence of αPDL1 (mAb42) or control isotype antibody stimulated with 0.1 μg/ml SEB for 48 hours and TNFα production was measured by MSD units. Data presented as means of triplicates *, $p<0.0005$.

DETAILED DESCRIPTION

[0036] The present invention provides humanized monoclonal antibodies specific against PD-L1, also known as B7H1. The antibodies were identified by a method of phage display antibody library selection by using proteoliposome-coupled-PD-L1 as the library selection target. These antibodies represent a new class of human monoclonal antibodies against PD-L1.

[0037] These anti-PD-L1 human monoclonal antibodies are referred to herein as "huPD-L1 antibodies".

[0038] Binding of PD-L1 to PD-1 negatively regulates T cell antigen-specific responses, which is critical for tolerance and prevention of autoimmunity and immunopathology. However, excessive PD-L1/PD-1 interaction, which can be caused by chronic antigenic stimulation, can result in inhibition of T cell antigen-specific responses and loss of T cells, which are characteristics of T cell exhaustion. T cell exhaustion is a state of T cell dysfunction that can arise in chronic infections and cancer. It is defined by poor effector function, sustained expression of inhibitory receptors and a transcriptional state distinct from that of functional effector or memory T cells. Exhaustion prevents management of infection and tumor progression.

[0039] PD-L1 overexpression has been detected in different cancers. For example, in breast cancer, PD-L1 is overexpressed and associated with high-risk prognostic factors. In renal cell carcinoma, PD-L1 is upregulated and increased expression of PD-1 has also been found in tumor infiltrating leukocytes. Anti-PD-L1 and anti-PD-1 antibodies have

demonstrated some clinical efficacy in phase I trials for renal cell carcinoma. Therapeutic agents that can bind to PD-1 or PD-L1 may be useful for specifically targeting tumor cells. Agents that are capable of blocking the PD-1/PD-L1 interaction may be even more useful in treating cancers that have induced T cell exhaustion to evade anti-tumor T cell activity. Use of such agents, alone or in combination with other anti-cancer therapeutics, can effectively target tumor cells that overexpress PD-L1 and increase anti-tumor T cell activity, thereby augmenting the immune response to target tumor cells.

[0040] PD-1 and PD-L1 can also be upregulated by T cells after chronic antigen stimulation, for example, by chronic infections. During chronic HIV infection, HIV-specific CD8+ T cells are functionally impaired, showing a reduced capacity to produce cytokines and effector molecules as well as a diminished ability to proliferate. PD-1 is highly expressed on HIV-specific CD8+ T cells of HIV infected individuals. Therefore, blocking this pathway may enhance the ability of HIV-specific T cells to proliferate and produce cytokines in response to stimulation with HIV peptides, thereby augmenting the immune response against HIV. Other chronic infections may also benefit from the use of PD-1/PD-L1 blocking agents, such as chronic viral, bacterial or parasitic infections.

[0041] The present invention provides a human monoclonal antibody that specifically binds PD-L1 proteins. Binding of the antibody of the present invention to PD-L1 interrupts the ligand's ability to bind to its receptor PD1. By a variety of mechanisms, the huPD-L1 antibody prevents the negative feedback mechanisms that inhibit T cell responses. In some cases, the huPD-L1 antibody prevents, inhibits or reverses T cell exhaustion. Administration of the huPD-L1 antibody may result in increased T cell proliferation, increased antigen-specific T cell activity, and increased production of effector cytokines. In some instances, the huPD-L1 antibody promotes or augments the antigen-specific immune response. This immune response may be mediated by effector T cells.

[0042] The huPD-L1 antibody is monovalent or bivalent and comprises a single or double chain. Functionally, the binding affinity of the huPD-L1 antibody is within the range of 10^{-5}M to 10^{-12} M. For example, the binding affinity of the huPD-L1 antibody is from 10^{-6} M to 10^{-12} M, from 10^{-7} M to 10^{-12} M, from 10^{-8} M to 10^{-12} M, from 10^{-9} M to 10^{-12} M, from 10^{-5} M to 10^{-11} M, from 10^{-6} M to 10^{-11} M, from 10^{-7} M to 10^{-11} M, from 10^{-8} M to 10^{-11} M, from 10^{-9} M to 10^{-11} M, from 10^{-10} M to 10^{-11} M, from 10^{-5} M to 10^{-10} M, from 10^{-6} M to 10^{-10} M, from 10^{-7} M to 10^{-10} M, from 10^{-8} M to 10^{-10} M, from 10^{-9} M to 10^{-10} M, from 10^{-5} M to 10^{-9} M, from 10^{-6} M to 10^{-9} M, from 10^{-7} M to 10^{-9} M, from 10^{-8} M to 10^{-9} M, from 10^{-5} M to

10^{-8} M, from 10^{-6} M to 10^{-8} M, from 10^{-7} M to 10^{-8} M, from 10^{-5} M to 10^{-7} M, from 10^{-6} M to 10^{-7} M or from 10^{-5} M to 10^{-6} M.

[0043] Furthermore, the antibody of the present invention comprises a therapeutic agent including, but not limited to, a toxin, a radiolabel, a siRNA, or a cytokine.

[0044] The huPD-L1 antibody is capable of inducing cell death. Cell death is induced by either direct or indirect mechanisms. For instance, PD-L1 binding by the huPD-L1 antibody can lead to complement-dependent cytotoxicity (CDC). Alternatively, the huPD-L1 antibody binds PD-L1, and leads to the recruitment of a second cell type that will kill the PD-L14-expressing target cell. Exemplary mechanisms by which the huPD-L1 antibody mediates cell death by recruitment of a second cell type include, but are not limited to, antibody-dependent cellular toxicity (ADCC) and antibody dependent cellular phagocytosis (ADCP). Target PD-L1-expressing cell types comprise tumor and T cells, such as activated T cells.

[0045] Fourteen unique monoclonal huPD-L1 antibodies were identified. These include Ab-14, Ab-16, Ab-22, Ab-30, Ab-31, Ab-32, Ab-38, Ab-42, Ab-46, Ab-50, Ab-52, Ab-55, Ab-56 and Ab-65.

[0046] The nucleic acid and amino acid sequence of the monoclonal huPD-L1 antibodies are provided below:

[0047]

Table 1A. Ab-14 Variable Region nucleic acid sequences
V_H chain of Ab-14 (SEQ ID NO:1)
CAGGTGCAGCTGGTGCAGTCTGGAGCTGAGGTGAAGAAGCCTGGGGCCTCAGTGAAGGTCTCCTGCAAGGCTTC TGGTTACACCTTTACCAGCTATGGTATCAGCTGGGTGCGACAGGCCCCTGGACAAGGGCTTGAGTGGATGGGAT GGATCAGCGCTTACAATGGTAACACAAACTATGCACAGAAGCTCCAGGGCAGAGTCACCATGACCACAGACACA TCCACGAGCACAGCCTACATGGAGCTGAGGAGCCTGAGATCTGACGACACGGCCGTGTATTACTGTGCGAGAGC TCTACCTAGTGGGACTATACTGGTCGGAGGTTGGTTCGACCCCTGGGGCCAGGGAACCCTGGTCACCGTCTCCT CA
V_L chain of Ab-14 (SEQ ID NO:3)
AATTTTATGCTGACTCAGCCCCACTCTGTGTCGGAGTCTCCGGGGAAGACGGTAACCATCTCCTGCACCCGCAG CAGTGGCAACATTGCCAGCAATTATGTGCAGTGGTACCAACAGCGCCCGGGCAGTGCCCCCACCACTGTGATCT ATGAGGATAACCAAAGACCCTCTGGGGTCCCTGATCGGTTCTCTGGCTCCATCGACAGCTCCTCCAACTCTGCC TCCCTCACCATCTCTGGACTGAAGACTGAGGACGAGGCTGACTACTACTGTCAGTCTTATGATAGCAGCAATCT TTGGGTGTTCGGCGGAGGGACCAAGCTGACCGTCCTA

[0048]

Table 1B. Ab-14 Variable Region amino acid sequences
V_H chain of Ab-14 (SEQ ID NO:2)
QVQLVQSGAEVKKPGASVKVSCKASGYTFTSYGISWVRQAPGQGLEWMGWISAYNGNTNYAQKLQGRVTMTTDT STSTAYMELRSLRSDDTAVYYCARALPSGTILVGGWFDPWGQGTLVTVSS
V_L chain of Ab-14 (SEQ ID NO:4)
NFMLTQPHSVSESPGKTVTISCTRSSGNIASNYVQWYQQRPGSAPTTVIYEDNQRPSGVPDRFSGSIDSSSNSA SLTISGLKTEDEADYYCQSYDSSNLWVFGGGTKLTVL

[0049]

Table 2A. Ab-16 Variable Region nucleic acid sequences
V_H chain of Ab-16 (SEQ ID NO:5)
GAGGTGCAGCTGGTGCAGTCTGGGGGAGGCGTGGTCCAGCCTGGGAGGTCCCTGAGACTCTCCTGTGCAGCCTC TGGATTCACCTTTAGCAGCTATGCCCTGAGCTGGGTCCGCCAGGCTCCAGGGAAGGGGCTGGAGTGGGTCTCAG CTATTAGTGGTGGTGGTGGTAGCACATACTACGCAGACTCCGTGAAGGGCCGGTTCACCATCTCCAGAGACAAT TCCAAGAACACGCTGTATCTGCAAATGAACAGCCTGAGAGCCGAGGACACGGCCGTATATTACTGTGCGAAAGA CGTGTTTCCAGAGACTTTTTCGATGAACTACGGTATGGACGTCTGGGGCCAAGGAACCCTGGTCACCGTCTCCT CA
V_L chain of Ab-16 (SEQ ID NO:7)
TCTTCTGAGCTGACTCAGGACCCTGCTGTGTCTGTGGCCTTGGGACAGACAGTCAGGATCACATGCCAAGGAGA CAGCCTCAGAAGCTATTATGCAAGCTGGTACCAGCAGAAGCCAGGACAGGCCCCTGTACTTGTCATCTATGGTA AAAACAACCGGCCCTCAGGGATCCCAGACCGATTCTCTGGCTCCAGCTCAGGAAACACAGCTTCCTTGACCATC ACTGGGGCTCAGGCGGAAGATGAGGCTGACTATTACTGTAACTCCCGGGACAGCAGTGGTAACCATTATGTCTT CGGAACTGGGACCAAGGTCACCGTCCTA

[0050]

Table 2B. Ab-16 Variable Region amino acid sequences
V_H chain of Ab-16 (SEQ ID NO:6)
EVQLVQSGGGVVQPGRSLRLSCAASGFTFSSYALSWVRQAPGKGLEWVSAISGGGGSTYYADSVKGRFTISRDN SKNTLYLQMNSLRAEDTAVYYCAKDVFPETFSMNYGMDVWGQGTLVTVSS
V_L chain of Ab-16 (SEQ ID NO:8)
SSELTQDPAVSVALGQTVRITCQGDSLRSYYASWYQQKPGQAPVLVIYGKNNRPSGIPDRFSGSSSGNTASLTI TGAQAEDEADYYCNSRDSSGNHYVFGTGTKVTVL

[0051]

Table 3A. Ab-22 Variable Region nucleic acid sequences
V_H chain of Ab-22 (SEQ ID NO:9)
CAGGTGCAGCTGGTGCAGTCTGGGGGAGGCGTGGTACAGCCTGGGGGGTCCCTGAGACTCTCCTGTGCAGCCTC TGGATTCACCTTTGATGATTATGCCATGCACTGGGTCCGTCAAGCTCCAGGGAAGGGTCTGGAGTGGGTCTCTC TTATTAGTGGGGATGGTGGTAGCACATACTATGCAGACTCTGTGAAGGGCCGATTCACCATCTCCAGAGACAAC AGCAAAAACTCCCTGTATCTGCAAATGAACAGTCTGAGAACTGAGGACACCGCCTTGTATTACTGTGCAAAAGT GCTCCTCCCCTGTAGTAGTACCAGCTGCTATGGAAGCGTCGGTGCTTTTGATATCTGGGGCCAAGGGACCACGG TCACCGTCTCCTCA
V_L chain of Ab-22 (SEQ ID NO:11)
TAGGACGATGAGCTCGGTCCCAGCTCCGAAGACATAATGATCACTATTATTATCCCACACCTGACAGTAATAAT CGGCCTCATCACCGGCTTCGACCCTGCTGATGGTCAGGGTGGCCGTGTTCCCAGAGTTGGAGCCAGAGAATCGC TCAGAGATCCCTGAGGGCCGGTCCCTATCAGAGTAGATGACCAACGCAGGGGCCTGGCCTGGCTTCTGCTGGTA CCAGTGCACACTCTTCCTTCCAATGTCGCTTCCCCCACAGGTAATCCTGGCCGTCTTTCCTGGGGCCACTGACA CTGAGGGTGCCTGAGTCAGCACAGGCAG

[0052]

Table 3B. Ab-22 Variable Region amino acid sequences
V_H chain of Ab-22 (SEQ ID NO:10)
QVQLVQSGGGVVQPGGSLRLSCAASGFTFDDYAMHWVRQAPGKGLEWVSLISGDGGSTYYADSVKGRFTISRDN SKNSLYLQMNSLRTEDTALYYCAKVLLPCSSTSCYGSVGAFDIWGQGTTVTVSS
V_L chain of Ab-22 (SEQ ID NO:12)
LPVLTQAPSVSVAPGKTARITCGGSDIGRKSVHWYQQKPGQAPALVIYSDRDRPSGISERFSGSNSGNTATLTI SRVEAGDEADYYCQVWDNNSDHYVFGAGTELIVL

[0053]

Table 4A. Ab-30 Variable Region nucleic acid sequences
V_H chain of Ab-30 (SEQ ID NO:13)

CAGGTGCAGCTGGTGCAGTCTGGGGGAAGTGTGGTACGGCCTGGGGAATCCCTCAGACTCTCCTGTGTAGCCTC TGGATTCATCTTTGATAATTATGACATGAGTTGGGTCCGCCAAGTTCCAGGGAAGGGGCTGGAGTGGGTCTCTC GTGTTAATTGGAATGGTGGTAGCACAACTTATGCAGACGCTGTGAAGGGCCGATTCACCATCTCCAGAGACAAC ACCAAGAACTCCCTGTATCTACAAATGAACAACCTGAGAGCCGAAGACACGGCCGTGTATTACTGTGTGCGCGA GTTTGTCGGTGCTTATGATCTCTGGGGCCAGGGGACCACGGTCACCGTCTCCTCA
V_L chain of Ab-30 (SEQ ID NO:15)
CAGTCTGCCCTGACTCAGCCTGCCTCCGTGTCTGGGTCTCCTGGACAGTCGATCACCATCTCCTGCACTGGAAC CAGCAGTGACGTTGGTGGTTATAACTATGTCTCCTGGTACCAACAACACCCAGGCAAAGCCCCCAAACTCATGA TTTATGATGTCAGTAATCGGCCCTCAGGGGTTTCTAATCGCTTCTCTGGCTCCAAGTCTGGCAACACGGCCTCC CTGACCATCTCTGGGCTCCAGGCTGAGGACGAGGCTGATTATTACTGCAGCTCATATACAAGCAGCACTCTGCC GTTCGGCGGAgGGACCAAGCTGACCGTCCTA

[0054]

Table 4B. Ab-30 Variable Region amino acid sequences
V_H chain of Ab-30 (SEQ ID NO:14)
QVQLVQSGGSVVRPGESLRLSCVASGFIFDNYDMSWVRQVPGKGLEWVSRVNWNGGSTTYADAVKGRFTISRDN TKNSLYLQMNNLRAEDTAVYYCVREFVGAYDLWGQGTTVTVSS
V_L chain of Ab-30 (SEQ ID NO:16)
QSALTQPASVSGSPGQSITISCTGTSSDVGGYNYVSWYQQHPGKAPKLMIYDVSNRPSGVSNRFSGSKSGNTAS LTISGLQAEDEADYYCSSYTSSTLPFGGGTKLTVL

[0055]

Table 5A. Ab-31 Variable Region nucleic acid sequences
V_H chain of Ab-31 (SEQ ID NO:17)
CAGGTGCAGCTGGTGCAGTCTGGGGCTGAGGTGAAGAAGCCAGGGGCCACAGTGAAGGTCTCCTGCAAGGTTTT TGGAGACACCTTCCGCGGCCTCTATATACACTGGGTGCGACAGGCCCCTGGACAAGGGCTTGAGTGGATGGGAG GGATCATCCCTATCTTTGGTACAGCAAACTACGCACAGAAGTTCCAGGGCAGAGTCACGATTACCACGGACGAA TCCACGAGCACAGCCTACATGGAGCTGAGCAGCCTGAGATCTGAGGACACGGCCGTGTATTACTGTGCGAGCGG ACTACGTTGGGGGATCTGGGGCTGGTTCGACCCCTGGGGCCAGGGCACCCTGGTCACCGTCTCCTCA
V_L chain of Ab-31 (SEQ ID NO:19)
GAAATTGTGTTGACGCAGTCTCCAGCCACCCTGTCTTTGTCTCCAGGGGAAAGAGCCACCCTCTCCTGCAGGGC CAGTCAGAGTATTGGCAACAGCTTAGCCTGGTACCAGCAGAAACCTGGCCAGGCTCCCAGGCTCCTCATGTATG GTGCATCCAGCAGGGCCACTGGCATCCCAGACAGGTTCAGTGGCAGTGGGGCTGGGACAGACTTCACTCTCACC ATCAGCAGCCTAGAGCCTGAAGATTTTGCAACGTATTACTGTCAGCAGCATACTATCCCAACATTCTCTTTCGG CCCTGGGACCAAAGTGGAAGTCAAA

[0056]

Table 5B. Ab-31 Variable Region amino acid sequences
V_H chain of Ab-31 (SEQ ID NO:18)
QVQLVQSGAEVKKPGATVKVSCKVFGDTFRGLYIHWVRQAPGQGLEWMGGIIPIFGTANYAQKFQGRVTITTDE STSTAYMELSSLRSEDTAVYYCASGLRWGIWGWFDPWGQGTLVTVSS
V_L chain of Ab-31 (SEQ ID NO:20)
EIVLTQSPATLSLSPGERATLSCRASQSIGNSLAWYQQKPGQAPRLLMYGASSRATGIPDRFSGSGAGTDFTLT ISSLEPEDFATYYCQQHTIPTFSFGPGTKVEVK

[0057]

Table 6A. Ab-32 Variable Region nucleic acid sequences
V_H chain of Ab-32 (SEQ ID NO:21)
GAGGTGCAGCTGGTGCAGTCTGGGGCTGAGCTGAAGAAGCCTGGGTCCTCGGTGAAGGTCTCCTGCAAGGCTTT TGGAGGCACCTTCAGTGACAATGCTATCAGCTGGGTGCGACAGGCCCCTGGACAAGGGCCTGAGTGGATGGGGG GCATCATTCCTATCTTTGGAAAACCAAACTACGCACAGAAGTTCCAGGGCAGAGTCACGATTACCGCGGACGAA TCCACGAGCACTGCCTACATGGTCCTGAGCAGCCTGAGATCTGAGGACACGGCCGTATATTACTGTGCGAGAAC TATGGTTCGGGGCTTTCTTGGGGTTATGGACGTCTGGGGCCAAGGGACCACGGTCACCGTCTCCTCA

V_L chain of Ab-32 (SEQ ID NO:23)
GATATTGTGATGACCCAGACTCCATCCTTCCTGTCCGCATCCATAGGAGACAGAGTCACCATCACTTGCCGGGC CAGTCAGGGCATTGGCAGTTATTTAGCCTGGTATCAGCAAAGACCAGGGGAAGCCCCTAAGCTCCTGATCTATG CTGCATCGACTTTGCAAAGTGGAGTCCCATCAAGGTTCAGCGGCAGTGGATCTGGGACGGACTTCACTCTCACA ATCAGCAACCTGCAGCCTGAAGATTTTGCAACTTATTACTGTCAACAGCTTAATAATTACCCGATCACCTTCGG CCAAGGGACACGACTGGAGATTAAA

[0058]

Table 6B. Ab-32 Variable Region amino acid sequences
V_H chain of Ab-32 (SEQ ID NO:22)
EVQLVQSGAELKKPGSSVKVSCKAFGGTFSDNAISWVRQAPGQGPEWMGGIIPIFGKPNYAQKFQGRVTITADE STSTAYMVLSSLRSEDTAVYYCARTMVRGFLGVMDVWGQGTTVTVSS
V_L chain of Ab-32 (SEQ ID NO:24)
DIVMTQTPSFLSASIGDRVTITCRASQGIGSYLAWYQQRPGEAPKLLIYAASTLQSGVPSRFSGSGSGTDFTLT ISNLQPEDFATYYCQQLNNYPITFGQGTRLEIK

[0059]

Table 7A. Ab-38 Variable Region nucleic acid sequences
V_H chain of Ab-38 (SEQ ID NO:25)
CAGGTGCAGCTGGTGCAGTCTGGGGGAGGCTTGGTACAGCCTGGGGGGTCCCTGAGACTCTCCTGTGCAGCCTC TGGATTCACCTTTAGCAGCTATGCCATGAGCTGGGTCCGCCAGGCTCCAGGGAAGGGGCTGGAGTGGGTCTCAG CTATTAGTGGTAGTGGTGGTAGCACATACTACGCAGACTCCGTGAAGGGCCGGTTCACCATCTCCAGAGACAAT TCCAAGAACACGCTGTATCTGCAAATGAACAGCCTGAGAGCCGAGGACACGGCCGTATATTACTGTGCGAAAGA TCAGTTCGTTACGATTTTTGGAGTGCCAAGATACGGTATGGACGTCTGGGGCCAAGGGACCACGGTCACCGTCT CCTCA
V_L chain of Ab-38 (SEQ ID NO:27)
CAGTCTGCCCTGACTCAGCCACCCTCAGTGTCCGTGTCCCCAGGACAGACAGCCAACATCCCCTGCTCTGGAGA TAAATTGGGGAATAAATATGCTTACTGGTATCAGCAGAAGCCAGGCCAGTCCCCTGTACTGCTCATCTATCAAG ATATCAAGCGGCCCTCAAGGATCCCTGAGCGATTCTCTGGCTCCAACTCTGCGGACACAGCCACTCTGACCATC AGCGGGACCCAGGCTATGGATGAGGCTGACTATTACTGTCAGACGTGGGACAACAGCGTGGTCTTCGGCGGCGG GACCAAGCTGACCGTCCTC

[0060]

Table 7B. Ab-38 Variable Region amino acid sequences
V_H chain of Ab-38 (SEQ ID NO:26)
QVQLVQSGGGLVQPGGSLRLSCAASGFTFSSYAMSWVRQAPGKGLEWVSAISGSGGSTYYADSVKGRFTISRDN SKNTLYLQMNSLRAEDTAVYYCAKDQFVTIFGVPRYGMDVWGQGTTVTVSS
V_L chain of Ab-38 (SEQ ID NO:28)
QSALTQPPSVSVSPGQTANIPCSGDKLGNKYAYWYQQKPGQSPVLLIYQDIKRPSRIPERFSGSNSADTATLTI SGTQAMDEADYYCQTWDNSVVFGGGTKLTVL

[0061]

Table 8A. Ab-42 Variable Region nucleic acid sequences
V_H chain of Ab-42 (SEQ ID NO:29)
CAGGTGCAGCTGGTGCAGTCTGGGGCTGAGGTGAAGAAGCCTGGGTCCTCGGTGAAGGTCTCCTGCAAGGCTTC TGGAGGCACCTTCAGCAGCTATGCTATCAGCTGGGTGCGACAGGCCCCTGGACAAGGGCTTGAGTGGATGGGAG GGATCATCCCTATCTTTGGTACAGCAAACTACGCACAGAAGTTCCAGGGCAGAGTCACGATTACCGCGGACAAA TCCACGAGCACAGCCTACATGGAGCTGAGCAGCCTGAGATCTGAGGACACGGCCGTCTATTACTGTGCGAGAGG GCGTCAAATGTTCGGTGCGGGAATTGATTTCTGGGGCCCGGGCACCCTGGTCACCGTCTCCTCA
V_L chain of Ab-42 (SEQ ID NO:31)
AATTTTATGCTGACTCAGCCCCACTCTGTGTCGGAGTCTCCGGGGAAGACGGTAACCATCTCCTGCACCCGCAG CAGTGGCAGCATTGACAGCAACTATGTGCAGTGGTACCAGCAGCGCCCGGGCAGCGCCCCCACCACTGTGATCT ATGAGGATAACCAAAGACCCTCTGGGGTCCCTGATCGGTTCTCTGGCTCCATCGACAGCTCCTCCAACTCTGCC TCCCTCACCATCTCTGGACTGAAGACTGAGGACGAGGCTGACTACTACTGTCAGTCTTATGATAGCAACAATCG TCATGTGATATTCGGCGGAGGGACCAAGCTGACCGTCCTA

[0062]

Table 8B. Ab-42 Variable Region amino acid sequences
V_H chain of Ab-42 (SEQ ID NO:30)
QVQLVQSGAEVKKPGSSVKVSCKASGGTFSSYAISWVRQAPGQGLEWMGGIIPIFGTANYAQKFQGRVTITADK STSTAYMELSSLRSEDTAVYYCARGRQMFGAGIDFWGPGTLVTVSS
V_L chain of Ab-42 (SEQ ID NO:32)
NFMLTQPHSVSESPGKTVTISCTRSSGSIDSNYVQWYQQRPGSAPTTVIYEDNQRPSGVPDRFSGSIDSSSNSA SLTISGLKTEDEADYYCQSYDSNNRHVIFGGGTKLTVL

[0063]

Table 9A. Ab-46 Variable Region nucleic acid sequences
V_H chain of Ab-46 (SEQ ID NO:33)
GAGGTGCAGCTGGTGGAGTCTGGGGCTGAAGTAAAGAAGCCTGGGTCCTCGGTGAAAGTCTCCTGCAAGGTTTC AGGAGGCACATTCGGCACCTATGCTCTCAACTGGGTGCGCCAGGCCCCTGGACAAGGGCTTGAGTGGATGGGAA GGATCGTCCCTCTCATTGGTCTAGTAAACTACGCACATAACTTTGAGGGCAGAATCTCGATTACCGCGGACAAG TCCACGGGCACAGCCTACATGGAACTGAGCAACCTGAGATCTGACGACACGGCCGTGTATTACTGTGCGAGAGA GGTCTACGGTGGTAACTCCGACTACTGGGGCCAGGGAACCCTGGTCACCGTCTCCTCA
V_L chain of Ab-46 (SEQ ID NO:35)
AATTTTATGCTGACTCAGCCCCACTCAGTGTCGGAGTCTCCGGGGAAGACGGTAACCATCTCCTGCACTCGCAG TAGTGGCAACATTGGCACCAACTATGTGCAGTGGTACCAGCAGCGCCCGGGCAGTGCCCCCGTCGCTTTGATCT ACGAGGATTATCGAAGACCCTCTGGGGTCCCTGATCGGTTCTCTGGCTCCATCGACAGCTCCTCCAACTCTGCC TCCCTCATCATCTCTGGACTGAAGCCTGAGGACGAGGCTGACTACTACTGTCAGTCTTATCATAGCAGCGGTTG GGAATTCGGCGGAGGGACCAAGCTGACCGTCCTC

[0064]

Table 9B. Ab-46 Variable Region amino acid sequences
V_H chain of Ab-46 (SEQ ID NO:34)
EVQLVESGAEVKKPGSSVKVSCKVSGGTFGTYALNWVRQAPGQGLEWMGRIVPLIGLVNYAHNFEGRISITADK STGTAYMELSNLRSDDTAVYYCAREVYGGNSDYWGQGTLVTVSS
V_L chain of Ab-46 (SEQ ID NO:36)
NFMLTQPHSVSESPGKTVTISCTRSSGNIGTNYVQWYQQRPGSAPVALIYEDYRRPSGVPDRFSGSIDSSSNSA SLIISGLKPEDEADYYCQSYHSSGWEFGGGTKLTVL

[0065]

Table 10A. Ab-50 Variable Region nucleic acid sequences
V_H chain of Ab-50 (SEQ ID NO:37)
CAGGTGCAGCTGGTGCAGTCTGGAGGTGAGGTGAAGAAGCCGGGGGCCTCAGTGAAGGTCTCCTGCAAGGCTTC TGGTTACACCTTGAGCAGTCATGGTATAACCTGGGTGCGACAGGCCCCTGGACAAGGGCTTGAGTGGATGGGAT GGATCAGCGCTCACAATGGTCACGCTAGCAATGCACAGAAGGTGGAGGACAGAGTCACTATGACTACTGACACA TCCACGAACACAGCCTACATGGAACTGAGGAGCCTGACAGCTGACGACACGGCCGTGTATTACTGTGCGAGAGT ACATGCTGCCCTCTACTATGGTATGGACGTCTGGGGCCAAGGAACCCTGGTCACCGTCTCCTCA
V_L chain of Ab-50 (SEQ ID NO:39)
CAGTCTGTGCTGACTCAGCCACCCTCGGTGTCAGTGGCCCCAGGACAGACGGCCAGGATTACCTGTGGGGGAAA CAACATTGGAAGTAAAGGTGTGCACTGGTATCAGCAGAAGCCAGGCCAGGCCCCTGTACTGGTCGTCTATGATG ATAGTGACCGGCCCTCAGGGATCCCTGAGCGATTCTCTGGCTCCAACTCTGGGAACACGGCCACCCTGACCATC AGCAGGGTCGAAGCCGGGGATGAGGCCGACTATTACTGTCAGGTGTGGGATAGTAGTAGTGATCATTGGGTGTT CGGCGGAGGGACCAAGCTGACCGTCCTA

[0066]

Table 10B. Ab-50 Variable Region amino acid sequences
V_H chain of Ab-50 (SEQ ID NO:38)
QVQLVQSGGEVKKPGASVKVSCKASGYTLSSHGITWVRQAPGQGLEWMGWISAHNGHASNAQKVEDRVTMTTDT STNTAYMELRSLTADDTAVYYCARVHAALYYGMDVWGQGTLVTVSS
V_L chain of Ab-50 (SEQ ID NO:40)
QSVLTQPPSVSVAPGQTARITCGGNNIGSKGVHWYQQKPGQAPVLVVYDDSDRPSGIPERFSGSNSGNTATLTI

SRVEAGDEADYYCQVWDSSSDHWVFGGGTKLTVL

[0067]

Table 11A. Ab-52 Variable Region nucleic acid sequences
V_H chain of Ab-52 (SEQ ID NO:41)
CAGGTGCAGCTGCAGGAGTCGGGGGGAGGCGTGGTGCAGCCTGGGAGGTCCCTGAGACTCTCCTGTTCAGCCTC TGGATTCACCTTCAGCAGACATGGCATGCACTGGGTCCGCCAGGCTCCAGGCAAGGGGCTGGAGTGGGTGGCAG TGATATCACATGATGGAAGTGTAAAATACTATGCAGACTCCATGAAGGGCCGATTCAGCATCTCCAGAGACAAT TCCAACAACACACTGTATCTCCAAATGGACAGCCTGAGAGCTGACGACACGGCCGTTTATTACTGTGCGAGAGG ACTGTCGTACCAGGTGTCGGGGTGGTTCGACCCCTGGGGCCAGGGCACCCTGGTCACCGTCTCCTCA
V_L chain of Ab-52 (SEQ ID NO:43)
AATTTTATGCTGACTCAGCCCCACTCTGTGTCGGAGTCTCCGGGGAAGACGGTAACCATCTCCTGCACCCGCAG CAGTGGCAGCATTGCCAGCAACTATGTGCAGTGGTACCAGCAGCGCCCGGGCAGTGCCCCCACCACTGTGATCT ATGAGGATAACCAAAGACCCTCTGGGGTCCCTGATCGGTTCTCTGGCTCCATCGACAGCTCCTCCAACTCTGCC TCCCTCACCATCTCTGGACTGAAGACTGAGGACGAGGCTGACTACTACTGTCAGTCTTATGATAGCACCACCCC TTCGGTGTTCGGCGGCGGGACCAAGCTGACCGTCCTA

[0068]

Table 11B. Ab-52 Variable Region amino acid sequences
V_H chain of Ab-52 (SEQ ID NO:42)
QVQLQESGGGVVQPGRSLRLSCSASGFTFSRHGMHWVRQAPGKGLEWVAVISHDGSVKYYADSMKGRFSISRDN SNNTLYLQMDSLRADDTAVYYCARGLSYQVSGWFDPWGQGTLVTVSS
V_L chain of Ab-52 (SEQ ID NO:44)
NFMLTQPHSVSESPGKTVTISCTRSSGSIASNYVQWYQQRPGSAPTTVIYEDNQRPSGVPDRFSGSIDSSSNSA SLTISGLKTEDEADYYCQSYDSTTPSVFGGGTKLTVL

[0069]

Table 12A. Ab-55 Variable Region nucleic acid sequences
V_H chain of Ab-55 (SEQ ID NO:45)
CAGGTGCAGCTGGTGCAGTCTGGAGCTGAGGTGAAGAAGCCTGGGGCCTCAGTGAAGGTCTCCTGCAAGGCTTC TGGTTACACCTTTACCAGCTATGGTATCAGCTGGGTGCGACAGGCCCCTGGACAAGGGCTTGAGTGGATGGGAT GGACCAGCCCTCATAATGGTCTCACAGCATTTGCACAGATCCTAGAGGGCCGAGTCACCATGACCACAGACACA TCCACGAACACAGCCTACATGGAATTGAGGAACCTGACATTTGATGACACGGCCGTTTATTTCTGTGCGAAAGT ACATCCTGTCTTCTCTTATGCGTTGGACGTCTGGGGCCAAGGCACCCTGGTCACCGTCTCCTCA
V_L chain of Ab-55 (SEQ ID NO:47)
AATTTTATGCTGACTCAGCCCCACTCTGTGTCGGAGTCCCCGGGGAAGACGGTAACCATCTCCTGCACCCGCAG CAGTGGCAGCATTGCCAGCAACTATGTACAGTGGTACCAGCAGCGCCCGGGCAGTTCCCCCACCACTGTGATCT ATGAAGATAACCAAAGACCCTCTGGGGTCCCTGATCGGTTCTCTGGCTCCATCGACACCTCCTCCAACTCTGCC TCCCTCACCATCTCTGGACTGAAGACTAAGGACGAGGCGGACTACTACTGTCAGTCTTATGATGGCATCACTGT GATTTTCGGCGGAGGGACCAAGTTGACCGTCCTA

[0070]

Table 12B. Ab-55 Variable Region amino acid sequences
V_H chain of Ab-55 (SEQ ID NO:46)
QVQLVQSGAEVKKPGASVKVSCKASGYTFTSYGISWVRQAPGQGLEWMGWTSPHNGLTAFAQILEGRVTMTTDT STNTAYMELRNLTFDDTAVYFCAKVHPVFSYALDVWGQGTLVTVSS
V_L chain of Ab-55 (SEQ ID NO:48)
NFMLTQPHSVSESPGKTVTISCTRSSGSIASNYVQWYQQRPGSSPTTVIYEDNQRPSGVPDRFSGSIDTSSNSA SLTISGLKTKDEADYYCQSYDGITVIFGGGTKLTVL

[0071]

Table 13A. Ab-56 Variable Region nucleic acid sequences
V_H chain of Ab-56 (SEQ ID NO:49)
GAGGTGCAGCTGGTGGAGTCTGGAGCTGAGGTGATGAACCCTGGGTCCTCGGTGAGGGTCTCCTGCAGGGGTTC TGGAGGCGACTTCAGTACCTATGCTTTCAGCTGGGTGCGACAGGCCCCTGGACAAGGGCTTGAGTGGATGGGAA

GGATCATCCCTATCCTTGGTATAGCAAACTACGCACAGAAGTTCCAGGGCAGGGTCACGATTACCGCGGACAAA TCCACGAGCACAGCCTACATGGAGCTGAGCAGCCTGAGATCTGACGATACGGCCGTGTATTACTGTGCGAGAGA TGGCTATGGTTCGGACCCGGTGCTATGGGGCCAGGGCACCCTGGTCACCGTCTCCTCA
V_L chain of Ab-56 (SEQ ID NO:51)
AATTTTATGCTGACTCAGCCCCACTCTGTGTCGGGGTCTCCGGGGAAGACGGTAACCCTCCCCTGCACCCGCAG CAGTGGCAGCATTGCCAGCCACTATGTCCAGTGGTACCAGCAGCGCCCGGGCAGTGCCCCCACCACTGTGATCT ATGAGGATAACAAGAGACCCTCTGGGGTCCCTGATCGGTTCTCTGGCTCCATCGACAGCTCCTCCAACTCTGCC TCCCTCAGCATCTCTGGACTGAAGACTGAGGACGAGGCTGACTACTACTGTCAGTCTTATGATAGCAGCAATCG TTGGGTGTTCGGCGGAGGGACCAAGCTGACCGTCCTA

[0072]

Table 13B. Ab-56 Variable Region amino acid sequences
V_H chain of Ab-56 (SEQ ID NO:50)
EVQLVESGAEVMNPGSSVRVSCRGSGGDFSTYAFSWVRQAPGQGLEWMGRIIPILGIANYAQKFQGRVTITADK STSTAYMELSSLRSDDTAVYYCARDGYGSDPVLWGQGTLVTVSS
V_L chain of Ab-56 (SEQ ID NO:52)
NFMLTQPHSVSGSPGKTVTLPCTRSSGSIASHYVQWYQQRPGSAPTTVIYEDNKRPSGVPDRFSGSIDSSSNSA SLSISGLKTEDEADYYCQSYDSSNRWVFGGGTKLTVL

[0073]

Table 14A. Ab-65 Variable Region nucleic acid sequences
V_H chain of Ab-65 (SEQ ID NO:53)
GAGGTGCAGCTGGTGCAGTCTGGAGCTGAGGTGAAGAAGCCTGGGGCCTCAGTGAAGGTCTCCTGCAAGGCTTC TGGTTACACCTTTACCAACTATGGTATCAGCTGGGTGCGACAGGCCCCTGGACAAGGGCTTGAGTGGATGGGAT GGATCAGCGCTTACAATGGTAACACAAACTATGCACAGAAGGTCCAGGGCAGAGTCACCATGACCACAGACACA TCCACGAGCACAGGCTACATGGAGCTGAGGAGCCTGAGATCTGACGACACGGCCGTGTATTACTGTGCGAGAGG AGATTTTCGGAAACCCTTTGACTACTGGGGCCAGGGAACCCTGGTCACCGTCTCCTCA
V_L chain of Ab-65 (SEQ ID NO:55)
CTGCCTGTGCTGACTCAGCCGGCTTCCCTCTCTGCATCCCCCGGAGCATCAGCCAGTCTCACCTGCACCTTACG CAGTGGCCTCAATGTTGGTTCCTACAGGATATACTGGTACCAGCAGAAGCCAGGGAGTCGTCCCCAGTATCTCC TGAACTACAAATCAGACTCAAATAAACAGCAGGCCTCTGGAGTCCCCAGCCGCTTCTCTGGATCCAAGGATGCT TCGGCCAATGCAGGGATTTTACTCATCTCCGGGCTCCAGTCTGAGGATGAGGCTGACTATTACTGTATGATTTG GTACAGCAGCGCTGTGGTATTCGGCGGAGGGACCAAGCTGACCGTCCTA

[0074]

Table 14B. Ab-65 Variable Region amino acid sequences
V_H chain of Ab-65 (SEQ ID NO:54)
EVQLVQSGAEVKKPGASVKVSCKASGYTFTNYGISWVRQAPGQGLEWMGWISAYNGNTNYAQKVQGRVTMTTDT STSTGYMELRSLRSDDTAVYYCARGDFRKPFDYWGQGTLVTVSS
V_L chain of Ab-65 (SEQ ID NO:56)
LPVLTQPASLSASPGASASLTCTLRSGLNVGSYRIYWYQQKPGSRPQYLLNYKSDSNKQQASGVPSRFSGSKDA SANAGILLISGLQSEDEADYYCMIWYSSAVVFGGGTKLTVL

[0075] The amino acid sequences of the heavy and light chain complementary determining regions of the huPD-L1 antibodies are shown in Table 15A and 15B below.

[0076]

Table 15A. Amino acid sequences of the complementary determining regions of the heavy chain.

Antibody	CDR1	SEQ ID	CDR2	SEQ ID	CDR3	SEQ ID

		NO:		NO:		NO:
Consensus	**SYAIS**	57	**WISPIGGSTNYAQKVQG**	70	**GLXXXXXXXXXXXXXXXDV**	85
Ab-14	SYGIS	58	WISAYNGNTNYAQKLED	71	ALPSGTILVGGWFDP	86
Ab-16	SYALS	59	AISGGGGSTYYADSVKD	72	DVFPETFSMNYGMDV	87
Ab-22	DYAMH	60	LISGDGGSTYYADSVKD	73	VLLPCSSTSCYGSVGAFDI	88
Ab-30	NYDMS	61	RVNWNGGSTTYADAVKD	74	EFVGAYDL	89
Ab-31	GLYIH	62	WIIPIFGTANYAQKFED	75	GLRWGIWGWFDP	90
Ab-32	DNAIS	63	WIIPIFGKPNYAQKFED	76	TMVRGFLGVMDV	91
Ab-38	SYAMS	64	AISGSGGSTYYADSVKD	77	DQFVTIFGVPRYGMDV	92
Ab-42	SYAIS	57	WIIPIFGTANYAQKFED	78	GRQMFGAGIDF	93
Ab-46	TYALN	65	RIVPLIGLVNYAHNFED	79	EVYGGNSDY	94
Ab-50	SHGIT	66	WISAHNGHASNAQKVED	80	VHAALYYGMDV	95
Ab-52	RHGMH	67	VISHDGSVKYYADSMKD	81	GLSYQVSGWFDP	96
Ab-55	SYGIS	58	WTSPHNGLTAFAQILED	82	VHPVFSYALDV	97
Ab-56	TYAFS	68	RIIPILGIANYAQKFED	83	DGYGSDPVL	98
Ab-65	NYGIS	69	WISAYNGNTNYAQKVED	84	GDFRKPFDY	99

[0077] **Table 15B. Amino acid sequences of the complementary determining regions of the light chain.**

Antibody	CDR1	SEQ ID NO:	CDR2	SEQ ID NO:	CDR3	SEQ ID NO:
Consensus	**TRSSGSIGSNYVQ**	100	**EDNQRPS**	115	**QSYDSSTWV**	126
Ab-14	TRSSGNIASNYVQ	101	EDNQRPS	115	QSYDSSNLWV	127
Ab-16	QGDSLRSYYAS	102	GKNNRPS	116	NSRDSSGNHYV	128
Ab-22	GGSDIGRKSVH	103	SDRDRPS	117	QVWDNNSDHYV	129
Ab-30	TGTSSDVGGYNYVS	104	DVSNRPS	118	SSYTSSTLP	130
Ab-31	RASQSIGNSLA	105	GASSRAT	119	QQHTIPTFS	131
Ab-32	RASQGIGSYLA	106	AASTLQS	120	QQLNNYPIT	132
Ab-38	SGDKLGNKYAY	107	QDIKRPS	121	QTWDNSVV	133
Ab-42	TRSSGSIDSNYVQ	108	EDNQRPS	115	QSYDSNNRHVI	134
Ab-46	TRSSGNIGTNYVQ	109	EDYRRPS	122	QSYHSSGWE	135
Ab-50	GGNNIGSKGVH	110	DDSDRPS	123	QVWDSSSDHWV	136
Ab-52	TRSSGSIASNYVQ	111	EDNQRPS	115	QSYDSTTPSV	137
Ab-55	TRSSGSIASNYVQ	112	EDNQRPS	115	QSYDGITVI	138
Ab-56	TRSSGSIASHYVQ	113	EDNKRPS	124	QSYDSSNRWV	139
Ab-65	TLRSGLNVGSYRIY	114	YKSDSNKQQAS	125	MIWYSSAVV	140

[0078] The huPD-L1 antibodies described herein bind to PD-L1. In one aspect, the huPD-L1 antibodies have high affinity and high specificity for PD-L1. In another aspect, the

huPD-L1 antibodies can bind the PD-1 receptor and prevent, inhibit, or block the ligand PD-L1 from binding its receptor PD-1. In some instances, the huPD-L1 antibodies may have some cross-reactivity with PD-L2. In some instances, the huPD-L1 antibodies do not exhibit any cross-reactivity with PD-L2. In some instances, the huPD-L1 antibodies bind to PD-L1 with higher affinity and/or higher specificity than to PD-L2.

[0079] The present invention also features antibodies that have a specified percentage identity or similarity to the amino acid or nucleotide sequences of the huPD-L1 antibodies described herein. For example, the antibodies may have 60% , 70%, 75%, 80%, 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or higher identity when compared a specified region or the full length of any one of the huPD-L1 antibodies described herein. Sequence identity or similarity to the nucleic acids and proteins of the present invention can be determined by sequence comparison and/or alignment by methods known in the art. For example, sequence comparison algorithms (i.e. BLAST or BLAST 2.0), manual alignment or visual inspection can be utilized to determine percent sequence identity or similarity for the nucleic acids and proteins of the present invention.

[0080] As to amino acid sequences, one of skill in the art will readily recognize that individual substitutions, deletions or additions to a nucleic acid, peptide, polypeptide, or protein sequence which alters, adds, deletes, or substitutes a single amino acid or a small percentage of amino acids in the encoded sequence is collectively referred to herein as a “conservatively modified variant”. In some embodiments the alteration results in the substitution of an amino acid with a chemically similar amino acid. Conservative substitution tables providing functionally similar amino acids are well known in the art. Such conservatively modified variants of the huPD-L1 antibody disclosed herein may exhibit increased cross-reactivity to PD-L2 in comparison to an unmodified huPD-L1 antibody.

Antibodies

[0081] As used herein, the term "antibody" refers to immunoglobulin molecules and immunologically active portions of immunoglobulin (Ig) molecules, *i.e.*, molecules that contain an antigen binding site that specifically binds (immunoreacts with) an antigen. By “specifically binds” or “immunoreacts with” is meant that the antibody reacts with one or more antigenic determinants of the desired antigen and does not react with other polypeptides. Antibodies include, but are not limited to, polyclonal, monoclonal, chimeric, dAb (domain antibody), single chain, F_{ab}, $F_{ab'}$ and $F_{(ab')2}$ fragments, scFvs, and F_{ab} expression libraries.

[0082] A single chain Fv ("scFv") polypeptide molecule is a covalently linked V_H:V_L heterodimer, which can be expressed from a gene fusion including V_H- and V_L-encoding genes linked by a peptide-encoding linker. (*See* Huston et al. (1988) Proc Nat Acad Sci USA 85(16):5879-5883). A number of methods have been described to discern chemical structures for converting the naturally aggregated, but chemically separated, light and heavy polypeptide chains from an antibody V region into an scFv molecule, which will fold into a three dimensional structure substantially similar to the structure of an antigen-binding site. *See, e.g.,* U.S. Patent Nos. 5,091,513; 5,132,405; and 4,946,778.

[0083] Very large naïve human scFv libraries have been and can be created to offer a large source of rearranged antibody genes against a plethora of target molecules. Smaller libraries can be constructed from individuals with infectious diseases in order to isolate disease-specific antibodies. (*See* Barbas et al., Proc. Natl. Acad. Sci. USA 89:9339-43 (1992); Zebedee et al., Proc. Natl. Acad. Sci. USA 89:3175-79 (1992)).

[0084] In general, antibody molecules obtained from humans relate to any of the classes IgG, IgM, IgA, IgE and IgD, which differ from one another by the nature of the heavy chain present in the molecule. Certain classes have subclasses as well, such as IgG_1, IgG_2, and others. Furthermore, in humans, the light chain may be a kappa chain or a lambda chain. The term "antigen-binding site," or "binding portion" refers to the part of the immunoglobulin molecule that participates in antigen binding. The antigen binding site is formed by amino acid residues of the N-terminal variable ("V") regions of the heavy ("H") and light ("L") chains. Three highly divergent stretches within the V regions of the heavy and light chains, referred to as "hypervariable regions," are interposed between more conserved flanking stretches known as "framework regions," or "FRs". Thus, the term "FR" refers to amino acid sequences which are naturally found between, and adjacent to, hypervariable regions in immunoglobulins. In an antibody molecule, the three hypervariable regions of a light chain and the three hypervariable regions of a heavy chain are disposed relative to each other in three dimensional space to form an antigen-binding surface. The antigen-binding surface is complementary to the three-dimensional surface of a bound antigen, and the three hypervariable regions of each of the heavy and light chains are referred to as "complementarity-determining regions," or "CDRs." CDRs for the VH and VL regions of the scFv antibodies are shown in Figure 2.

[0085] As used herein, the term “epitope” includes any protein determinant capable of specific binding to an immunoglobulin, a scFv, or a T-cell receptor. Epitopic determinants

usually consist of chemically active surface groupings of molecules such as amino acids or sugar side chains and usually have specific three dimensional structural characteristics, as well as specific charge characteristics. For example, antibodies may be raised against N-terminal or C-terminal peptides of a polypeptide.

[0086] As used herein, the terms "immunological binding," and "immunological binding properties" refer to the non-covalent interactions of the type which occur between an immunoglobulin molecule and an antigen for which the immunoglobulin is specific. The strength, or affinity of immunological binding interactions can be expressed in terms of the dissociation constant (K_d) of the interaction, wherein a smaller K_d represents a greater affinity. Immunological binding properties of selected polypeptides can be quantified using methods well known in the art. One such method entails measuring the rates of antigen-binding site/antigen complex formation and dissociation, wherein those rates depend on the concentrations of the complex partners, the affinity of the interaction, and geometric parameters that equally influence the rate in both directions. Thus, both the "on rate constant" (K_{on}) and the "off rate constant" (K_{off}) can be determined by calculation of the concentrations and the actual rates of association and dissociation. (*See* Nature 361:186-87 (1993)). The ratio of K_{off} /K_{on} enables the cancellation of all parameters not related to affinity, and is equal to the dissociation constant K_d. (*See, generally*, Davies et al. (1990) Annual Rev Biochem 59:439-473). An antibody of the present invention is said to specifically bind to a PD-L1 epitope when the equilibrium binding constant (K_d) is ≤10 μM, preferably ≤ 10 nM, more preferably ≤ 10 nM, and most preferably ≤ 100 pM to about 1 pM, as measured by assays such as radioligand binding assays or similar assays known to those skilled in the art.

[0087] An PD-L1 protein of the invention, or a derivative, fragment, analog, homolog or ortholog thereof, may be utilized as an immunogen in the generation of antibodies that immunospecifically bind these protein components. A PD-L1 protein or a derivative, fragment, analog, homolog, or ortholog thereof, coupled to a proteoliposome may be utilized as an immunogen in the generation of antibodies that immunospecifically bind these protein components.

[0088] Those skilled in the art will recognize that it is possible to determine, without undue experimentation, if a human monoclonal antibody has the same specificity as a human monoclonal antibody of the invention by ascertaining whether the former prevents the latter from binding to PD-L1. If the human monoclonal antibody being tested competes with the human monoclonal antibody of the invention, as shown by a decrease in binding by the

human monoclonal antibody of the invention, then it is likely that the two monoclonal antibodies bind to the same, or to a closely related, epitope.

[0089] Another way to determine whether a human monoclonal antibody has the specificity of a human monoclonal antibody of the invention is to pre-incubate the human monoclonal antibody of the invention with the PD-L1 protein, with which it is normally reactive, and then add the human monoclonal antibody being tested to determine if the human monoclonal antibody being tested is inhibited in its ability to bind PD-L1. If the human monoclonal antibody being tested is inhibited then, in all likelihood, it has the same, or functionally equivalent, epitopic specificity as the monoclonal antibody of the invention. Screening of human monoclonal antibodies of the invention can be also carried out by utilizing PD-L1 and determining whether the test monoclonal antibody is able to neutralize PD-L1.

[0090] Various procedures known within the art may be used for the production of polyclonal or monoclonal antibodies directed against a protein of the invention, or against derivatives, fragments, analogs homologs or orthologs thereof. (*See*, *for example,* Antibodies: A Laboratory Manual, Harlow E, and Lane D, 1988, Cold Spring Harbor Laboratory Press, Cold Spring Harbor, NY, incorporated herein by reference).

[0091] Antibodies can be purified by well-known techniques, such as affinity chromatography using protein A or protein G, which provide primarily the IgG fraction of immune serum. Subsequently, or alternatively, the specific antigen which is the target of the immunoglobulin sought, or an epitope thereof, may be immobilized on a column to purify the immune specific antibody by immunoaffinity chromatography. Purification of immunoglobulins is discussed, for example, by D. Wilkinson (The Scientist, published by The Scientist, Inc., Philadelphia PA, Vol. 14, No. 8 (April 17, 2000), pp. 25-28).

[0092] The term "monoclonal antibody" or “MAb” or "monoclonal antibody composition", as used herein, refers to a population of antibody molecules that contain only one molecular species of antibody molecule consisting of a unique light chain gene product and a unique heavy chain gene product. In particular, the complementarity determining regions (CDRs) of the monoclonal antibody are identical in all the molecules of the population. MAbs contain an antigen binding site capable of immunoreacting with a particular epitope of the antigen characterized by a unique binding affinity for it.

[0093] Monoclonal antibodies can be prepared using hybridoma methods, such as those described by Kohler and Milstein, Nature, 256:495 (1975). In a hybridoma method, a

mouse, hamster, or other appropriate host animal, is typically immunized with an immunizing agent to elicit lymphocytes that produce or are capable of producing antibodies that will specifically bind to the immunizing agent. Alternatively, the lymphocytes can be immunized *in vitro*.

[0094] The immunizing agent will typically include the protein antigen, a fragment thereof or a fusion protein thereof. Generally, either peripheral blood lymphocytes are used if cells of human origin are desired, or spleen cells or lymph node cells are used if non-human mammalian sources are desired. The lymphocytes are then fused with an immortalized cell line using a suitable fusing agent, such as polyethylene glycol, to form a hybridoma cell (Goding, Monoclonal Antibodies: Principles and Practice, Academic Press, (1986) pp. 59-103). Immortalized cell lines are usually transformed mammalian cells, particularly myeloma cells of rodent, bovine and human origin. Usually, rat or mouse myeloma cell lines are employed. The hybridoma cells can be cultured in a suitable culture medium that preferably contains one or more substances that inhibit the growth or survival of the unfused, immortalized cells. For example, if the parental cells lack the enzyme hypoxanthine guanine phosphoribosyl transferase (HGPRT or HPRT), the culture medium for the hybridomas typically will include hypoxanthine, aminopterin, and thymidine ("HAT medium"), which substances prevent the growth of HGPRT-deficient cells.

[0095] Preferred immortalized cell lines are those that fuse efficiently, support stable high level expression of antibody by the selected antibody-producing cells, and are sensitive to a medium such as HAT medium. More preferred immortalized cell lines are murine myeloma lines, which can be obtained, for instance, from the Salk Institute Cell Distribution Center, San Diego, California and the American Type Culture Collection, Manassas, Virginia. Human myeloma and mouse-human heteromyeloma cell lines also have been described for the production of human monoclonal antibodies. (*See* Kozbor, J. Immunol., 133:3001 (1984); Brodeur et al., Monoclonal Antibody Production Techniques and Applications, Marcel Dekker, Inc., New York, (1987) pp. 51-63)).

[0096] The culture medium in which the hybridoma cells are cultured can then be assayed for the presence of monoclonal antibodies directed against the antigen. Preferably, the binding specificity of monoclonal antibodies produced by the hybridoma cells is determined by immunoprecipitation or by an *in vitro* binding assay, such as radioimmunoassay (RIA) or enzyme-linked immunoabsorbent assay (ELISA). Such techniques and assays are known in the art. The binding affinity of the monoclonal antibody

can, for example, be determined by the Scatchard analysis of Munson and Pollard, Anal. Biochem., 107:220 (1980). Moreover, in therapeutic applications of monoclonal antibodies, it is important to identify antibodies having a high degree of specificity and a high binding affinity for the target antigen.

[0097] After the desired hybridoma cells are identified, the clones can be subcloned by limiting dilution procedures and grown by standard methods. (*See* Goding, Monoclonal Antibodies: Principles and Practice, Academic Press, (1986) pp. 59-103). Suitable culture media for this purpose include, for example, Dulbecco's Modified Eagle's Medium and RPMI-1640 medium. Alternatively, the hybridoma cells can be grown *in vivo* as ascites in a mammal.

[0098] The monoclonal antibodies secreted by the subclones can be isolated or purified from the culture medium or ascites fluid by conventional immunoglobulin purification procedures such as, for example, protein A-Sepharose, hydroxylapatite chromatography, gel electrophoresis, dialysis, or affinity chromatography.

[0099] Monoclonal antibodies can also be made by recombinant DNA methods, such as those described in U.S. Patent No. 4,816,567. DNA encoding the monoclonal antibodies of the invention can be readily isolated and sequenced using conventional procedures (*e.g.*, by using oligonucleotide probes that are capable of binding specifically to genes encoding the heavy and light chains of murine antibodies). The hybridoma cells of the invention serve as a preferred source of such DNA. Once isolated, the DNA can be placed into expression vectors, which are then transfected into host cells such as simian COS cells, Chinese hamster ovary (CHO) cells, or myeloma cells that do not otherwise produce immunoglobulin protein, to obtain the synthesis of monoclonal antibodies in the recombinant host cells. The DNA also can be modified, for example, by substituting the coding sequence for human heavy and light chain constant domains in place of the homologous murine sequences (*see* U.S. Patent No. 4,816,567; Morrison, Nature 368, 812-13 (1994)) or by covalently joining to the immunoglobulin coding sequence all or part of the coding sequence for a non-immunoglobulin polypeptide. Such a non-immunoglobulin polypeptide can be substituted for the constant domains of an antibody of the invention, or can be substituted for the variable domains of one antigen-combining site of an antibody of the invention to create a chimeric bivalent antibody.

[00100] Fully human antibodies are antibody molecules in which the entire sequence of both the light chain and the heavy chain, including the CDRs, arise from human genes.

Such antibodies are termed "humanized antibodies", "human antibodies", or "fully human antibodies" herein. Human monoclonal antibodies can be prepared by using trioma technique; the human B-cell hybridoma technique (*see* Kozbor, et al., 1983 Immunol Today 4: 72); and the EBV hybridoma technique to produce human monoclonal antibodies (*see* Cole, et al., 1985 In: MONOCLONAL ANTIBODIES AND CANCER THERAPY, Alan R. Liss, Inc., pp. 77-96). Human monoclonal antibodies may be utilized and may be produced by using human hybridomas (*see* Cote, et al., 1983. Proc Natl Acad Sci USA 80: 2026-2030) or by transforming human B-cells with Epstein Barr Virus *in vitro* (see Cole, et al., 1985 In: MONOCLONAL ANTIBODIES AND CANCER THERAPY, Alan R. Liss, Inc., pp. 77-96).

[00101] In addition, human antibodies can also be produced using additional techniques, including phage display libraries. (*See* Hoogenboom and Winter, J. Mol. Biol., 227:381 (1991); Marks et al., J. Mol. Biol., 222:581 (1991)). Similarly, human antibodies can be made by introducing human immunoglobulin loci into transgenic animals, *e.g.*, mice in which the endogenous immunoglobulin genes have been partially or completely inactivated. Upon challenge, human antibody production is observed, which closely resembles that seen in humans in all respects, including gene rearrangement, assembly, and antibody repertoire. This approach is described, for example, in U.S. Patent Nos. 5,545,807; 5,545,806; 5,569,825; 5,625,126; 5,633,425; 5,661,016, and in Marks et al., Bio/Technology 10, 779-783 (1992); Lonberg et al., Nature 368 856-859 (1994); Morrison, Nature 368, 812-13 (1994); Fishwild et al, Nature Biotechnology 14, 845-51 (1996); Neuberger, Nature Biotechnology 14, 826 (1996); and Lonberg and Huszar, Intern. Rev. Immunol. 13 65-93 (1995).

[00102] Human antibodies may additionally be produced using transgenic nonhuman animals which are modified so as to produce fully human antibodies rather than the animal's endogenous antibodies in response to challenge by an antigen. (*See* PCT publication WO94/02602). The endogenous genes encoding the heavy and light immunoglobulin chains in the nonhuman host have been incapacitated, and active loci encoding human heavy and light chain immunoglobulins are inserted into the host's genome. The human genes are incorporated, for example, using yeast artificial chromosomes containing the requisite human DNA segments. An animal which provides all the desired modifications is then obtained as progeny by crossbreeding intermediate transgenic animals containing fewer than the full complement of the modifications. The preferred embodiment of such a nonhuman animal is a mouse, and is termed the Xenomouse™ as disclosed in PCT publications WO 96/33735

and WO 96/34096. This animal produces B cells which secrete fully human immunoglobulins. The antibodies can be obtained directly from the animal after immunization with an immunogen of interest, as, for example, a preparation of a polyclonal antibody, or alternatively from immortalized B cells derived from the animal, such as hybridomas producing monoclonal antibodies. Additionally, the genes encoding the immunoglobulins with human variable regions can be recovered and expressed to obtain the antibodies directly, or can be further modified to obtain analogs of antibodies such as, for example, single chain Fv (scFv) molecules.

[00103] An example of a method of producing a nonhuman host, exemplified as a mouse, lacking expression of an endogenous immunoglobulin heavy chain is disclosed in U.S. Patent No. 5,939,598. It can be obtained by a method, which includes deleting the J segment genes from at least one endogenous heavy chain locus in an embryonic stem cell to prevent rearrangement of the locus and to prevent formation of a transcript of a rearranged immunoglobulin heavy chain locus, the deletion being effected by a targeting vector containing a gene encoding a selectable marker; and producing from the embryonic stem cell a transgenic mouse whose somatic and germ cells contain the gene encoding the selectable marker.

[00104] One method for producing an antibody of interest, such as a human antibody, is disclosed in U.S. Patent No. 5,916,771. This method includes introducing an expression vector that contains a nucleotide sequence encoding a heavy chain into one mammalian host cell in culture, introducing an expression vector containing a nucleotide sequence encoding a light chain into another mammalian host cell, and fusing the two cells to form a hybrid cell. The hybrid cell expresses an antibody containing the heavy chain and the light chain.

[00105] In a further improvement on this procedure, a method for identifying a clinically relevant epitope on an immunogen and a correlative method for selecting an antibody that binds immunospecifically to the relevant epitope with high affinity, are disclosed in PCT publication WO 99/53049.

[00106] The antibody can be expressed by a vector containing a DNA segment encoding the single chain antibody described above.

[00107] These can include vectors, liposomes, naked DNA, adjuvant-assisted DNA, gene gun, catheters, *etc*. Vectors include chemical conjugates such as described in WO 93/64701, which has targeting moiety (*e.g.* a ligand to a cellular surface receptor), and a nucleic acid binding moiety (*e.g.* polylysine), viral vector (*e.g.* a DNA or RNA viral vector),

fusion proteins such as described in PCT/US 95/02140 (WO 95/22618) which is a fusion protein containing a target moiety (*e.g.* an antibody specific for a target cell) and a nucleic acid binding moiety (*e.g.* a protamine), plasmids, phage, etc. The vectors can be chromosomal, non-chromosomal or synthetic.

[00108] Preferred vectors include viral vectors, fusion proteins and chemical conjugates. Retroviral vectors include moloney murine leukemia viruses. DNA viral vectors are preferred. These vectors include pox vectors such as orthopox or avipox vectors, herpesvirus vectors such as a herpes simplex I virus (HSV) vector (*see* Geller, A. I. et al., J. Neurochem, 64:487 (1995); Lim, F., et al., in DNA Cloning: Mammalian Systems, D. Glover, Ed. (Oxford Univ. Press, Oxford England) (1995); Geller, A. I. et al., Proc Natl. Acad. Sci.: U.S.A. 90:7603 (1993); Geller, A. I., et al., Proc Natl. Acad. Sci USA 87:1149 (1990), Adenovirus Vectors (*see* LeGal LaSalle et al., Science, 259:988 (1993); Davidson, et al., Nat. Genet 3:219 (1993); Yang, et al., J. Virol. 69:2004 (1995) and Adeno-associated Virus Vectors (*see* Kaplitt, M. G.. et al., Nat. Genet. 8:148 (1994).

[00109] Pox viral vectors introduce the gene into the cells cytoplasm. Avipox virus vectors result in only a short term expression of the nucleic acid. Adenovirus vectors, adeno-associated virus vectors and herpes simplex virus (HSV) vectors are preferred for introducing the nucleic acid into neural cells. The adenovirus vector results in a shorter term expression (about 2 months) than adeno-associated virus (about 4 months), which in turn is shorter than HSV vectors. The particular vector chosen will depend upon the target cell and the condition being treated. The introduction can be by standard techniques, *e.g.* infection, transfection, transduction or transformation. Examples of modes of gene transfer include *e.g.*, naked DNA, $CaPO_4$ precipitation, DEAE dextran, electroporation, protoplast fusion, lipofection, cell microinjection, and viral vectors.

[00110] The vector can be employed to target essentially any desired target cell. For example, stereotaxic injection can be used to direct the vectors (*e.g.* adenovirus, HSV) to a desired location. Additionally, the particles can be delivered by intracerebroventricular (icv) infusion using a minipump infusion system, such as a SynchroMed Infusion System. A method based on bulk flow, termed convection, has also proven effective at delivering large molecules to extended areas of the brain and may be useful in delivering the vector to the target cell. (*See* Bobo et al., Proc. Natl. Acad. Sci. USA 91:2076-2080 (1994); Morrison et al., Am. J. Physiol. 266:292-305 (1994)). Other methods that can be used include catheters,

intravenous, parenteral, intraperitoneal and subcutaneous injection, and oral or other known routes of administration.

[00111] These vectors can be used to express large quantities of antibodies that can be used in a variety of ways. For example, to detect the presence of PD-L1 in a sample. The antibody can also be used to try to bind to and disrupt a PD-L1 activity.

[00112] Techniques can be adapted for the production of single-chain antibodies specific to an antigenic protein of the invention (*see e.g.*, U.S. Patent No. 4,946,778). In addition, methods can be adapted for the construction of F_{ab} expression libraries (*see e.g.*, Huse, et al., 1989 Science 246: 1275-1281) to allow rapid and effective identification of monoclonal F_{ab} fragments with the desired specificity for a protein or derivatives, fragments, analogs or homologs thereof. Antibody fragments that contain the idiotypes to a protein antigen may be produced by techniques known in the art including, but not limited to: (i) an $F_{(ab')2}$ fragment produced by pepsin digestion of an antibody molecule; (ii) an F_{ab} fragment generated by reducing the disulfide bridges of an $F_{(ab')2}$ fragment; (iii) an F_{ab} fragment generated by the treatment of the antibody molecule with papain and a reducing agent and (iv) F_v fragments.

[00113] Heteroconjugate antibodies are also within the scope of the present invention. Heteroconjugate antibodies are composed of two covalently joined antibodies. Such antibodies have, for example, been proposed to target immune system cells to unwanted cells (*see* U.S. Patent No. 4,676,980), and for treatment of HIV infection (*see* WO 91/00360; WO 92/200373; EP 03089). It is contemplated that the antibodies can be prepared *in vitro* using known methods in synthetic protein chemistry, including those involving crosslinking agents. For example, immunotoxins can be constructed using a disulfide exchange reaction or by forming a thioether bond. Examples of suitable reagents for this purpose include iminothiolate and methyl-4-mercaptobutyrimidate and those disclosed, for example, in U.S. Patent No. 4,676,980.

[00114] It can be desirable to modify the antibody of the invention with respect to effector function, so as to enhance, *e.g.*, the effectiveness of the antibody in treating cancer. For example, cysteine residue(s) can be introduced into the Fc region, thereby allowing interchain disulfide bond formation in this region. The homodimeric antibody thus generated can have improved internalization capability and/or increased complement-mediated cell killing and antibody-dependent cellular cytotoxicity (ADCC). (*See* Caron et al., J. Exp Med., 176: 1191-1195 (1992) and Shopes, J. Immunol., 148: 2918-2922 (1992)). Alternatively, an

antibody can be engineered that has dual Fc regions and can thereby have enhanced complement lysis and ADCC capabilities. (*See* Stevenson et al., Anti-Cancer Drug Design, 3: 219-230 (1989)).

[00115] The invention also pertains to immunoconjugates comprising an antibody conjugated to a cytotoxic agent such as a toxin (*e.g.*, an enzymatically active toxin of bacterial, fungal, plant, or animal origin, or fragments thereof), or a radioactive isotope (*i.e.*, a radioconjugate).

[00116] Enzymatically active toxins and fragments thereof that can be used include diphtheria A chain, nonbinding active fragments of diphtheria toxin, exotoxin A chain (from Pseudomonas aeruginosa), ricin A chain, abrin A chain, modeccin A chain, alpha-sarcin, Aleurites fordii proteins, dianthin proteins, Phytolaca americana proteins (PAPI, PAPII, and PAP-S), momordica charantia inhibitor, curcin, crotin, sapaonaria officinalis inhibitor, gelonin, mitogellin, restrictocin, phenomycin, enomycin, and the tricothecenes. A variety of radionuclides are available for the production of radioconjugated antibodies. Examples include ^{212}Bi, ^{131}I, ^{131}In, ^{90}Y, and ^{186}Re.

[00117] Conjugates of the antibody and cytotoxic agent are made using a variety of bifunctional protein-coupling agents such as N-succinimidyl-3-(2-pyridyldithiol) propionate (SPDP), iminothiolane (IT), bifunctional derivatives of imidoesters (such as dimethyl adipimidate HCL), active esters (such as disuccinimidyl suberate), aldehydes (such as glutareldehyde), bis-azido compounds (such as bis (p-azidobenzoyl) hexanediamine), bis-diazonium derivatives (such as bis-(p-diazoniumbenzoyl)-ethylenediamine), diisocyanates (such as tolyene 2,6-diisocyanate), and bis-active fluorine compounds (such as 1,5-difluoro-2,4-dinitrobenzene). For example, a ricin immunotoxin can be prepared as described in Vitetta et al., Science 238: 1098 (1987). Carbon-14-labeled 1-isothiocyanatobenzyl-3-methyldiethylene triaminepentaacetic acid (MX-DTPA) is an exemplary chelating agent for conjugation of radionucleotide to the antibody. (*See* WO94/11026).

[00118] Those of ordinary skill in the art will recognize that a large variety of possible moieties can be coupled to the resultant antibodies or to other molecules of the invention. (*See, for example*, "Conjugate Vaccines", Contributions to Microbiology and Immunology, J. M. Cruse and R. E. Lewis, Jr (eds), Carger Press, New York, (1989), the entire contents of which are incorporated herein by reference).

[00119] Coupling may be accomplished by any chemical reaction that will bind the two molecules so long as the antibody and the other moiety retain their respective activities. This linkage can include many chemical mechanisms, for instance covalent binding, affinity binding, intercalation, coordinate binding and complexation. The preferred binding is, however, covalent binding. Covalent binding can be achieved either by direct condensation of existing side chains or by the incorporation of external bridging molecules. Many bivalent or polyvalent linking agents are useful in coupling protein molecules, such as the antibodies of the present invention, to other molecules. For example, representative coupling agents can include organic compounds such as thioesters, carbodiimides, succinimide esters, diisocyanates, glutaraldehyde, diazobenzenes and hexamethylene diamines. This listing is not intended to be exhaustive of the various classes of coupling agents known in the art but, rather, is exemplary of the more common coupling agents. (*See* Killen and Lindstrom, Jour. Immun. 133:1335-2549 (1984); Jansen et al., Immunological Reviews 62:185-216 (1982); and Vitetta et al., Science 238:1098 (1987)). Preferred linkers are described in the literature. (*See*, *for example*, Ramakrishnan, S. et al., Cancer Res. 44:201-208 (1984) describing use of MBS (M-maleimidobenzoyl-N-hydroxysuccinimide ester). *See also*, U.S. Patent No. 5,030,719, describing use of halogenated acetyl hydrazide derivative coupled to an antibody by way of an oligopeptide linker. Particularly preferred linkers include: (i) EDC (1-ethyl-3-(3-dimethylamino-propyl) carbodiimide hydrochloride; (ii) SMPT (4-succinimidyloxycarbonyl-alpha-methyl-alpha-(2-pridyl-dithio)-toluene (Pierce Chem. Co., Cat. (21558G); (iii) SPDP (succinimidyl-6 [3-(2-pyridyldithio) propionamido]hexanoate (Pierce Chem. Co., Cat #21651G); (iv) Sulfo-LC-SPDP (sulfosuccinimidyl 6 [3-(2-pyridyldithio)-propianamide] hexanoate (Pierce Chem. Co. Cat. #2165-G); and (v) sulfo-NHS (N-hydroxysulfo-succinimide: Pierce Chem. Co., Cat. #24510) conjugated to EDC.

[00120] The linkers described above contain components that have different attributes, thus leading to conjugates with differing physio-chemical properties. For example, sulfo-NHS esters of alkyl carboxylates are more stable than sulfo-NHS esters of aromatic carboxylates. NHS-ester containing linkers are less soluble than sulfo-NHS esters. Further, the linker SMPT contains a sterically hindered disulfide bond, and can form conjugates with increased stability. Disulfide linkages, are in general, less stable than other linkages because the disulfide linkage is cleaved *in vitro*, resulting in less conjugate available. Sulfo-NHS, in particular, can enhance the stability of carbodimide couplings. Carbodimide couplings (such

as EDC) when used in conjunction with sulfo-NHS, forms esters that are more resistant to hydrolysis than the carbodimide coupling reaction alone.

[00121] The antibodies disclosed herein can also be formulated as immunoliposomes. Liposomes containing the antibody are prepared by methods known in the art, such as described in Epstein et al., Proc. Natl. Acad. Sci. USA, 82: 3688 (1985); Hwang et al., Proc. Natl Acad. Sci. USA, 77: 4030 (1980); and U.S. Pat. Nos. 4,485,045 and 4,544,545. Liposomes with enhanced circulation time are disclosed in U.S. Patent No. 5,013,556.

[00122] Particularly useful liposomes can be generated by the reverse-phase evaporation method with a lipid composition comprising phosphatidylcholine, cholesterol, and PEG-derivatized phosphatidylethanolamine (PEG-PE). Liposomes are extruded through filters of defined pore size to yield liposomes with the desired diameter. Fab' fragments of the antibody of the present invention can be conjugated to the liposomes as described in Martin et al., J. Biol. Chem., 257: 286-288 (1982) via a disulfide-interchange reaction.

Use of Antibodies Against PD-L1

[00123] Antibodies specifically binding a PD-L1 protein or a fragment thereof of the invention can be administered for the treatment of cancer or other proliferative disorders. Many cancers overexpress PD-L1 and the upregulation of PD-L1 is associated with high risk prognostic factors. Overexpression fo PD-L1 in tumor cells can also indicate a mechanism by which the tumor cells evade anti-tumor immunity, such as by inducing T cell exhaustion. Such cancers include renal cell carcinoma and breast cancer. Other exemplary cancers are those cancers that are associated with or utilize T cell exhaustion to evade anti-tumor T cell activity. Use of the antibody of the invention can enhance the ability of tumor antigen-specific T cells to proliferate and produce cytokines in response to stimulation with tumor antigen peptides, thereby augmenting T cell activity or anti-tumor immune response.

[00124] Antibodies specifically binding a PD-L1 protein or fragment thereof of the invention can be administered for the treatment of a chronic infection. Such chronic infections include, for example, viral, bacterial and parasitic infections. An exemplary chronic viral infection is HIV. During chronic HIV infection, HIV-specific CD8+ T cells are functionally impaired, showing a reduced capacity to produce cytokines and effector molecules as well as a diminished ability to proliferate. PD-1 is highly expressed on HIV-specific CD8+ T cells of HIV infected individuals. Use of the antibody of the invention can enhance the ability of HIV-specific T cells to proliferate and produce cytokines in response to

stimulation with HIV peptides, thereby augmenting T cell activity or anti-viral immune response.

[00125] Antibodies of the invention, including bi-specific, polyclonal, monoclonal, humanized and fully human antibodies, may be used as therapeutic agents. Such agents will generally be employed to treat or prevent cancer in a subject, increase vaccine efficiency or augment a natural immune response. An antibody preparation, preferably one having high specificity and high affinity for its target antigen, is administered to the subject and will generally have an effect due to its binding with the target. Administration of the antibody may abrogate or inhibit or interfere with an activity of the PD-L1 protein.

[00126] Antibodies of the invention are capable of inducing cell death. Cell death is induced by either direct or indirect mechanisms. For instance, PD-L1 binding by the huPD-L1 antibody can lead to complement-dependent cytotoxicity (CDC). Alternatively, the huPD-L1 antibody binds PD-L1, and leads to the recruitment of a second cell type that will kill the PD-L14-expressing target cell. Exemplary mechanisms by which the huPD-L1 antibody mediates cell death by recruitment of a second cell type include, but are not limited to, antibody-dependent cellular toxicity (ADCC) and antibody dependent cellular phagocytosis (ADCP). Target PD-L1-expressing cell types comprise tumor and T cells, for example, activated T cells.

[00127] Antibodies specifically binding a PD-L1 protein or fragment thereof of the invention can be administered for the treatment of a cancer or chronic infection in the form of pharmaceutical compositions. Principles and considerations involved in preparing therapeutic compositions comprising the antibody, as well as guidance in the choice of components are provided, for example, in Remington: The Science And Practice Of Pharmacy 19th ed. (Alfonso R. Gennaro, et al., editors) Mack Pub. Co., Easton, Pa., 1995; Drug Absorption Enhancement: Concepts, Possibilities, Limitations, And Trends, Harwood Academic Publishers, Langhorne, Pa., 1994; and Peptide And Protein Drug Delivery (Advances In Parenteral Sciences, Vol. 4), 1991, M. Dekker, New York.

[00128] A therapeutically effective amount of an antibody of the invention relates generally to the amount needed to achieve a therapeutic objective. As noted above, this may be a binding interaction between the antibody and its target antigen that, in certain cases, interferes with the functioning of the target. The amount required to be administered will furthermore depend on the binding affinity of the antibody for its specific antigen, and will also depend on the rate at which an administered antibody is depleted from the free volume

other subject to which it is administered. Common ranges for therapeutically effective dosing of an antibody or antibody fragment of the invention may be, by way of nonlimiting example, from about 0.1 mg/kg body weight to about 50 mg/kg body weight. Common dosing frequencies may range, for example, from twice daily to once a week.

[00129] Where antibody fragments are used, the smallest inhibitory fragment that specifically binds to the binding domain of the target protein is preferred. For example, based upon the variable-region sequences of an antibody, peptide molecules can be designed that retain the ability to bind the target protein sequence. Such peptides can be synthesized chemically and/or produced by recombinant DNA technology. (*See*, *e.g.*, Marasco et al., Proc. Natl. Acad. Sci. USA, 90: 7889-7893 (1993)). The formulation can also contain more than one active compound as necessary for the particular indication being treated, preferably those with complementary activities that do not adversely affect each other. Alternatively, or in addition, the composition can comprise an agent that enhances its function, such as, for example, a cytotoxic agent, cytokine, chemotherapeutic agent, or growth-inhibitory agent. Such molecules are suitably present in combination in amounts that are effective for the purpose intended.

[00130] The active ingredients can also be entrapped in microcapsules prepared, for example, by coacervation techniques or by interfacial polymerization, for example, hydroxymethylcellulose or gelatin-microcapsules and poly-(methylmethacrylate) microcapsules, respectively, in colloidal drug delivery systems (for example, liposomes, albumin microspheres, microemulsions, nano-particles, and nanocapsules) or in macroemulsions.

[00131] The formulations to be used for *in vivo* administration must be sterile. This is readily accomplished by filtration through sterile filtration membranes.

[00132] Sustained-release preparations can be prepared. Suitable examples of sustained-release preparations include semipermeable matrices of solid hydrophobic polymers containing the antibody, which matrices are in the form of shaped articles, *e.g.*, films, or microcapsules. Examples of sustained-release matrices include polyesters, hydrogels (for example, poly(2-hydroxyethyl-methacrylate), or poly(vinylalcohol)), polylactides (U.S. Pat. No. 3,773,919), copolymers of L-glutamic acid and γ ethyl-L-glutamate, non-degradable ethylene-vinyl acetate, degradable lactic acid-glycolic acid copolymers such as the LUPRON DEPOT ™ (injectable microspheres composed of lactic acid-glycolic acid copolymer and leuprolide acetate), and poly-D-(-)-3-hydroxybutyric

acid. While polymers such as ethylene-vinyl acetate and lactic acid-glycolic acid enable release of molecules for over 100 days, certain hydrogels release proteins for shorter time periods.

[00133] An antibody according to the invention can be used as an agent for detecting the presence of PD-L1 (or a protein or a protein fragment thereof) in a sample. Preferably, the antibody contains a detectable label. Antibodies can be polyclonal, or more preferably, monoclonal. An intact antibody, or a fragment thereof (*e.g.*, F_{ab}, scFv, or $F_{(ab)2}$) can be used. The term "labeled", with regard to the probe or antibody, is intended to encompass direct labeling of the probe or antibody by coupling (*i.e.*, physically linking) a detectable substance to the probe or antibody, as well as indirect labeling of the probe or antibody by reactivity with another reagent that is directly labeled. Examples of indirect labeling include detection of a primary antibody using a fluorescently-labeled secondary antibody and end-labeling of a DNA probe with biotin such that it can be detected with fluorescently-labeled streptavidin. The term "biological sample" is intended to include tissues, cells and biological fluids isolated from a subject, as well as tissues, cells and fluids present within a subject. Included within the usage of the term “biological sample”, therefore, is blood and a fraction or component of blood including blood serum, blood plasma, or lymph. That is, the detection method of the invention can be used to detect an analyte mRNA, protein, or genomic DNA in a biological sample *in vitro* as well as *in vivo*. For example, *in vitro* techniques for detection of an analyte mRNA includes Northern hybridizations and *in situ* hybridizations. *In vitro* techniques for detection of an analyte protein include enzyme linked immunosorbent assays (ELISAs), Western blots, immunoprecipitations, and immunofluorescence. *In vitro* techniques for detection of an analyte genomic DNA include Southern hybridizations. Procedures for conducting immunoassays are described, for example in “ELISA: Theory and Practice: Methods in Molecular Biology”, Vol. 42, J. R. Crowther (Ed.) Human Press, Totowa, NJ, 1995; “Immunoassay”, E. Diamandis and T. Christopoulus, Academic Press, Inc., San Diego, CA, 1996; and “Practice and Theory of Enzyme Immunoassays”, P. Tijssen, Elsevier Science Publishers, Amsterdam, 1985. Furthermore, *in vivo* techniques for detection of an analyte protein include introducing into a subject a labeled anti-analyte protein antibody. For example, the antibody can be labeled with a radioactive marker whose presence and location in a subject can be detected by standard imaging techniques.

[00134] Antibodies directed against a PD-L1 protein (or a fragment thereof) may be used in methods known within the art relating to the localization and/or quantitation of a PD-

L1 protein (*e.g.*, for use in measuring levels of the PD-L1 protein within appropriate physiological samples, for use in diagnostic methods, for use in imaging the protein, and the like). In a given embodiment, antibodies specific to a PD-L1 protein, or derivative, fragment, analog or homolog thereof, that contain the antibody derived antigen binding domain, are utilized as pharmacologically active compounds (referred to hereinafter as "Therapeutics").

[00135] An antibody specific for a PD-L1 protein of the invention can be used to isolate a PD-L1 polypeptide by standard techniques, such as immunoaffinity, chromatography or immunoprecipitation. Antibodies directed against a PD-L1 protein (or a fragment thereof) can be used diagnostically to monitor protein levels in tissue as part of a clinical testing procedure, *e.g.*, to, for example, determine the efficacy of a given treatment regimen. Detection can be facilitated by coupling (*i.e.*, physically linking) the antibody to a detectable substance. Examples of detectable substances include various enzymes, prosthetic groups, fluorescent materials, luminescent materials, bioluminescent materials, and radioactive materials. Examples of suitable enzymes include horseradish peroxidase, alkaline phosphatase, β-galactosidase, or acetylcholinesterase; examples of suitable prosthetic group complexes include streptavidin/biotin and avidin/biotin; examples of suitable fluorescent materials include umbelliferone, fluorescein, fluorescein isothiocyanate, rhodamine, dichlorotriazinylamine fluorescein, dansyl chloride or phycoerythrin; an example of a luminescent material includes luminol; examples of bioluminescent materials include luciferase, luciferin, and aequorin, and examples of suitable radioactive material include ^{125}I, ^{131}I, ^{35}S or ^{3}H.

Pharmaceutical compositions

[00136] The antibodies or agents of the invention (also referred to herein as "active compounds"), and derivatives, fragments, analogs and homologs thereof, can be incorporated into pharmaceutical compositions suitable for administration. Such compositions typically comprise the antibody or agent and a pharmaceutically acceptable carrier. As used herein, the term "pharmaceutically acceptable carrier" is intended to include any and all solvents, dispersion media, coatings, antibacterial and antifungal agents, isotonic and absorption delaying agents, and the like, compatible with pharmaceutical administration. Suitable carriers are described in the most recent edition of Remington's Pharmaceutical Sciences, a standard reference text in the field, which is incorporated herein by reference. Preferred examples of such carriers or diluents include, but are not limited to, water, saline, ringer's solutions, dextrose solution, and 5% human serum albumin. Liposomes and non-aqueous

vehicles such as fixed oils may also be used. The use of such media and agents for pharmaceutically active substances is well known in the art. Except insofar as any conventional media or agent is incompatible with the active compound, use thereof in the compositions is contemplated. Supplementary active compounds can also be incorporated into the compositions.

[00137] A pharmaceutical composition of the invention is formulated to be compatible with its intended route of administration. Examples of routes of administration include parenteral, *e.g.*, intravenous, intradermal, subcutaneous, oral (*e.g.*, inhalation), transdermal (*i.e.,* topical), transmucosal, and rectal administration. Solutions or suspensions used for parenteral, intradermal, or subcutaneous application can include the following components: a sterile diluent such as water for injection, saline solution, fixed oils, polyethylene glycols, glycerine, propylene glycol or other synthetic solvents; antibacterial agents such as benzyl alcohol or methyl parabens; antioxidants such as ascorbic acid or sodium bisulfite; chelating agents such as ethylenediaminetetraacetic acid (EDTA); buffers such as acetates, citrates or phosphates, and agents for the adjustment of tonicity such as sodium chloride or dextrose. The pH can be adjusted with acids or bases, such as hydrochloric acid or sodium hydroxide. The parenteral preparation can be enclosed in ampoules, disposable syringes or multiple dose vials made of glass or plastic.

[00138] Pharmaceutical compositions suitable for injectable use include sterile aqueous solutions (where water soluble) or dispersions and sterile powders for the extemporaneous preparation of sterile injectable solutions or dispersion. For intravenous administration, suitable carriers include physiological saline, bacteriostatic water, Cremophor EL™ (BASF, Parsippany, N.J.) or phosphate buffered saline (PBS). In all cases, the composition must be sterile and should be fluid to the extent that easy syringeability exists. It must be stable under the conditions of manufacture and storage and must be preserved against the contaminating action of microorganisms such as bacteria and fungi. The carrier can be a solvent or dispersion medium containing, for example, water, ethanol, polyol (for example, glycerol, propylene glycol, and liquid polyethylene glycol, and the like), and suitable mixtures thereof. The proper fluidity can be maintained, for example, by the use of a coating such as lecithin, by the maintenance of the required particle size in the case of dispersion and by the use of surfactants. Prevention of the action of microorganisms can be achieved by various antibacterial and antifungal agents, for example, parabens, chlorobutanol, phenol, ascorbic acid, thimerosal, and the like. In many cases, it will be preferable to include isotonic

agents, for example, sugars, polyalcohols such as manitol, sorbitol, sodium chloride in the composition. Prolonged absorption of the injectable compositions can be brought about by including in the composition an agent which delays absorption, for example, aluminum monostearate and gelatin.

[00139] Sterile injectable solutions can be prepared by incorporating the active compound in the required amount in an appropriate solvent with one or a combination of ingredients enumerated above, as required, followed by filtered sterilization. Generally, dispersions are prepared by incorporating the active compound into a sterile vehicle that contains a basic dispersion medium and the required other ingredients from those enumerated above. In the case of sterile powders for the preparation of sterile injectable solutions, methods of preparation are vacuum drying and freeze-drying that yields a powder of the active ingredient plus any additional desired ingredient from a previously sterile-filtered solution thereof.

[00140] Oral compositions generally include an inert diluent or an edible carrier. They can be enclosed in gelatin capsules or compressed into tablets. For the purpose of oral therapeutic administration, the active compound can be incorporated with excipients and used in the form of tablets, troches, or capsules. Oral compositions can also be prepared using a fluid carrier for use as a mouthwash, wherein the compound in the fluid carrier is applied orally and swished and expectorated or swallowed. Pharmaceutically compatible binding agents, and/or adjuvant materials can be included as part of the composition. The tablets, pills, capsules, troches and the like can contain any of the following ingredients, or compounds of a similar nature: a binder such as microcrystalline cellulose, gum tragacanth or gelatin; an excipient such as starch or lactose, a disintegrating agent such as alginic acid, Primogel, or corn starch; a lubricant such as magnesium stearate or Sterotes; a glidant such as colloidal silicon dioxide; a sweetening agent such as sucrose or saccharin; or a flavoring agent such as peppermint, methyl salicylate, or orange flavoring.

[00141] For administration by inhalation, the compounds are delivered in the form of an aerosol spray from pressured container or dispenser which contains a suitable propellant, *e.g.*, a gas such as carbon dioxide, or a nebulizer.

[00142] Systemic administration can also be by transmucosal or transdermal means. For transmucosal or transdermal administration, penetrants appropriate to the barrier to be permeated are used in the formulation. Such penetrants are generally known in the art, and include, for example, for transmucosal administration, detergents, bile salts, and fusidic acid

derivatives. Transmucosal administration can be accomplished through the use of nasal sprays or suppositories. For transdermal administration, the active compounds are formulated into ointments, salves, gels, or creams as generally known in the art.

[00143] The compounds can also be prepared in the form of suppositories (*e.g.*, with conventional suppository bases such as cocoa butter and other glycerides) or retention enemas for rectal delivery.

[00144] In one embodiment, the active compounds are prepared with carriers that will protect the compound against rapid elimination from the body, such as a controlled release formulation, including implants and microencapsulated delivery systems. Biodegradable, biocompatible polymers can be used, such as ethylene vinyl acetate, polyanhydrides, polyglycolic acid, collagen, polyorthoesters, and polylactic acid. Methods for preparation of such formulations will be apparent to those skilled in the art. The materials can also be obtained commercially from Alza Corporation and Nova Pharmaceuticals, Inc. Liposomal suspensions (including liposomes targeted to infected cells with monoclonal antibodies to viral antigens) can also be used as pharmaceutically acceptable carriers. These can be prepared according to methods known to those skilled in the art, for example, as described in U.S. Patent No. 4,522,811.

[00145] It is especially advantageous to formulate oral or parenteral compositions in dosage unit form for ease of administration and uniformity of dosage. Dosage unit form as used herein refers to physically discrete units suited as unitary dosages for the subject to be treated; each unit containing a predetermined quantity of active compound calculated to produce the desired therapeutic effect in association with the required pharmaceutical carrier. The specification for the dosage unit forms of the invention are dictated by and directly dependent on the unique characteristics of the active compound and the particular therapeutic effect to be achieved, and the limitations inherent in the art of compounding such an active compound for the treatment of individuals.

[00146] The pharmaceutical compositions can be included in a container, pack, or dispenser together with instructions for administration.

<u>Diagnostic Assays</u>

[00147] The huPD-L1 antibody of the invention, when joined to a detectable moiety, provides a way for detecting “cancerous tissue” or tissue subject to aberrant cell proliferation and therefore at risk for cancer. In addition to tissue that becomes cancerous due to an *in situ* neoplasm, for example, the antibody-detectable moiety conjugates also provides a method of

detecting cancerous metastatic tissue present in distal organs and/or tissues. Thus such tissue may be detected by contacting tissue suspected of being cancerous with the antibody-detectable moiety under appropriate conditions to cause the detectable moiety to be detected in cancerous tissue, thereby detecting the presence of cancerous tissue.

[00148] The huPD-L1 antibody of the invention, when joined to a detectable moiety, provides a way for detecting T cell exhaustion in a subject suffering from a cancer or a chronic infection. For example, the huPD-L1 antibody can be used to detect the levels of PD-L1 in a subject, wherein the levels in comparison to a reference level can indicate whether the subject is suffering from T cell exhaustion. Thus, this method can also be used to determine whether or not treatment using the huPD-L1 antibody to augment the immune response by reversing or inhibiting T cell exhaustion would be beneficial to the subject.

[00149] The detectable moieties can be conjugated directly to the antibodies or fragments, or indirectly by using, for example, a fluorescent secondary antibody. Direct conjugation can be accomplished by standard chemical coupling of, for example, a fluorophore to the antibody or antibody fragment, or through genetic engineering. Chimeras, or fusion proteins can be constructed which contain an antibody or antibody fragment coupled to a fluorescent or bioluminescent protein. For example, Casadei, et al., describe a method of making a vector construct capable of expressing a fusion protein of aequorin and an antibody gene in mammalian cells.

[00150] As used herein, the term "labeled", with regard to the probe or antibody, is intended to encompass direct labeling of the probe or antibody by coupling (*i.e.*, physically linking) a detectable substance to the probe or antibody, as well as indirect labeling of the probe or antibody by reactivity with another reagent that is directly labeled. Examples of indirect labeling include detection of a primary antibody using a fluorescently-labeled secondary antibody and end-labeling of a DNA probe with biotin such that it can be detected with fluorescently-labeled streptavidin. The term "biological sample" is intended to include tissues, cells and biological fluids isolated from a subject (such as a biopsy), as well as tissues, cells and fluids present within a subject. That is, the detection method of the invention can be used to detect cancer, a cancer cell, or a cancer-associated cell (such as a stromal cell associated with a tumor or cancer cell) in a biological sample *in vitro* as well as *in vivo*. For example, *in vitro* techniques for detection of PD-L1 include enzyme linked immunosorbent assays (ELISAs), Western blots, immunoprecipitations, and

immunofluorescence. Furthermore, *in vivo* techniques for detection of PD-L1 include introducing into a subject a labeled anti-PD-L1 antibody. For example, the antibody can be labeled with a radioactive marker whose presence and location in a subject can be detected by standard imaging techniques. In embodiments, the invention provides a non-invasive method of detecting a tumor or cancer cell in a subject. The subject is administered an antibody or scFv antibody of the invention, where the antibody is linked to a detectable moiety (*i.e.*, any moiety capable of being detected by, *e.g.,* fluorescent, chemical, chemiluminescent, radioactive, or other means known in the art), the antibody is allowed to localize to the tumor then is detected by observation of the detectable moiety.

[00151] In the case of "targeted" conjugates, that is, conjugates which contain a targeting moiety--a molecule or feature designed to localize the conjugate within a subject or animal at a particular site or sites, localization refers to a state when an equilibrium between bound, "localized", and unbound, "free" entities within a subject has been essentially achieved. The rate at which such equilibrium is achieved depends upon the route of administration. For example, a conjugate administered by intravenous injection to localize thrombi may achieve localization, or accumulation at the thrombi, within minutes of injection. On the other hand, a conjugate administered orally to localize an infection in the intestine may take hours to achieve localization. Alternatively, localization may simply refer to the location of the entity within the subject or animal at selected time periods after the entity is administered. By way of another example, localization is achieved when an moiety becomes distributed following administration.

[00152] In all of the above cases, a reasonable estimate of the time to achieve localization may be made by one skilled in the art. Furthermore, the state of localization as a function of time may be followed by imaging the detectable moiety (e.g., a light-emitting conjugate) according to the methods of the invention, such as with a photodetector device. The "photodetector device" used should have a high enough sensitivity to enable the imaging of faint light from within a mammal in a reasonable amount of time, and to use the signal from such a device to construct an image.

[00153] In cases where it is possible to use light-generating moieties which are extremely bright, and/or to detect light-generating fusion proteins localized near the surface of the subject or animal being imaged, a pair of "night-vision" goggles or a standard high-sensitivity video camera, such as a Silicon Intensified Tube (SIT) camera (e.g., from

Hammamatsu Photonic Systems, Bridgewater, N.J.), may be used. More typically, however, a more sensitive method of light detection is required.

[00154] In extremely low light levels the photon flux per unit area becomes so low that the scene being imaged no longer appears continuous. Instead, it is represented by individual photons which are both temporally and spatially distinct form one another. Viewed on a monitor, such an image appears as scintillating points of light, each representing a single detected photon. By accumulating these detected photons in a digital image processor over time, an image can be acquired and constructed. In contrast to conventional cameras where the signal at each image point is assigned an intensity value, in photon counting imaging the amplitude of the signal carries no significance. The objective is to simply detect the presence of a signal (photon) and to count the occurrence of the signal with respect to its position over time.

[00155] At least two types of photodetector devices, described below, can detect individual photons and generate a signal which can be analyzed by an image processor. Reduced-Noise Photodetection devices achieve sensitivity by reducing the background noise in the photon detector, as opposed to amplifying the photon signal. Noise is reduced primarily by cooling the detector array. The devices include charge coupled device (CCD) cameras referred to as “backthinned”, cooled CCD cameras. In the more sensitive instruments, the cooling is achieved using, for example, liquid nitrogen, which brings the temperature of the CCD array to approximately –120°C. “Backthinned” refers to an ultra-thin backplate that reduces the path length that a photon follows to be detected, thereby increasing the quantum efficiency. A particularly sensitive backthinned cryogenic CCD camera is the “TECH 512”, a series 200 camera available from Photometrics, Ltd. (Tucson, Ariz.).

[00156] “Photon amplification devices” amplify photons before they hit the detection screen. This class includes CCD cameras with intensifiers, such as microchannel intensifiers. A microchannel intensifier typically contains a metal array of channels perpendicular to and co-extensive with the detection screen of the camera. The microchannel array is placed between the sample, subject, or animal to be imaged, and the camera. Most of the photons entering the channels of the array contact a side of a channel before exiting. A voltage applied across the array results in the release of many electrons from each photon collision.

The electrons from such a collision exit their channel of origin in a “shotgun” pattern, and are detected by the camera.

[00157] Even greater sensitivity can be achieved by placing intensifying microchannel arrays in series, so that electrons generated in the first stage in turn result in an amplified signal of electrons at the second stage. Increases in sensitivity, however, are achieved at the expense of spatial resolution, which decreases with each additional stage of amplification. An exemplary microchannel intensifier-based single-photon detection device is the C2400 series, available from Hamamatsu.

[00158] Image processors process signals generated by photodetector devices which count photons in order to construct an image which can be, for example, displayed on a monitor or printed on a video printer. Such image processors are typically sold as part of systems which include the sensitive photon-counting cameras described above, and accordingly, are available from the same sources. The image processors are usually connected to a personal computer, such as an IBM-compatible PC or an Apple Macintosh (Apple Computer, Cupertino, Calif.), which may or may not be included as part of a purchased imaging system. Once the images are in the form of digital files, they can be manipulated by a variety of image processing programs (such as “ADOBE PHOTOSHOP”, Adobe Systems, Adobe Systems, Mt. View, Calif.) and printed.

[00159] In one embodiment, the biological sample contains protein molecules from the test subject. One preferred biological sample is a peripheral blood leukocyte sample isolated by conventional means from a subject.

[00160] The invention also encompasses kits for detecting the presence of PD-L1 or a PD-L1-expressing cell in a biological sample. For example, the kit can comprise: a labeled compound or agent capable of detecting a cancer or tumor cell (*e.g.*, an anti-PD-L1 scFv or monoclonal antibody) in a biological sample; means for determining the amount of PD-L1 in the sample; and means for comparing the amount of PD-L1 in the sample with a standard. The standard is, in some embodiments, a non-cancer cell or cell extract thereof. The compound or agent can be packaged in a suitable container. The kit can further comprise instructions for using the kit to detect cancer in a sample.

<u>Bi-specific Antibodies</u>

[00161] A bi-specific antibody (bsAb) is an antibody comprising two variable domains or scFv units such that the resulting antibody recognizes two different antigens. The present

invention provides for bi-specific antibodies that recognize PD-L1 and a second antigen. Exemplary second antigens include tumor associated antigens, cytokines and cell surface receptors. In some embodiments, the second antigen can be CAIX (carbonic anhydrase IX, or G250), IL-10 or CCR4. In some embodiments, the second antigen can be a cell surface receptor, wherein the cell surface receptor is CCR4, IL21R, BTLA, HVEM or TIM3.

[00162] A bi-specific antibody of the present invention comprises a heavy chain and a light chain combination or scFv of the huPD-L1 antibodies disclosed herein.

Construction of bi-specific antibodies

[00163] Bi-specific antibodies of the present invention can be constructed using methods known art. In some embodiments, the bi-specific antibody is a single polypeptide wherein the two scFv fragments are joined by a long linker polypeptide, of sufficient length to allow intramolecular association between the two scFv units to form an antibody. In other embodiments, the bi-specific antibody is more than one polypeptide linked by covalent or non-covalent bonds.

[00164] In another embodiment, the bi-specific antibody is constructed using the "knob into hole" method (Ridgway et al., Protein Eng 7:617-621 (1996)). In this method, the Ig heavy chains of the two different variable domains are reduced to selectively break the heavy chain pairing while retaining the heavy-light chain pairing. The two heavy-light chain heterodimers that recognize two different antigens are mixed to promote heteroligation pairing, which is mediated through the engineered "knob into holes" of the CH3 domains, as shown in Figure 5 and 6A.

[00165] In another embodiment, the bi-specific antibody can be constructed through exchange of heavy-light chain dimers from two or more different antibodies to generate a hybrid antibody where the first heavy-light chain dimer recognizes PD-L1 and the second heavy-light chain dimer recognizes a second antigen. The mechanism for heavy-light chain dimer is similar to the formation of human IgG4, which also functions as a bispecific molecule. Dimerization of IgG heavy chains is driven by intramolecular force, such as the pairing the CH3 domain of each heavy chain and disulfide bridges. Presence of a specific amino acid in the CH3 domain (R409) has been shown to promote dimer exchange and construction of the IgG4 molecules. Heavy chain pairing is also stabilized further by interheavy chain disulfide bridges in the hinge region of the antibody. Specifically, in IgG4, the hinge region contains the amino acid sequence Cys-Pro-Ser-Cys (in comparison to the stable IgG1 hinge region which contains the sequence Cys-Pro-Pro-Cys) at amino acids 226-

230. This sequence difference of Serine at position 229 has been linked to the tendency of IgG4 to form novel intrachain disulfides in the hinge region (Van der Neut Kolfschoten, M. et al., 2007, *Science* 317:1554-1557 and Labrijn, A.F. et al, 2011, *Journal of immunol* 187:3238-3246).

[00166] Therefore, bi-specific antibodies of the present invention can be created through introduction of the R409 residue in the CH3 domain and the Cys-Pro-Ser-Cys sequence in the hinge region of antibodies that recognize PD-L1 or a second antigen, so that the heavy-light chain dimers exchange to produce an antibody molecule with one heavy-light chain dimer recognizing PD-L1 and the second heavy-light chain dimer recognizing a second antigen, wherein the second antigen is any antigen disclosed herein. Preferably, the bi-specific antibody contains one anti-PD-L1 heavy-light chain dimer conjugated to one anti-CAIX (carbonic anhydrase IX, or 250) heavy-light chain dimer, as discussed in Examples 3 and 4. Heavy-light chain dimer exchange can also be enhanced with addition of a reducing agent, such as reduced glutathione, to promote the exchange. Known IgG4 molecules may also be altered such that the heavy and light chains recognize PD-L1 or a second antigen, as disclosed herein. Use of this method for constructing the bi-specific antibodies of the present invention may be beneficial due to the intrinsic characteristic of IgG4 molecules wherein the Fc region differs from other IgG subtypes in that it interacts poorly with effector systems of the immune response, such as complement and Fc receptors expressed by certain white blood cells. This specific property makes these IgG4-based bi-specific antibodies attractive for therapeutic applications, in which the antibody is required to bind the target(s) and functionally alter the signaling pathways associated with the target(s), however not trigger effector activities.

[00167] In some embodiments, mutations are introduced to the constant regions of the bsAb such that the antibody dependent cell-mediated cytotoxicity (ADCC) activity of the bsAb is altered. Such examples are depicted in Figure 6B. For example, the mutation is an LALA mutation in the CH2 domain. In one aspect, the bsAb contains mutations on one scFv unit of the heterodimeric bsAb, which reduces the ADCC activity. In another aspect, the bsAb contains mutations on both chains of the heterodimeric bsAb, which completely ablates the ADCC activity. For example, the mutations introduced one or both scFv units of the bsAb are LALA mutations in the CH2 domain. These bsAbs with variable ADCC activity can be optimized such that the bsAbs exhibits maximal selective killing towards cells that

express one antigen that is recognized by the bsAb, however exhibits minimal killing towards the second antigen that is recognized by the bsAb.

Exemplary second antigens

[00168] The present invention provides for bi-specific antibodies that recognize PD-L1 and a second antigen.

[00169] In some embodiments, the second antigen is a tumor associated antigen. In some embodiments, the tumor associated antigen is carbonic anhydrase IX (CAIX). For example, a CAIX/PD-L1 bi-specific antibody may be constructed, comprising one heavy and one light chain combination of the huPD-L1 antibodies described herein and one heavy and one light chain combination that recognizes CAIX (Figure 5A). CAIX has been described as a prognostic marker for disease progression and a target for immunotherapy with IL-2. CAIX is a tumor-associated antigen that is highly expressed in cancers, such as renal cell carcinoma. In some cases, activation of the PD1/PD-L1 axis by tumor cells may induce T cell exhaustion for certain tumor-specific antigens, such as CAIX, whereby tumor cells expressing CAIX escape recognition by the immune system. The bsAb targeting both CAIX and PD-L1 serves as a novel cancer therapeutic. Treatment with a CAIX/PD-L1 bsAb would inhibit or reverse the PD-1/PD-L1-mediated T-cell exhaustion for CAIX, and promote tumor surveillance and an immune response against CAIX-expressing tumor cells. For example, treatment with a CAIX/PD-L1 bsAb would promote an antigen-specific immune response against tumor cells, where the antigen targeted is CAIX.

[00170] In some instances, mutations may be introduced to the CAIX or the PD-L1 chains at the constant regions (e.g., CH2 domain) to reduce ADCC activity (Figure 5B). In some instances, mutations may be introduced into both the CAIX and the PD-L1 chains in the constant regions to completely ablate ADCC activity. Mutated bsAbs with variable ADCC activity can be assayed by methods known in the art for their specificity of killing tumor cells. Preferably, the CAIX/PD-L1 bsAb will exhibit maximal selective killing of CAIX-expressing tumor cells but exhibits only minimal killing of PD-L1-expressing endogenous peripheral blood mononuclear cells (PBMCs).

[00171] In some embodiments, the second antigen is a cell surface receptor, wherein the cell surface receptor is interleukin 21 receptor (IL21R). For example, an IL21R/PD-L1 bi-specific antibody may be constructed, comprising one heavy and one light chain combination of the huPD-L1 antibodies described herein and one heavy and one light chain combination that binds IL21R in an agonistic manner. The cytokine IL21 is secreted by CD4+ T helper

cells and binds to IL21R to promote several immune activation pathways, particularly, promoting the antigen-specific cytotoxicity of CTLs and the maturation, proliferation and cytotoxicity of NK cells (Sondergaard, H et al., 2009 *Tissue antigens* 74:467-479; Kasaian, M. T. et al, 2002 *Immunity* 16:559-569; and Coquet, J. M. et al, 2008 *J Immunol* 178:2827-2834). In particular, IL21 has been shown to promote activation and cytotoxic capacity against melanoma antigens (Li, Y. et al, 2005, *J Immunol* 175:2261-2269). Systemic administration IL21 has been explored for use in treating cancer and has shown some efficacy (Schmidt, H. et al, 2010 *Clinical cancer research*, 16: 5312-5319), however, some data has also shown detrimental and undesirable side effects (Grunwald, V. et al, 2011, *Acta Oncol* 50:121-126).

[00172] The IL21R/PD-L1 bsAb of the present invention binds to IL21R in an agonistic manner, thereby acting as a mimic or surrogate for the cytokine IL21. Binding by the IL21R/PD-L1 bsAb can result in activation of the IL21R-mediated pathways and subsequent promotion of antigen-specific cytotoxic immune responses against tumor cells that have induced PD-L1-mediated T-cell exhaustion. One particular benefit of treatment with the bi-specific antibody of the invention is the localization of the IL21R activation to areas where PD-1/PD-L1-mediated T cell exhaustion has occurred, for example, in the tumor microenvironment, for example near or within tumors that have induced T cell exhaustion to evade anti-tumor immune responses. In this manner, the ILR/PD-L1 bsAb promotes anti-tumor immune response in a two-pronged mechanism: 1) by reversing or inhibiting PD-1/PD-L1-mediated T cell exhaustion, and 2) by promoting IL21/IL21R-mediated activation of cytotoxic immune response, thereby inducing antigen-specific or anti-tumor immune responses and cytotoxicity.

[00173] The IL21R/PD-L1 bi-specific antibody may also have use in a vaccine for vaccination of a subject, or as a vaccine adjuvant. In the germinal center reaction (GCR), in which high-affinity antibody-secreting plasma cells and memory B cells that ensure sustained immune protection and rapid recall responses against previously encountered foreign antigens are produced, PD1 is expressed by follicular T helper cells (TFH) while PDL1 is expressed by germinal center B cells (Crotty, S. et al, 2011, *Annual review of Immunology*, 29:621-623). Overexpression of PD1 or PD-L1 inhibits the expansion of antibody-producing B cells. Use of the IL21R/PD-L1 bi-specific antibody, wherein the PD-L1 portion of the antibody inhibits the P D1/PD-L1 axis, would result in the preferential expansion of only high affinity antibodies. As IL-21 strongly promotes the transition of antigen-specific B cells to antibody-

secreting plasma cells, as well as the formation and persistence of TFH activity (Crotty, S. et al, 2011, *Annual review of Immunology*, 29:621-623), a bsAb against PDL1 with IL21 surrogate or agonistic activity could act as a GCR-specific adjuvant towards promotion of high affinity antibody production against particular antigens, such as antigens administered through a vaccine, or antigens of infectious agents.

[00174] In some embodiments, the second antigen is BTLA (B and T lymphocyte attenuator protein) or HVEM (Herpesvirus entry mediator, also known as TNFRSF14). For example, a BTLA/PD-L1 bi-specific antibody may be constructed, comprising one heavy and one light chain combination of the huPD-L1 antibodies described herein and one heavy and one light chain combination that binds BTLA. For example, an HVEM/PD-L1 bi-specific antibody may be constructed, comprising one heavy and one light chain combination of the huPD-L1 antibodies described herein and one heavy and one light chain combination that binds HVEM, preferably, to those regions of HVEM that mediate binding with BTLA. BTLA binding to HVEM results in T cell inhibition, similar to PD1/PD-L1 interactions. The BTLA/PD-L1 or HVEM/PD-L1 bi-specific antibodies of the present invention would inhibit or prevent the association between BTLA and HVEM, and prevent BTLA/HVE-mediated T cell inhibition. BTLA inhibition promotes tumor-specific T cell responses. Therefore, treatment with the bi-specific antibodies of the present invention results in the simultaneous blockade of two distinct inhibitor pathways that limit T cell activity.

[00175] In some instances, the HVEM/PD-L1 bsAb antibody would also prevent the association between HVEM and another HVEM ligand, CD160, which has been shown to be an agonist for the survival of B cell chronic lymphocytic leukemia (BCLL) cells, and strongly inhibits CD4+ T-cell activation and function. Treatment with the HVEM/PD-L1 bi-specific antibody that also prevents HVEM/CD160 binding of the present invention results in the simultaneous blockade of two inhibitor pathways that limit T cell activity and inhibition of CD160-mediated tumor survival pathways.

[00176] In some embodiments, the second antigen is TIM3 (T-cell immunoglobulin and mucin domain 3). For example, a TIM3/PD-L1 bi-specific antibody may be constructed, comprising one heavy and one light chain combination of the huPD-L1 antibodies described herein and one heavy and one light chain combination that binds TIM3. In one aspect, the antibodies of the present invention prevent or inhibit TIM3 binding with galectin-9 (GAL9). Interaction between TIM3 and GAL9 results in inhibition of T cell function and activation of macrophages (Sakuishi, K. et al, 2010, *The Journal of experimental medicine,* 207:2187-2194

and Zhang, Y. et al, 2012, *Journal of leukocyte biology,* 91: 189-196). TIM3 has also been shown to promote the activity of myeloid derived suppressor cells (MDSC) (Dardalhon, V. et al., 2012 *Journal of leukocyte biology*, 185:1383-1392). Therefore, treatment with a bispecific antibody of the present invention, such as the TIM3/PD-L1 bsAb, would reverse and prevent T-cell exhaustion, promote tumor surveillance and inhibit generation of MDSC. Use of the TIM3/PD-L1 bsAb may be particularly beneficial for treatment of subjects that suffer from cancers with polymorphisms in TIM3, such as renal cell carcinoma and metastatic renal cell carcinoma.

[00177] The bi-specific antibodies disclosed herein may be useful in treatment of diseases or medical conditions, for example, cancer. The bi-specific antibodies of the present invention may be particularly useful in diseases or medical conditions that are associated with T cell exhaustion. In some cases, the bi-specific antibodies disclosed herein may be used as a vaccine for promoting antigen-specific immune responses. The bi-specific antibodies of the present invention will target tumors that induce T-cell exhaustion.

Methods of Treatment

[00178] The invention provides for both prophylactic and therapeutic methods of treating a subject at risk of (or susceptible to) a cancer, or other cell proliferation-related diseases or disorders. Such diseases or disorders include but are not limited to, *e.g.*, those diseases or disorders associated with aberrant expression of PD-L1. For example, the methods are used to treat, prevent or alleviate a symptom of renal cell carcinoma or breast cancer. Alternatively, the methods are used to treat, prevent or alleviate a symptom of a cancer in which PD-L1 plays a negative regulatory role in T cell response. Alternatively, the methods are used to treat, prevent or alleviate a symptom of a solid tumor such as breast cancer, lung cancer, ovarian cancer, prostate cancer, colon cancer, cervical cancer, brain cancer, liver cancer, pancreatic cancer or stomach cancer. Alternatively, the methods are used to treat, prevent or alleviate a symptom of a cancer that has metastasized.

[00179] The invention provides for both prophylactic and therapeutic methods of treating a subject at risk of (or susceptible to) a chronic viral, bacterial or parasitic infection. Particularly, the invention provides for both prophylactic and therapeutic methods of treating a subject at risk of (or susceptible to) HIV infection or AIDS.

[00180] The invention also provides for therapeutic methods for both prophylactic and therapeutic methods of treating a subject at risk of a disease or disorder or condition associated with T-cell exhaustion or a risk of developing T-cell exhaustion. The invention

also provides for therapeutic methods for both prophylactic and therapeutic methods of treating a subject at risk of a disease or disorder or condition associated with T-cell exhaustion or a risk of developing T-cell exhaustion. Such diseases or disorder include, but are not limited to HIV, AIDS, and chronic bacterial, viral or parasitic infections. Other such chronic infections include those caused by, for example, hepatitis B virus (HBV), hepatitis C virus (HCV), herpes simplex virus 1 (HSV-1), *H. pylori*, or *Toxoplasma gondii*.

[00181] Accordingly, in one aspect, the invention provides methods for preventing, treating or alleviating a symptom cancer or a cell proliferative disease or disorder in a subject by administering to the subject a monoclonal antibody or scFv antibody of the invention. For example, a huPD-L1 antibody may be administered in therapeutically effective amounts.

[00182] Subjects at risk for cancer or cell proliferation-related diseases or disorders include patients who have a family history of cancer or a subject exposed to a known or suspected cancer-causing agent. Administration of a prophylactic agent can occur prior to the manifestation of cancer such that the disease is prevented or, alternatively, delayed in its progression.

[00183] In another aspect, tumor cell growth is inhibited or suppressor T cell activity is decreased by contacting a cell with a PD-L1 antibody of the invention. The cell is any cell that expresses PD-L1. For example the cell is T cell.

[00184] Also included in the invention are methods of increasing or enhancing an immune response to an antigen. An immune response is increased or enhanced by administering to the subject a monoclonal antibody or scFv antibody of the invention. The immune response is augmented for example by augmenting antigen specific T effector function. The antigen is a viral (e.g. HIV), bacterial, parasitic or tumor antigen. The immune response is a natural immune response. By natural immune response is meant an immune response that is a result of an infection. The infection is a chronic infection. Increasing or enhancing an immune response to an antigen can be measured by a number of methods known in the art. For example, an immune response can be measured by measuring any one of the following: T cell activity, T cell proliferation, T cell activation, production of effector cytokines, and T cell transcriptional profile.

[00185] Alternatively, the immune response is a response induced due to a vaccination. Accordingly, in another aspect the invention provides a method of increasing vaccine efficiency by administering to the subject a monoclonal antibody or scFv antibody of the

invention and a vaccine. The antibody and the vaccine are administered sequentially or concurrently. The vaccine is a tumor vaccine a bacterial vaccine or a viral vaccine.

Combinatory Methods

[00186] The invention provides treating cancer in a patient by administering two antibodies that bind to the same epitope of the PD-L1 protein or, alternatively, two different epitopes of the PD-L1 protein. Alternatively, the cancer is treated by administering a first antibody that binds to PD-L1 and a second antibody that binds to a protein other than PD-L1. For example, the other protein other than PD-L1 may include, but is not limited to, CAIX, CCR4 and IL-10. For example, the other protein other than PD-L1 is a tumor-associated antigen.

[00187] In some embodiments, the invention provides administration of a huPD-L1 antibody alone or with an additional antibody that recognizes another protein other than PD-L1, with cells that are capable of effecting or augmenting an immune response. For example, these cells may be peripheral blood mononuclear cells (PBMC), or any cell type that is found in PBMC, e.g., cytotoxic T cells, macrophages, and natural killer (NK) cells.

[00188] Additionally, the invention provides administration of an antibody that binds to the PD-L1 protein and an anti-neoplastic agent, such a small molecule, a growth factor, a cytokine or other therapeutics including biomolecules such as peptides, peptidomimetics, peptoids, polynucleotides, lipid-derived mediators, small biogenic amines, hormones, neuropeptides, and proteases. Small molecules include, but are not limited to, inorganic molecules and small organic molecules. Suitable growth factors or cytokines include an IL-2, GM-CSF, IL-12, and TNF-alpha. Small molecule libraries are known in the art. (See, Lam, Anticancer Drug Des., 12:145, 1997.)

[00189] The invention will be further described in the following examples, which do not limit the scope of the invention described in the claims.

EXAMPLES

[00190] **EXAMPLE 1: GENERATION OF HUMAN MABS AGAINST PD-L1**

[00191] Human mAbs against human PD-L1 were generated by panning against a 27-billion member human scFv phage display library. Using full length PD-L1 in the form of paramagnetic proteoliposomes (PMPL), which assure proper orientation of the extracellular domain of PD-L1 for presentation to the library, 14 unique scFv-phage were identified that bind PD-L1. Human IgG constructs were constructed for these 14 unique scFv-phage: Ab-

14, Ab-16, Ab-22, Ab-30, Ab-31, Ab-32, Ab-38, Ab-42, Ab-46, Ab-50, Ab-52, Ab-55, Ab-56 and Ab-65.

[00192] EXAMPLE 2: CHARACTERIZATION OF HUPD-L1 MABS BINDING TO PD-L1

[00193] Binding analysis of huPD-L1 antibodies were performed using PD-L1-expressing cells. Four types of cells were tested, including parental cell line 300.19, and transfected cell lines expressing human PD-L1 (hPD-L1), human PD-L2 (HPD-L2), and human C-type lectin domain family 2 member (hCLEC2D). The binding assays were performed in duplicate, with the results summarized below in Tables 16-19 and Figure 2. Antibody affinity was tested by using four antibody concentrations: 10 μg/ml, 1 μg/ml, 0.1 μg/ml and 0.01 μg/ml. GF1538 and GF1757 antibodies were used as controls: GF1538 is a humanized Ab against hPD-L1, and GF1757 is a humanized Ab against hPD-L2. The secondary antibody utilized was PE-goat anti-human IgG. All values represent mean fluorescence intensity (GMFI), as detected by FACs analysis.

[00194] Results from the binding assay show that the tested huPD-L1 antibodies show highly affinity and specificity for binding PD-L1. Use of the parental cell line 300.19, which does not express human PD-L1, as a control established the basal or non-specific fluorescence (Table 16 and Figure 2, top left). Staining of 300.19 cells that express human PD-L1 by transfection with huPD-L1 antibodies showed significantly higher mean fluorescence intensity (MFI), demonstrating that the antibodies can bind to PD-L1. Significant FMI values, even at the lowest dilution of 0.01 μg/ml of antibody, demonstrated the high affinity of the huPD-L1 for binding PD-L1 protein (Tables 17A, 17B and Figure 2, top right). HuPD-L1 antibodies demonstrated some capacity to bind human PD-L2 or CLEC2D when expressed in 300.19 cells, as demonstrated by Tables 18A, 18B, 19A, 19B and Figure 2, bottom two graphs). However, the MFI values obtained for staining of PD-L2 and CLE2D were not as high as the values obtained for PD-L1. Moreover, at the lower dilutions, the MFI values were not significantly higher than the basal levels, indicating that the huPD-L1 antibodies did not have high affinity or specificity for PD-L2 or CLE2D. Although a few huPD-L1 antibodies, for example Ab-42, demonstrated some cross-reactivity with PD-L2, the antibodies have significantly higher affinity and specificity for PD-L1.

[00195] Table 16. Staining using huPD-L1 on untransfected 300.19 cells

	Cells PE-A Mean

Well	huPD-L1 Ab #		approx 10 ug/ml	1 ug/ml	0.1 ug/ml	0.01 ug/ml
A1	14		358	186	91	73
A2	16		269	105	79	67
A3	30		75	78	74	66
A4	31		324	137	82	73
A5	42		771	315	119	82
A6	50		145	95	70	71
A7	52		324	124	85	72
A8	55		110	75	74	69
A9	1538	GF anti-hPD-L1	79	72	75	71
A10	1757	GF anti-hPD-L2	108	74	70	71
A11	control hIgG		260	87	74	73
A12	Wash		81	73	73	73

[00196] **Table 17A. Staining using huPD-L1 on hPD-L1-transfected 300.19 cells, assay 1 results.**

			Cells PE-A Mean			
Well	**huPD-L1 Ab #**		**approx 10 ug/ml**	**1 ug/ml**	**0.1 ug/ml**	**0.01 ug/ml**
E1	14		41500	23277	8095	1653
E2	16		31837	8277	1866	386
E3	30		47645	37503	17479	3509
E4	31		53790	44199	12498	2826
E5	42		53583	40923	14869	2084
E6	50		49437	42087	15083	2690
E7	52		28372	21430	5614	1073
E8	55		24422	9653	2298	543
E9	1538	GF anti-hPD-L1	64961	60765	27366	5091
E10	1757	GF anti-hPD-L2	154	60	56	54
E11	control hIgG		195	64	52	51
E12	wash		52	51	49	51

[00197] **Table 17B. Staining using huPD-L1 on hPD-L1-transfected 300.19 cells, assay 2 results.**

			Cells PE-A Mean			
Well	**huPD-L1 Ab #**		**approx 10 ug/ml**	**1 ug/ml**	**0.1 ug/ml**	**0.01 ug/ml**
E1	14		77893	53207	17715	3700
E2	16		60182	46561	14101	4218
E3	30		67252	61219	39104	7722
E4	31		76388	70951	37698	6830
E5	42	best	76824	72143	49449	11559
E6	50		69598	63446	28694	5198
E7	52		37203	34863	14718	2689

E8	55		42433	26528	8911	1758
E9	1538 hPD-L1		83021	85450	47677	8164
E11	1757 hPD-L2		122	72	63	57
E10	con hIgG		283	88	71	81

[00198] **Table 18A. Staining using huPD-L1 on hPD-L2-transfected 300.19 cells, assay 1 results.**

			Cells PE-A Mean			
Well	**huPD-L1 Ab #**		**approx 10 ug/ml**	**1 ug/ml**	**0.1 ug/ml**	**0.01 ug/ml**
A1	14		137	464	173	67
A2	16		678	224	80	53
A3	30		60	52	49	50
A4	31		795	239	91	82
A5	42		1417	668	254	72
A6	50		444	149	88	51
A7	52		813	190	79	55
A8	55		132	69	55	51
A9	1538	GF anti-hPD-L1	60	50	62	51
A10	1757	GF anti-hPD-L2	104849	105994	36970	4810
A11	control hIgG		481	176	60	92
A12	wash		69	51	52	60

[00199] **Table 18B. Staining using huPD-L1 on hPD-L2-transfected 300.19 cells, assay 2 results.**

		Cells PE-A Mean			
huPD-L1 Ab #		**approx 10 ug/ml**	**1 ug/ml**	**0.1 ug/ml**	**0.01 ug/ml**
14		1394	847	295	133
16		1502	858	317	129
30		112	78	74	67
31		1573	834	182	87
42		2310	1496	761	213
50		680	386	220	79
52		1467	656	182	85
55		440	200	82	69
1538 hPD-L1		78	70	97	74
1757 hPD-L2		132613	117381	66269	11160
con hIgG		443	118	77	71

[00200] **Table 19A. Staining using huPD-L1 on hCLEC2D-transfected 300.19 cells, assay 1.**

[00201]

			Cells PE-A Mean			
Well	**huPD-L1 Ab #**		**approx 10 ug/ml**	**1 ug/ml**	**0.1 ug/ml**	**0.01 ug/ml**
E1	14		3629	1924	793	245
E2	16		2371	898	294	154
E3	30		283	161	153	144
E4	31		2954	870	360	153
E5	42		4669	2318	748	234
E6	50		1957	869	327	167
E7	52		3138	1105	298	166
E8	55		944	297	171	139
E9	1538	GF anti-hPD-L1	199	143	141	137
E10	1757	GF anti-hPD-L2	343	163	141	137
E11	control hIgG		1541	351	175	146
E12	wash		139	141	142	136

[00202] **Table 19B. Staining using huPD-L1 on hCLEC2- transfected 300.19 cells, assay 2.**

			Cells PE-A Mean			
Well	**huPD-L1 Ab #**		**approx 10 ug/ml**	**1 ug/ml**	**0.1 ug/ml**	**0.01 ug/ml**
E1	14		3629	1924	793	245
E2	16		2371	898	294	154
E3	30		283	161	153	144
E4	31		2954	870	360	153
E5	42		4669	2318	748	234
E6	50		1957	869	327	167
E7	52		3138	1105	298	166
E8	55		944	297	171	139
E9	1538	GF anti-hPD-L1	199	143	141	137
E10	1757	GF anti-hPD-L2	343	163	141	137
E11	control hIgG		1541	351	175	146
E12	wash		139	141	142	136

[00203] **EXAMPLE 2: CHARACTERIZATION OF ANTI-PD-L1 PHAGE-ANTIBODIES BLOCKING PD/PD-L1 BINDING**

[00204] A competitive FACS analysis was performed to characterize the inhibition of hPD1 binding to hPD-L1 by anti-PD-L1 phage antibodies. All anti-hPD-L1 antibodies were

in phage-scFv form. 293T cells were transfected with a vector encoding human PD-L1 fused to human Fc region for expression of hPD-L1-Fc. In this assay, 10^{12} plaque-forming units (pfu) of phage-scFvs were mixed with about 0.25 mg/ml of soluble hPD-1-hFc fusion protein and then added to the hPD-L1-expressing 293T cells. After washing, the cells were incubated with FITC-anti-human IgG antibody and analyzed by FACS to measure the binding of hPD1-hFc to hPD-L1 on the cell surface.

[00205] Fluorescence values were obtained by FACS analysis and used to generate a percentage of inhibition of binding of hPD-1 to hPD-L1+ cells. These values are displayed in Figure 3. Almost all anti-PD-L1 phage scFvs demonstrated some ability to inhibit the binding of hPD-1 to hPD-L1. Particularly, Ab-14, Ab-16, Ab-30, Ab-31, Ab-42, Ab-50, Ab-52, and Ab-55 phage-scFvs demonstrated significant inhibition of hPD-1/hPD-L1 binding.

[00206] **EXAMPLE 3: CHARACTERIZATION OF HUPD-L1 SOLUBLE MABS BLOCKING PD/PD-L1 BINDING**

[00207] A competitive FACS analysis was performed to characterize the inhibition of hPD1 binding to hPD-L1 by the soluble huPD-L1 antibodies of the present invention. All huPD-L1 antibodies were tested for their ability to inhibit the binding of hPD1-IgG fusion protein with hPD-L1-expressing 300.19 cells. In this assay, 50,000 cells expressing hPD-L1 were pre-incubated with the huPD-L1 or control antibodies for 30 minutes at the following concentrations: 10 μg/ml, 1 μg/ml, 0.1 μg/ml and 0.01 μg/ml. After the pre- incubation, 0.125 μg of human PD1 fused to mouse IgG2α was added to the cells and incubated for another 30 minutes. The cells were washed twice, and then 0.125 μg of goat anti-mouse IgG2α-PE antibody was added to the cells. After 30 minutes of incubation, the cells were washed twice and then analyzed by FACS. The values obtained from FACS analysis are represented as mean fluorescence intensity units (MFI) and are summarized in Table 20. MFI values from Table 20 are used to generate a percent inhibition of hPD1 binding with hPD-L1+ cells for Figure 4. As shown herein, all of the tested huPD-L1 soluble antibodies demonstrated nearly complete inhibition of hPD1 binding with hPD-L1 at 10 μg/ml. At successively lower concentrations, most of the soluble antibodies still demonstrated very good inhibition of PD1/PD-L1 binding, particularly Ab-42 and Ab-50.

[00208] **Table 20. Results from blocking of PD-1 binding using huPD-L1**

			Cells PE-A Mean			
Well	**huPD-L1 Ab #**		**10 ugml**	**1 ug/ml**	**0.1 ug/ml**	**0.01 ug/ml**

A1	14		696	11991	14859	15840
A2	16		1092	6660	11080	14783
A3	30		244	661	4775	11585
A4	31		157	623	7934	11908
A5	42		139	142	3137	11797
A6	50		207	156	2795	12517
A7	52		380	2070	6746	11856
A8	55		150	3625	9137	12926
A9	1538	GF anti-hPD-L1	143	148	198	10145
A10	1757	GF anti-hPD-L2	10922	11447	11197	13167
A11	control hIgG		11355	11664	11571	12274
A12	wash		10339	10274	9842	12305
E1	wash neg control		155	137	130	
E4	mIgG2a neg control		128	207	131	
average positive control			11363			
average negative control			155			

[00209] **EXAMPLE 4: FORMATION OF BI-SPECIFIC ANTIBODIES**

[00210] Based on the role of the hinge in generating half-monomers of IgG_4 molecules, it was hypothesized that that introducing charged mutations in the hinge region of human IgG_1 may not only facilitate half-monomer exchange but also potentially stabilize the bi-specific molecule. This example demonstrates that the combination of hinge and CH3 mutations increase bi-specific antibody formation.

[00211] To further stabilize the heterodimer formation, an oppositely charged mutation was further replaced in CH3 domain, which is a concept of “Knobs-into-holes”. Formation of bispecific antibodies was achieved in the following steps. First, the two parental antibodies carrying the bispecific mutations were expressed and purified separately. Then the antibodies were mixed in the presence of a mild reducing agent. The mild reduction of the antibodies caused dissociation of the antibodies into two monomers, each with a variable heavy and light chain. The monomers were then mixed together, followed by an oxidation step which causes the formation of bi-specific antibody molecules.

[00212] Bi-specific antibodies that recognize PD-L1 and G250 (Carbonic Anhydrase IX) were generated. Anti-G250 parental (G37 wild type, G37WT) and engineered (G37 KIHA) antibodies were generated and purified by two independent vectors. G37 KIHA, which conforms to a “knob-in-hole” concept and carried bispecific mutations, was altered in the sequence of immunoglobulin hinge region.

[00213] To understand the dissociation activity of G37 KIHA, antibodies were mixed in the presence of mild reducing agent to interrupt to form antibody monomer by glutathione (GSH) in different concentrations (Figure 1A). Addition of increasing concentration of reducing agent (GSH) caused dissociation of the antibody into monomers, as indicated by the increase in the levels of the lower molecular weight species in Figure 1A.

[00214] Similarly, Anti-PD-L1 parental (PD-L1 wild type, PDL-1WT) and engineered (PD-L1 KIHB) antibodies were generated and purified by two independent vectors. Using different concentrations of GSH verified the appropriate condition to obtain anti-PD-L1 monomers (Figure 7B). The best condition of GSH concentration was selected and resulting anti-PD-L1 monomers were incubated with the G37 KIHA monomers. The formation of bispecific antibody was observed at the same size of wild type IgG, indicating that antibodies containing two heavy-light chain monomers were generated, one PD-L1-specific monomer and one G250-specific monomer.

[00215] **EXAMPLE 5: BI-SPECIFIC ANTIBODY FUNCTION**

[00216] The bi-specific antibodies generated in Example 3 were next tested for their ability to recognize both antigens, for example, PD-L1 and G250. The function of the PD-L1 and G250 bi-specific antibodies was tested using flow cytometry. $CAIX^{+}PD\text{-}L1^{-}$ SKRC-52 express CAIX (250) but not PD-L1, and therefore it was expected that cells could be recognized by only anti-CAIX antibody G37 and not PD-L1. To avoid the saturation of antibody binding, the concentration of antibody was low and in a reduced dose manner. Indeed, parental anti-G37 recognized SKRC-52 cells, while parental anti-PD-L1 did not (levels same as control). The bi-specific antibody, containing conjugated anti-G37 and anti-PD-L1 monomers, recognized SKRC-52 cells in a half reduced concentration compared to the parental G37 antibody, thereby demonstrating the functionality of a bi-specific antibody generated using the methods and antibodies described herein.

[00217] **EXAMPLE 6: FUNCTIONAL CHARACTERIZATION OF MAB42**

[00218] Further functional characterization of the monoclonal antibody against PD-L1 (mAb42) was performed. Peripheral blood mononuclear cells (PBMCs) were cultured from 4 different healthy donors (D1-D4). PBMCs were cultured either in the presence of mAb42 or in the presence of a control isotype IgG antibody. PMBCs were stimulated with 0.1μg/ml SEB (staphylococcal enterotoxin B) and TNFα production was measured using MSD (Meso Scale Delivery). Sample analyses were performed in triplicate.

[00219] As shown in Figure 8, culturing PBMCs in the presence of mAb42 caused an increase in production of TNFα in response to SEB when cultured in the presence of anti-PD-L1 antibody (mAb42) compared to control antibody in all four donor samples. Furthermore, the increase in TNFα production was statistically significant, with a $p<0.0005$. Thus, treatment with anti-PD-L1 antibody augments the immune response in response to an antigen, or infection, in humans.

OTHER EMBODIMENTS

[00220] While the invention has been described in conjunction with the detailed description thereof, the foregoing description is intended to illustrate and not limit the scope of the invention, which is defined by the scope of the appended claims. Other aspects, advantages, and modifications are within the scope of the following claims.

What is claimed is:

1. An isolated humanized monoclonal antibody having:

a. a heavy chain with three CDRs comprising the amino acid sequences SYGIS (SEQ ID NO:57), WISAYNGNTNYAQKLED (SEQ ID NO:70), and ALPSGTILVGGWFDP (SEQ ID NO:86) respectively and a light chain with three CDRs comprising the amino acid sequences TRSSGNIASNYVQ (SEQ ID NO:101), EDNQRPS (SEQ ID NO:115), and QSYDSSNLWV (SEQ ID NO:127) respectively;

b. a heavy chain with three CDRs comprising the amino acid sequences SYALS (SEQ ID NO:58), AISGGGGSTYYADSVKD (SEQ ID NO:71), and DVFPETFSMNYGMDV (SEQ ID NO:87) respectively and a light chain with three CDRs comprising the amino acid sequences QGDSLRSYYAS (SEQ ID NO:102), GKNNRPS (SEQ ID NO:116), and NSRDSSGNHYV (SEQ ID NO:128) respectively;

c. a heavy chain with three CDRs comprising the amino acid sequences DYAMH (SEQ ID NO:60), LISGDGGSTYYADSVKD (SEQ ID NO:73), and VLLPCSSTSCYGSVGAFDI (SEQ ID NO:88) respectively and a light chain with three CDRs comprising the amino acid sequences GGSDIGRKSVH (SEQ ID NO:103), SDRDRPS (SEQ ID NO:117), and QVWDNNSDHYV (SEQ ID NO:129) respectively;

d. a heavy chain with three CDRs comprising the amino acid sequences NYDMS (SEQ ID NO:61), RVNWNGGSTTYADAVKD (SEQ ID NO:74), and EFVGAYDL (SEQ ID NO:89) respectively and a light chain with three CDRs comprising the amino acid sequences TGTSSDVGGYNYVS (SEQ ID NO:104), DVSNRPS (SEQ ID NO:118), and SSYTSSTLP (SEQ ID NO:130) respectively;

e. a heavy chain with three CDRs comprising the amino acid sequences GLYIH (SEQ ID NO:62), WIIPIFGTANYAQKFED (SEQ ID NO:75), and GLRWGIWGWFDP (SEQ ID NO:90) respectively and a light chain with

three CDRs comprising the amino acid sequences RASQSIGNSLA (SEQ ID NO:105), GASSRAT (SEQ ID NO:119), and QQHTIPTFS (SEQ ID NO:131) respectively;

f. a heavy chain with three CDRs comprising the amino acid sequences DNAIS (SEQ ID NO:63), WIIPIFGKPNYAQKFED (SEQ ID NO:76), and TMVRGFLGVMDV (SEQ ID NO:91) respectively and a light chain with three CDRs comprising the amino acid sequences RASQGIGSYLA (SEQ ID NO:106), AASTLQS (SEQ ID NO:120), and QQLNNYPIT (SEQ ID NO:132) respectively;

g. a heavy chain with three CDRs comprising the amino acid sequences SYAMS (SEQ ID NO:64), AISGSGGSTYYADSVKD (SEQ ID NO:77), and DQFVTIFGVPRYGMDV (SEQ ID NO:92) respectively and a light chain with three CDRs comprising the amino acid sequences SGDKLGNKYAY (SEQ ID NO:107), QDIKRPS (SEQ ID NO:121), and QTWDNSVV (SEQ ID NO:133) respectively;

h. a heavy chain with three CDRs comprising the amino acid sequences SYAIS (SEQ ID NO:57), WIIPIFGTANYAQKFED (SEQ ID NO:78), and GRQMFGAGIDF (SEQ ID NO:93) respectively and a light chain with three CDRs comprising the amino acid sequences TRSSGSIDSNYVQ (SEQ ID NO:108), EDNQRPS (SEQ ID NO:115), and QSYDSNNRHVI (SEQ ID NO:134) respectively;

i. a heavy chain with three CDRs comprising the amino acid sequences TYALN (SEQ ID NO:65), RIVPLIGLVNYAHNFED (SEQ ID NO:79), and EVYGGNSDY (SEQ ID NO:94) respectively and a light chain with three CDRs comprising the amino acid sequences TRSSGNIGTNYVQ (SEQ ID NO:109), EDYRRPS (SEQ ID NO:122), and QSYHSSGWE (SEQ ID NO:135) respectively;

j. a heavy chain with three CDRs comprising the amino acid sequences SHGIT (SEQ ID NO:66), WISAHNGHASNAQKVED (SEQ ID NO:80), and VHAALYYGMDV (SEQ ID NO:95) respectively and a light chain with three

CDRs comprising the amino acid sequences GGNNIGSKGVH (SEQ ID NO:110), DDSDRPS (SEQ ID NO:123), and QVWDSSSDHWV (SEQ ID NO:136) respectively;

k. a heavy chain with three CDRs comprising the amino acid sequences RHGMH (SEQ ID NO:67), VISHDGSVKYYADSMKD (SEQ ID NO:81), and GLSYQVSGWFDP (SEQ ID NO:96) respectively and a light chain with three CDRs comprising the amino acid sequences TRSSGSIASNYVQ (SEQ ID NO:111), EDNQRPS (SEQ ID NO:115), and QSYDSTTPSV (SEQ ID NO:137) respectively;

l. a heavy chain with three CDRs comprising the amino acid sequences SYGIS (SEQ ID NO:58), WTSPHNGLTAFAQILED (SEQ ID NO:82), and VHPVFSYALDV (SEQ ID NO:97) respectively and a light chain with three CDRs comprising the amino acid sequences TRSSGSIASNYVQ (SEQ ID NO:112), EDNQRPS (SEQ ID NO:115), and QSYDGITVI (SEQ ID NO:138) respectively;

m. a heavy chain with three CDRs comprising the amino acid sequences TYAFS (SEQ ID NO:68), RIIPILGIANYAQKFED (SEQ ID NO:83), and DGYGSDPVL (SEQ ID NO:98) respectively and a light chain with three CDRs comprising the amino acid sequences TRSSGSIASHYVQ (SEQ ID NO:113), EDNKRPS (SEQ ID NO:124), and QSYDSSNRWV (SEQ ID NO:139) respectively; or

n. a heavy chain with three CDRs comprising the amino acid sequences NYGIS (SEQ ID NO:69), WISAYNGNTNYAQKVED (SEQ ID NO:84), and GDFRKPFDY (SEQ ID NO:99) respectively and a light chain with three CDRs comprising the amino acid sequences TLRSGLNVGSYRIY (SEQ ID NO:114), YKSDSNKQQAS (SEQ ID NO:125), and MIWYSSAVV (SEQ ID NO:140) respectively;

wherein said antibody binds human PD-L1.

2. The antibody of claim 1, wherein said antibody is monovalent or bivalent.

3. The antibody of claim 1, wherein said antibody is a single chain antibody.

4. A single chain antibody comprising:

a. a V_H nucleotide sequence comprising SEQ ID NO: 1 and a V_L nucleotide sequence comprising SEQ ID NO: 3;

b. a V_H nucleotide sequence comprising SEQ ID NO: 5 and a V_L nucleotide sequence comprising SEQ ID NO:7;

c. a V_H nucleotide sequence comprising SEQ ID NO: 9 and a V_L nucleotide sequence comprising SEQ ID NO: 11;

d. a V_H nucleotide sequence comprising SEQ ID NO: 13 and a V_L nucleotide sequence comprising SEQ ID NO: 15;

e. a V_H nucleotide sequence comprising SEQ ID NO: 17 and a V_L nucleotide sequence comprising SEQ ID NO:19;

f. a V_H nucleotide sequence comprising SEQ ID NO: 21 and a V_L nucleotide sequence comprising SEQ ID NO:23;

g. a V_H nucleotide sequence comprising SEQ ID NO: 25 and a V_L nucleotide sequence comprising SEQ ID NO:27;

h. a V_H nucleotide sequence comprising SEQ ID NO: 29 and a V_L nucleotide sequence comprising SEQ ID NO:31;

i. a V_H nucleotide sequence comprising SEQ ID NO: 33 and a V_L nucleotide sequence comprising SEQ ID NO:35;

j. a V_H nucleotide sequence comprising SEQ ID NO: 37 and a V_L nucleotide sequence comprising SEQ ID NO:39;

k. a V_H nucleotide sequence comprising SEQ ID NO: 41 and a V_L nucleotide sequence comprising SEQ ID NO:43;

l. a V_H nucleotide sequence comprising SEQ ID NO: 45 and a V_L nucleotide sequence comprising SEQ ID NO:47;

m. a V_H nucleotide sequence comprising SEQ ID NO: 49 and a V_L nucleotide sequence comprising SEQ ID NO:51; or

n. a V_H nucleotide sequence comprising SEQ ID NO: 53 and a V_L nucleotide sequence comprising SEQ ID NO:55.

5. A single chain antibody comprising

a. a V_H amino acid sequence comprising SEQ ID NO: 2 and a V_L amino acid sequence comprising SEQ ID NO: 4;

b. a V_H amino acid sequence comprising SEQ ID NO: 6 and a V_L amino acid sequence comprising SEQ ID NO: 8;

c. a V_H amino acid sequence comprising SEQ ID NO: 10 and a V_L amino acid sequence comprising SEQ ID NO: 12;

d. a V_H amino acid sequence comprising SEQ ID NO: 14 and a V_L amino acid sequence comprising SEQ ID NO: 16;

e. a V_H amino acid sequence comprising SEQ ID NO: 18 and a V_L amino acid sequence comprising SEQ ID NO: 20;

f. a V_H amino acid sequence comprising SEQ ID NO: 22 and a V_L amino acid sequence comprising SEQ ID NO: 24;

g. a V_H amino acid sequence comprising SEQ ID NO: 26 and a V_L amino acid sequence comprising SEQ ID NO: 28;

h. a V_H amino acid sequence comprising SEQ ID NO: 30 and a V_L amino acid sequence comprising SEQ ID NO: 32;

i. a V_H amino acid sequence comprising SEQ ID NO: 34 and a V_L amino acid sequence comprising SEQ ID NO: 36;

j. a V_H amino acid sequence comprising SEQ ID NO: 38 and a V_L amino acid sequence comprising SEQ ID NO: 40;

k. a V_H amino acid sequence comprising SEQ ID NO: 42 and a V_L amino acid sequence comprising SEQ ID NO: 44;

l. a V_H amino acid sequence comprising SEQ ID NO: 46 and a V_L amino acid sequence comprising SEQ ID NO: 48;

m. a V_H amino acid sequence comprising SEQ ID NO: 50 and a V_L amino acid sequence comprising SEQ ID NO: 52; or

n. a V_H amino acid sequence comprising SEQ ID NO: 54 and a V_L amino acid sequence comprising SEQ ID NO: 56.

6. The antibody of claim 1, wherein said antibody has a binding affinity within the range of 10^{-5} M to 10^{-12} M.

7. The antibody according to claim 1 wherein said antibody is a bi-specific antibody that also binds to a tumor-associated antigen, a cytokine or a cell surface receptor.

8. The antibody according to claim 7 wherein said tumor-associated antigen is CAIX.

9. The antibody according to claim 7, wherein said cytokine is IL-10.

10. The antibody according to claim 7, wherein said cell surface receptor is CCR4, IL21R, BTLA, HVEM or TIM3.

11. The antibody according to any one of claims 1-8 linked to a therapeutic agent.

12. The antibody of claim 9, wherein said therapeutic agent is a toxin, a radiolabel, a siRNA, a small molecule, or a cytokine.

13. A cell producing the antibody of any one of claims 1-12.

14. A method of selectively killing a tumor cell comprising contacting said cell with the antibody of any one of claims 1-12.

15. The method of claim 14, wherein said selective killing occurs by antibody-dependent cellular toxicity (ADCC), complement-dependent cytotoxicity (CDC), antibody dependent cellular phagocytosis (ADCP).

16. The method of claim 14, wherein said tumor cell expresses PD-L1.

17. A method of preventing or reversing T cell exhaustion comprising administering to a subject in need thereof a composition comprising an antibody of any one of claims 1-12.

18. A method of augmenting an immune response to an antigen comprising administering to a subject in need thereof a composition comprising an antibody of any one of claims 1-12.

19. The method of claim 18, wherein said antigen is a viral antigen, a bacterial antigen or a tumor associated antigen.

20. The method of claim 19, wherein said viral antigen is HIV.

21. The method of claim 19, wherein said tumor associated antigen is CAIX.

22. The method of claim 18, wherein said antibody is administered prior to or after exposure to the antigen.

23. The method of claim 17 or 18, wherein said administration of said antibody causes an increase in antigen specific T cell activity.

24. The method of claim 23, wherein said T-cell is an effector T cell.

25. A method of treating or alleviating a symptom of cancer, comprising administering to a subject in need thereof a composition comprising an antibody according to any one of claims 1-12.

26. The method of claim 25, wherein said cancer is renal cell carcinoma or breast cancer.

27. The method of claim 25, wherein said cancer is a cancer in which PD-L1 is overexpressed.

28. The method of claim 25, wherein said cancer is a cancer that induces T cell exhaustion.

29. A method of treating or alleviating a symptom of a chronic viral infection, comprising administering to a subject in need thereof a composition comprising an antibody according to any one of claims 1-12.

30. The method of claim 29, wherein said chronic viral infection is an HIV infection.

31. The method of claim 29, wherein said chronic viral infection is a viral infection that induces T cell exhaustion.

32. A nucleic acid sequence comprising the nucleic acid sequence of SEQ ID NO: 1, 3, 5, 7, 9, 11, 13. 15. 17, 19, 21, 23, 25, 27, 29, 31, 33, 35, 37, 39, 41, 43, 45, 47, 49, 51, 53 or 55.

33. A nucleic acid sequence encoding the polypeptide of SEQ ID NO: 2, 4, 6, 8, 10, 12, 14, 16, 18, 20, 22, 24, 26, 28, 30, 32, 34, 36, 38, 40, 42, 44, 46, 48, 50, 52, 54 or 56.

34. A polypeptide comprising the amino acid sequence of SEQ ID NO: 2, 4, 6, 8, 10, 12, 14, 16, 18, 20, 22, 24, 26, 28, 30, 32, 34, 36, 38, 40, 42, 44, 46, 48, 50, 52, 54 or 56.

35. A vector comprising the nucleic acid claims 32 or 33.

36. A cell comprising the vector of claim 35.

FIG. 1

VH:

```
          1   FW1              CDR1       FW2            CDR2            FW3                    CDR3        FW4        Family
          1                      31   36              50 A   66           ABC          95  ABCDEFGHIJK  103           IGHV
Consensus QVQLVQSGAEVKKPGASVKVSCKASGFTFS SYAIS WVRQAPGQGLEWMG WISPIGGSTNYAQKVQG RVTIT.DNSTNTAYMELSSLRSDDTAVYYCAR GLXXXXXXXXXXXXXXXDV WGQGTLVTVSS
50   ........G.................Y.L. .HG.T .............. ...AHN.HASN....ED ...M.T.T.........R..TA.......... VHAALYYGM---------.. ........... 1-18*01
55   ..........................Y..T ..G.. .............. .T..HN.L.AF..ILE. ...M.T.T.........RN.TF.....F..K VHPVFSYAL---------.. ........... 1-18*01
14   ..........................Y..T ..G.. .............. ...AYN.N......L.. ...M.T.T..S......R.............. A.PSGTILVGGWF-----.P ........... 1-18*01
65   E.........................Y..T N.G.. .............. ...AYN.N......... ...M.T.T..S.G...R.............. .DFRKPF-----------.Y ........... 1-18*01
46   E...E.........S........V..G..G T..LN .............. R.V.LI.LV...HNFE. .IS..A.K..G.......N............. EVYGGNS-----------.Y ........... 1-69*09
31   ................T......VF.D..R GLY.H .............. ..I..F.TA....F.. .....T.E..S...........E........S ..RWGIWGWF--------.P ........... 1-69*01
32   E.........L....S........F.G... DN... .........P.... ..I..F.KP....F.. .....A.E..S....V......E......... TMVRGFLGVM--------.. .....T..... 1-69*01
56   E....E.....MN.....R...RG..GD.. T..F. .............. R.I..L.IA....F.. .....A.K..S..................... DGYGSDP-----------VL ........... 1-69*09
42   ..........................G... ..... .............. ..I..F.TA....F.. .....A.K..S...........E......... .RQMFGAGI---------.F ..P........ 1-69*06
52   ....QE..GG.VQ..R.LRL..S....... RHGMH .......K....VA V..HD.SVKY..DSMK. .FS.SR...N..L.LQMD...A.......... ..SYQVSGWF--------.P ........... 3-30*03
30   ........GS.VR..E.LRL..V....I.D N.DM. ....V..K....VS RVNWN....T..DA.K. .F..SR..TK.SL.LQMNN..AE...L...V. EFVGAY-----------.L .....T..... 3-20*01
22   ........GG.VQ..G.LRL..A......D D..MH .......K....VS L..GD....Y..DS.K. .F..SR...K.SL.LQMN...TE........K V.LPCSSTSCYGSVGAF.I .....T..... 3-43*02
16   E.......GG.VQ..R.LRL..A....... ...L. .......K....VS A..GG....Y..DS.K. .F..SR...K..L.LQMN...AE........K DVFPETFSMNYGM----.. ........... 3-23*04
38   ........GGLVQ..G.LRL..A....... ...M. .......K....VS A..GS....Y..DS.K. .F..SR...K..L.LQMN...AE........K DQFVTIFGVPRYGM---.. .....T..... 3-23*04
```

Key: "." AA matches to consensus, "X" no consensus AA, and "-" is a space (i.e. no AA)

VL:

```
             FW1              CDR1          FW2            CDR2                 FW3                        CDR3    FW4       Family
         1                 24  ABC  35           50    ABCD57      AB            89    AB   98
Consensus    NFMLTQ-PHSVSESPGKTVTISC TRSSGS-IGSNYVQ WYQQRPGSAPTLVIY EDNQR----PS GVPDRFSGSIDSSSNSASLTISGLKTEDEADYYC QSYDSST--WV FGGGTKLTVL
50  QSV.....P...VA..Q.AR.T. GGNN--....KG.H ....K..Q..V..V. D.SD....... .I.E.....N--.G.T.T...RVEAG........ .VW...SDH.. .......... IGLV3-21*02
55  ....................... ....G..A...... ........S..T... ........... ..........T..............K........ ....GI...VI .......... IGLV6-57*01
14  .....Q................. ....GN..A..... ...........T... ........... .................................. ......NL... .......... IGLV6-57*01
65  LPV..Q..A.L.A...ASASLT. .LR.GLNV..YRIY ....K...R.QYLLN YKSDSNKQQA. ...S.....K.A.A.AGI.L...QS......... MIWY..A..V. .......... IGLV5-45*01
46  .....Q................. ....GN...T.... ..........VAL.. ..YR....... ...................I.....P........ ...H..G...E .......... IGLV6-57*01
31  EIV..QS.ATL.LS..ERA.L.. RA.Q--.SIG.SLA ....K..Q..R.LM. GASS.....AT .I......G--AGTDFT...S.EP..F.T..... .QHTIP...FS ..P...VE.K IGKV3-20*01
32  DIVM.QT.SFL.A.I.DR...T. RA.Q--.GIGS.LA .......E..K.L.. AASTL....Q. ...S.....G--.GTDFT...N.QP..F.T.... .QLNNYP..IT ..Q..R.EIK IGKV1-9*01
56  .....Q.....G........LP. ....G..A.H.... ...........T... ...K....... ...................S.............. ......NR... .......... IGLV6-57*01
42  .....Q................. ....G..D...... ...........T... ........... .................................. .....NNRHVI .......... IGLV6-57*01
52  .....Q................. ....G..A...... ...........T... ........... .................................. .....T.P.S. .......... IGLV6-57*01
30  QSA..Q..A..G..QSI...... .GT.SDVG.Y...S ....H..K..K.M.. DVSN....... ..SN.....K--.G.T.......QA......... S..T.....LP .......... IGLV2-14*01
22  LPV..Q.AP...VA....AR.T. GG.D--...RKS.H ....K..Q..A.... S.RD....... .ISE.....N--.G.T.T...RVEAG........ .VW.NNSDHY. ..A..E.I.. IGLV3-12*01
16  SSE..Q.DPA..VAL.Q..R.T. QGD.--.LR.Y.AS ....K..Q..V.... GK.N....... .I.......S--.G.T.....T.AQA........ N.R...GNHY. ..T...V... IGLV3-19*01
38  QSA..Q..P...V...Q.AN.P. SGDK--.L.NK.AY ....K..QS.V.L.. Q.IK....... RI.E.....N--.ADT.T.....TQAM....... .TW.N.-..V. .......... IGLV3-1*01
```

Key: "." AA matches to consensus, "X" no consensus AA, and "-" is a space (i.e. no AA)

FIG. 1 continued

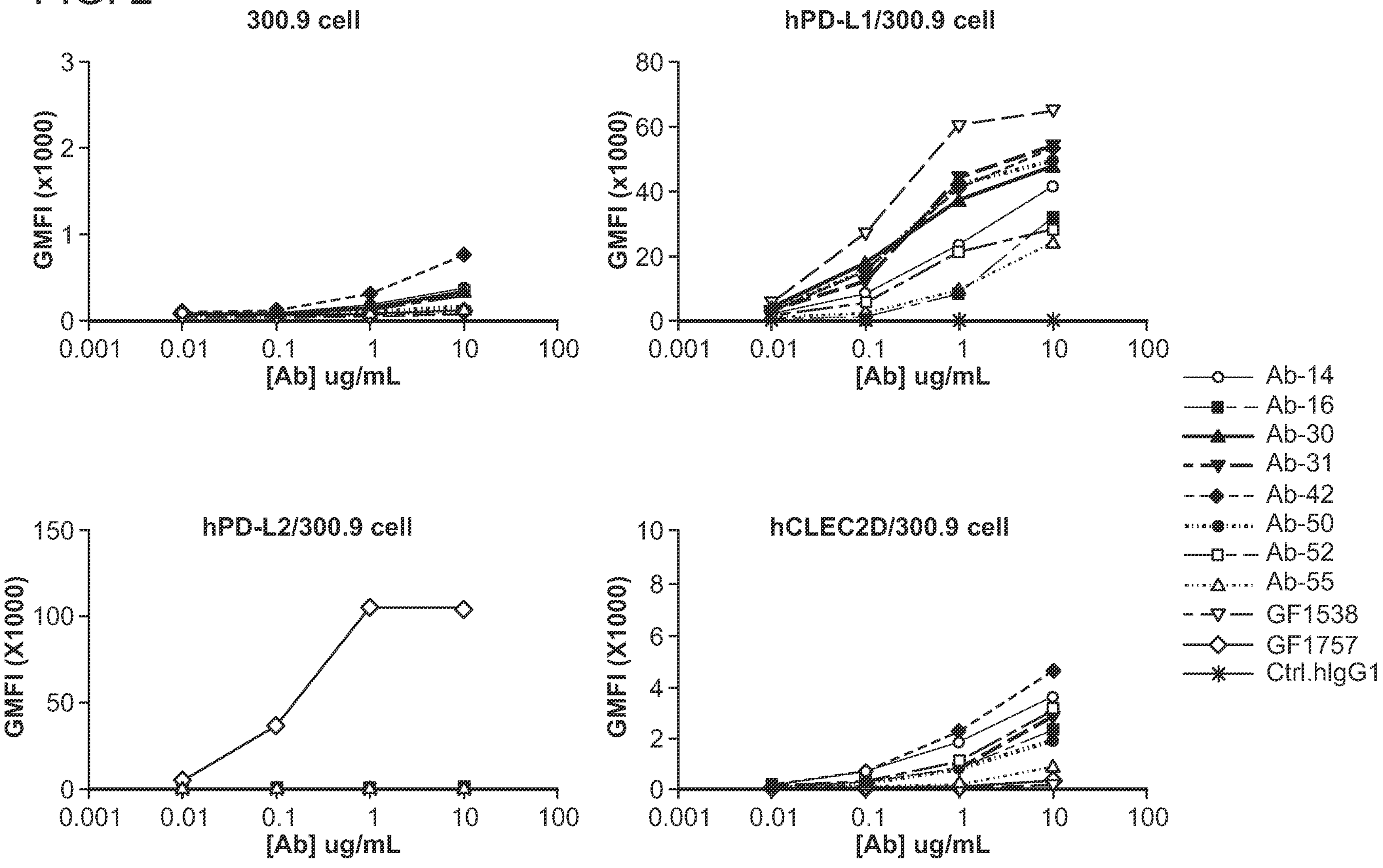


FIG. 2
300.9 cell
GMFI (x1000)
[Ab] ug/mL
hPD-L1/300.9 cell
GMFI (x1000)
[Ab] ug/mL
hPD-L2/300.9 cell
GMFI (X1000)
[Ab] ug/mL
hCLEC2D/300.9 cell
GMFI (X1000)
[Ab] ug/mL
0.001
0.01
0.1
1
10
100
Ab-14
Ab-16
Ab-30
Ab-31
Ab-42
Ab-50
Ab-52
Ab-55
GF1538
GF1757
Ctrl.hIgG1

FIG. 3

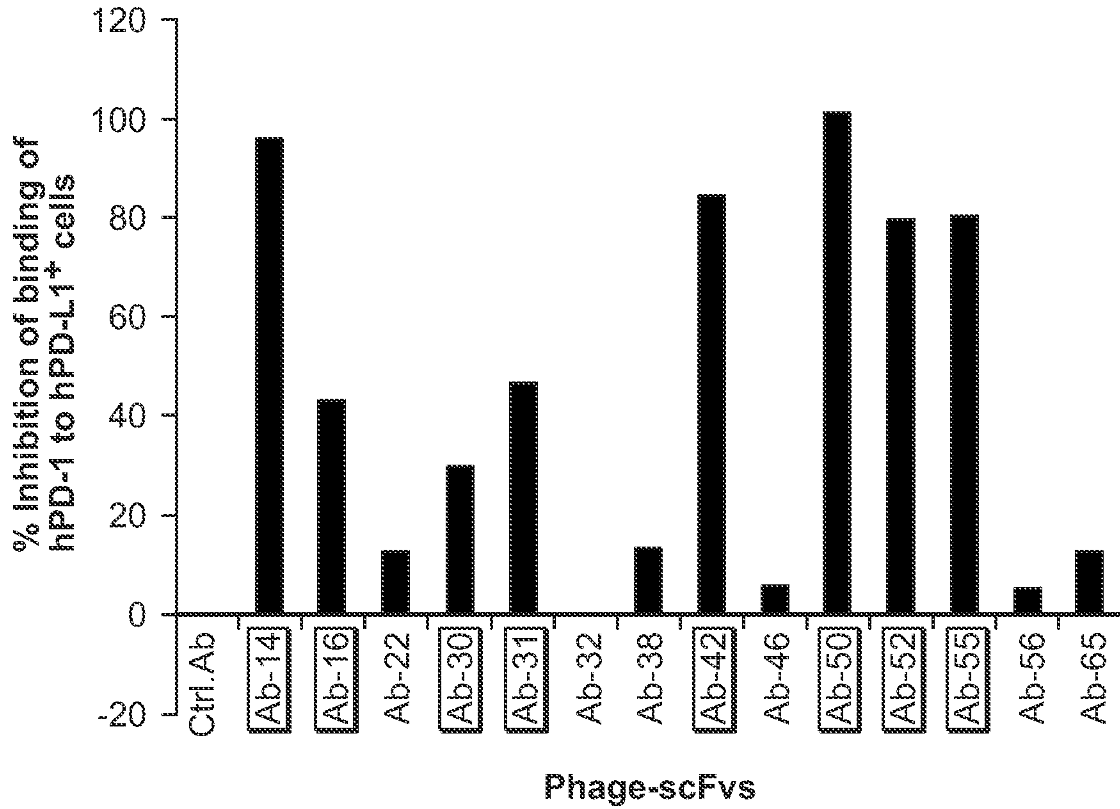
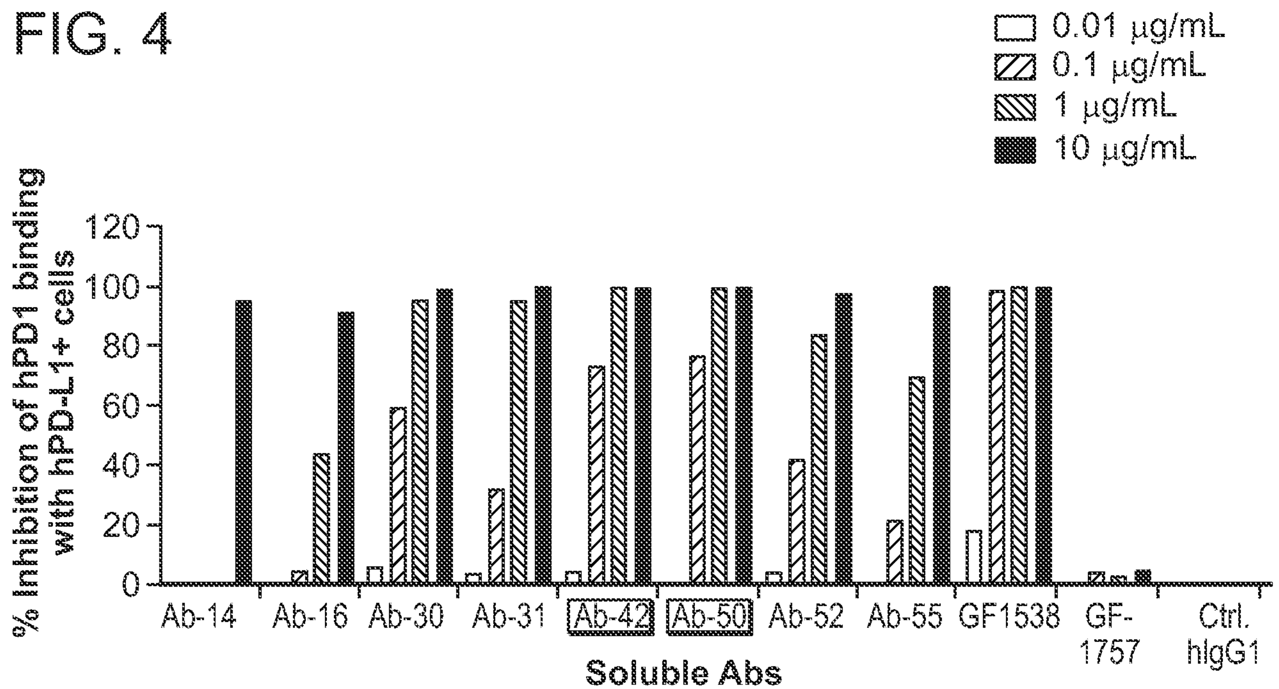


FIG. 4



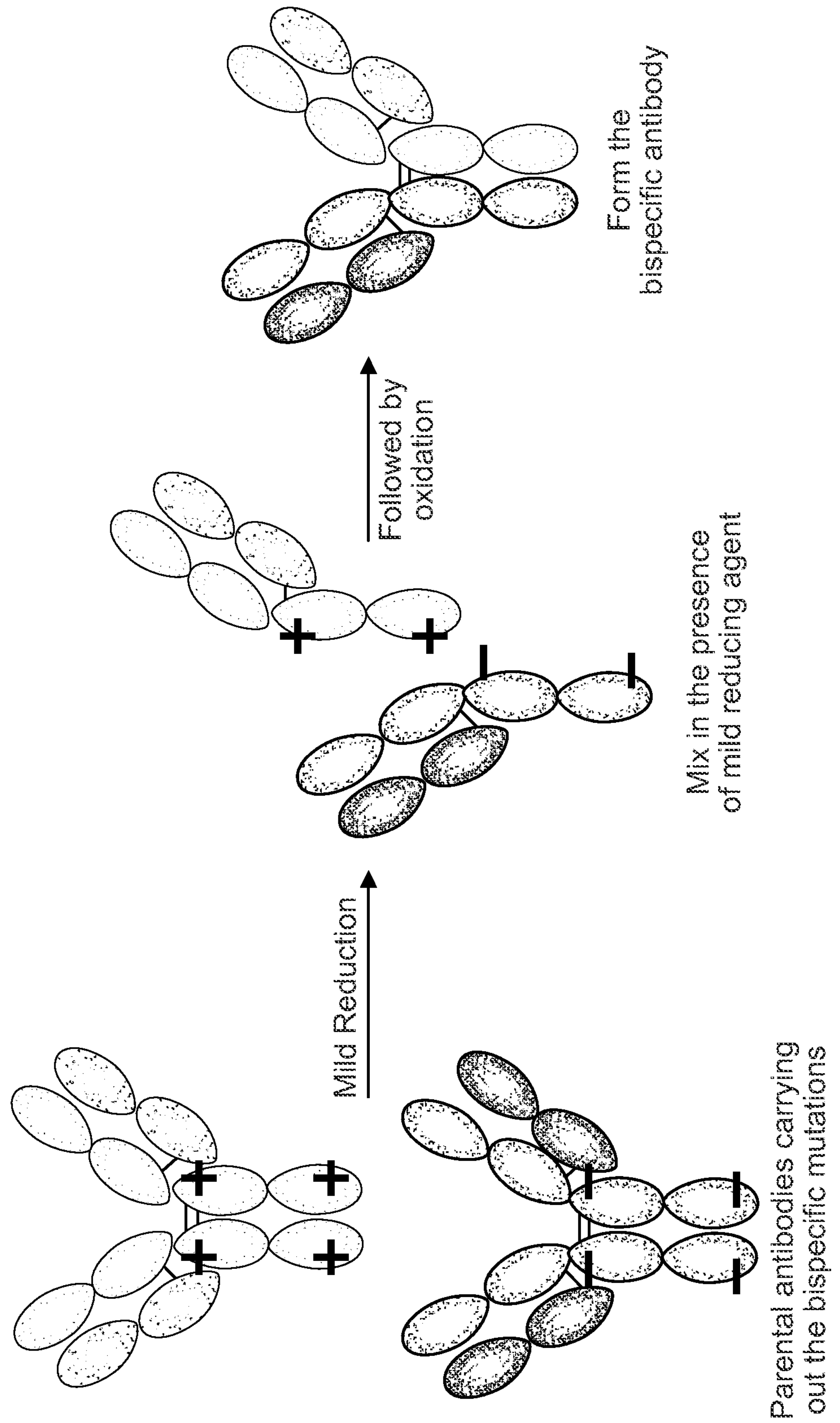


FIG. 5

FIG. 6A

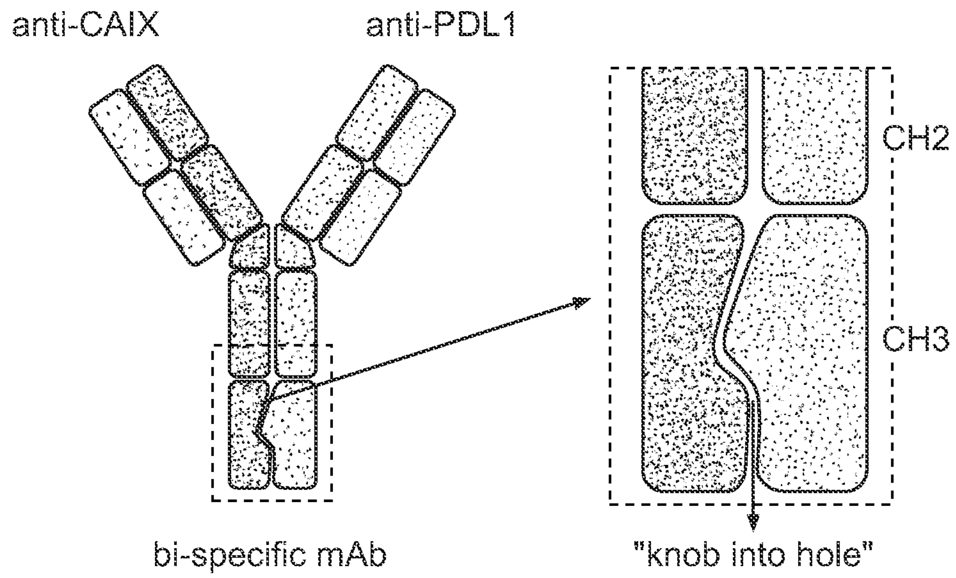


FIG. 6B

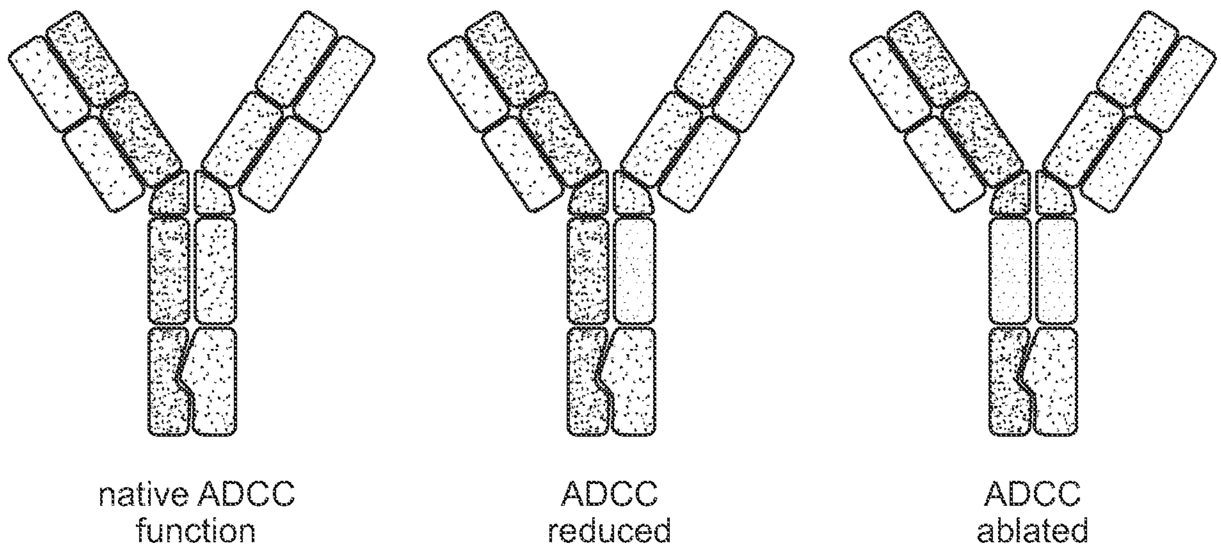


FIG. 7A

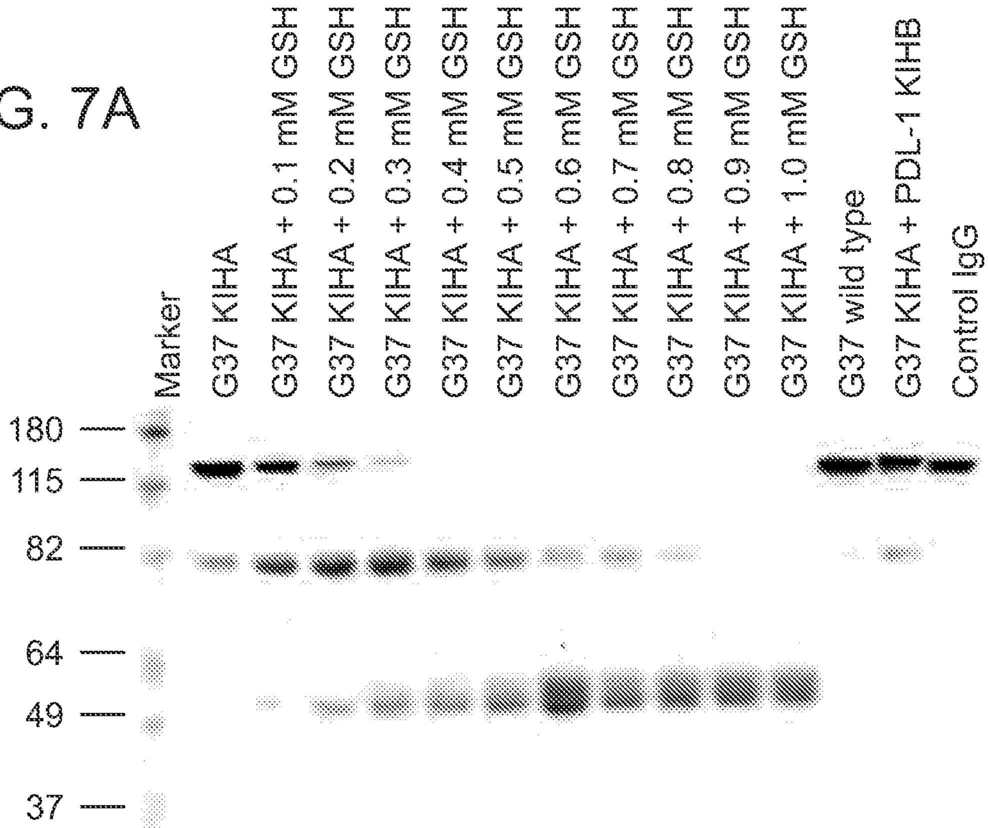
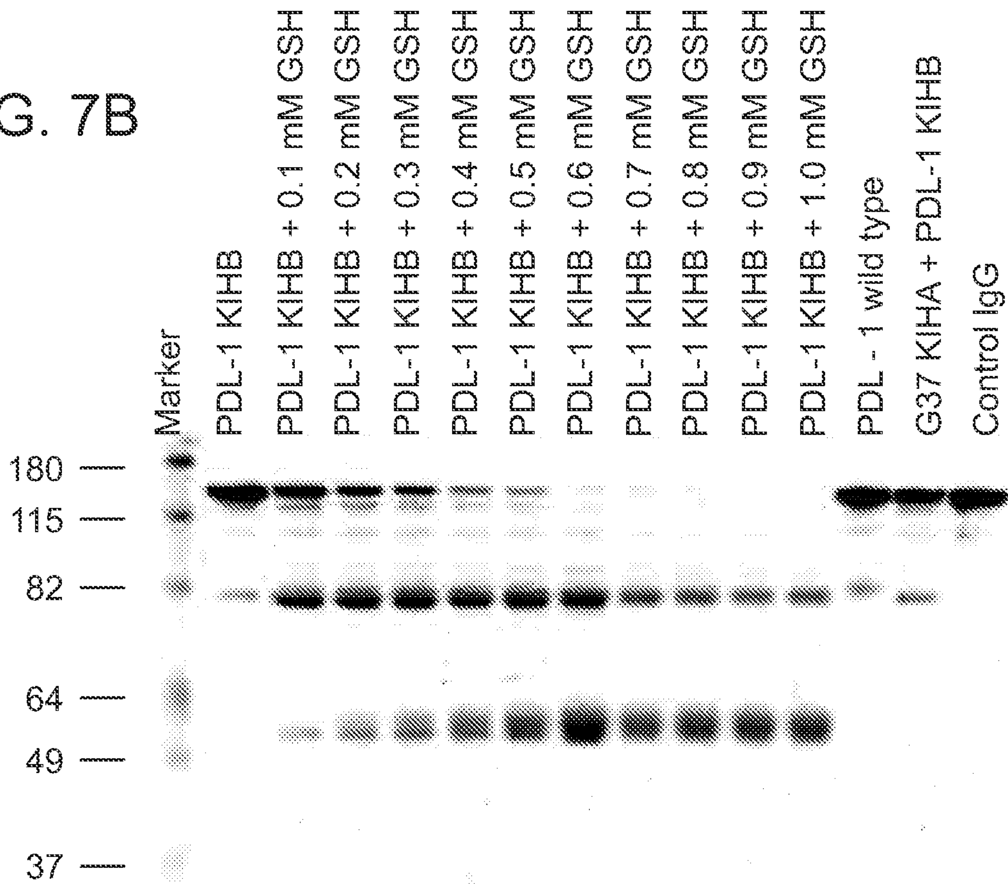


FIG. 7B



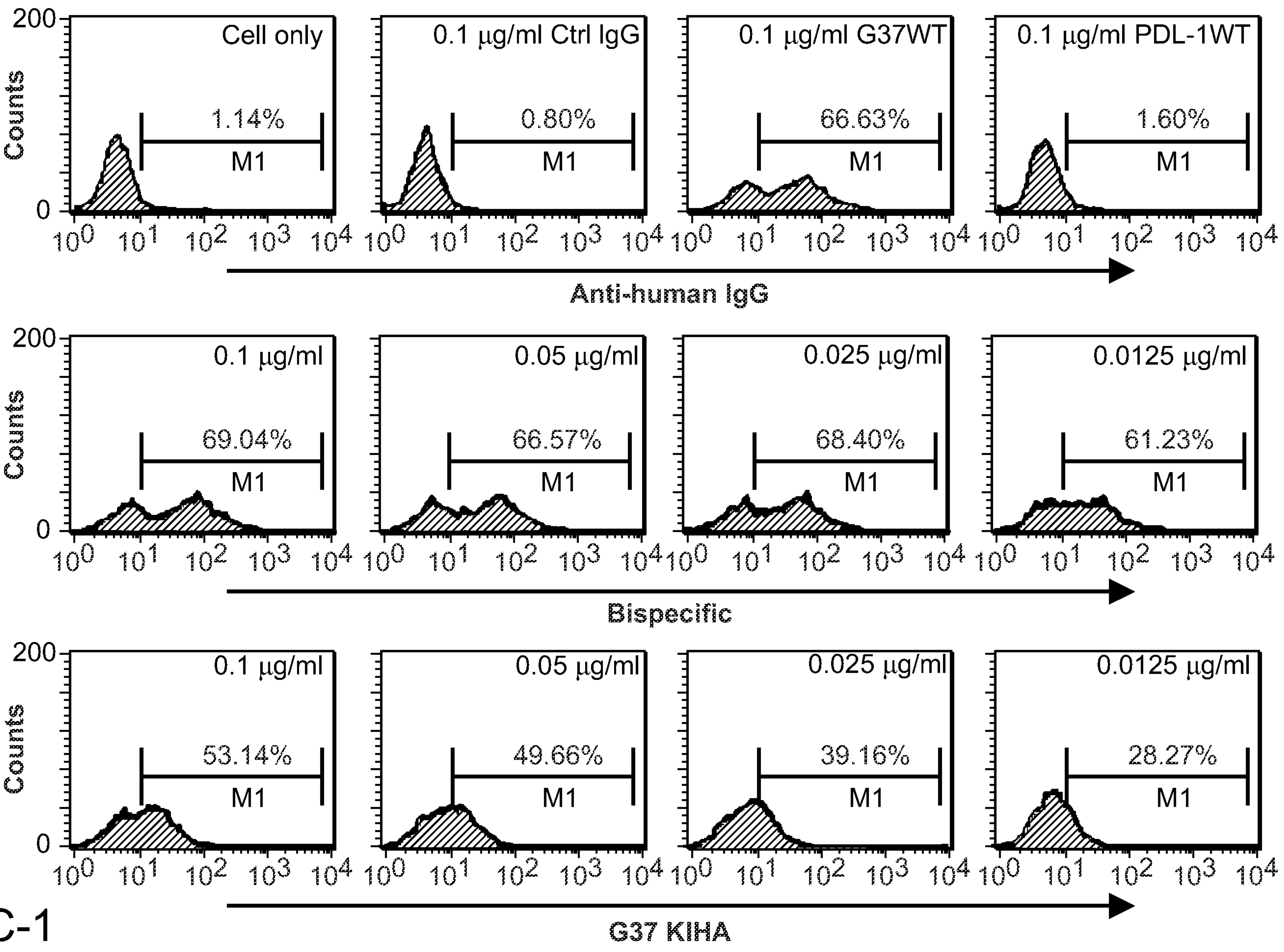


FIG. 7C-1

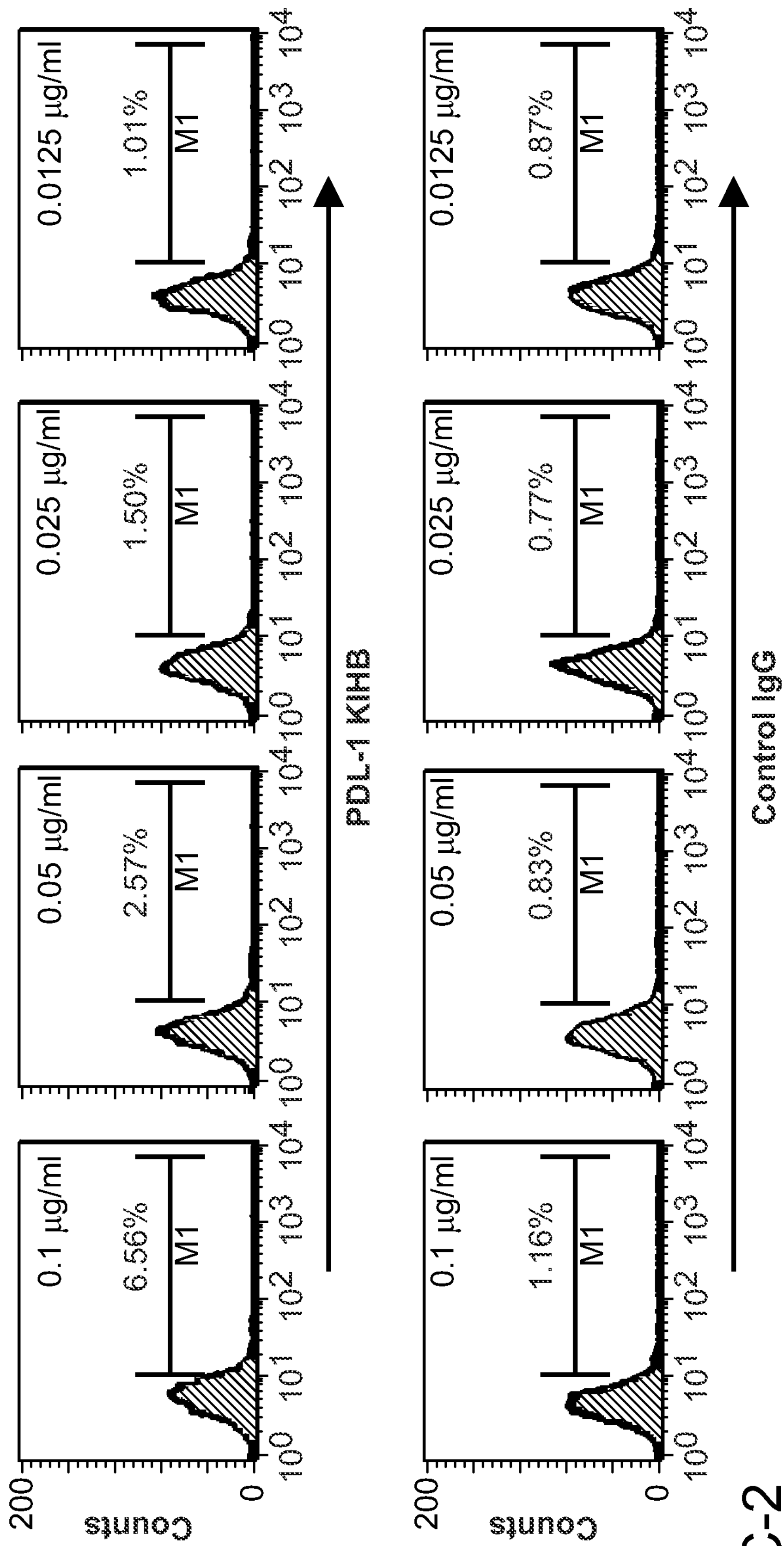


FIG. 7C-2

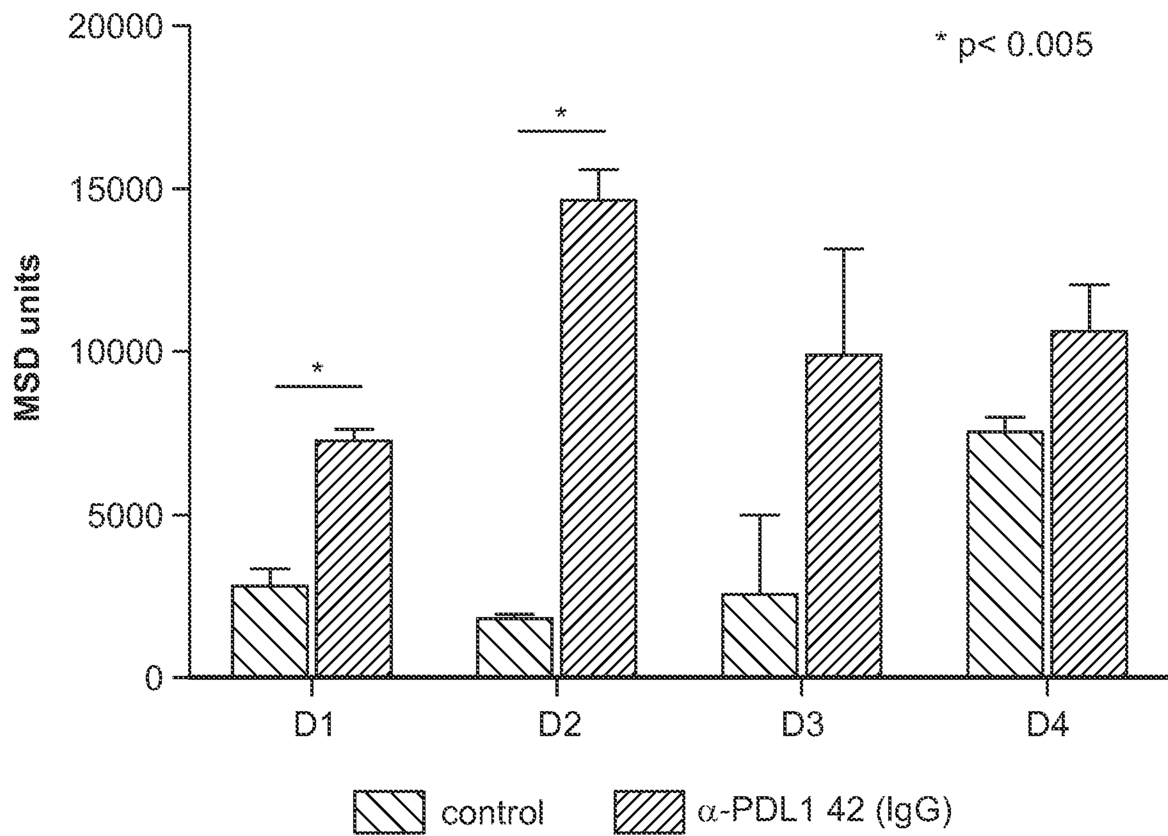


FIG. 8