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(54) **Title:** PD-1 AGONIST AND METHOD OF USING SAME

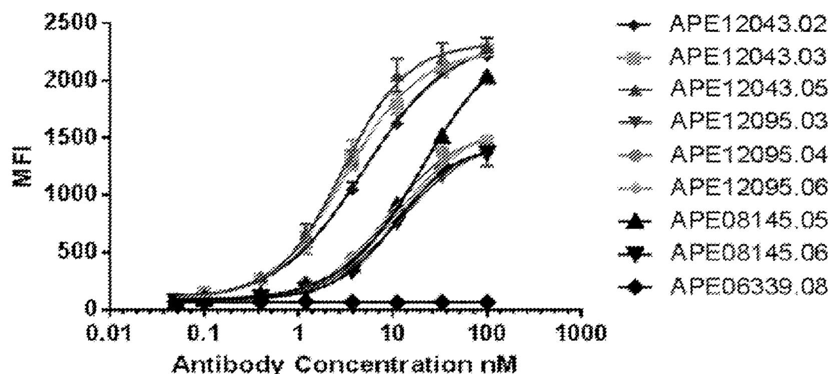

Figure 1

(57) **Abstract:** Provided is a PD-1-binding agent comprising an immunoglobulin heavy chain polypeptide and immunoglobulin light chain polypeptide, as well as related compositions and methods for making and using same.

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[Continued on next page]

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PD-1 AGONIST AND METHOD OF USING SAME

CROSS-REFERENCE TO RELATED APPLICATIONS

[0001] This patent application claims priority to U.S. provisional patent application 62/857,699 filed June 5, 2019; U.S. provisional patent application 62/863,193 filed June 18, 2019; and U.S. provisional patent application 62/983,512 filed February 28, 2020, the entire disclosures of which are hereby incorporated by reference.

BACKGROUND OF THE INVENTION

[0002] Programmed Death 1 (PD-1) (also known as Programmed Cell Death 1) is a type I transmembrane protein of 268 amino acids originally identified by subtractive hybridization of a mouse T cell line undergoing apoptosis (Ishida et al., *Embo J.*, *11*: 3887-95 (1992)). PD-1 is a member of the CD28/CTLA-4 family of T-cell regulators, and is reported to be expressed on activated T-cells, B-cells, and myeloid lineage cells (Greenwald et al., *Annu. Rev. Immunol.*, *23*: 515-548 (2005); and Sharpe et al., *Nat. Immunol.*, *8*: 239-245 (2007)).

[0003] Two ligands for PD-1 have been identified, PD ligand 1 (PD-L1) and PD ligand 2 (PD-L2), both of which belong to the B7 protein superfamily (Greenwald et al., *supra*). PD-L1 is expressed in a variety of cell types, including cells of the lung, heart, thymus, spleen, and kidney (see, e.g., Freeman et al., *J. Exp. Med.*, *192*(7): 1027-1034 (2000); and Yamazaki et al., *J. Immunol.*, *169*(10): 5538-5545 (2002)). PD-L1 expression is upregulated on macrophages and dendritic cells (DCs) in response to lipopolysaccharide (LPS) and GM-CSF treatment, and on T-cells and B-cells upon signaling via T-cell and B-cell receptors. PD-L1 also is expressed in a variety of murine and human tumor cell lines (see, e.g., Iwai et al., *Proc. Natl. Acad. Sci. USA*, *99*(19): 12293-12297 (2002); and Blank et al., *Cancer Res.*, *64*(3): 1140-1145 (2004)). In contrast, PD-L2 exhibits a more restricted expression pattern and is expressed primarily by antigen presenting cells (e.g., dendritic cells and macrophages), and some tumor cell lines (see, e.g., Latchman et al., *Nat. Immunol.*, *2*(3): 261-238 (2001)).

[0004] PD-1 negatively regulates T-cell activation, and this inhibitory function is linked to an immunoreceptor tyrosine-based switch motif (ITSM) in the cytoplasmic domain (see, e.g., Greenwald et al., *supra*; and Parry et al., *Mol. Cell. Biol.*, *25*: 9543-9553 (2005)). PD-L1-induced clustering of PD-1 has been found to induce recruitment of the SHP2 phosphatase which preferentially dephosphorylates CD28, suppressing T cell function (Hui et

al., *Science, 355*: 1428-1433 (2017)). PD-1 deficiency can lead to autoimmunity. For example, C57BL/6 PD-1 knockout mice have been shown to develop a lupus-like syndrome (see, e.g., Nishimura et al., *Immunity, 11*: 141-1151 (1999)). In humans, a single nucleotide polymorphism in the PD-1 gene is associated with higher incidences of systemic lupus erythematosus, type 1 diabetes, rheumatoid arthritis, and progression of multiple sclerosis (see, e.g., Nielsen et al., *Tissue Antigens*, *62*(6): 492-497 (2003); Bertsias et al., *Arthritis Rheum.*, *60*(1): 207-218 (2009); Ni et al., *Hum. Genet.*, *121*(2): 223-232 (2007); Tahoori et al., *Clin. Exp. Rheumatol.*, *29*(5): 763-767 (2011); and Kroner et al., *Ann. Neurol.*, *58*(1): 50-57 (2005)).

[0005] Despite recent advances in inhibiting PD-1 activity to treat various types of cancer and for immunopotentiation (e.g., to treat infectious diseases) there is a need for a PD-1-binding agent (e.g., an antibody) that binds PD-1 with high affinity which promotes negative signaling and functions as a PD-1 agonist.

BRIEF SUMMARY OF THE INVENTION

[0006] The invention provides an agonistic PD-1 binding agent. In one embodiment, the PD-1 binding agent comprises an immunoglobulin heavy chain variable region and an immunoglobulin light chain variable region, wherein the immunoglobulin heavy chain variable region comprises: a CDR1 comprising SEQ ID NO: 1; a CDR2 comprising SEQ ID NO: 2; and a CDR3 comprising SEQ ID NO: 3; and the immunoglobulin light chain variable region comprises a CDR1 comprising SEQ ID NO: 4; a CDR2 comprising SEQ ID NO: 5; and a CDR3 comprising SEQ ID NO: 6.

[0007] Also provided is an anti-PD-1 binding agent comprising an immunoglobulin heavy chain variable region with at least 80%, 85% or 90% sequence identity to any one of SEQ ID NOs: 24-33, or a heavy chain variable region comprising at least the CDR regions of SEQ ID NOs: 24-33, and/or an immunoglobulin light chain variable region with at least 80%, 85% or 90% sequence identity to SEQ ID NO: 34 or 35, or a light chain variable region comprising at least the CDR regions of SEQ ID NO: 34 or 35.

[0008] In another aspect, the PD-1 binding agent comprises an immunoglobulin heavy chain variable region and an immunoglobulin light chain variable region, wherein the immunoglobulin heavy chain variable region comprises: a CDR1 comprising SEQ ID NO: 7; a CDR2 comprising SEQ ID NO: 8; and a CDR3 comprising SEQ ID NO: 9; and the

immunoglobulin light chain variable region comprises a CDR1 comprising SEQ ID NO: 10; a CDR2 comprising SEQ ID NO: 11; and a CDR3 comprising SEQ ID NO: 12.

[0009] Also provided is an anti-PD-1 binding agent comprising an immunoglobulin heavy chain variable region with at least 80%, 85% or 90% sequence identity to any one of SEQ ID NOs: 43-47 or 61-63, or a heavy chain variable region comprising at least the CDR regions thereof, and/or an immunoglobulin light chain variable region with at least 80%, 85% or 90% sequence identity to SEQ ID NOs: 48-50, or a light chain variable region comprising at least the CDR regions thereof.

[0010] In addition, the invention provides isolated or purified nucleic acid sequences encoding the foregoing immunoglobulin polypeptides, vectors comprising such nucleic acid sequences, isolated PD-1-binding agents comprising the foregoing immunoglobulin polypeptides, nucleic acid sequences encoding such PD-1-binding agents, vectors comprising such nucleic acid sequences, isolated cells comprising such vectors, compositions comprising such PD-1-binding agents or such vectors with a pharmaceutically acceptable carrier, and methods inhibiting an immune response and treating inflammatory or autoimmune disorders in mammals by administering effective amounts of such compositions to mammals.

BRIEF DESCRIPTION OF THE SEVERAL VIEWS OF THE DRAWING(S)

[0011] Figure 1 is a graph depicting the results of binding of anti-PD-1 antibodies to HEK 293 cells stably transfected with human PD-1.

[0012] Figure 2 is a graph depicting the results of binding of anti-PD-1 antibodies to HEK 293 cells stably transfected with cynomolgus monkey PD-1.

[0013] Figure 3 is a graph depicting the results of binding of anti-PD-1 antibodies to 2-day anti-CD3/anti-CD28 activated human peripheral blood $CD4^+$ T cells.

[0014] Figures 4-7 are graphs showing testing results of anti-PD-1 antibodies competing with either PD-L1-Fc or PD-L2-Fc for binding to PD-1 CHO-K1 cells.

[0015] Figure 4 is a graph depicting the results of a competition assay, which illustrates the ability of anti-PD-1 antibodies to compete with PD-L1-Fc for binding to CHO-K1 cells stably transfected with human PD-1.

[0016] Figure 5 is a graph depicting the results of a competition assay, which illustrates the ability of anti-PD-1 antibodies to compete with PD-L1-Fc for binding to CHO-K1 cells stably transfected with human PD-1.

[0017] Figure 6 is a graph depicting the results of a competition assay, which illustrates the ability of anti-PD-1 antibodies to compete with PD-L2-Fc for binding to CHO-K1 cells stably transfected with human PD-1.

[0018] Figure 7 is a graph depicting the results of a competition assay, which illustrates the ability of anti-PD-1 antibodies to compete with PD-L2-Fc for binding to CHO-K1 cells stably transfected with human PD-1.

[0019] Figure 8A is a graph depicting the agonist activity performance of anti-PD-1 antibodies in a bead-based $CD4^+$ T cell agonist assay using a 2:1 bead to cell ratio.

[0020] Figure 8B is a graph depicting the agonist activity performance of anti-PD-1 antibodies in a bead-based $CD4^+$ T cell agonist assay using a 1:1 bead to cell ratio.

[0021] Figure 9A is a graph depicting the agonist activity performance of anti-PD-1 antibodies in a bead-based $CD4^+$ T cell agonist assay using a 4:1 bead to cell ratio.

[0022] Figure 9B is a graph depicting the agonist activity performance of anti-PD-1 antibodies in a bead-based $CD4^+$ T cell agonist assay using a 2:1 bead to cell ratio.

[0023] Figure 9C is a graph depicting the agonist activity performance of anti-PD-1 antibodies in a bead-based $CD4^+$ T cell agonist assay using a 1:1 bead to cell ratio.

[0024] Figure 10A is a graph depicting the mean % inhibition of IFNγ production across multiple donors in a bead-based $CD4^+$ T cell agonist assay for an anti-PD-1 antibody.

[0025] Figure 10B is a chart providing a description of anti-PD-1 antibodies, % inhibition of IFNγ, and number of donors included in Figure 10A.

[0026] Figure 11A is a graph depicting the mean % inhibition of IFNγ production across multiple donors in a bead-based $CD4^+$ T cell agonist assay for an anti-PD-1 antibody.

[0027] Figure 11B is a graph depicting the mean % inhibition of IFNγ production across the same donors in a bead-based $CD4^+$ T cell agonist assay for a reference PD-1 agonist, PD-L1-Fc.

[0028] Figure 11C is a chart providing the candidate antibodies, description of the antibodies, % inhibition of IFNγ, and number of donors included in Figures 11A and 11B.

[0029] Figure 12A is a graph depicting the agonist potency of anti-PD-1 antibodies in inhibiting IL-2 production in a plate-based human PBMC agonist assay (donor #747).

[0030] Figure 12B is a graph depicting the agonist potency of an anti-PD-1 antibody and PD-L1-Fc in inhibiting IL-2 production in a plate-based human PBMC agonist assay (donor #500).

[0031] Figure 13A is a graph depicting the agonist potency of an anti-PD-1 antibody and PD-L1-Fc in inhibiting IL-2 production in a plate-based human PBMC agonist assay (frozen donor #500).

[0032] Figure 13B is a graph depicting the agonist potency of an anti-PD-1 antibody and PD-L1-Fc in inhibiting IL-2 production in a plate-based human PBMC agonist assay (frozen donor #500).

[0033] Figure 14A is a graph depicting the agonist potency of an anti-PD-1 antibody and PD-L1-Fc in inhibiting IL-2 production in a plate-based human PBMC agonist assay (frozen donor #1202).

[0034] Figure 14B is a graph depicting the agonist potency of an anti-PD-1 antibody and PD-L1-Fc in inhibiting IL-2 production in a plate-based human PBMC agonist assay (frozen donor #1202).

[0035] Figure 15A is a graph depicting the observed agonist activity of a PD-L1-Fc tetramer in a whole human blood tetanus recall assay and lack of agonist activity of nivolumab in the presence of blocking anti-PD-L1/anti-PD-L2.

[0036] Figure 15B is a graph depicting the observed agonist activity of PD-1 agonist antibodies in a whole human blood tetanus recall assay in the presence of blocking anti-PD-L1/anti-PD-L2.

[0037] Figure 15C is a graph depicting the effect of a WT IgG1 anti-PD-1 agonist antibody (closed triangular data points) on IFNγ in a whole human blood tetanus recall assay.

[0038] Figure 15D is a graph depicting the effect of an IgG2 isotype anti-PD-1 antibody (open triangular data points) on IFNγ in a whole human blood tetanus recall assay.

[0039] Figure 16A is a schematic of the xenogeneic NSG/Hu-PBMC mouse model for the Graft vs. Host Disease study described in Example 8, in accordance with embodiments of the invention.

[0040] Figure 16B is a schematic showing the timeline, dosing schedule, and model groups of the NSG/Hu-PBMC Graft vs. Host Disease study described in Example 8, in accordance with embodiments of the invention.

[0041] Figure 16C is a graph depicting the results of the time to ≥10% body weight loss of the NSG/Hu-PBMC Graft vs. Host Disease study in Example 8 for an anti-PD-1 antibody.

[0042] Figure 16D is a graph depicting the results of the time to ≥10% body weight loss of the NSG/Hu-PBMC Graft vs. Host Disease study in Example 8 for an anti-PD-1 antibody.

[0043] Figure 17A is a graph depicting the pharmacokinetic properties in cynomolgus monkeys after a 10 mg/kg intravenous or subcutaneous single dose of an anti-PD-1 antibody.

[0044] Figure 17B is a graph depicting the pharmacokinetic properties in cynomolgus monkeys after a 10 mg/kg intravenous or subcutaneous single dose of an anti-PD-1 antibody.

[0045] Figure 18A is a graph depicting the $CD3^+$ T-cell PD-1 receptor occupancy in cynomolgus monkeys after a 10 mg/kg intravenous or subcutaneous single dose of an anti-PD-1 antibody.

[0046] Figure 18B is a graph depicting the $CD3^+$ T-cell PD-1 receptor occupancy in cynomolgus monkeys after a 10 mg/kg intravenous or subcutaneous single dose of an anti-PD-1 antibody.

[0047] Figure 19A is an SDS-PAGE gel showing the results of immunoblotting of PD-1 immunoprecipitates with either anti-PD-1 (top), anti-SHP2 (middle), or anti-SHP1 (bottom).

[0048] Figure 19B is a graph depicting the densitometry quantification of the immunoblot shown in Figure 19A.

[0049] Figure 20A is a ribbon-model illustration of the crystal structure of human PD-1 extracellular domain (black) docked with a space-filling model of the crystal structure of human PD-L1 extracellular binding domain (gray). The molecule is oriented with the membrane-proximal region of PD-1 at the bottom left,

[0050] Figure 20B is a ribbon-model illustration of the crystal structure of human PD-1 extracellular domain (black) docked with a space-filling model of the crystal structure of human PD-L1 extracellular binding domain (gray). The molecule is rotated by 90° as compared to the view of the molecule shown in Figure 20A, showing the membrane-proximal region of PD-1 at the bottom center.

[0051] Figure 21A is a graph depicting the effect of IgG1 3.7C6 anti-PD-1 antibody on secreted IFNγ in PBMCs from alopecia areata donors stimulated with keratinocyte antigens as compared to IgG1 isotype.

[0052] Figure 21B is a graph depicting the effect of PD-L1-IgG1 Fc tetramer on secreted IFNγ in PBMCs from alopecia areata donors stimulated with keratinocyte antigens as compared to IgG1 isotype tetramer.

[0053] Figure 21C is a graph depicting the effect of IgG1 3.7C6 anti-PD-1 antibody on the number of IFNγ spotforming cells (SFCs) in PBMCs isolated from alopecia areata donors stimulated with keratinocyte antigens.

[0054] Figure 21D is a graph depicting the effect of PD-L1 IgG1-Fc tetramer on the number of IFNγ spotforming cells (SFCs) in PBMCs isolated from alopecia areata donors stimulated with keratinocyte antigens.

[0055] Figure 22A is a graph depicting the effect of IgG1 3.7C6 anti-PD-1 antibody on secreted INFγ in a Tetanus Toxoid-specific antigen-recall assay as compared to IgG1 isotype.

[0056] Figure 22B is a graph depicting the effect of IgG1 3.7C6 anti-PD-1 antibody on secreted IL-17A in a Tetanus Toxoid-specific antigen-recall assay as compared to IgG1 isotype.

[0057] Figure 23A is a graph depicting the effect of IgG1 3.7C6 anti-PD-1 antibody on secreted IFNγ in PBMCs from alopecia areata donors stimulated with melanocyte antigens as compared to igG1 isotype.

[0058] Figure 23B is a graph depicting the effect of PD-L1-IgG1-Fc tetramer on secreted IFNγ in PBMCs from alopecia areata donors stimulated with melanocyte antigens as compared to IgG1 isotype tetramer.

[0059] Figure 23C is a graph depicting the effect of IgG1 3.7C6 anti-PD-1 antibody on the number of IFNγ SFCs in PBMCs isolated from alopecia areata donors stimulated with melanocyte antigens.

[0060] Figure 23D is a graph depicting the effect of PD-L1 IgG1-Fc tetramer on the number of IFNγ SFCs in PBMCs isolated from alopecia areata donors stimulated with melanocyte antigens.

[0061] Figure 24A is a schematic of the xenogeneic NSG/Hu-PBMC mouse model for the Graft vs. Host Disease study described in Example 15, in accordance with the embodiments of the invention.

[0062] Figure 24B is a schematic showing the timeline, dosing schedule, and model groups of the NSG/Hu-PBMC Graft vs. Host Disease study described in Example 15, in accordance with the embodiments of the invention.

[0063] Figure 24C is a graph depicting the results of the time to death of the NSG/Hu-PBMC Graft vs. Host Disease study in Example 15 for anti-PD-1 agonist IgG1 antibody 3.7C6.

[0064] Figure 24D is a graph depicting the results of the percent body weight change from start of study of individual animals for isotype control during the time of the study.

[0065] Figure 24E is a graph depicting the results of the percent body weight change from start of study of individual animals for anti-PD-1 agonist IgG1 antibody 3.7C6 at 30mg/kg dosage during the time of the study.

[0066] Figure 24F is a graph depicting the results of the percent body weight change from start of study of individual animals for anti-PD-1 agonist IgG1 antibody 3.7C6 at 10 mg/kg dosage during the time of the study.

[0067] Figure 24G is a graph depicting the results of the percent body weight change from start of study of individual animals for anti-PD-1 agonist IgG1 antibody 3.7C6 at 3 mg/kg dosage during the time of the study.

[0068] Figure 24H is a graph depicting the results of the percent body weight change from start of study of individual animals for positive control CTLA-4-Ig during the time of the study.

DETAILED DESCRIPTION OF THE INVENTION

[0069] The invention provides a PD-1 binding agent. As discussed above, programmed death 1 (PD-1) (also known as programmed cell death 1) is a 268 amino acid type I transmembrane protein (Ishida et al., *supra*). PD-1 is a member of the CD28/CTLA-4 family of T-cell regulators and is reported to be expressed on activated T-cells, B-cells, and myeloid lineage cells (Greenwald et al., *supra*; and Sharpe et al., *supra*). PD-1 includes an extracellular IgV domain followed by short extracellular stalk, a transmembrane region and an intracellular tail. The PD-1 intracellular tail contains two phosphorylation sites located in an immunoreceptor tyrosine-based inhibitory motif and an immunoreceptor tyrosine-based switch motif, which when phosphorylated function to negatively regulate T-cell receptor signaling (see, e.g., Ishida et al., *supra*; and Blank et al., *supra*) by recruiting tyrosine phosphatases.

[0070] In some embodiments, the PD-1 binding agent provided herein is agonistic, meaning that the PD-1 binding agent binds to PD-1 but does not significantly inhibit binding of PD-1 to PD-1 ligand, thereby maintaining the ability of PD-1 to negatively regulate T-cell receptor signaling. According to certain embodiments, the PD-1 binding agents provided herein can induce or stimulate the ability of PD-1 to negatively regulate T-cell-receptor signaling and suppress an immune response. In a particular embodiment, there is provided a PD-1 binding agent that binds PD-1 at an epitope comprising, consisting essentially of, or

consisting of, residues 33-41 of human PD1 (sequence: NPPTFSPAL) and/or 96-110 of human PD-1 (sequence: RVTQLPNGRDFHMSV).

[0071] The PD-1 binding agent comprises an immunoglobulin heavy chain variable region and an immunoglobulin light chain variable region, each of which comprise three complementarity determining regions (CDRs), usually referred to as CDR1, CDR2, or CDR3. The CDR regions also can be referred to using an "H" or "L" in the nomenclature to denote the heavy or light chain, respectively, i.e., CDRH1, CDRH2, CDRH3, CDRL1, CDRL2, or CDRL3. The CDRs of a given Ig sequence can be determined by any of several conventional numbering schemes, such as Kabat, Chothia, Martin (Enhanced Chothia), IGMT, or AHo (see, e.g., Kabat, et al., *Sequences of Proteins of Immunological Interest*, U.S. Department of Health and Human Services, NIH (1991); Chothia, et al., *Canonical Structures for the Hypervariable Regions of Immunoglobulins*, J. Mol. Biol., 196:901-917 (1987); Al-Lazikani et al., *Standard Conformations for the Canonical Structures of Immunoglobulins*, J. Mol. Biol., 273:927 – 948 (1997); Abhinandan et al., *Analysis and Improvements to Kabat and Structurally Correct Numbering of Antibody Variable Domains*, Mol. Immunol., 45: 3832 – 3839 (2008); Lefranc et al., *The IMGT unique numbering for immunoglobulins, T cell Receptors and Ig-like domains*, The Immunologist, 7: 132-136 (1999); Lefranc et al., *IMGT unique numbering for immunoglobulin and T cell receptor variable domains and I superfamily V-like domains*, Dev. Comp. Immunol., 27: 55 – 77 (2003); and Honegger et al., *Yet another numbering scheme for immunoglobulin variable domains: an automatic modeling and analysis tool*, J. Mol. Biol. 309: 657 – 670 (2001).

[0072] According to one aspect of the invention, the immunoglobulin heavy chain variable region of the PD-1 binding agent comprises a CDR1 comprising SEQ ID NO: 1; a CDR2 comprising SEQ ID NO: 2; and a CDR3 comprising SEQ ID NO: 3; and the immunoglobulin light chain variable region comprises a CDR1 comprising SEQ ID NO: 4; a CDR2 comprising SEQ ID NO: 5; and a CDR3 comprising SEQ ID NO: 6. In some embodiments, the heavy chain CDR1 comprises any one of SEQ ID NOs: 13-18. In addition, or alternatively, some embodiments of the heavy chain CDR3 comprises any one of SEQ ID NOs: 19-21. Furthermore, the light chain CDR1 can comprise SEQ ID NO: 22 or 23.

[0073] In particular embodiments, the PD-1 binding agent can comprise an immunoglobulin heavy chain variable region of any one of SEQ ID NOs: 24-33, or an amino acid sequence with at least 80% , 85%, or 90% sequence identity (e.g., at least 80%, at least 81%, at least 82%, at least 83%, at least 84%, at least 85%, at least 86%, at least 87%, at least

88%, at least 89%, at least 90%, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98%, at least 99%, or 100% sequence identity) to any one of SEQ ID NOs: 24-33. In other embodiments, the PD-1 binding agent comprises an immunoglobulin heavy chain variable region comprising the CDRs of any of SEQ ID NOs: 24-33, wherein the CDRs are as provided above or as determined in accordance with any of the various known immunoglobulin numbering schemes (e.g., Kabat, Chothia, Martin (Enhanced Chothia), IGMT, or AHo). Optionally, the immunoglobulin heavy chain variable region comprising the CDRs of any of SEQ ID NOs: 24-33 also has an amino acid sequence with at least 80% , 85%, or 90% sequence identity (e.g., at least 80%, at least 81%, at least 82%, at least 83%, at least 84%, at least 85%, at least 86%, at least 87%, at least 88%, at least 89%, at least 90%, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98%, at least 99%, or 100% sequence identity) to any of SEQ ID NOs: 24-33.

[0074] In addition to the Ig heavy chain variable region described above, or alternatively, the anti-PD-1 binding agent can comprise an immunoglobulin light chain variable region of SEQ ID NO: 34 or 35, or an amino acid sequence with at least 80% , 85%, or 90% sequence identity (e.g., at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98%, at least 99%, or 100% sequence identity) to SEQ ID NOs: 34 or 35. In other embodiments, the PD-1 binding agent comprises an immunoglobulin light chain variable region comprising the CDRs of SEQ ID NO: 34 or 35, wherein the CDRs are as provided above or as determined in accordance with any of the various known immunoglobulin numbering schemes (e.g., Kabat, Chothia, Martin (Enhanced Chothia), IGMT, or AHo). Optionally, the immunoglobulin light chain variable region comprising the CDRs of SEQ ID NO: 34 or 35 also has an amino acid sequence with at least 80% , 85% , or 90% sequence identity (e.g., at least 80%, at least 81%, at least 82%, at least 83%, at least 84%, at least 85%, at least 86%, at least 87%, at least 88%, at least 89%, at least 90%, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98%, at least 99%, or 100% sequence identity) to SEQ ID NO: 34 or 35.

[0075] According to one embodiment, the PD-1 binding agent can comprise an immunoglobulin heavy chain variable region of SEQ ID NO: 29 or an amino acid sequence with at least 80% , 85% , or 90% sequence identity (e.g., at least 80%, at least 81%, at least 82%, at least 83%, at least 84%, at least 85%, at least 86%, at least 87%, at least 88%, at least 89%, at least 90%, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least

96%, at least 97%, at least 98%, at least 99%, or 100% sequence identity) thereto; or an immunoglobulin heavy chain variable region comprising at least the CDRs of SEQ ID NO: 29, wherein the CDR regions are as provided above (e.g., CDR1 - SEQ ID NO: 15, CDR2 - SEQ ID NO: 2, and CDR3 - SEQ ID NO: 20) or as determined in accordance with any of the various known immunoglobulin numbering schemes (e.g., Kabat, Chothia, Martin (Enhanced Chothia), IGMT, or AHo); and an immunoglobulin light chain variable region of SEQ ID NO: 35 or an amino acid sequence with at least 80% , 85% , or 90% sequence identity (e.g., at least 80%, at least 81%, at least 82%, at least 83%, at least 84%, at least 85%, at least 86%, at least 87%, at least 88%, at least 89%, at least 90%, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98%, at least 99%, or 100% sequence identity) thereto, or an immunoglobulin light chain variable region comprising at least the CDRs of SEQ ID NO: 35; wherein the CDR regions are as provided above (e.g., CDR1 - SEQ ID NO: 23, CDR2 - SEQ ID NO: 5, and CDR3 - SEQ ID NO: 6) or as determined in accordance with any of the various known immunoglobulin numbering schemes (e.g., Kabat, Chothia, Martin (Enhanced Chothia), IGMT, or AHo). In some embodiments, the antibody comprises a heavy chain variable region of SEQ ID NO: 29 and light chain variable region of SEQ ID NO: 35, or at least the CDRs thereof as determined by Kabat. In some embodiments, the antibody comprises a heavy chain variable region of SEQ ID NO: 29 and light chain variable region of SEQ ID NO: 35, or at least the CDRs thereof as determined by Chothia. In some embodiments, the antibody comprises a heavy chain variable region of SEQ ID NO: 29 and light chain variable region of SEQ ID NO: 35, or at least the CDRs thereof as determined by Martin. In some embodiments, the antibody comprises a heavy chain variable region of SEQ ID NO: 29 and light chain variable region of SEQ ID NO: 35, or at least the CDRs thereof as determined by IGMT. In some embodiments, the antibody comprises a heavy chain variable region of SEQ ID NO: 29 and light chain variable region of SEQ ID NO: 35, or at least the CDRs thereof as determined by AHo. By way of further example, the anti-PD-1 binding agent can comprise an immunoglobulin heavy chain comprising SEQ ID NO: 36 and an immunoglobulin light chain comprising SEQ ID NO: 37, or an amino acid sequence with at least 80% , 85% , or 90% sequence identity (e.g., at least 80%, at least 81%, at least 82%, at least 83%, at least 84%, at least 85%, at least 86%, at least 87%, at least 88%, at least 89%, at least 90%, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98%, at least 99%, or 100% sequence identity) to SEQ ID NO: 36 and 37, respectively, optionally wherein the sequence retains the

heavy chain and light chain CDRs of SEQ ID NO: 36 and 37, respectively, wherein the CDRs are as provided above or as determined in accordance with any of the various known immunoglobulin numbering schemes (e.g., Kabat, Chothia, Martin (Enhanced Chothia), IGMT, or AHo).

[0076] According to another embodiment, the PD-1 binding agent can comprise an immunoglobulin heavy chain variable region of SEQ ID NO: 24 or an amino acid sequence with at least 80% , 85% , or 90% sequence identity (e.g., at least 80%, at least 81%, at least 82%, at least 83%, at least 84%, at least 85%, at least 86%, at least 87%, at least 88%, at least 89%, at least 90%, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98%, at least 99%, or 100% sequence identity) thereto; or an immunoglobulin heavy chain variable region comprising at least the CDRs of SEQ ID NO: 24, wherein the CDR regions are as provided above (e.g., CDR1 - SEQ ID NO: 13, CDR2 - SEQ ID NO: 2, and CDR3 - SEQ ID NO: 19) or as determined in accordance with any of the various known immunoglobulin numbering schemes (e.g., Kabat, Chothia, Martin (Enhanced Chothia), IGMT, or AHo); and an immunoglobulin light chain variable region of SEQ ID NO: 34 or an amino acid sequence with at least 80% , 85% , or 90% sequence identity (e.g., at least 80%, at least 81%, at least 82%, at least 83%, at least 84%, at least 85%, at least 86%, at least 87%, at least 88%, at least 89%, at least 90%, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98%, at least 99%, or 100% sequence identity) thereto, or an immunoglobulin light chain variable region comprising at least the CDRs of SEQ ID NO: 34; wherein the CDR regions are as provided above (e.g., CDR1 - SEQ ID NO: 22, CDR2 - SEQ ID NO: 5, and CDR3 - SEQ ID NO: 6) or as determined in accordance with any of the various known immunoglobulin numbering schemes (e.g., Kabat, Chothia, Martin (Enhanced Chothia), IGMT, or AHo). In some embodiments, the antibody comprises a heavy chain variable region of SEQ ID NO: 24 and light chain variable region of SEQ ID NO: 34, or at least the CDRs thereof as determined by Kabat. In some embodiments, the antibody comprises a heavy chain variable region of SEQ ID NO: 24 and light chain variable region of SEQ ID NO: 34, or at least the CDRs thereof as determined by Chothia. In some embodiments, the antibody comprises a heavy chain variable region of SEQ ID NO: 24 and light chain variable region of SEQ ID NO: 34, or at least the CDRs thereof as determined by Martin. In some embodiments, the antibody comprises a heavy chain variable region of SEQ ID NO: 24 and light chain variable region of SEQ ID NO: 34, or at least the CDRs thereof as determined by IGMT. In some embodiments, the

antibody comprises a heavy chain variable region of SEQ ID NO: 24 and light chain variable region of SEQ ID NO: 34, or at least the CDRs thereof as determined by AHo.

[0077] According to one embodiment, the PD-1 binding agent can comprise an immunoglobulin heavy chain variable region of SEQ ID NO: 30 or an amino acid sequence with at least 80% , 85% , or 90% sequence identity (e.g., at least 80%, at least 81%, at least 82%, at least 83%, at least 84%, at least 85%, at least 86%, at least 87%, at least 88%, at least 89%, at least 90%, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98%, at least 99%, or 100% sequence identity) thereto; or an immunoglobulin heavy chain variable region comprising at least the CDRs of SEQ ID NO: 30, wherein the CDR regions are as provided above (e.g., CDR1 - SEQ ID NO: 15, CDR2 - SEQ ID NO: 2, and CDR3 - SEQ ID NO: 21) or as determined in accordance with any of the various known immunoglobulin numbering schemes (e.g., Kabat, Chothia, Martin (Enhanced Chothia), IGMT, or AHo); and an immunoglobulin light chain variable region of SEQ ID NO: 35 or an amino acid sequence with at least 80% , 85% , or 90% sequence identity (e.g., at least 80%, at least 81%, at least 82%, at least 83%, at least 84%, at least 85%, at least 86%, at least 87%, at least 88%, at least 89%, at least 90%, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98%, at least 99%, or 100% sequence identity) thereto, or an immunoglobulin light chain variable region comprising at least the CDRs of SEQ ID NO: 35; wherein the CDR regions are as provided above (e.g., CDR1 - SEQ ID NO: 23, CDR2 - SEQ ID NO: 5, and CDR3 - SEQ ID NO: 6) or as determined in accordance with any of the various known immunoglobulin numbering schemes (e.g., Kabat, Chothia, Martin (Enhanced Chothia), IGMT, or AHo). In some embodiments, the antibody comprises a heavy chain variable region of SEQ ID NO: 30 and light chain variable region of SEQ ID NO: 35, or at least the CDRs thereof as determined by Kabat. In some embodiments, the antibody comprises a heavy chain variable region of SEQ ID NO: 30 and light chain variable region of SEQ ID NO: 35, or at least the CDRs thereof as determined by Chothia. In some embodiments, the antibody comprises a heavy chain variable region of SEQ ID NO: 30 and light chain variable region of SEQ ID NO: 35, or at least the CDRs thereof as determined by Martin. In some embodiments, the antibody comprises a heavy chain variable region of SEQ ID NO: 30 and light chain variable region of SEQ ID NO: 35, or at least the CDRs thereof as determined by IGMT. In some embodiments, the antibody comprises a heavy chain variable region of SEQ ID NO: 30 and light chain variable region of SEQ ID NO: 35, or at least the CDRs thereof as determined by AHo.

[0078] According to another aspect, the anti-PD-1 binding agent comprises an immunoglobulin heavy chain variable region comprising: a CDR1 comprising SEQ ID NO: 7; a CDR2 comprising SEQ ID NO: 8; and a CDR3 comprising SEQ ID NO: 9; and an immunoglobulin light chain variable region comprising a CDR1 comprising SEQ ID NO: 10; a CDR2 comprising SEQ ID NO: 11; and a CDR3 comprising SEQ ID NO: 12. In some embodiments, the heavy chain CDR1 comprises any of SEQ ID NOs: 57-60. In some embodiments, the heavy chain CDR2 comprises any one of SEQ ID NOs: 38-42.

[0079] In some embodiments, the PD-1 binding agent comprises an immunoglobulin heavy chain variable region of any one of SEQ ID NOs: 43-47 or 61-63, or an amino acid sequence with at least 80% , 85% , or 90% sequence identity (e.g., at least 80%, at least 81%, at least 82%, at least 83%, at least 84%, at least 85%, at least 86%, at least 87%, at least 88%, at least 89%, at least 90%, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98%, at least 99%, or 100% sequence identity) to any one of SEQ ID NOs: 43-47 or 61-63. In some embodiments, the PD-1 binding agent comprises an immunoglobulin heavy chain variable region comprising the CDRs of any of SEQ ID NOs: 43-47 or 61-63, wherein the CDRs are as provided above or as determined in accordance with any of the various known immunoglobulin numbering schemes (e.g., Kabat, Chothia, Martin (Enhanced Chothia), IGMT, or AHo). Optionally, the immunoglobulin heavy chain variable region comprising the CDRs of any of SEQ ID NOs: 43-47 or 61-63 also has an amino acid sequence with at least 80% , 85% , or 90% sequence identity (e.g., at least 80%, at least 81%, at least 82%, at least 83%, at least 84%, at least 85%, at least 86%, at least 87%, at least 88%, at least 89%, at least 90%, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98%, at least 99%, or 100% sequence identity) to any one of SEQ ID NOs: 43-47 or 61-63.

[0080] In addition to the Ig heavy chain variable region described above (e.g., SEQ ID NOs: 43-47 or 61-63), or alternatively, the anti-PD-1 binding agent can comprise an immunoglobulin light chain variable region of any of SEQ ID NOs: 48-50, or an amino acid sequence with at least 80% , 85% , or 90% sequence identity (e.g., at least 80%, at least 81%, at least 82%, at least 83%, at least 84%, at least 85%, at least 86%, at least 87%, at least 88%, at least 89%, at least 90%, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98%, at least 99%, or 100% sequence identity) to any one of SEQ ID NOs: 48-50. In other embodiments, the PD-1 binding agent comprises an immunoglobulin light chain variable region comprising the CDRs of any of SEQ ID NOs: 48-

50, wherein the CDRs are as provided above or as determined in accordance with any of the various known immunoglobulin numbering schemes (e.g., Kabat, Chothia, Martin (Enhanced Chothia), IGMT, or AHo). Optionally, the immunoglobulin light chain variable region comprising the CDRs of any of SEQ ID NOs: 48-50 also has an amino acid sequence with at least 80% , 85% , or 90% sequence identity (e.g., at least 80%, at least 81%, at least 82%, at least 83%, at least 84%, at least 85%, at least 86%, at least 87%, at least 88%, at least 89%, at least 90%, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98%, at least 99%, or 100% sequence identity) to any of SEQ ID NOs: 48-50.

[0081] In a particular embodiment, the anti-PD-1 binding agent comprises an immunoglobulin heavy chain variable region of SEQ ID NO: 47, or an amino acid sequence with at least 80% , 85% , or 90% sequence identity (e.g., ., at least 80%, at least 81%, at least 82%, at least 83%, at least 84%, at least 85%, at least 86%, at least 87%, at least 88%, at least 89%, at least 90%, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98%, at least 99%, or 100% sequence identity) to SEQ ID NO: 47; or an immunoglobulin heavy chain variable region comprising at least the CDRs of SEQ ID NO: 47, wherein the CDR regions are as provided above (e.g., CDR1 - SEQ ID NO: 57, CDR2 - SEQ ID NO: 42, and CDR3 - SEQ ID NO: 9) or as determined in accordance with any of the various known immunoglobulin numbering schemes (e.g., Kabat, Chothia, Martin (Enhanced Chothia), IGMT, or AHo); and an immunoglobulin light chain variable region of SEQ ID NO: 49, or an amino acid sequence with at least 80% , 85% , or 90% sequence identity (e.g., at least 80%, at least 81%, at least 82%, at least 83%, at least 84%, at least 85%, at least 86%, at least 87%, at least 88%, at least 89%, at least 90%, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98%, at least 99%, or 100% sequence identity) to SEQ ID NO: 49; or an immunoglobulin heavy chain variable region comprising at least the CDRs of SEQ ID NO: 49; wherein the CDR regions are as provided above (e.g., CDR1 - SEQ ID NO: 10, CDR2 - SEQ ID NO: 11, and CDR3 - SEQ ID NO: 12) or as determined in accordance with any of the various known immunoglobulin numbering schemes (e.g., Kabat, Chothia, Martin (Enhanced Chothia), IGMT, or AHo). In some embodiments, the antibody comprises a heavy chain variable region of SEQ ID NO: 47 and light chain variable region of SEQ ID NO: 49, or at least the CDRs thereof as determined by Kabat. In some embodiments, the antibody comprises a heavy chain variable region of SEQ ID NO: 47 and light chain variable region of SEQ ID

NO: 49, or at least the CDRs thereof as determined by Chothia. In some embodiments, the antibody comprises a heavy chain variable region of SEQ ID NO: 47 and light chain variable region of SEQ ID NO: 49, or at least the CDRs thereof as determined by Martin. In some embodiments, the antibody comprises a heavy chain variable region of SEQ ID NO: 47 and light chain variable region of SEQ ID NO: 49, or at least the CDRs thereof as determined by IGMT. In some embodiments, the antibody comprises a heavy chain variable region of SEQ ID NO: 47 and light chain variable region of SEQ ID NO: 49, or at least the CDRs thereof as determined by AHo. By way of further example, the anti-PD-1 binding agent can comprise an immunoglobulin heavy chain comprising SEQ ID NO: 51 and an immunoglobulin light chain comprising SEQ ID NO: 52, or an amino acid sequence with at least 80% , 85% , or 90% sequence identity (e.g., at least 80%, at least 81%, at least 82%, at least 83%, at least 84%, at least 85%, at least 86%, at least 87%, at least 88%, at least 89%, at least 90%, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98%, at least 99%, or 100% sequence identity) to SEQ ID NOs: 51 and 52, optionally wherein the sequence retains the heavy chain and light chain CDRs of SEQ ID NOs: 51 and 52 as provided above or as determined in accordance with any of the various known immunoglobulin numbering schemes (e.g., Kabat, Chothia, Martin (Enhanced Chothia), IGMT, or AHo).

[0082] In another embodiment, the anti-PD-1 binding agent comprises an immunoglobulin heavy chain variable region of SEQ ID NO: 46, or an amino acid sequence with at least 80% , 85% , or 90% sequence identity (e.g., ., at least 80%, at least 81%, at least 82%, at least 83%, at least 84%, at least 85%, at least 86%, at least 87%, at least 88%, at least 89%, at least 90%, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98%, at least 99%, or 100% sequence identity) to SEQ ID NO: 46; or an immunoglobulin heavy chain variable region comprising at least the CDRs of SEQ ID NO: 46, wherein the CDR regions are as provided above (e.g., CDR1 - SEQ ID NO: 57, CDR2 - SEQ ID NO: 41, and CDR3 - SEQ ID NO: 9) or as determined in accordance with any of the various known immunoglobulin numbering schemes (e.g., Kabat, Chothia, Martin (Enhanced Chothia), IGMT, or AHo); and an immunoglobulin light chain variable region of SEQ ID NO: 50, or an amino acid sequence with at least 80% , 85% , or 90% sequence identity (e.g., at least 80%, at least 81%, at least 82%, at least 83%, at least 84%, at least 85%, at least 86%, at least 87%, at least 88%, at least 89%, at least 90%, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98%, at least

99%, or 100% sequence identity) to SEQ ID NO: 50; or an immunoglobulin heavy chain variable region comprising at least the CDRs of SEQ ID NO: 50; wherein the CDR regions are as provided above (e.g., CDR1 - SEQ ID NO: 10, CDR2 - SEQ ID NO: 11, and CDR3 - SEQ ID NO: 12) or as determined in accordance with any of the various known immunoglobulin numbering schemes (e.g., Kabat, Chothia, Martin (Enhanced Chothia), IGMT, or AHo). In some embodiments, the antibody comprises a heavy chain variable region of SEQ ID NO: 46 and light chain variable region of SEQ ID NO: 50, or at least the CDRs thereof as determined by Kabat. In some embodiments, the antibody comprises a heavy chain variable region of SEQ ID NO: 46 and light chain variable region of SEQ ID NO: 50, or at least the CDRs thereof as determined by Chothia. In some embodiments, the antibody comprises a heavy chain variable region of SEQ ID NO: 46 and light chain variable region of SEQ ID NO: 50, or at least the CDRs thereof as determined by Martin. In some embodiments, the antibody comprises a heavy chain variable region of SEQ ID NO: 46 and light chain variable region of SEQ ID NO: 50, or at least the CDRs thereof as determined by IGMT. In some embodiments, the antibody comprises a heavy chain variable region of SEQ ID NO: 46 and light chain variable region of SEQ ID NO: 50, or at least the CDRs thereof as determined by AHo.

[0083] Sequence "identity," as described herein, can be determined by comparing a nucleic acid or amino acid sequence of interest to a reference nucleic acid or amino acid sequence. The percent identity is the number of nucleotides or amino acid residues that are the same (i.e., that are identical) as between the sequence of interest and the reference sequence divided by the length of the longest sequence (i.e., the length of either the sequence of interest or the reference sequence, whichever is longer). A number of mathematical algorithms for obtaining the optimal alignment and calculating identity between two or more sequences are known and incorporated into a number of available software programs. Examples of such programs include CLUSTAL-W, T-Coffee, and ALIGN (for alignment of nucleic acid and amino acid sequences), BLAST programs (e.g., BLAST 2.1, BL2SEQ, and later versions thereof) and FASTA programs (e.g., FASTA3x, FASTM, and SSEARCH) (for sequence alignment and sequence similarity searches). Sequence alignment algorithms also are disclosed in, for example, Altschul et al., *J. Molecular Biol.*, *215*(3): 403-410 (1990), Beigert et al., *Proc. Natl. Acad. Sci. USA*, *106*(10): 3770-3775 (2009), Durbin et al., eds., *Biological Sequence Analysis: Probalistic Models of Proteins and Nucleic Acids*, Cambridge University Press, Cambridge, UK (2009), Soding, *Bioinformatics*, *21*(7): 951-960 (2005),

Altschul et al., *Nucleic Acids Res.*, *25*(17): 3389-3402 (1997), and Gusfield, *Algorithms on Strings, Trees and Sequences*, Cambridge University Press, Cambridge UK (1997)).

[0084] Variation in sequence identity can be accomplished through addition, substitution, or deletion of one or more amino acid residues. An amino acid "replacement" or "substitution" refers to the replacement of one amino acid at a given position or residue by another amino acid at the same position or residue within a polypeptide sequence. The amino acid replacement or substitution can be conservative, semi-conservative, or non-conservative depending upon whether the substitution is by an amino acid residue that has similar properties to the residue being replaced. A functional way to define common properties between individual amino acids is to analyze the normalized frequencies of amino acid changes between corresponding proteins of homologous organisms (Schulz and Schirmer, *Principles of Protein Structure*, Springer-Verlag, New York (1979)). According to such analyses, groups of amino acids may be defined where amino acids within a group exchange preferentially with each other, and therefore resemble each other most in their impact on the overall protein structure (Schulz and Schirmer, *supra*).

[0085] Amino acids can be broadly grouped as "aromatic" or "aliphatic." An aromatic amino acid includes an aromatic ring. Examples of "aromatic" amino acids include histidine (H or His), phenylalanine (F or Phe), tyrosine (Y or Tyr), and tryptophan (W or Trp). Non-aromatic amino acids are broadly grouped as "aliphatic." Examples of "aliphatic" amino acids include glycine (G or Gly), alanine (A or Ala), valine (V or Val), leucine (L or Leu), isoleucine (I or Ile), methionine (M or Met), serine (S or Ser), threonine (T or Thr), cysteine (C or Cys), proline (P or Pro), glutamic acid (E or Glu), aspartic acid (A or Asp), asparagine (N or Asn), glutamine (Q or Gln), lysine (K or Lys), and arginine (R or Arg).

[0086] Aliphatic amino acids may be sub-divided into four sub-groups. The "large aliphatic non-polar sub-group" consists of valine, leucine, and isoleucine. The "aliphatic slightly-polar sub-group" consists of methionine, serine, threonine, and cysteine. The "aliphatic polar/charged sub-group" consists of glutamic acid, aspartic acid, asparagine, glutamine, lysine, and arginine. The "small-residue sub-group" consists of glycine and alanine. The group of charged/polar amino acids may be sub-divided into three sub-groups: the "positively-charged sub-group" consisting of lysine and arginine, the "negatively-charged sub-group" consisting of glutamic acid and aspartic acid, and the "polar sub-group" consisting of asparagine and glutamine.

[0087] Aromatic amino acids may be sub-divided into two sub-groups: the "nitrogen ring sub-group" consisting of histidine and tryptophan and the "phenyl sub-group" consisting of phenylalanine and tyrosine.

[0088] Examples of conservative amino acid substitutions include substitutions of amino acids within the sub-groups described above, for example, lysine for arginine and vice versa such that a positive charge may be maintained, glutamic acid for aspartic acid and vice versa such that a negative charge may be maintained, serine for threonine such that a free -OH can be maintained, and glutamine for asparagine such that a free $-NH_2$ can be maintained. "Semi-conservative mutations" include amino acid substitutions of amino acids within the same groups listed herein, but not within the same sub-group. For example, the substitution of aspartic acid for asparagine, or asparagine for lysine, involves amino acids within the same group, but different sub-groups. "Non-conservative mutations" involve amino acid substitutions between different groups, for example, lysine for tryptophan, or phenylalanine for serine, etc.

[0089] In some embodiments, the PD-1-binding agent can comprise, consist essentially of, or consist of the immunoglobulin heavy and light chain variable region or full heavy and light chain polypeptides provided herein. The isolated PD-1-binding agent can be any type of molecule or construct comprising at least the specified immunoglobulin heavy and light chain variable regions. Thus, the PD-1 binding agent can be, for instance, a whole immunoglobulin or antibody, as described herein, or an antigen-binding (PD-1 binding) immunoglobulin or antibody "fragment." The term "fragment" used with respect to an antibody or immunoglobulin means any molecule or construct that comprises some part of an immunoglobulin or antibody and binds the target antigen. Such a fragment will generally comprise at least the parts of the heavy and light chain variable regions including the CDRs, and may also include parts of the constant regions, optionally along with other elements that are not normally part of an immunoglobulin or antibody (e.g., linkers, etc.). Examples of such "fragments" include, but are not limited to, (i) a Fab fragment, which is a monovalent fragment consisting of the V_L, V_H, C_L, and CH_1 domains, (ii) a $F(ab')_2$ fragment, which is a bivalent fragment comprising two Fab fragments linked by a disulfide bridge at the hinge region, (iii) a Fv fragment consisting of the V_L and V_H domains of a single arm of an antibody, (iv) a Fab' fragment, which results from breaking the disulfide bridge of an $F(ab')_2$ fragment using mild reducing conditions; (v) a diabody; (vi) a single-chain variable region (scFv), and (vii) a disulfide-stabilized Fv fragment (dsFv).

[0090] In some embodiments, the PD-1-binding agent comprises an immunoglobulin heavy chain constant region, such as a fragment crystallizable (F_c) region or portion thereof. The Fc region can be of any Ig class/subclass (IgA (IgA1, IgA2), IgD, IgE, IgG (IgG1, IgG2, IgG3 and IgG4),IgM , including variants thereof. In a particular embodiment, the PD-1 binding agent comprises an Fc region that binds an Fc receptor of an antigen-presenting cell (e.g., dendritic cell, macrophage, Langerhans cell, or B cell). The Fc receptor can be an Fcγ receptor (FcγR), such as FcγRI (CD64), FcγRIIA (CD32), FcγRIIB (CD32), FcγRIIIA (CD16a), FcγRIIIB (CD16b). In one embodiment, the PD-1 binding agent comprises an Fc region that binds FcγR, such as IgG1. Thus, in some embodiments, the PD-1 binding agent is a "whole" or "complete" Ig (i.e., an antibody). In additional embodiments, the PD-1 binding agent is an IgG antibody,particularly an IgG1 antibody.

[0091] The isolated PD-1-binding agent also can be an antibody conjugate. In this respect, the isolated PD-1-binding agent can be a conjugate comprising the PD-1-binding agent (e.g., anti-PD-1 antibody or antibody fragment) and another biologically active moiety. For example, the PD-1-binding agent can be conjugated to a peptide, a fluorescent molecule, or a chemotherapeutic agent, particularly an agent useful in suppressing an immune response.

[0092] The isolated PD-1-binding agent can be, or can be obtained from, a human antibody, a non-human antibody, or a chimeric antibody. By "chimeric" is meant an antibody or fragment thereof comprising both human and non-human regions. Preferably, the isolated PD-1-binding agent is a humanized antibody. A "humanized" antibody is a monoclonal antibody comprising a human antibody scaffold and at least one CDR obtained or derived from a non-human antibody. Non-human antibodies include antibodies isolated from any non-human animal, such as, for example, a rodent (e.g., a mouse or rat). A humanized antibody can comprise, one, two, or three CDRs obtained or derived from a non-human antibody. In a preferred embodiment of the invention, CDRH3 of the inventive PD-1-binding agent is obtained or derived from a mouse monoclonal antibody, while the remaining variable regions and constant region of the inventive PD-1-binding agent are obtained or derived from a human monoclonal antibody.

[0093] A human antibody, a non-human antibody, a chimeric antibody, or a humanized antibody can be obtained by any means, including via *in vitro* sources (e.g., a hybridoma or a cell line producing an antibody recombinantly) and *in vivo* sources (e.g., rodents). Methods for generating antibodies are known in the art and are described in, for example, Köhler and Milstein, *Eur. J. Immunol.*, *5*: 511-519 (1976); Harlow and Lane (eds.), *Antibodies: A*

Laboratory Manual, CSH Press (1988); and Janeway et al. (eds.), *Immunobiology, 5th Ed.*, Garland Publishing, New York, NY (2001); Starkie et al., *PLoS One,* 11(3): e0152282 (2016)). In certain embodiments, a human antibody or a chimeric antibody can be generated using a transgenic animal (e.g., a mouse) wherein one or more endogenous immunoglobulin genes are replaced with one or more human immunoglobulin genes. Examples of transgenic mice wherein endogenous antibody genes are effectively replaced with human antibody genes include, but are not limited to, the Medarex HUMAB-MOUSE™, the Kirin TC MOUSE™, and the Kyowa Kirin KM-MOUSE™ (see, e.g., Lonberg, *Nat. Biotechnol.*, *23(9)*: 1117-25 (2005), and Lonberg, *Handb. Exp. Pharmacol.*, *181*: 69-97 (2008)). A humanized antibody can be generated using any suitable method known in the art (see, e.g., An, Z. (ed.), *Therapeutic Monoclonal Antibodies: From Bench to Clinic*, John Wiley & Sons, Inc., Hoboken, New Jersey (2009)), including, e.g., grafting of non-human CDRs onto a human antibody scaffold (see, e.g., Kashmiri et al., *Methods*, *36*(1): 25-34 (2005); and Hou et al., *J. Biochem.*, *144*(1): 115-120 (2008)). In one embodiment, a humanized antibody can be produced using the methods described in, e.g., U.S. Patent Application Publication 2011/0287485 A1.

[0094] The PD-1 binding agent can have any suitable affinity for human PD-1. The term "affinity" refers to the equilibrium constant for the reversible binding of two agents and is expressed as the dissociation constant (K_D). Affinity of a binding agent to a ligand, such as affinity of an antibody for an epitope, can be, for example, from about 1 picomolar (pM) to about 100 micromolar (μM) (e.g., from about 1 picomolar (pM) to about 1 nanomolar (nM), from about 1 nM to about 1 micromolar (μM), or from about 1 μM to about 100 μM). In one embodiment, the PD-1-binding agent can bind to an PD-1 protein with a K_D less than or equal to 1 nM (e.g., 0.9 nM, 0.8 nM, 0.7 nM, 0.6 nM, 0.5 nM, 0.4 nM, 0.3 nM, 0.2 nM, 0.1 nM, 0.05 nM, 0.025 nM, 0.01 nM, 0.001 nM, or a range defined by any two of the foregoing values). In another embodiment, the PD-1-binding agent can bind to PD-1 with a K_D less than or equal to 200 pM (e.g., 190 pM, 175 pM, 150 pM, 125 pM, 110 pM, 100 pM, 90 pM, 80 pM, 75 pM, 60 pM, 50 pM, 40 pM, 30 pM, 25 pM, 20 pM, 15 pM, 10 pM, 5 pM, 1 pM, or a range defined by any two of the foregoing values). In some embodiments, the PD-1 binding agent is cross-reactive with cynomolgus PD-1 with an affinity in any of the foregoing ranges discussed with respect to human PD-1. Immunoglobulin affinity for an antigen or epitope of interest can be measured using any art-recognized assay. Such methods include, for example, fluorescence activated cell sorting (FACS), separable beads (e.g., magnetic

beads), surface plasmon resonance (SPR), solution phase competition (KinExA®), antigen panning, and/or ELISA (see, e.g., Janeway et al. (eds.), *Immunobiology*, 5th ed., Garland Publishing, New York, NY, 2001).

[0095] The PD-1 binding agent binds PD-1, but preferably does not completely inhibit the ability of PD-1 to negatively regulate an immune response or, in some cases, does not substantially inhibit the ability of PD-1 to negatively regulate an immune response or even enhances the ability of PD-1 to negatively regulate an immune response. In some embodiments, the PD-1 binding agent does not completely block binding between PD-1 and PD-L1, or, preferably, does not substantially reduce binding between PD-1 and PD-L1. Assessment of the degree to which a PD-1 binding agent inhibits PD-1 regulation of an immune response or PD-1 binding to PD-L1 can be performed using assays such as those set forth in the examples or other assays known in the art. In some embodiments, the PD-1 binding agent inhibits PD-1 binding to PD-L1 by no more than about 80%, by no more than about 75%, by no more than about 70%, by no more than about 65%, by no more than about 60%, by no more than about 55%, by no more than about 50%, by no more than about 45%, by no more than about 40%, by no more than about 35%, by no more than about 30%, by no more than about 25%, by no more than about 20%, by no more than about 15%, by no more than about 10%.

Methods of Use/Treatment

[0096] The invention provides a method of suppressing an immune response, particularly a T-cell mediated immune response, in a mammal by administering to the mammal the PD-1 binding agent described herein. The invention further provides a method of treating a disease or disorder in which a decrease in PD-1 activity (e.g., a decrease in PD-1 signaling through decreased PD-L1 binding, such as a decrease in negative regulation of the immune system) causes or contributes to the pathological effects of the disease, or any disease or disorder in which an increase in PD-1 activity (e.g., an increase in PD-1 signaling through PD-L1 binding, such as an increase in negative regulation of the immune system) would have a therapeutic benefit, which method comprises administering to a mammal the PD-1 binding agent described herein to reduce or eliminate any symptom of the disorder, or prevent or inhibit the onset of such symptoms. Negative regulation of the immune system as used herein in synonymous with immunosuppression. It will be appreciated that the PD-1 binding agent can be administered prior to the onset of symptoms in some instances (e.g., prior to

exposure to an antigen that triggers an immune response) so as to prevent, suppress, or reduce the severity of an immune response upon introduction of the antigen.

[0097] The disease or disorder can be an inflammatory or autoimmune disorder. Examples of inflammatory or autoimmune disorders include, for example, infections (viral, bacterial, fungal and parasitic), endotoxic shock associated with infection, arthritis, rheumatoid arthritis, asthma, Chronic obstructive pulmonary disease (COPD), pelvic inflammatory disease, Behcet disease, Alzheimer's Disease, inflammatory bowel disease including Crohn's disease and ulcerative colitis, Peyronie's Disease, coeliac disease, gallbladder disease, Pilonidal disease, peritonitis, psoriasis, psoriatic arthritis, vasculitis, antineutrophil cytoplasmic antibody-associated (ANCA) vasculitis, surgical adhesions, stroke, Type I Diabetes, lyme disease, arthritis, meningoencephalitis, autoimmune uveitis, immune mediated inflammatory disorders of the central and peripheral nervous system such as multiple sclerosis, lupus (such as systemic lupus erythematosus and chronic discoid lupus erythematosus) and Guillain-Barr syndrome, Atopic dermatitis, polymyositis, dermatomyositis, autoimmune hepatitis, fibrosing alveolitis, Grave's disease, IgA nephropathy, idiopathic thrombocytopenic purpura, Meniere's disease, pemphigus, pemphigoid, primary biliary cholangitis, hepatitis, sarcoidosis, scleroderma (localized scleroderma, systemic scleroderma, and progressive systemic scleroderma), Ganulomatosis with polyangiitis, other autoimmune disorders, cholangitis, pancreatitis, trauma (surgery), graft-versus-host disease, transplant rejection, heart disease including ischaemic diseases such as myocardial infarction as well as atherosclerosis, periarteritis nodosa (polyarteritis nodosa and microscopic polyangiitis), allergic granulomatous angiitis, hypersensitivity angiitis, aortitis syndrome (Takayasu arteritis), temporal arteritis, intravascular coagulation, bone resorption, osteoporosis, osteoarthritis, periodontitis and hypochlorhydia, Still's disease, Cogan's syndrome, RS3PE, polymyalgia rheumatica, fibromyalgia syndrome, antiphospholipid antibody syndrome, eosinophilic fasciitis, Guillain-Barre syndrome, myasthenia gravis, chronic atrophic gastritis, Goodpasture's syndrome, rapidly progressive glomerulonephritis, megaloblastic anemia, hemolytic anemia, autoimmune neutropenia, Hashimoto's thyroiditis, autoimmune adrenal insufficiency, primary hypothyroidism, idiopathic Addison's disease (chronic adrenal insufficiency), herpes gestationis, linear IgA bullous skin disease, epidermolysis bullosa acquisita, alopecia areata, vitiligo, Harada disease, autoimmune optic neuropathy, idiopathic azoospermia, recurrent fetal loss, or infertility related to lack of fetal-maternal tolerance.

[0098] In some embodiments, the disease or disorder is Giant Cell Arteritis, Polymyalgia Rheumatica, Primary Sjögren's Syndrome, TNF-refractory Rheumatoid Arthritis, Alopecia Areata, Primary Biliary Cholangitis (PBC), Graft vs Host Disease (GvHD), Vitiligo, ANCA Vasculitis, Type 1 Diabetes, or Noninfectious Uveitis.

[0099] An "immune response" can entail, for example, antibody production and/or the activation of immune effector cells (e.g., T-cells), production of inflammatory cytokines, or any of the indications or disorders described herein or otherwise known in the art. As used herein, the terms "treatment," "treating," and the like refer to obtaining a desired pharmacologic and/or physiologic effect. Preferably, the effect is therapeutic, i.e., the effect partially or completely cures a disease and/or adverse symptom attributable to the disease. To this end, the inventive method comprises administering a "therapeutically effective amount" of the PD-1-binding agent. A "therapeutically effective amount" refers to an amount effective, at dosages and for periods of time necessary, to achieve a desired therapeutic result. The therapeutically effective amount may vary according to factors such as the disease state, age, sex, and weight of the individual, and the ability of the PD-1-binding agent to elicit a desired response in the individual.

[00100] Alternatively, the pharmacologic and/or physiologic effect may be prophylactic, i.e., the effect completely or partially prevents a disease or symptom thereof. In this respect, the inventive method comprises administering a "prophylactically effective amount" of the PD-1-binding agent. A "prophylactically effective amount" refers to an amount effective, at dosages and for periods of time necessary, to achieve a desired prophylactic result (e.g., prevention of disease onset).

[00101] The PD-1 binding agent can be part of a composition suitable for administration to a mammal. Preferably, the composition is a pharmaceutically acceptable (e.g., physiologically acceptable) composition, which comprises a carrier, preferably a pharmaceutically acceptable (e.g., physiologically acceptable) carrier, and the inventive amino acid sequences, antigen-binding agent, or vector. Any suitable carrier can be used within the context of the invention, and such carriers are well known in the art. The choice of carrier will be determined, in part, by the particular site to which the composition may be administered and the particular method used to administer the composition. The composition also can comprise any other excipient used in the formulation of therapeutic molecules (e.g., proteins or antibodies), particularly parenteral formulations, including, for instance, buffers, tonicity modifiers, stabilizers, surfactants and the like. The composition optionally can be

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

[00102] A typical dose of the PD-1 binding agent can be, for example, in the range of 1 pg/kg to 20 mg/kg of animal or human body weight; however, doses below or above this exemplary range are within the scope of the invention. The daily parenteral dose can be about 0.00001 μg/kg to about 20 mg/kg of total body weight (e.g., about 0.001 μg /kg, about 0.1 μg /kg , about 1 μg /kg, about 5 μg /kg, about 10 μg/kg, about 100 μg /kg, about 500 μg/kg, about 1 mg/kg, about 5 mg/kg, about 10 mg/kg, or a range defined by any two of the foregoing values), preferably from about 0.1 μg/kg to about 10 mg/kg of total body weight (e.g., about 0.5 μg/kg, about 1 μg/kg, about 50 μg/kg, about 150 μg/kg, about 300 μg/kg, about 750 μg/kg, about 1.5 mg/kg, about 5 mg/kg, or a range defined by any two of the foregoing values), more preferably from about 1 μg/kg to 5 mg/kg of total body weight (e.g., about 3 μg/kg, about 15 μg/kg, about 75 μg/kg, about 300 μg/kg, about 900 μg/kg, about 2 mg/kg, about 4 mg/kg, or a range defined by any two of the foregoing values), and even more preferably from about 0.5 to 15 mg/kg body weight per day (e.g., about 1 mg/kg, about 2.5 mg/kg, about 3 mg/kg, about 6 mg/kg, about 9 mg/kg, about 11 mg/kg, about 13 mg/kg, or a range defined by any two of the foregoing values). Therapeutic or prophylactic efficacy can be monitored by periodic assessment of treated patients. For repeated administrations over several days or longer, depending on the condition, the treatment can be repeated until a desired suppression of disease symptoms occurs. However, other dosage regimens may be useful and are within the scope of the invention. The desired dosage can be delivered by a single bolus administration of the composition, by multiple bolus administrations of the composition, or by continuous infusion administration of the composition.

[00103] The PD-1-binding agent can be administered to a mammal using standard administration techniques, including oral, intravenous, intraperitoneal, subcutaneous, pulmonary, transdermal, intramuscular, intranasal, buccal, sublingual, or suppository administration. The composition preferably is suitable for parenteral administration. The term “parenteral,” as used herein, includes intravenous, intramuscular, subcutaneous, rectal, vaginal, and intraperitoneal administration. More preferably, the composition is administered

to a mammal using peripheral systemic delivery by intravenous, intraperitoneal, or subcutaneous injection.

[00104] Once administered to a mammal (e.g., a human), the biological activity of the inventive PD-1-binding agent can be measured by any suitable method known in the art. For example, the biological activity can be assessed by determining the stability of a particular PD-1-binding agent. In one embodiment of the invention, the PD-1-binding agent (e.g., an antibody) has an *in vivo* half-life between about 30 minutes and 45 days (e.g., about 30 minutes, about 45 minutes, about 1 hour, about 2 hours, about 4 hours, about 6 hours, about 10 hours, about 12 hours, about 1 day, about 5 days, about 10 days, about 15 days, about 25 days, about 35 days, about 40 days, about 45 days, or a range defined by any two of the foregoing values). In another embodiment, the PD-1-binding agent has an *in vivo* half-life between about 2 hours and 20 days (e.g., about 5 hours, about 10 hours, about 15 hours, about 20 hours, about 2 days, about 3 days, about 7 days, about 12 days, about 14 days, about 17 days, about 19 days, or a range defined by any two of the foregoing values). In another embodiment, the PD-1-binding agent has an *in vivo* half-life between about 10 days and about 40 days (e.g., about 10 days, about 13 days, about 16 days, about 18 days, about 20 days, about 23 days, about 26 days, about 29 days, about 30 days, about 33 days, about 37 days, about 38 days, about 39 days, about 40 days, or a range defined by any two of the foregoing values).

[00105] The PD-1-binding agent of the invention may be administered alone or in combination with other active agents or drugs. For example, the PD-1-binding agent can be administered in combination with other agents for the treatment or prevention of the diseases disclosed herein. In this respect, the PD-1-binding agent can be used in combination with at least one other inflammatory or autoimmune disorder inhibiting agent including, for example, other monoclonal antibodies, disease-killing viruses, gene therapy, and adoptive T-cell transfer, and/or surgery. The inventive PD-1-binding agent described herein can also be used in combination with at least one other immunosuppressive agent, including, for example, methotrexate, corticosteroids, and other small molecule agents used to treat autoimmune and inflammatory disease. When the inventive method treats an infectious disease, the PD-1-binding agent can be administered in combination with at least one anti-bacterial agent or at least one anti-viral agent. In this respect, the anti-bacterial agent can be any suitable antibiotic known in the art. The anti-viral agent can be any vaccine of any suitable type that specifically targets a particular virus (e.g., live-attenuated vaccines, subunit vaccines,

recombinant vector vaccines, and small molecule anti-viral therapies (e.g., viral replication inhibitors and nucleoside analogs).

[00106] In addition to therapeutic uses, the PD-1-binding agent described herein can be used in diagnostic or research applications. In this respect, the PD-1-binding agent can be used in a method to diagnose a cancer or infectious disease. In a similar manner, the PD-1-binding agent can be used in an assay to monitor PD-1 protein levels in a subject being tested for a disease or disorder that is associated with abnormal PD-1 expression. Research applications include, for example, methods that utilize the PD-1-binding agent and a label to detect a PD-1 protein in a sample, e.g., in a human body fluid or in a cell or tissue extract. The PD-1-binding agent can be used with or without modification, such as covalent or non-covalent labeling with a detectable moiety. For example, the detectable moiety can be a radioisotope (e.g., ^{3}H, ^{14}C, ^{32}P, ^{35}S, or ^{125}I), a fluorescent or chemiluminescent compound (e.g., fluorescein isothiocyanate, rhodamine, or luciferin), an enzyme (e.g., alkaline phosphatase, beta-galactosidase, or horseradish peroxidase), or prosthetic groups. Any method known in the art for separately conjugating an antigen-binding agent (e.g., an antibody) to a detectable moiety may be employed in the context of the invention (see, e.g., Hunter et al., *Nature*, *194*: 495-496 (1962); David et al., *Biochemistry*, *13*: 1014-1021 (1974); Pain et al., *J. Immunol. Meth.*, *40*: 219-230 (1981); and Nygren, *J. Histochem. Cytochem.*, *30*: 407-412 (1982)).

[00107] PD-1 protein levels can be measured using the inventive PD-1-binding agent by any suitable method known in the art. Such methods include, for example, radioimmunoassay (RIA), and FACS. Normal or standard expression values of PD-1 protein can be established using any suitable technique, e.g., by combining a sample comprising, or suspected of comprising, a PD-1 polypeptide with a PD-1-specific antibody under conditions suitable to form an antigen-antibody complex. The antibody is directly or indirectly labeled with a detectable substance to facilitate detection of the bound or unbound antibody. Suitable detectable substances include various enzymes, prosthetic groups, fluorescent materials, luminescent materials, and radioactive materials (see, e.g., Zola, *Monoclonal Antibodies: A Manual of Techniques*, CRC Press, Inc. (1987)). The amount of PD-1 polypeptide expressed in a sample is then compared with a standard value.

[00108] The PD-1-binding agent can be provided in a kit, i.e., a packaged combination of reagents in predetermined amounts with instructions for performing a diagnostic assay. If the PD-1-binding agent is labeled with an enzyme, the kit desirably includes substrates and

cofactors required by the enzyme (e.g., a substrate precursor which provides a detectable chromophore or fluorophore). In addition, other additives may be included in the kit, such as stabilizers, buffers (e.g., a blocking buffer or lysis buffer), and the like. The relative amounts of the various reagents can be varied to provide for concentrations in solution of the reagents which substantially optimize the sensitivity of the assay. The reagents may be provided as dry powders (typically lyophilized), including excipients which on dissolution will provide a reagent solution having the appropriate concentration.

Nucleic Acids, Cells, Methods of Production

[00109] The invention also provides one or more isolated or purified nucleic acid sequences that encode the PD-1 binding agent or individual heavy or light chain immunoglobulin polypeptides thereof. Thus, in one embodiment, the nucleic acid encodes an immunoglobulin light chain variable region or full immunoglobulin light chain as provided herein. In another embodiment, the nucleic acid encodes an immunoglobulin heavy chain variable region or full immunoglobulin light chain as provided herein. In yet another embodiment, the nucleic acid encodes both an immunoglobulin light chain variable region or full immunoglobulin light chain, and an immunoglobulin heavy chain variable region or full immunoglobulin heavy chain, as provided herein. Examples of a nucleic acid sequence encoding an immunoglobulin heavy chain are provided by SEQ ID NOs: 53 and 55, which encode the heavy chain variable regions of SEQ ID NOs: 29 and 47, respectively, and the full heavy and light chains of SEQ ID NOs: 36 and 51, respectively. Examples of a nucleic acid sequence encoding an immunoglobulin light chain are provided by SEQ ID NOs: 54 and 56, which encode the light chain variable region of SEQ ID NOs: 35 and 49, respectively, and the full heavy and light chains of SEQ ID NOs: 37 and 52, respectively.

[00110] The terms “nucleic acid” and “nucleic acid sequence” are intended to encompass a polymer of DNA or RNA, i.e., a polynucleotide, which can be single-stranded or double-stranded and which can contain non-natural or altered nucleotides. The terms “nucleic acid” and “polynucleotide” as used herein refer to a polymeric form of nucleotides of any length, either ribonucleotides (RNA) or deoxyribonucleotides (DNA). These terms refer to the primary structure of the molecule, and thus include double- and single-stranded DNA, and double- and single-stranded RNA. The terms include, as equivalents, analogs of either RNA or DNA made from nucleotide analogs and modified polynucleotides such as, though not limited to, methylated and/or capped polynucleotides. Nucleic acids are typically linked via

phosphate bonds to form nucleic acid sequences or polynucleotides, though many other linkages are known in the art (e.g., phosphorothioates, boranophosphates, and the like).

[00111] The nucleic acid can be part of a vector. The vector can be, for example, a plasmid, episome, cosmid, viral vector (e.g., retroviral or adenoviral), or phage. Suitable vectors and methods of vector preparation are well known in the art (see, e.g., Sambrook et al., *Molecular Cloning, a Laboratory Manual, 3rd edition*, Cold Spring Harbor Press, Cold Spring Harbor, N.Y. (2001), and Ausubel et al., *Current Protocols in Molecular Biology*, Greene Publishing Associates and John Wiley & Sons, New York, N.Y. (1994)).

[00112] In addition to the nucleic acid sequence encoding the immunoglobulin heavy and/or light chains, the vector can comprise expression control sequences, such as promoters, enhancers, polyadenylation signals, transcription terminators, internal ribosome entry sites (IRES), and the like, that provide for the expression of the coding sequence in a host cell. Exemplary expression control sequences are known in the art and described in, for example, Goeddel, *Gene Expression Technology: Methods in Enzymology*, Vol. 185, Academic Press, San Diego, Calif. (1990).

[00113] A large number of promoters, including constitutive, inducible, and repressible promoters, from a variety of different sources are well known in the art. Representative sources of promoters include for example, virus, mammal, insect, plant, yeast, and bacteria, and suitable promoters from these sources are readily available, or can be made synthetically, based on sequences publicly available, for example, from depositories such as the ATCC as well as other commercial or individual sources. Promoters can be unidirectional (i.e., initiate transcription in one direction) or bi-directional (i.e., initiate transcription in either a 3' or 5' direction). Non-limiting examples of promoters include, for example, the T7 bacterial expression system, pBAD (araA) bacterial expression system, the cytomegalovirus (CMV) promoter, the SV40 promoter, the RSV promoter. Inducible promoters include, for example, the Tet system (U.S. Patents 5,464,758 and 5,814,618), the Ecdysone inducible system (No et al., *Proc. Natl. Acad. Sci.*, *93*: 3346-3351 (1996)), the T-REX™ system (Invitrogen, Carlsbad, CA), LACSWITCH™ system (Stratagene, San Diego, CA), and the Cre-ERT tamoxifen inducible recombinase system (Indra et al., *Nuc. Acid. Res.*, *27*: 4324-4327 (1999); *Nuc. Acid. Res.*, *28*: e99 (2000); U.S. Patent 7,112,715; and Kramer & Fussenegger, *Methods Mol. Biol.*, *308*: 123-144 (2005)).

[0100] The term "enhancer" as used herein, refers to a DNA sequence that increases transcription of, for example, a nucleic acid sequence to which it is operably linked.

Enhancers can be located many kilobases away from the coding region of the nucleic acid sequence and can mediate the binding of regulatory factors, patterns of DNA methylation, or changes in DNA structure. A large number of enhancers from a variety of different sources are well known in the art and are available as or within cloned polynucleotides (from, e.g., depositories such as the ATCC as well as other commercial or individual sources). A number of polynucleotides comprising promoters (such as the commonly-used CMV promoter) also comprise enhancer sequences. Enhancers can be located upstream, within, or downstream of coding sequences.

[0101] The vector also can comprise a selectable marker gene. The term “selectable marker gene,” as used herein, refers to a nucleic acid sequence that allow cells expressing the nucleic acid sequence to be specifically selected for or against, in the presence of a corresponding selective agent. Suitable selectable marker genes are known in the art and described in, e.g., International Patent Application Publications WO 1992/008796 and WO 1994/028143; Wigler et al., *Proc. Natl. Acad. Sci. USA*, *77*: 3567-3570 (1980); O'Hare et al., *Proc. Natl. Acad. Sci. USA*, *78*: 1527-1531 (1981); Mulligan & Berg, *Proc. Natl. Acad. Sci. USA*, *78*: 2072-2076 (1981); Colberre-Garapin et al., *J. Mol. Biol.*, *150*: 1-14 (1981); Santerre et al., *Gene*, *30*: 147-156 (1984); Kent et al., *Science*, *237*: 901-903 (1987); Wigler et al., *Cell*, *11*: 223-232 (1977); Szybalska & Szybalski, *Proc. Natl. Acad. Sci. USA*, *48*: 2026-2034 (1962); Lowy et al., *Cell*, *22*: 817-823 (1980); and U.S. Patents 5,122,464 and 5,770,359.

[0102] In some embodiments, the vector is an “episomal expression vector” or “episome,” which is able to replicate in a host cell, and persists as an extrachromosomal segment of DNA within the host cell in the presence of appropriate selective pressure (see, e.g., Conese et al., *Gene Therapy*, *11*: 1735-1742 (2004)). Representative commercially available episomal expression vectors include, but are not limited to, episomal plasmids that utilize Epstein Barr Nuclear Antigen 1 (EBNA1) and the Epstein Barr Virus (EBV) origin of replication (oriP). The vectors pREP4, pCEP4, pREP7, and pcDNA3.1 from Invitrogen (Carlsbad, CA) and pBK-CMV from Stratagene (La Jolla, CA) represent non-limiting examples of an episomal vector that uses T-antigen and the SV40 origin of replication in lieu of EBNA1 and oriP.

[0103] Other suitable vectors include integrating expression vectors, which may randomly integrate into the host cell’s DNA, or may include a recombination site to enable the specific recombination between the expression vector and the host cell’s chromosome. Such integrating expression vectors may utilize the endogenous expression control sequences

of the host cell's chromosomes to effect expression of the desired protein. Examples of vectors that integrate in a site specific manner include, for example, components of the flp-in system from Invitrogen (Carlsbad, CA) (e.g., pcDNA™5/FRT), or the cre-lox system, such as can be found in the pExchange-6 Core Vectors from Stratagene (La Jolla, CA). Examples of vectors that randomly integrate into host cell chromosomes include, for example, pcDNA3.3 (when introduced in the absence of T-antigen) from ThermoFisher (Carlsbad, CA), UCOE from Millipore (Billerica, MA), and pCI or pFN10A (ACT) FLEXI™ from Promega (Madison, WI).

[0104] Viral vectors also can be used. Representative commercially available viral expression vectors include, but are not limited to, the adenovirus-based Per.C6 system available from Crucell, Inc. (Leiden, The Netherlands), the lentiviral-based pLP1 from ThermoFisher (Carlsbad, CA), and the retroviral vectors pFB-ERV plus pCFB-EGSH from Agilent (Stratagene, La Jolla, CA).

[0105] Nucleic acid sequences encoding the inventive amino acid sequences can be provided to a cell on the same vector (i.e., in *cis*). A unidirectional promoter can be used to control expression of each nucleic acid sequence. In another embodiment, a combination of bidirectional and unidirectional promoters can be used to control expression of multiple nucleic acid sequences. Nucleic acid sequences encoding the inventive amino acid sequences alternatively can be provided to the population of cells on separate vectors (i.e., in *trans*). Each of the nucleic acid sequences in each of the separate vectors can comprise the same or different expression control sequences. The separate vectors can be provided to cells simultaneously.

[0106] The vector(s) comprising the nucleic acid(s) encoding the inventive amino acid sequences can be introduced into a host cell that is capable of expressing the polypeptides encoded thereby, including any suitable prokaryotic or eukaryotic cell. As such, the invention provides an *in vitro* cell or cell line comprising the inventive vector. The invention also provides an *in vitro* cell or cell line that expresses the immunoglobulin heavy and/or light chain polypeptides, or expresses the PD-1 binding agent. Preferred host cells are those that can be easily and reliably grown, have reasonably fast growth rates, have well characterized expression systems, and can be transformed or transfected easily and efficiently.

[0107] Examples of suitable prokaryotic cells include, but are not limited to, cells from the genera *Bacillus* (such as *Bacillus subtilis* and *Bacillus brevis*), *Escherichia* (such as *E.*

coli), *Pseudomonas*, *Streptomyces*, *Salmonella*, and *Erwinia*. Particularly useful prokaryotic cells include the various strains of *Escherichia coli* (e.g., K12, HB101 (ATCC No. 33694), DH5α, DH10, MC1061 (ATCC No. 53338), and CC102).

[0108] In some embodiments, the vector is introduced into a eukaryotic cell. Suitable eukaryotic cells are known in the art and include, for example, yeast cells, insect cells, and mammalian cells. Examples of suitable yeast cells include those from the genera *Kluyveromyces*, *Pichia*, *Rhino-sporidium*, *Saccharomyces*, and *Schizosaccharomyces*. Preferred yeast cells include, for example, *Saccharomyces cerivisae* and *Pichia pastoris*.

[0109] Suitable insect cells are described in, for example, Kitts et al., *Biotechniques*, *14*: 810-817 (1993); Lucklow, *Curr. Opin. Biotechnol.*, *4*: 564-572 (1993); and Lucklow et al., *J. Virol.*, *67*: 4566-4579 (1993). Preferred insect cells include Sf-9 and HI5 (Invitrogen, Carlsbad, CA).

[0110] In some embodiments, mammalian cells are utilized in the invention. A number of suitable mammalian host cells are known in the art, and many are available from the American Type Culture Collection (ATCC, Manassas, VA). Examples of suitable mammalian cells include, but are not limited to, Chinese hamster ovary cells (CHO) (e.g., ATCC No. CCL61), CHO DHFR-cells (e.g., Urlaub et al., *Proc. Natl. Acad. Sci. USA*, *97*: 4216-4220 (1980)), human embryonic kidney (HEK) 293 or 293T cells (e.g., ATCC No. CRL1573), and 3T3 cells (e.g., ATCC No. CCL92). Other suitable mammalian cell lines are the monkey COS-1 (e.g., ATCC No. CRL1650) and COS-7 cell lines (e.g., ATCC No. CRL1651), as well as the CV-1 cell line (e.g., ATCC No. CCL70). Further exemplary mammalian host cells include primate cell lines and rodent cell lines, including the mouse cell line NS0 a derivative of the mouse myeloma line MOPC21 (e.g. Tysabri), and transformed cell lines. Normal diploid cells, cell strains derived from in vitro culture of primary tissue, as well as primary explants, are also suitable. Other suitable mammalian cell lines include, but are not limited to, mouse neuroblastoma N2A cells, HeLa, mouse L-929 cells, and BHK or HaK hamster cell lines, all of which are available from the ATCC. Methods for selecting suitable mammalian host cells and methods for transformation, culture, amplification, screening, and purification of cells are known in the art.

[0111] In some embodiments, the mammalian cell is a human cell. For example, the mammalian cell can be a human lymphoid or lymphoid derived cell line, such as a cell line of pre-B lymphocyte origin. Examples of human lymphoid cells lines include, without

limitation, RAMOS (e.g., CRL-1596), Daudi (e.g., CCL-213), EB-3 (e.g., CCL-85), Raji cells (e.g., CCL-86), and derivatives thereof.

[0112] A nucleic acid sequence encoding the inventive amino acid sequence may be introduced into a cell by any suitable technique, such as by "transfection," "transformation," or "transduction." "Transfection," "transformation," or "transduction," as used herein, refer to the introduction of one or more exogenous polynucleotides into a host cell by using physical or chemical methods. Many transfection techniques are known in the art and include, for example, calcium phosphate DNA co-precipitation (see, e.g., Murray E.J. (ed.), *Methods in Molecular Biology*, *Vol. 7*, *Gene Transfer and Expression Protocols*, Humana Press (1991)); DEAE-dextran; electroporation; cationic liposome-mediated transfection; tungsten particle-facilitated microparticle bombardment (Johnston, *Nature*, *346*: 776-777 (1990)); and strontium phosphate DNA co-precipitation (Brash et al., *Mol. Cell Biol.*, *7*: 2031-2034 (1987)). Phage or viral vectors can be introduced into host cells, after growth of infectious particles in suitable packaging cells, many of which are commercially available.

[0113] The nucleic acids and cells can be used for any purpose, such as for the manufacture of the PD-1 binding agent described herein. In this respect, the invention provides a method of preparing the PD-1 binding agent comprising culturing a cell comprising a nucleic acid encoding the heavy and/or light immunoglobulin polypeptides of the PD-1 binding agent. Phrased differently, the method comprises expressing a nucleic acid encoding the immunoglobulin heavy and/or light chains of the PD-1 binding agent in a cell. It will be appreciated that the immunoglobulin heavy and light chains can be expressed from a single nucleic acid in a given cell, or the immunoglobulin heavy and light chains can be expressed from separate nucleic acids in the same cells. The method can further comprise harvesting and/or purifying the PD-1 binding agent from the cell or cell culture media using known techniques.

[0114] The following examples further illustrate the invention but, of course, should not be construed as in any way limiting its scope.

EXAMPLES

[0115] The following examples describe particular anti-PD-1 antibody heavy chain polypeptide and light chain polypeptide sequences, according to embodiments of the invention. The antibodies used in these examples are as set forth below.

[0116] 437M5-112 antibodies were derived from single cell PCR on sorted PD-1 binding IgG switched B cells from an immunized mouse spleen. 3.7C6 antibodies were derived from a mouse hybridoma generated by standard fusion techniques from spleen cells of an immunized mouse. The antibodies were humanized using standard techniques described herein. The final optimized antibodies were expressed in CHO cells. The antibody sequences are summarized in Tables 1A, 1B, and 1C wherein “H” and “L” chains refer to heavy and light chains, respectively, and CDRs are as determined to include amino acids according to both Kabat and IMGT definitions (Table 1B) or according to Kabat or IMGT for certain antibodies (Tables 1C).

Table 1A

AKA 437M5-112	**Expression Conditions**	**H Chain Variable Region**	**L Chain Variable Region**
APE12044		SEQ ID NO: 30	SEQ ID NO: 35
APE11844		SEQ ID NO: 24	SEQ ID NO: 34
APE12043	ExpiCHO-S™ transient	SEQ ID NO: 29	SEQ ID NO: 35
APE12538	CHO-S sorted stable pool	SEQ ID NO: 29 (Full H Chain SEQ ID NO: 36)	SEQ ID NO: 35 (Full L Chain SEQ ID NO: 37)
AKA 3.7C6	**Expression Conditions**	**H Chain Variable Region**	**L Chain Variable Region**
APE12093		SEQ ID NO: 46	SEQ ID NO: 50
APE12095	ExpiCHO-S™ transient	SEQ ID NO: 47	SEQ ID NO: 49
APE12537	CHO-S sorted stable pool	SEQ ID NO: 47 (Full H Chain SEQ ID NO: 51)	SEQ ID NO: 49 (Full L Chain SEQ ID NO: 52)
APE12890	CHO-S	SEQ ID NO: 47 (Full H Chain SEQ ID NO: 51)	SEQ ID NO: 49 (Full L Chain SEQ ID NO: 52)

Table 1B

Antibody	CDR Sequences (SEQ ID NO)					
	CDRH1	CDRH2	CDRH3	CDRL1	CDRL2	CDRL3
APE12044	15	2	21	23	5	6
APE11844	13	2	19	22	5	6
APE12043	15	2	20	23	5	6
APE12538	15	2	20	23	5	6
APE12093	57	41	9	10	11	12
APE12095	57	42	9	10	11	12
APE12537	57	42	9	10	11	12
APE12890	57	42	9	10	11	12

Table 1C

3.7C6 (APE12095, APE12537, APE12890)	**SEQ ID NO**	**Kabat Sequence**
CDRH1	64	DYSMH
CDRH2	65	WINIETYYPTYADQFKG
CDRH3	66	DYYGRFYYAMDY
CDRL1	67	TASSSVSSSYFH
CDRL2	68	STSNLAS
CDRL3	69	HQYHRSPLT
3.7C6 (APE12095, APE12537, APE12890)		**IMGT Sequence**
CDRH1	70	NYTFTDYS
CDRH2	71	INIETYYP
CDRH3	72	ARDYYGRFYYAMDY
CDRL1	73	SSVSSSY
CDRL2	74	STS
CDRL3	75	HQYHRSPLT
437M5 (APE12043 and APE12538)		**Kabat Sequence**
CDRH1	76	SYSMH
CDRH2	77	YINPSSGFTNYIQKFRD
CDRH3	78	DYYGRYYYVMDY
CDRL1	79	TASSSVSSSFLH
CDRL2	80	STSDLAS
CDRL3	81	HQYHRSPLT
437M5 (APE12043 and APE12538)		**IGMT Sequence**
CDRH1	82	GYTFTSYS
CDRH2	83	INPSSGFT
CDRH3	84	ARDYYGRYYYVMDY

CDRL1	85	SSVSSSF
CDRL2	86	STS
CDRL3	87	HQYHRSPLT

EXAMPLE 1

[0117] This example demonstrates that the antibodies disclosed herein exhibit saturation binding to human and cynomolgus monkey PD-1 expressed in stably transfected HEK 293 cells.

[0118] HEK 293 cells were stably transfected to express either human PD-1 or cynomolgus monkey PD-1. Cells were harvested by Accutase^{TM} treatment (Innovative Cell Technologies, San Diego, CA), the cells expressing cynomolgus monkey PD-1 were treated with the lipophilic fluorescent dye Vybrant® DiD (ThermoFisher Scientific, Carlsbad, CA), and then mixed with an equal number of unlabeled HEK 293 cells expressing human PD-1. Cells (2×10^5 total per sample) were stained with the indicated concentrations of each antibody for 40 min at 4°C with gentle shaking, centrifuged and washed once. Cells were fixed in 2% paraformaldehyde in phosphate-buffered saline (PBS) for 10 min at room temperature, washed, and antibodies detected with Phycoerythrin (PE)-conjugated goat anti-human kappa (Southern Biotechnology, Birmingham, AL) for 15 min at 4°C with gentle shaking. Cells were washed, resuspended, and bound antibody fluorescence quantified on a BD FACSArray^{TM} (BD Biosciences, San Jose, CA). Data were analyzed for median fluorescence intensity (MFI) using FlowJo® analysis software (FlowJo, LLC). EC_{50} values were determined in GraphPad Prism 5.0 (GraphPad Software) using a log(agonist) vs. response – Variable slope (4 parameters) curve fit. The results are shown in Figs. 1 and 2, and the EC_{50} values are set forth in Table 2 (human) and Table 3 (cynomolgus monkey). APE06339 is a human IgG1 isotype control antibody specific for hen egg lysozyme. APE08145 is a reference anti-PD-1antibody.

Table 2.

Antibody	**Type**	**EC_{50} (nM)**
APE12043.02	437M5-112	4.88
APE12043.03	437M5-112	3.13
APE12043.05	437M5-112	2.95
APE12095.03	3.7C6	11.92

APE12095.04	3.7C6	9.04
APE12095.06	3.7C6	11.82
APE08145.05	Reference Anti-PD1	20.80
APE08145.06	Reference Anti-PD1	10.91
APE06339.08	IgG1 Isotype Control	No binding

Table 3.

Antibody	**Type**	**EC_{50} (nM)**
APE12043.02	437M5-112	3.69
APE12043.03	437M5-112	2.69
APE12043.05	437M5-112	2.59
APE12095.03	3.7C6	7.99
APE12095.04	3.7C6	6.17
APE12095.06	3.7C6	7.47
APE08145.05	Reference Anti-PD1	91.14
APE08145.06	Reference Anti-PD1	65.68
APE06339.08	IgG1 Isotype Control	No binding

EXAMPLE 2

[0119] This example demonstrates that the antibodies disclosed herein bind to 2-day anti-CD3/anti-CD28 activated human peripheral blood $CD4^+$ T cells.

[0120] Primary human peripheral blood $CD4^+$ T cells were prepared using magnetic bead separation ($CD4^+$ T Cell Isolation Kit, Miltenyi Biotec, Auburn, CA) of peripheral blood mononuclear cells (PBMCs) and activated for 48 hours with plastic-coated anti-CD3 and anti-CD28 in 6-well plates. Cells (1×10^5 per sample) were washed and stained in V-bottom 96-well plates with the indicated concentrations of each antibody for 30 min at 4°C with gentle shaking, centrifuged and washed once. Cells were fixed in 4% paraformaldehyde in PBS for 10 min at room temperature, washed, and antibodies detected with Alexa Fluor 647-conjugated F(ab')2 goat anti-human IgG Fc (Jackson ImmunoResearch, West Grove, PA) for 10 min at 4°C. Cells were washed, resuspended, and bound antibody fluorescence quantified on a BD FACSArray™ (BD Biosciences, San Jose, CA). Data were analyzed for geometric mean fluorescence intensity (MFI) using FlowJo® analysis software (FlowJo, LLC). EC_{50} values were determined in GraphPad Prism 7.02 (GraphPad Software) using a log(agonist) vs. response – Variable slope (4 parameters) curve fit. The results are shown in Fig. 3, and

the EC_{50} values are set forth in Table 4. APE10787 is a human IgG1 positive control antibody specific for PD-1, and APE06339 is a human IgG1 isotype control antibody specific for hen egg lysozyme.

Table 4.

Antibody	**Description**	**EC_{50} (nM)**
APE10787	Anti-PD-1 Positive Control	0.36
APE06339.08	IgG1 Isotype Control	No binding
APE12043.03	437M5-112	2.99
APE12043.05	437M5-112	3.06
APE12095.04	3.7C6	39.07
APE12095.06	3.7C6	53.87

EXAMPLE 3

[0121] This example documents the degree to which the antibodies disclosed herein compete with PD-L1 and PD-L2 for binding to human PD-1 transfected CHO-K1 cells.

[0122] Competition assays were performed to test competition for PD-1 binding between anti-PD-1 antibodies and PD-L1-Fc or PD-L2-Fc constructs. As shown in Figures 4-7, the tested antibody shows moderate competition with PD-L1 (~70% maximum inhibition) and strong competition with PD-L2; Another tested antibody shows weak/minimal competition with PD-L1 (~15% maximum inhibition) and moderate competition with PD-L2 (~70% maximum inhibition).

[0123] CHO-K1 cells were stably transfected to express human PD-1 and a high level expressing clone was selected. Cells were harvested by Accutase™ treatment (Innovative Cell Technologies, San Diego, CA), and placed in U-bottom 96-well plates (2×10^5 cells/well). For testing PD-L1 competition antibodies were serially diluted and pre-mixed with DyLight 650 (DyL650)-labeled human PD-L1-mouse IgG1 Fc fusion protein (Abcam, Cambridge, MA) (10 nM final concentration DyL650- PD-L1-Fc and antibody concentrations as indicated in Figs. 4 and 5). After incubation for 10 min on ice the antibody/DyL650-PD-L1-Fc mixtures were added to the cells for 30 min at 4°C with gentle shaking. Cells were centrifuged, washed once, resuspended in buffer containing propidium iodide, and bound PD-L1-Fc fluorescence quantified on a BD FACSArray™ (BD Biosciences, San Jose, CA). Data were analyzed for PD-L1 geometric median fluorescence intensity (MFI) using FlowJo® analysis software (FlowJo, LLC). IC_{50} values were

determined in GraphPad Prism 7.02 (GraphPad Software) using a log(agonist) vs. response – Variable slope (4 parameters) curve fit. The results are shown in Figs. 4 and 5, and the resulting IC_{50} values are set forth in Tables 4-5. APE10787 (“10787”) is a human IgG1 positive control antagonist antibody specific for PD-1, and APE06339 (“06339.08”) is a human IgG1 isotype control antibody specific for hen egg lysozyme. APE08145 (“08145.05” and “08145.06”) is a reference antibody. APE12043 (“12043.02” and “12043.03”) is the 437M5-112 anti-PD-1 antibody described in Example 1. APE12095 (“12095.03” and “12095.04”) is the 3.7C6 anti-PD-1 antibody described in Example 1.

[0124] A CHO-K1 cell clone stably expressing high levels of human PD-1 was harvested by Accutase™ treatment (Innovative Cell Technologies, San Diego, CA), and placed in U-bottom 96-well plates (2 x 10^5 cells/well). For testing PD-L2 competition antibodies were serially diluted and pre-mixed with DyL650-labeled human PD-L2-mouse IgG1 Fc fusion protein (Abcam, Cambridge, MA) (10 nM final concentration DyL650- PD-L2-Fc and antibody concentrations as indicated in Figs. 6 and 7). After incubation for 10 min on ice the antibody/DyL650-PD-L2-Fc mixtures were added to the cells for 30 min at 4°C with gentle shaking. Cells were centrifuged, washed once, resuspended in buffer containing propidium iodide, and bound PD-L2-Fc fluorescence quantified on a BD FACSArray™ (BD Biosciences, San Jose, CA). Data were analyzed for PD-L2 geometric median fluorescence intensity (MFI) using FlowJo® analysis software (FlowJo, LLC). IC_{50} values were determined in GraphPad Prism 7.02 (GraphPad Software) using a log(agonist) vs. response – Variable slope (4 parameters) curve fit. The results are shown in Figs. 6 and 7, and the resulting IC_{50} values are set forth in Tables 6-7. APE10787 (“10787”) is a human IgG1 positive control antagonist antibody specific for PD-1, and APE06339 (“06339.08”) is a human IgG1 isotype control antibody specific for hen egg lysozyme. APE08145 (“08145.05” and “08145.06”) is a reference antibody. APE12043 (“12043.02” and “12043.03”) is the 437M5-112 anti-PD-1 antibody described in Example 1. APE12095 (“12095.03” and “12095.04”) is the 3.7C6 anti-PD-1 antibody described in Example 1.

Table 4. PD-L1-Fc Competition

Antibody	**Type**	**IC_{50} (nM)**
APE10787	Anti-PD-1 Positive Control	1.89
APE06339.08	IgG1 Isotype Control	No competition
APE08145.05	Reference Anti-PD-1	75.2
APE08145.06	Reference Anti-PD-1	119.1
APE12043.02	437M5-11	11.6
APE12043.03	437M5-11	12.2

Table 5. PD-L1-Fc Competition

Antibody	**Type**	**IC_{50} (nM)**
APE10787	Anti-PD-1 Positive Control	1.59
APE06339.08	IgG1 Isotype Control	No competition
APE12095.03	3.7C6	495.1
APE12095.04	3.7C6	632.1

Table 6. PD-L2-Fc Competition

Antibody	**Type**	**IC_{50} (nM)**
APE10787	Anti-PD-1 Positive Control	2.53
APE06339.08	IgG1 Isotype Control	No competition
APE08145.05	Reference Anti-PD-1	No competition
APE08145.06	Reference Anti-PD-1	No competition
APE12043.02	M5-11	5.8
APE12043.03	M5-11	5.5

Table 7. PD-L2-Fc Competition

Antibody	Type	IC_{50} (nM)
APE10787	Anti-PD-1 Positive Control	1.78
APE06339.08	IgG1 Isotype Control	No competition
APE12095.03	3.7C6	30.4
APE12095.04	3.7C6	20.0

EXAMPLE 4

[0125] This example demonstrates that the antibodies disclosed herein show consistent agonist activity in bead-based and plate-based agonist assays.

[0126] For bead-based agonist assays Dynabeads® M-280 Tosylactivated (Invitrogen – Life Technologies, Carlsbad, CA) were coupled according to the manufacturer's instructions with anti-CD3 (10 µg), anti-PD-1 or PD-L1-Fc (40 µg), and a negative control antibody binding hen egg lysozyme (50 µg) for a total of 100 µg protein coupled. Extent of bead coupling was quantified by flow cytometry. Primary human peripheral blood $CD4^+$ T cells were prepared using magnetic bead separation ($CD4^+$ T Cell Isolation Kit, Miltenyi Biotec, Auburn, CA) of PBMCs. Purified $CD4^+$ T cells (1×10^5 cells/well) were incubated with different numbers of beads as indicated, (4:1, 2:1, or 1:1 ratios of beads:T cells) in the presence of soluble anti-CD28 (eBioscience; 250 ng/ml, 100 ng/ml or 50 ng/ml as indicated) for 72 hours. Secreted IFNγ in culture supernatants was quantified by ELISA (R&D Systems, Minneapolis, MN). As shown in Figures 8A and 8B, and summarized in Table 8, the anti-PD-1 antibodies disclosed herein (437M5-112 and 3.7C6) demonstrated consistent inhibitory (agonist) activity in the bead assay that was comparable to PD-L1-Fc.

[0127] As shown in Figures 9A-9C, the 3.7C6 variants APE12093 and APE12095 were the best agonists in the bead-based assay, with stronger inhibition as compared with the PD-L1-Fc. The 437M5-112 variants APE12043 and APE12044 had improved agonist activity compared to the parent antibody APE11844.

Table 8.

Candidate Antibody	Number of donors tested	% Inhibition of IFNγ (Mean ± SEM)
437M5-112 (APE12043)	7	83 ± 6
3.7C6 (APE12095)	6	77± 7
PD-L1-Fc	10	83 ± 3

[0128] Inhibition of IFNγ production by the anti-PD-1 antibodies disclosed herein across donors tested in the bead-based agonist assay is shown in Figures 10A-10B and Figures 11A-11C.

[0129] For plate-based agonist assays 96-well plates were sequentially coated with anti-CD3 (0.3 μg/ml) overnight at 4°C, wells aspirated and washed with PBS, and then subjected to a second coating overnight at 4°C with various concentrations of anti-PD-1 antibody or PD-L1-Fc as indicated in Figs. 12-14. Fresh or frozen human PBMCs were cultured in the presence of phytohemagglutinin (PHA; 2 μg/ml) for 48 hours, harvested, washed to remove PHA, and cultured overnight in the presence of IL-2. Cells were harvested, washed and incubated in the anti-CD3/anti-PD-1 coated wells (1 x 10^5 cells/well) in the presence of human gamma globulin (100 μg/ml) for 48 hours. Secreted IL-2 in culture supernatants was quantified by ELISA (R&D Systems, Minneapolis, MN). Inhibition of IL-2 production by the PD-1 antibodies across three PBMC donors is shown in Figures 12A-12B, 13A-13B, and 14A-14B. Inhibition of IL-2 production by the anti-PD-1 antibodies disclosed herein was comparable to that induced by PD-L1-Fc.

EXAMPLE 5

[0130] This example demonstrates that the anti-PD-1 antibodies disclosed herein displayed agonist antibody activity in solution in the presence of blocking anti-PD-L1/anti-PD-L2.

[0131] Whole blood from tetanus toxoid immunized donors was diluted 1:3 and cultured for 4 days in U-bottom 96-well plates in the presence of tetanus toxoid (Astarte Biologics, Bothell, WA; 5 μg/ml), anti-PD-L1 + anti-PD-L2 (BioLegend, San Diego, CA; 2 μg/ml each), and the indicated concentrations of tetramer PD-L1-Fc, anti-PD-1 IgG1 antibodies described herein (3.7C6 APE12095; 437M5-112 APE12043), or control human IgG1. Secreted IFNγ in culture supernatants was quantified by ELISA (R&D Systems, Minneapolis,

MN). In this tetanus toxoid recall response whole blood assay, potent agonist antibody activity of the anti-PD-1 antibodies described herein was observed in the presence of blocking anti-PD-L1/anti-PD-L2, as shown in Figures 15A (positive and negative controls) and 15B (anti-PD-1 antibodies).

[0132] The IgG1 3.7C6 anti-PD-1 antibody was compared to the same antibody prepared as a human IgG2 (Figures 15C and 15D). The anti-PD-1 IgG2 version of the antibody had identical activated T cell binding as anti-PD-1 IgG1, but did not show agonist activity. IgG2, IgG4, or IgG1(L234A, L235A) isotypes of the antibody also lacked agonist activity, which demonstrates a requirement for FcγR engagement/ antibody clustering for functional agonist activity.

EXAMPLE 6

[0133] This example demonstrates that the anti-PD-1 antibodies provided herein reduce an immune response in whole blood in a concentration-dependent manner.

[0134] Human whole blood, stimulated with an appropriate antigen *in vitro*, will elicit a specific T cell recall immune response when the donor has previously experienced exposure to the antigen of interest. The immune response can be gauged by IFN-γ and IL-17A levels.

[0135] Healthy human donors (N=6) were prescreened for *in vitro* recall responsiveness to the antigen tetanus toxoid, which the donors were likely previously exposed to during the course of common tetanus vaccinations. Whole blood from the donors was cultured for 96 hours in the presence of tetanus toxoid and either anti-PD-1 3.7C6 antibody (APE12890) or an irrelevant human IgG1 isotype control. After 96 hours of culture, supernatant was assayed for the presence of the cytokines IFN-γ and IL-17A using cytokine detection kits (Meso Scale Diagnostics, Rockville, MD). The results are provided in Figures 22A and 22B.

[0136] All donors responded to tetanus toxoid-specific stimulation by producing significant quantities of IFN-γ and IL-17A, although some donors responded more robustly than others. 3.7C6 antibody reduced secretion of both IFN-γ and IL-17A in a concentration-dependent manner, relative to the IgG1 isotype control antibody, as shown in Figures 22A and 22B. The median IC_{50} and mean $IC_{50} \pm SD$ of 3.7C6 in the human whole blood tetanus toxoid recall assay was determined to be 0.053 nM and 0.091 ± 0.115 nM, respectively for IFN-γ inhibition, and 0.097 nM and 0.119 ± 0.098 nM, respectively for IL-17A inhibition.

EXAMPLE 7

[0137] This example demonstrates the binding kinetics (affinities) and thermal stability of the antibodies disclosed herein.

[0138] 3.7C6 (APE12095.06 and APE12537.01) showed comparable binding kinetics by surface plasmon resonance (SPR) to human and cynomolgus monkey PD-1. The tight binding kinetics approached the limits for the instrument. The SPR data agree well with equilibrium binding affinities for APE12095 determined by kinetic exclusion assay (KinExA®). The final affinity measurements were: KinExA® K_D for human PD-1: 75 pM; and KinExA® K_D for cynomolgus PD-1: 450 pM.

[0139] 437M5-112 (APE12043.05 and APE12538.01) also showed very comparable binding kinetics by SPR to human and cynomolgus monkey PD-1. The tight binding kinetics approached the limits for the instrument. The SPR data agree well with equilibrium binding affinities for APE12043 determined by KinExA®. The final affinity measurements were: KinExA® K_D for human PD-1: 51 pM; and KinExA® K_D for cynomolgus PD-1: 210 pM. A summary of K_D measurements on the anti-PD-1 antibodies disclosed herein by Surface Plasmon Resonance and KinExA® are set forth in Table 9.

Table 9.

Antibody/Lot	**Human PD-1 K_D (SPR)**	**Cynomolgus PD-1 K_D (SPR)**	**Human PD-1 K_D (KinExA)**	**Cynomolgus PD-1 K_D (KinExA)**
APE12043.05	31 pM	90 pM		
APE12538.01	51 pM	217 pM		
APE12043.04			51 pM	210 pM
APE12095.06	51 pM	244 pM		
APE12537.01	43 pM	445 pM		
APE12095.04			75 pM	450 pM

[0140] The APE12537 antibody binding affinity and thermal stability was compared to that of a similar antibody designated “030-13263/ 030-13264.” APE12537 differed from 030-13263/ 030-13264 by two mutations in the heavy chain: A52aI and D62Q by Kabat numbering (A53I and D63Q using the positions in the sequence listing). The results, provided in Table 10, show that these mutations increased binding affinity and thermal stability.

Table 10

Antibody	Heavy Chain Variable Region (SEQ ID)	Light Chain Variable Region (SEQ ID)	K_D Human PD-1 (nM)	K_D Cynomolgus PD-1 (nM)	Fab Tm (°C)
Mouse chimeric	--	--	4.3	n.d.	n.d.
030-13263/ 030-13264 (CDR-grafted)	43	49	0.186	1.02 ± 0.08 N=2	61.2 ± 0.3 N=3
APE12537 (CDR-grafted and optimized)	47	49	0.060	0.64 ± 0.10 N=2	66.1

[0141] K_D measurements for screening by Surface Plasmon Resonance (SPR) were performed on a Biacore T200 (GE Healthcare Life Sciences, Pittsburgh, PA), and kinetic constants were fit globally using a 1:1 binding model. Biotinylated human or cynomolgus monkey PD-1 extracellular domain monomer was captured at a 1 nM concentration on a Biacore Sensor chip SA (GE Healthcare Life Sciences, Pittsburgh, PA), with a carboxymethylated dextran surface pre-immobilized with streptavidin. The captured antigen level was targeted to yield a low response to prevent avidity effects on the dissociation rate. Tm measurements were determined by fluorescence-based thermal shift and differential scanning calorimetry.

EXAMPLE 8

[0142] This example demonstrates that the anti-PD-1 antibodies disclosed herein show efficacy *in vivo* in a xenogeneic NSG/Hu-PBMC Graft vs. Host Disease (GvHD) model.

[0143] A xenogeneic NSG/Hu-PBMC GvHD model testing the efficacy of the anti-PD-1 antibodies disclosed herein was performed at The Jackson Laboratory JAX® In Vivo Pharmacology Services (Sacramento, CA). NOD-*scid* $IL2r\gamma^{null}$ (NSG) mice were irradiated with 1 Gy followed by intravenous injection of 3 x 10^6 human PBMCs in each mouse as illustrated in Fig. 16A. Antibodies were dosed intraperitoneally at 10 mg/kg twice weekly for 4 weeks starting the day following PBMC injection, and belatacept biosimilar was dosed intraperitoneally at 75 µg/mouse three times weekly for 4 weeks. Dosing regimens and dose groups in the study are shown in Fig. 16B. Disease was monitored three times weekly by

body weight loss, death, and GvHD scores measuring: weight loss, activity, fur texture, paleness, and posture. Animals exhibiting more than 10% body weight loss were disease monitored daily, and animals exhibiting more than 20% body weight loss from starting weight were euthanized.

[0144] The 3.7C6 PD-1 agonist antibody (APE12095) disclosed herein showed statistically significant efficacy vs. isotype control in time to 10% body weight loss (Fig. 16C). The 437M5-112 anti-PD-1 agonist antibody disclosed herein also showed statistically significant efficacy vs. isotype control in time to 10% body weight loss (Fig. 16D). Responses to both anti-PD-1 antibodies were bimodal with a proportion of the animals in each group fully surviving in the study (Figures 16C and 16D).

EXAMPLE 9

[0145] This example demonstrates the study design of the cynomolgus monkey single dose Pharmacokinetics and Tolerability study.

[0146] The study design of the cynomolgus monkey single dose Pharmacokinetics and Tolerability study is set forth in Table 11. Assessments during the study were as follows:

- Clinical Pathology, pre-dose (twice), Days 2, 6, 22, and 35 (Charles River Laboratories (CRL))
- Blood FACS panels – major leukocyte populations and 20 T cell subsets, pre-dose (twice), Days 2, 6, 22, and 35 (CRL)
  - B, T, NK, monocyte
  - CD4, CD8, T Central Memory, T Effector Memory, PD-1^{+}, activated CD4^{+} and CD8^{+}, Treg
- Receptor occupancy, Days 4, 14, 28, 35
- PK sample analysis, anti-drug antibody analysis pre-dose & Day 35
- Serum cytokine analyses (17-plex: IL-1β, IL-1Ra, IL-2, IL-4, IL-5, IL-6, IL-8, IL-10, IL-12p40, IL-13, IL-17a, G-CSF, GM-CSF, IFNγ, MIP1β, MIP1α, TNF-α), pre-dose, 4 h and 24 h, Days 7 and 35
- PK parameter analysis using Phoenix® WinNonlin® (Certara, USA).

[0147] Both PD-1 antibodies showed well-behaved pharmacokinetic properties with detectable drug levels in all animals at day 28 (Figures 17A and 17B). The study was completed on day 35. The doses were well-tolerated, with no adverse clinical signs or

changes in clinical pathology observed. Results of the study are set forth in Tables 11 and 12.

Table 11. Study Design of the Cynomolgus Monkey Single Dose Pharmacokinetics and Tolerability Study.

Group	Dose (mg/kg)	Number of male animals	Test article	Route of Administration
1	10	3	437M5-112 stable CHO-S pool	Intravenous (IV)
2	10	3	437M5-112 stable CHO-S pool	Subcutaneous (SC)
3	10	3	3.7C6 stable CHO-S pool	Intravenous (IV)
4	10	3	3.7C6 stable CHO-S pool	Subcutaneous (SC)

Table 12. Analysis of Pharmacokinetic Parameters, Cytokine and Anti-Drug Antibody Responses.

Parameter*	Units	IV (Group 1)	SC (Group 2)	IV (Group 3)	SC (Group 4)
Half-life	hr	63.1 (11.6)	91.3 (4.9)	127.9 (7.4)	115.2 (57.8)
Tmax†	hr	0.67 (0.29)	37.67 (22.2)	0.50 (0)	28.83 (19.70)
Cmax	ng/mL	417,616 (62,049)	51,210 (4.163)	342,115 (31,785)	46,365 (14,645)
C0	ng/mL	439,299 (15,116)		392234 (36,564)	
AUCall	hr* ng/mL	13,422,916 (2,068,923)	8,265,256 (353,998)	12,199,665 (2,639,065)	8,518,430 (1,899,319)
AUCINF_obs	hr* ng/mL	13,473,226 (2,100,523)	8,272,411 (357,502)	12,263,340 (2,686,651)	8,621,009 (1,985,163)
Cavg	ng/mL	16,043 (2,499)	9,840 (421)	14,536 (3,128)	10,1412 (2,260)
CLss or CL/F	mL/hr/kg	0.753 (0.108)	1.211 (0.052)	0.842 (0.163)	1.22 (0.31)
Vz or Vz/F	mL/kg	69.8 (21.3)	159.3 (5.7)	154.3 (22.3)	185.6 (65.6)
Vss_obs	mL/kg	55.7 (18.9)		98.7 (15.4)	
F	%		61.4		70.3

*Mean values (± SD), 3 animals/group. †Tmax, time to Cmax; Cmax, maximum concentration; C0, initial concentration; AUCall, area under the curve to last observation; AUCINF_obs, AUC to infinity based on last observation; Cavg, average concentration; CLss, clearance at steady state; Vz, terminal phase volume of distribution; Vss obs, steady-state volume of distribution based on last observation; F, percent bioavailability.

Table 13. Analysis of Anti-Drug Antibody (ADA) Responses and Cytokines

Animal	**Antibody APE12538.01 Cohort**	**Pre-Dose ADA**	**Day 36 ADA**	**Day 36 ADA Titer**
1001	10mg/kg IV	Negative	Positive	320
1002	10mg/kg IV	Negative	Positive	80
1003	10mg/kg IV	Negative	Positive	320
2001	10mg/kg SC	Negative	Positive	20
2002	10mg/kg SC	Negative	Positive	320
2003	10mg/kg SC	Negative	Positive	20
Animal	**Antibody APE12537.01 Cohort**	**Pre-Dose ADA**	**Day 36 ADA**	**Day 36 ADA Titer**
3001	10mg/kg IV	Negative	Negative	
3002	10mg/kg IV	Negative	Negative	
3003	10mg/kg IV	Negative	Positive	64
4001	10mg/kg SC	Negative	Negative	
4002	10mg/kg SC	Negative	Negative	
4003	10mg/kg SC	Negative	Positive	128

[0148] As shown, all 6 animals dosed with Antibody APE12538.01 had measureable/low titer anti-drug antibody at Day 36. Two of 6 animals dosed with Antibody APE12537.01 had measurable/low titer anti-drug antibody at Day 36. There were no meaningful changes in any cytokine evaluated. Further as shown in Figs. 18A and 18B, sustained receptor occupancy through Day 14 was observed in all animals except #1001 (dosed IV with 437M5-112); some occupancy was found in most animals through Day 28.

EXAMPLE 10

[0149] This example demonstrates that the anti-PD-1 antibody disclosed herein induced recruitment of the phosphatase SHP2 to the PD-1 cytoplasmic domain in PD-1 transfected Jurkat cells.

[0150] Antibody 3.7C6 (APE12890) or a human IgG1 isotype control antibody recognizing hen egg lysozyme and a constant amount of anti-CD3 (UCHT1 clone; BioLegend, San Diego, CA) were coupled to magnetic beads (Dynabeads™ M-280 Tosylated; Invitrogen™/ThermoFisher Scientific). The anti-PD-1 antibody on beads mimics FcγR engagement by the antibody on antigen presenting cells. Stable human PD-1 transfected Jurkat cells were stimulated with the indicated beads for either 2 minutes or 10 minutes, cells lysed, and PD-1 immunoprecipitated by the addition of 3.7C6 coupled beads.

Immunoprecipitates were analyzed by SDS-PAGE followed by immunoblotting with either anti-PD-1 (Figure 19A, top), anti-SHP2 (Figure 19A, middle), or anti-SHP1 (Figure 19A, bottom). In this signaling assay, after PD-1 Jurkat cell activation with anti-CD3, the 3.7C6 antibody described herein, but not an isotype control antibody, induced recruitment of the phosphatase SHP2 but not SHP1 to the PD-1 cytoplasmic domain, as shown in Figures 19A (immunoblot) and 19B (densitometry quantification of the immunoblot). In the presence of anti-CD3 coated beads, the PD-1 antagonist antibody nivolumab (used at 100 nM in solution) did not induce either SHP2 or SHP1 recruitment to PD-1, as shown in Figures 19A-B. No SHP recruitment was found with soluble nivolumab. In combination with T-cell activation and CD28 co-stimulation, antibody 3.7C6 also reduced ZAP70 and LAT phosphorylation (data not shown). Antibody 3.7C6 had no effect on signaling pathways in the absence of T-cell activation.

EXAMPLE 11

[0151] This example demonstrates that the epitope on human PD-1 that is bound by the 3.7C6 antibody disclosed herein is on the opposite face of PD-1 from the PD-L1 binding site.

[0152] Hydrogen-deuterium exchange mapping of the peptides on PD-1 bound by the 3.7C6 antibody (APE12537) disclosed herein was performed at Biomotif AB (Danderyd, Sweden) using recombinant human PD-1 monomer. The structure of PD-1 is publically available through the Protein Data Bank (PDB) operated by the National Center for Biotechnology Information (Bethesda, MD) at accession 4ZqK (see, also, Zak, K. M. et al., 2015, Structure 23:2341-2348; and PDB accession 5GGR (see, also, Lee, J. Y. et al., 2016, Nat Commun., 7:13354). One major peptide, labeled "HDX mapped β-hairpin", in Figures 20A and 20B, was protected from hydrogen-deuterium exchange by the 3.7C6 antibody. "HDX mapped β-hairpin" was comprised of amino acids 96-110 of PD-1, which amino acids have the sequence RVTQLPNGRDFHMSV. Another major peptide was comprised of amino acids 33-41 of PD-1, which amino acids have the sequence NPPTFSPAL. Figures 20A and 20B show a ribbon model of the crystal structure of human PD-1 extracellular domain (black) docked with a space-filling model of the crystal structure of human PD-L1 extracellular binding domain (light gray) (PD-1 and PD-L1 structures from the NCBI PDB). The molecules are oriented with the membrane-proximal region of PD-1 at the bottom left (Figure 20A), and rotated by 90° showing the membrane-proximal region of PD-1 at the bottom center (Figure 20B). Human PD-1 monomers containing different sets of mutations in

defined surface regions were expressed, and the binding of the 3.7C6 antibody (APE12095) disclosed herein was evaluated by surface plasmon resonance. Mutations in the region labeled "PD-1 triple point mutant" in Figures 20A and 20B completely abolished binding of the 3.7C6 antibody disclosed herein. Mutations in the top of the loop labeled "HDX mapped β-hairpin" in Figures 20A and 20B did not affect binding of the 3.7C6 antibody disclosed herein. A combination of the hydrogen-deuterium exchange and PD-1 mutational mapping demonstrated that the 3.7C6 PD-1 agonist antibody disclosed herein showed binding to the region delineated in Figure 20B by the dotted circle, which is on the opposite face of PD-1 from the PD-L1 binding site.

EXAMPLE 12

[0153] This example demonstrates that the anti-PD-1 antibody disclosed herein inhibited production of IFNγ in peripheral blood mononuclear cells (PBMCs) from alopecia areata donors stimulated with keratinocyte peptide antigens.

[0154] Alopecia areata is hair loss mediated by the immune system. Hair loss results when immune privilege of the hair follicle is broken down by keratinocyte and melanocyte antigen-specific T cells producing IFNγ. T cells infiltrate hair follicle root sheaths. Activated T cells produce excessive IFNγ. Major histocompatibility complex class I and II molecules are abnormally expressed resulting in subsequent destruction of hair follicle cells and hair loss.

[0155] PBMCs were isolated from blood of alopecia areata donors and cultured (2×10^5 cells/well) in plates in the presence of keratinocyte peptide antigens (peptide antigen pools were as described by Wang et al., *J Invest Dermatol.* 2016 Aug;136(8):1617-1626), and the indicated concentrations of tetramer PD-L1-IgG1 Fc, IgG1 3.7C6 anti-PD-1 antibody (APE12890), or control IgG1 isotype tetramer, or control IgG1 isotype. After five days, the cells were washed and incubated for an additional 20 hours in an ELISpot plate to detect the number of IFNγ secreting cells. The results from each donor and treatment group were normalized to the untreated wells to allow statistical comparison of the data from 12 donors for treatment and negative controls.

[0156] As shown in Figure 21A, the IgG1 3.7C6 anti-PD-1 antibody as compared to the control IgG1 isotype inhibited IFNγ production in a concentration-dependent manner. As shown in Figure 21B, the positive control PD-L1-IgG1 Fc tetramer as compared to the IgG1 isotype tetramer inhibited IFNγ production compared to control IgG1 isotype tetramer in a

concentration-dependent manner. Both the anti-PD-1 antibody described herein and PD-L1-IgG1 Fc tetramer significantly inhibited the number of IFNγ secreting cells at concentrations at or above 1 nM ($p<0.001$), as shown in Figures 21C and 21D.

EXAMPLE 13

[0157] This example demonstrates that the anti-PD-1 antibody disclosed herein inhibited production of IFNγ in peripheral blood mononuclear cells (PBMCs) from alopecia areata donors stimulated with melanocyte peptide antigens.

[0158] Alopecia areata is hair loss mediated by the immune system. Hair loss and/or loss of hair pigmentation results when immune privilege of the hair follicle is broken down by keratinocyte and melanocyte antigen-specific T cells producing IFNγ. T cells infiltrate hair follicle root sheaths. Activated T cells produce excessive IFNγ. Major histocompatibility complex class I and II molecules are abnormally expressed resulting in subsequent destruction of hair follicle cells and hair loss. A similar melanocyte-specific T cell response in the skin, results in the destruction of melanocytes in vitiligo.

[0159] PBMCs were isolated from blood of alopecia areata donors and cultured (2 x 105 cells/well) in plates in the presence of melanocyte peptide antigens (peptide antigen pools were as described by Wang et al., J Invest Dermatol. 2016 Aug 136(8):1617-1626), and the indicated concentrations of tetramer PD-L1-IgG1 Fc, IgG1 3.7C6 anti-PD-1 antibody (APE12890), or control IgG1 isotype tetramer, or control IgG1 isotype. After five days, secreted IFNγ in culture supernatants was quantified by Meso Scale Discovery (Meso Scale Diagnostics, Rockville, MD). The results from each donor and treatment were normalized to the untreated wells to allow statistical comparison of the data from 12 donors for treatment and negative controls. Additionally, after 5 days, the cells were washed and incubated for an additional 20 hours in an ELISpot assay to detect the number of IFNγ secreting cells. The results from each donor and treatment were normalized to the untreated wells to allow statistical comparison of the data from 12 donors for treatment and negative controls. The results are presented in Figs. 23A-23D.

[0160] As shown in Figure 23A, the IgG1 3.7C6 anti-PD-1 antibody as compared to the control IgG1 isotype inhibited IFNγ production in a concentration-dependent manner. As shown in Figure 23B the positive control PD-L1-IgG1 Fc tetramer as compared to the IgG1 isotype tetramer inhibited IFNγ production compared to control IgG1 isotype tetramer in a concentration-dependent manner. Both the anti-PD-1 antibody described herein and PD-L1-

IgG1 Fc tetramer significantly inhibited IFNγ production at concentrations at or above 100 nM (p<0.001). Both the anti-PD-1 antibody described herein and PD-L1-IgG1 Fc tetramer significantly reduced the number of IFNγ secreting cells at concentrations at or above 10 nM (p<0.001) as shown in Figures 23C and 23D.

EXAMPLE 14

[0161] This example demonstrates that the anti-PD-1 antibody disclosed herein show efficacy *in vivo* in a xenogeneic NSG/Hu-PBMC Graft v. Host Disease (GvHD) model at a dosage of 3 mg/kg.

[0162] A xenogeneic NSG/Hu-PBMC GvHD model testing the efficacy of the anti-PD01 antibody disclosed herein was performed at The Jackson Laboratory, Sacramento, CA). NOD-*scid* $IL2r\gamma^{null}$ (NSG) mice were irradiated with 1 Gy followed by intravenous injection of 0.9 x 10^7 human PBMCs in each mouse as illustrated in Fig. 24A. Antibodies were dosed intraperitoneally at 30 mg/kg, 10 mg/kg, or 3 mg/kg twice weekly for 4 weeks starting the day following PBMC injection. A fourth group was dosed with an irrelevant isotype control antibody at 30 mg/kg twice weekly and a fifth group was dosed with CTLA-4-IgG, a known positive control for efficacy in the model, at 75 µg/mouse, three times a week. Dosing regimens and dose groups in the study are shown in Fig. 24B. Disease was monitored three times weekly for by weight loss, death, and GvHD scores measuring: weight loss, activity, fur texture, paleness, and posture. Animals exhibiting more than 10% body weight loss were disease monitored daily, and animals exhibiting more than 20% body weight loss from starting weight were euthanized.

[0163] The 3.7C6 PD-1 agonist antibody (APE12890) disclosed herein showed statistically significant efficacy vs. isotype control in survival, increasing median survival time (Fig. 24C). Individual animals' percent of starting body weight over the course of the study are shown in Figs. 24D, 24E, 24F, 24G, and 24H when dosed with isotype control IgG1 at 30 mg/kg, Anti-PD-1 agonist IgG1 (3.7C6) at 30 mg/kg, Anti-PD-1 agonist IgG1 (3.7C6) at 10 mg/kg, Anti-PD-1 agonist IgG1 (3.7C6) at 3 mg/kg, and CTLA-4-Ig (positive control) at 75µg/dose, respectively.

[0164] There was not a significant difference in survival between the anti-PD-1 agonist IgG1 (3.7C6) 30 mg/kg and anti-PD-1 agonist IgG1 (3.7C6) 3 mg/kg dose groups. This suggests that efficacy in the GvHD model may be obtained at doses less than 3 mg/kg.

[0165] All references, including publications, patent applications, and patents, cited herein are hereby incorporated by reference to the same extent as if each reference were individually and specifically indicated to be incorporated by reference and were set forth in its entirety herein.

[0166] The use of the terms "a" and "an" and "the" and "at least one" and similar referents in the context of describing the invention (especially in the context of the following claims) are to be construed to cover both the singular and the plural, unless otherwise indicated herein or clearly contradicted by context. The use of the term "at least one" followed by a list of one or more items (for example, "at least one of A and B") is to be construed to mean one item selected from the listed items (A or B) or any combination of two or more of the listed items (A and B), unless otherwise indicated herein or clearly contradicted by context. The terms "comprising," "having," "including," and "containing" are to be construed as open-ended terms (i.e., meaning "including, but not limited to,") unless otherwise noted. Recitation of ranges of values herein are merely intended to serve as a shorthand method of referring individually to each separate value falling within the range, unless otherwise indicated herein, and each separate value is incorporated into the specification as if it were individually recited herein. All methods described herein can be performed in any suitable order unless otherwise indicated herein or otherwise clearly contradicted by context. The use of any and all examples, or exemplary language (e.g., "such as") provided herein, is intended merely to better illuminate the invention and does not pose a limitation on the scope of the invention unless otherwise claimed. No language in the specification should be construed as indicating any non-claimed element as essential to the practice of the invention.

[0167] Preferred embodiments of this invention are described herein, including the best mode known to the inventors for carrying out the invention. Variations of those preferred embodiments may become apparent to those of ordinary skill in the art upon reading the foregoing description. The inventors expect skilled artisans to employ such variations as appropriate, and the inventors intend for the invention to be practiced otherwise than as specifically described herein. Accordingly, this invention includes all modifications and equivalents of the subject matter recited in the claims appended hereto as permitted by applicable law. Moreover, any combination of the above-described elements in all possible

variations thereof is encompassed by the invention unless otherwise indicated herein or otherwise clearly contradicted by context.

CLAIM(S):

1. An anti-PD-1 binding agent comprising an immunoglobulin heavy chain variable region and an immunoglobulin light chain variable region, wherein

(a) the immunoglobulin heavy chain variable region comprises:
a CDR1 comprising SEQ ID NO: 1;
a CDR2 comprising SEQ ID NO: 2; and
a CDR3 comprising SEQ ID NO: 3;
and the immunoglobulin light chain variable region comprises
a CDR1 comprising SEQ ID NO: 4;
a CDR2 comprising SEQ ID NO: 5; and
a CDR3 comprising SEQ ID NO: 6;

(b) the immunoglobulin heavy chain variable region comprises at least 80% sequence identity to any one of SEQ ID NOs: 24-33, and/or comprises at least the CDR regions of any one of SEQ ID NOs: 24-33, and the immunoglobulin light chain variable region comprises at least 80% sequence identity to SEQ ID NO: 34 or 35, and/or comprises at least the CDR regions of any one of SEQ ID NO: 34 or 35;

(c) the immunoglobulin heavy chain variable region comprises:
a CDR1 comprising SEQ ID NO: 7;
a CDR2 comprising SEQ ID NO: 8; and
a CDR3 comprising SEQ ID NO: 9;
and the immunoglobulin light chain variable region comprises
a CDR1 comprising SEQ ID NO: 10;
a CDR2 comprising SEQ ID NO: 11; and
a CDR3 comprising SEQ ID NO: 12;

or

(d) the immunoglobulin heavy chain variable region comprises at least 80% sequence identity to any one of SEQ ID NOs: 43-47 or 61-63, and/or comprises at least the CDR regions of any one of SEQ ID NOs: 43-47 or 61-63, and the immunoglobulin light chain

variable region comprises at least 80% sequence identity to SEQ ID NOs: 48-50, and/or comprises at least the CDR regions of any one of SEQ ID NOs: 48-50.

2. The anti-PD-1 binding agent of claim 1, wherein the immunoglobulin heavy chain variable region comprises at least 90% sequence identity to any one of SEQ ID NOs: 24-33, and/or comprises at least the CDR regions of any one of SEQ ID NOs: 24-33.

3. The anti-PD-1 binding agent of claim 1, comprising the immunoglobulin heavy chain variable region of any one of SEQ ID NOs: 24-33.

4. The anti-PD-1 binding agent of any of claims 1-3, wherein the immunoglobulin light chain variable region comprises at least 90% sequence identity to SEQ ID NO: 34 or 35, and/or comprises at least the CDR regions of any one of SEQ ID NO: 34 or 35.

5. The anti-PD-1 binding agent of any of claims 1-3, comprising the immunoglobulin light chain variable region of SEQ ID NO: 34 or 35.

6. The anti-PD-1 binding agent of claim 1, wherein the immunoglobulin heavy chain variable region comprises:

a CDR1 comprising SEQ ID NO: 1;

a CDR2 comprising SEQ ID NO: 2; and

a CDR3 comprising SEQ ID NO: 3;

and the immunoglobulin light chain variable region comprises

a CDR1 comprising SEQ ID NO: 4;

a CDR2 comprising SEQ ID NO: 5; and

a CDR3 comprising SEQ ID NO: 6.

7. The anti-PD-1 binding agent of claim 6, wherein the immunoglobulin heavy chain variable region CDR1 comprises any one of SEQ ID NOs: 13-18.

8. The anti-PD-1 binding agent of claim 6 or 7, wherein the immunoglobulin heavy chain variable region CDR3 comprises any one of SEQ ID NOs: 19-21.

9. The anti-PD-1 binding agent of any one of claims 5-8, wherein the immunoglobulin light chain variable region CDR1 comprises SEQ ID NO: 22 or 23.

10. The anti-PD-1 binding agent of claim 4, wherein the immunoglobulin heavy chain variable region comprises:

a CDR1 comprising SEQ ID NO: 15;

a CDR2 comprising SEQ ID NO: 2; and

a CDR3 comprising SEQ ID NO: 20;

and the immunoglobulin light chain variable region comprises

a CDR1 comprising SEQ ID NO: 23;

a CDR2 comprising SEQ ID NO: 5; and

a CDR3 comprising SEQ ID NO: 6;

or

wherein the immunoglobulin heavy chain variable region comprises:

a CDR1 comprising SEQ ID NO: 15;

a CDR2 comprising SEQ ID NO: 2; and

a CDR3 comprising SEQ ID NO: 21;

and the immunoglobulin light chain variable region comprises

a CDR1 comprising SEQ ID NO: 23;

a CDR2 comprising SEQ ID NO: 5; and

a CDR3 comprising SEQ ID NO: 6;

or

wherein the immunoglobulin heavy chain variable region comprises:

a CDR1 comprising SEQ ID NO: 13;

a CDR2 comprising SEQ ID NO: 2; and

a CDR3 comprising SEQ ID NO: 19;

and the immunoglobulin light chain variable region comprises

a CDR1 comprising SEQ ID NO: 23;

a CDR2 comprising SEQ ID NO: 5; and

a CDR3 comprising SEQ ID NO: 6.

11. The anti-PD-1 binding agent of claim 1, wherein the immunoglobulin heavy chain variable region comprises at least 90% sequence identity to SEQ ID NO: 29, or comprises at least the CDR regions of SEQ ID NO: 29, and the immunoglobulin light chain variable region comprises at least 90% sequence identity to SEQ ID NO: 35, or comprises at least the CDR regions of SEQ ID NO: 35;

or wherein the immunoglobulin heavy chain variable region comprises at least 90% sequence identity to SEQ ID NO: 30, or comprises at least the CDR regions of SEQ ID NO: 30, and the immunoglobulin light chain variable region comprises at least 90% sequence identity to SEQ ID NO: 35, or comprises at least the CDR regions of SEQ ID NO: 35;

or wherein the immunoglobulin heavy chain variable region comprises at least 90% sequence identity to SEQ ID NO: 24, or comprises at least the CDR regions of SEQ ID NO: 24, and the immunoglobulin light chain variable region comprises at least 90% sequence identity to SEQ ID NO: 34, or comprises at least the CDR regions of SEQ ID NO: 34.

12. The anti-PD-1 binding agent of claim 11, wherein the immunoglobulin heavy chain variable region comprises SEQ ID NO: 29, and the immunoglobulin light chain variable region comprises SEQ ID NO: 35;

or wherein the immunoglobulin heavy chain variable region comprises SEQ ID NO: 30, and the immunoglobulin light chain variable region comprises SEQ ID NO: 35;

or wherein the immunoglobulin heavy chain variable region comprises SEQ ID NO: 24, and the immunoglobulin light chain variable region comprises SEQ ID NO: 34.

13. The anti-PD-1 binding agent of claim 1, wherein the immunoglobulin heavy chain variable region comprises at least 90% sequence identity to any one of SEQ ID NOs: 43-47 or 61-63, or comprises at least the CDR regions of any one of SEQ ID NOs: 43-47 or 61-63.

14. The anti-PD-1 binding agent of claim 1 or 13, wherein the immunoglobulin light chain variable region comprises at least 90% sequence identity to SEQ ID NOs: 48-50, or comprises at least the CDR regions of any one of SEQ ID NOs: 48-50.

15. The anti-PD-1 binding agent of claim 13 or 14, wherein the immunoglobulin heavy chain comprises any one of SEQ ID NOs: 43-47 or 61-63.

16. The anti-PD-1 binding agent of any of claims 13-15, comprising the immunoglobulin light chain variable region of any one of SEQ ID NOs: 48-50.

17. The anti-PD-1 binding agent of claim 1, wherein the immunoglobulin heavy chain variable region comprises:

a CDR1 comprising SEQ ID NO: 7;

a CDR2 comprising SEQ ID NO: 8; and

a CDR3 comprising SEQ ID NO: 9;

and the immunoglobulin light chain variable region comprises

a CDR1 comprising SEQ ID NO: 10;

a CDR2 comprising SEQ ID NO: 11; and

a CDR3 comprising SEQ ID NO: 12.

18. The anti-PD-1 binding agent of claim 17, wherein the immunoglobulin heavy chain variable region CDR1 comprises any one of SEQ ID NO: 57-60.

19. The anti-PD-1 binding agent of claim 17 or 18, wherein the immunoglobulin heavy chain variable region CDR2 comprises any one of SEQ ID NOs: 38-42.

20. The anti-PD-1 binding agent of claim 17, wherein the immunoglobulin heavy chain variable region comprises:

a CDR1 comprising SEQ ID NO: 57;

a CDR2 comprising SEQ ID NO: 42; and

a CDR3 comprising SEQ ID NO: 9;

and the immunoglobulin light chain variable region comprises

a CDR1 comprising SEQ ID NO: 10;

a CDR2 comprising SEQ ID NO: 11; and

a CDR3 comprising SEQ ID NO: 12;

or

wherein the immunoglobulin heavy chain variable region comprises:

a CDR1 comprising SEQ ID NO: 57;

a CDR2 comprising SEQ ID NO: 41; and

a CDR3 comprising SEQ ID NO: 9;

and the immunoglobulin light chain variable region comprises

a CDR1 comprising SEQ ID NO: 10;

a CDR2 comprising SEQ ID NO: 11; and

a CDR3 comprising SEQ ID NO: 12.

21. The anti-PD-1 binding agent of claim 1, wherein the immunoglobulin heavy chain variable region comprises at least 90% sequence identity to SEQ ID NO: 47, or comprises at least the CDR regions of SEQ ID NO: 47;

and the immunoglobulin light chain variable region comprises at least 90% sequence identity to SEQ ID NO: 49, or comprises at least the CDR regions of SEQ ID NO: 49;

or wherein the immunoglobulin heavy chain variable region comprises at least 90% sequence identity to SEQ ID NO: 46, or comprises at least the CDR regions of SEQ ID NO: 46;

and the immunoglobulin light chain variable region comprises at least 90% sequence identity to SEQ ID NO: 50, or comprises at least the CDR regions of SEQ ID NO: 50.

22. The anti-PD-1 binding agent of claim 21, comprising the immunoglobulin heavy chain variable region of SEQ ID NO: 47, and the immunoglobulin light chain variable region of SEQ ID NO: 49;

or comprising the immunoglobulin heavy chain variable region of SEQ ID NO: 46, and the immunoglobulin light chain variable region of SEQ ID NO: 50.

23. The anti-PD-1 binding agent of any one of claims 1-22, wherein the anti-PD-1 binding agent is an antibody, an antibody conjugate, or an antigen-binding fragment thereof.

24. The anti-PD-1 binding agent of any one of claims 1-23, wherein the anti-PD-1 binding agent is a $F(ab')_2$, Fab', Fab, Fv, scFv, dsFv, or a single chain binding polypeptide.

25. The anti-PD-1 binding agent of any one of claims 1-24, wherein the anti-PD-1 binding agent comprises an IgG Fc region that binds an Fc receptor on an antigen presenting cell.

26. The anti-PD-1 binding agent of any one of claims 1-25, wherein the anti-PD-1 binding agent comprises an Fc region of IgG1 or other Fc region that binds FcγR.

27. The anti-PD-1 binding agent of claim 1, comprising an immunoglobulin heavy chain comprising SEQ ID NO: 36 and an immunoglobulin light chain comprising SEQ ID NO: 37.

28. The anti-PD-1 binding agent of claim 1, comprising an immunoglobulin heavy chain comprising SEQ ID NO: 51 and an immunoglobulin light chain comprising SEQ ID NO: 52.

29. A pharmaceutical composition comprising (a) anti-PD-1 binding agent of any one of claims 1-28, and (b) a pharmaceutically acceptable carrier.

30. A method of inhibiting an immune response in a mammal, which method comprises administering the anti-PD-1 binding agent of any of claims 1-28 or pharmaceutical composition of claim 29 to the mammal.

31. A method of treating an inflammatory or autoimmune disorder in a mammal, which method comprises administering the anti-PD-1 binding agent of any of claims 1-28 or pharmaceutical composition of claim 29 to a mammal with an inflammatory or autoimmune disorder, whereupon the disorder is treated.

32. The method of claim 31, wherein the inflammatory or autoimmune disorder is Primary Biliary Cholangitis (PBC), Graft vs Host Disease (GvHD), Vitiligo, ANCA Vasculitis, Type 1 Diabetes, or Noninfectious Uveitis..

33. A nucleic acid encoding the immunoglobulin heavy chain and/or immunoglobulin light chain of the anti-PD-1 binding agent of any of claims 1-28, optionally in a vector.

34. A cell that expresses the anti-PD-1 binding agent of any of claims 1-28.

35. A method of preparing the anti-PD-1 binding agent of any one of claims 1-28, the method comprising expressing in a cell a nucleic acid sequence encoding the immunoglobulin heavy chain and a nucleic acid sequence encoding the immunoglobulin light chain of the anti-PD-1 binding agent of any of claims 1-28.

36. The anti-PD-1 binding agent of any of claims 1-28, or the pharmaceutical composition of claim 29, for inhibiting an immune response in a mammal.

37. The anti-PD-1 binding agent of any of claims 1-28, or the pharmaceutical composition of claim 29, for treating an inflammatory or autoimmune disorder in a mammal.

32. The anti-PD-1 binding agent or pharmaceutical composition of claim 37, wherein the inflammatory or autoimmune disorder is Primary Biliary Cholangitis (PBC), Graft vs Host Disease (GvHD), Vitiligo, ANCA Vasculitis, Type 1 Diabetes, or Noninfectious Uveitis.

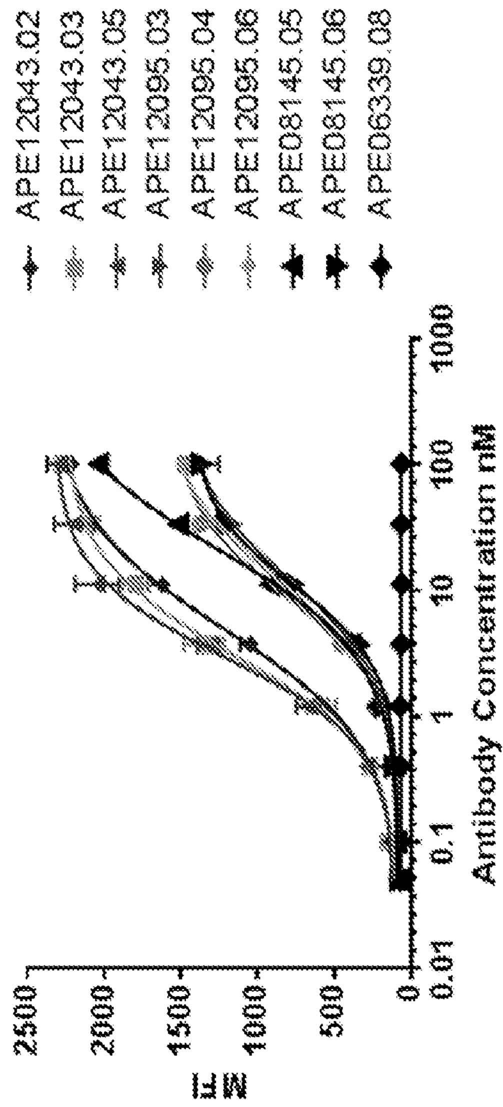


Figure 1

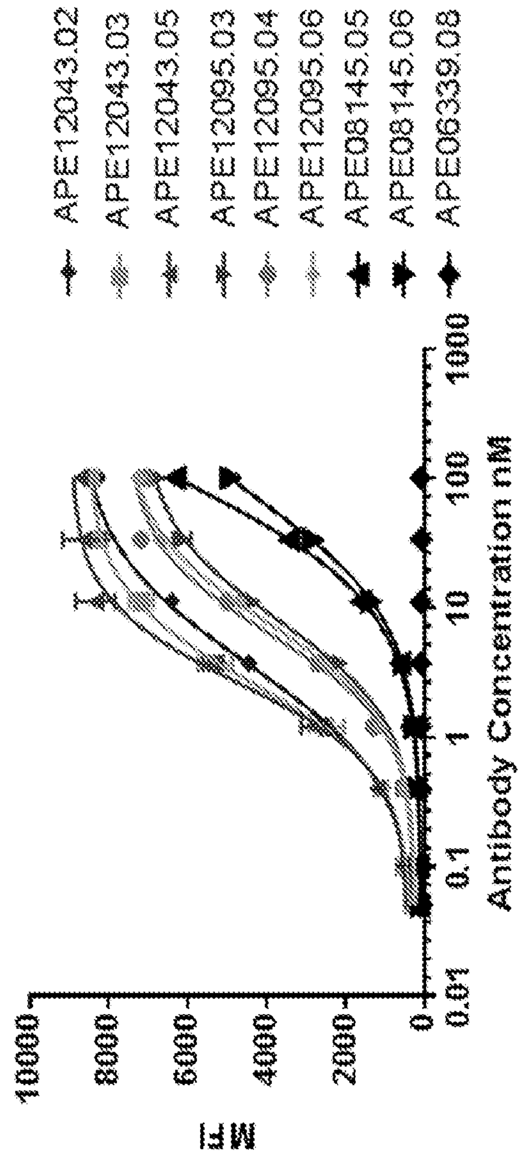


Figure 2

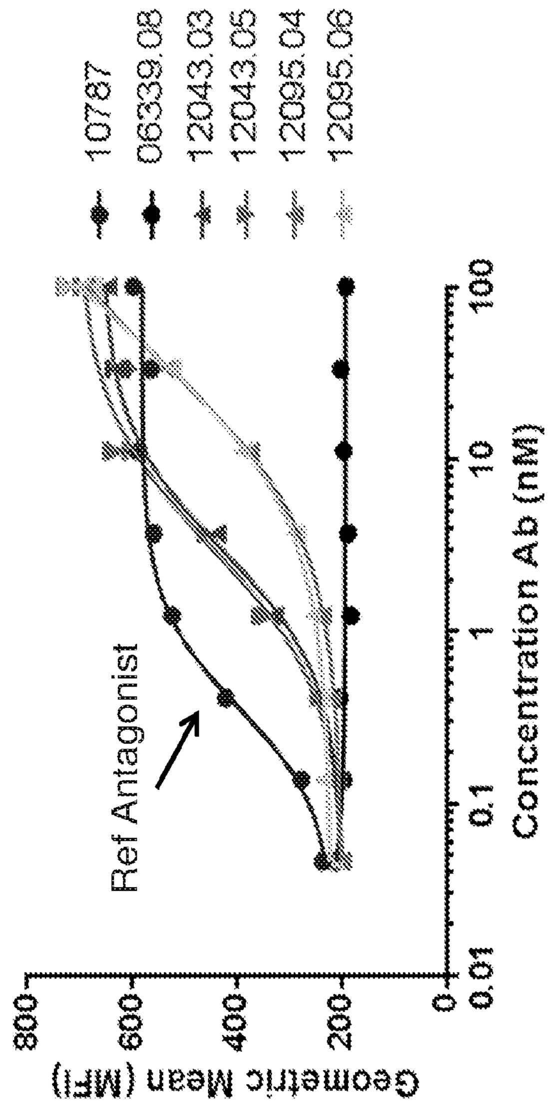


Figure 3

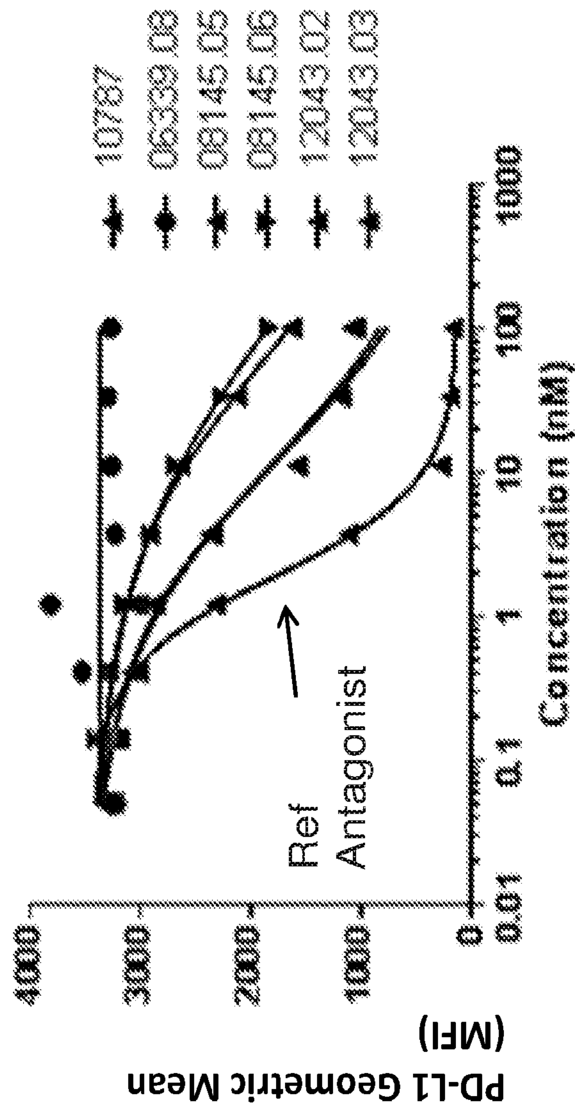


Figure 4

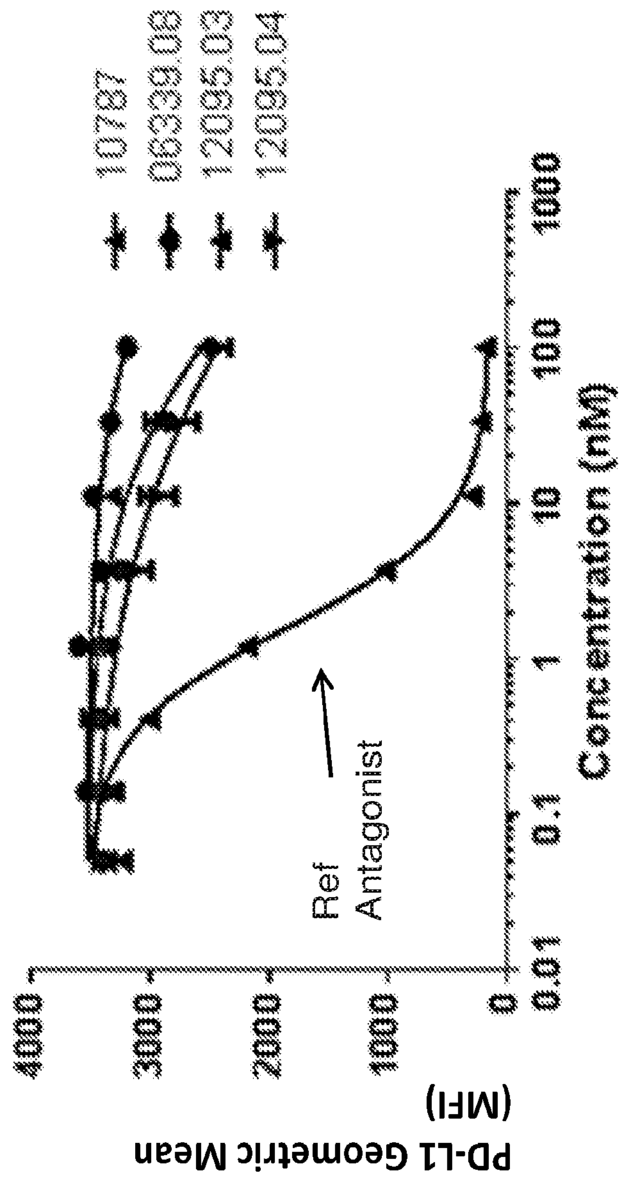


Figure 5

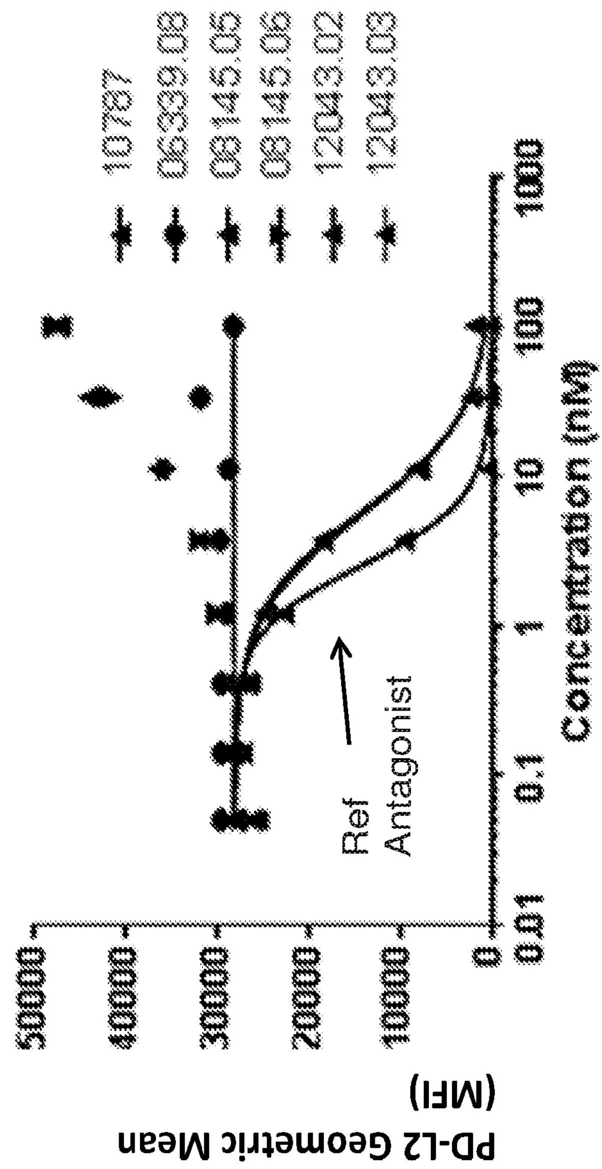


Figure 6

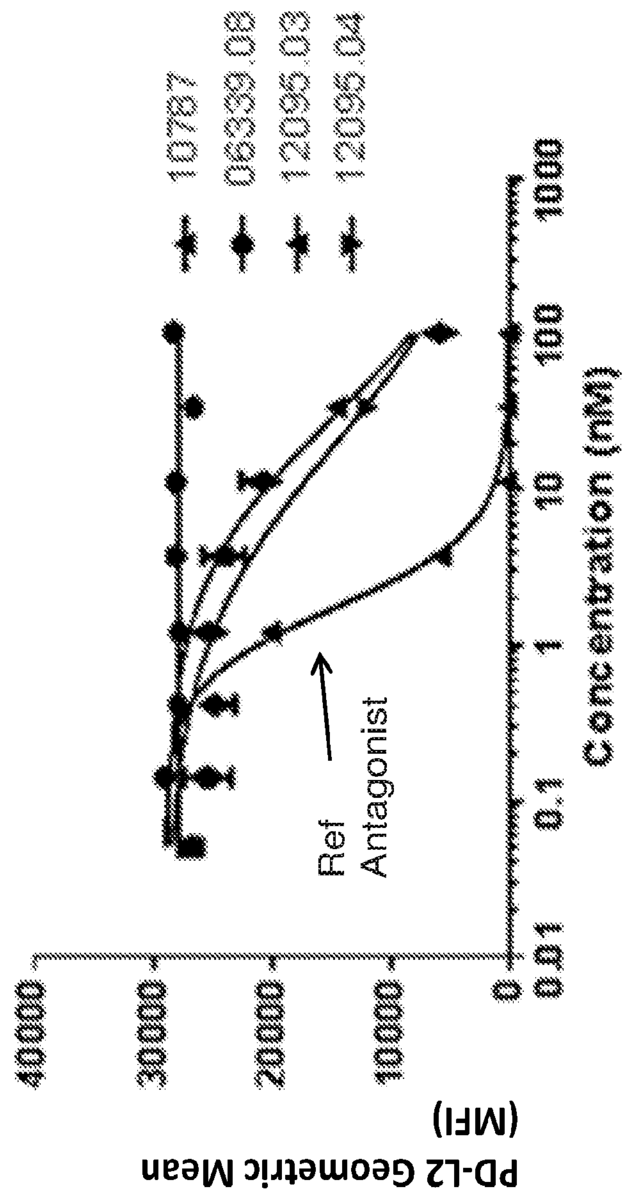


Figure 7

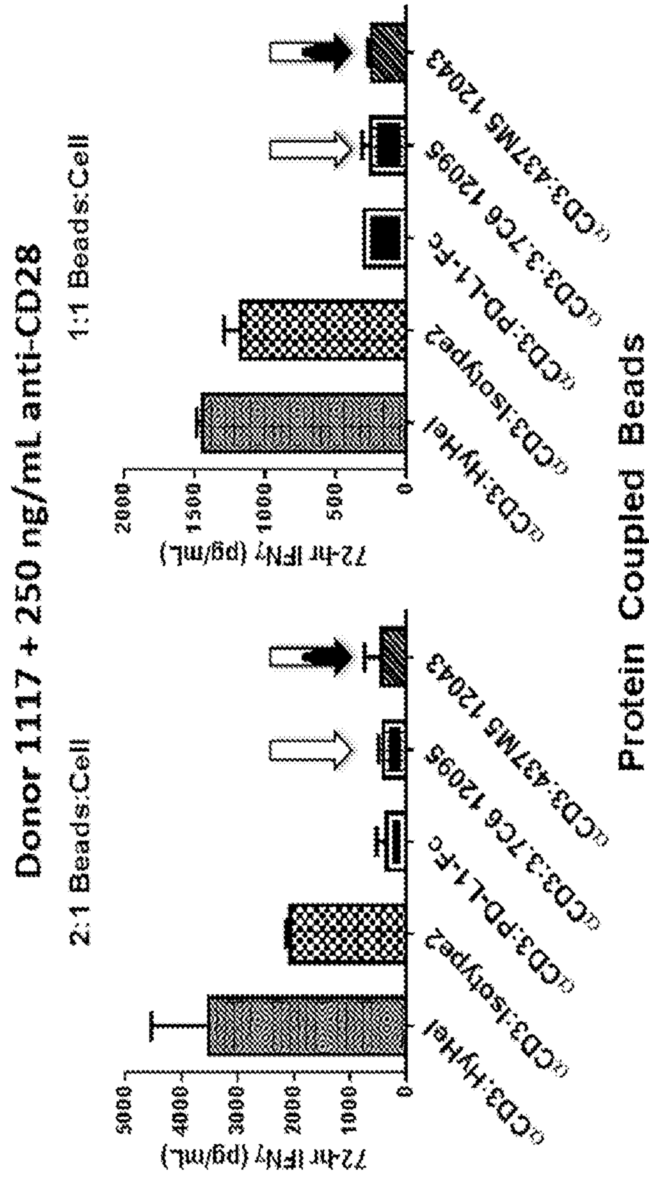


Figure 8A

Figure 8B

Donor 1118

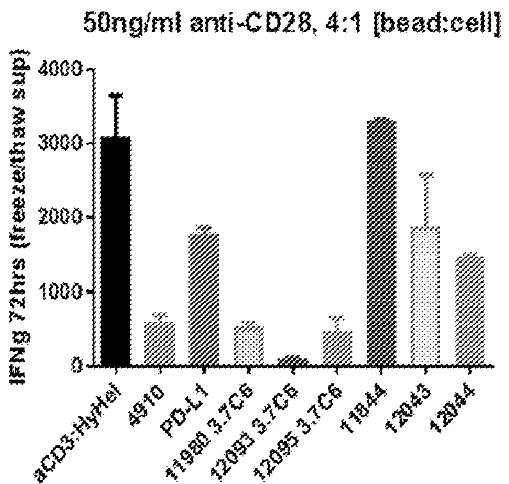


Figure 9A

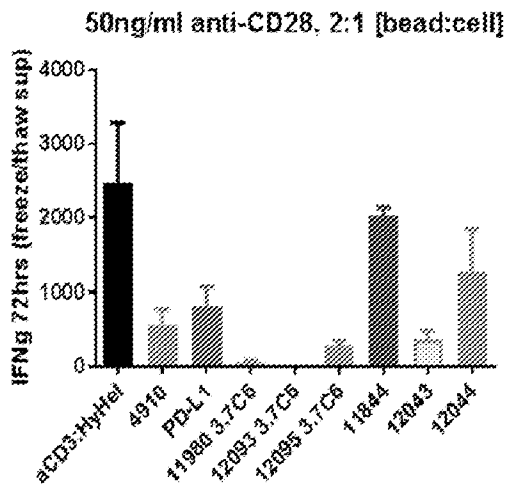


Figure 9B

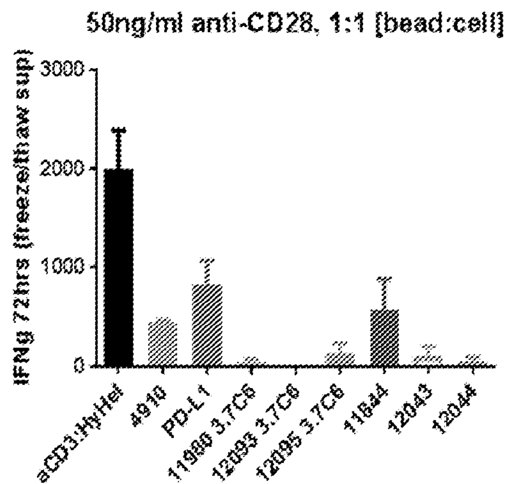


Figure 9C

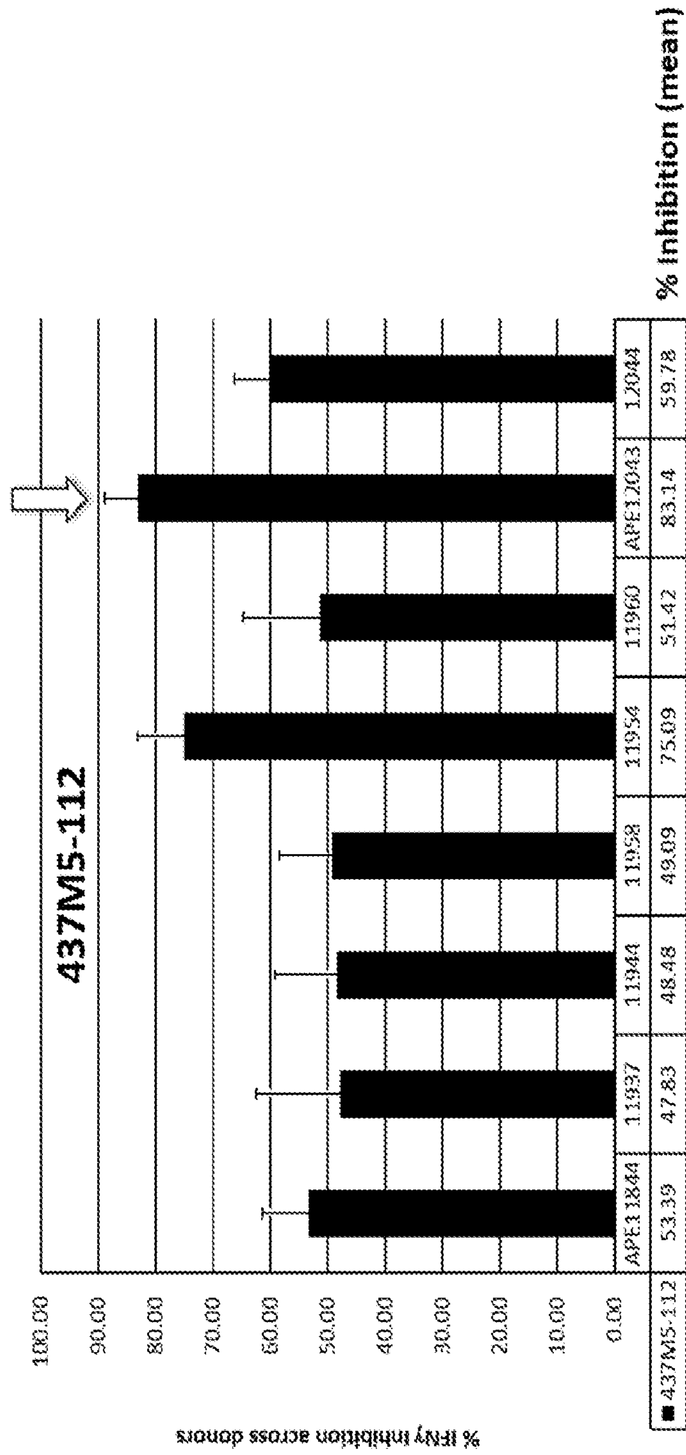


Figure 10A

437M5-112	Description	% IFNγ Inihibition Mean +/- SEM	Donors (N)
APE11844	CDR-grafted 437M5-112 R71A,T73K,I75S/Parental	53.39 ± 7.92	8
APE11937	437M5-112 N31S,T33S,R71A,T73K,I75S,G99R/parental	47.83 ± 14.72	3
APE11944	437M5-112 N31S,T33S,R71A,T73K,I75S,G99R,V100cA/parental	48.48 ± 10.61	3
APE11954	437M5-112 N31S,R71A,T73K,I75S,G99R/parental	75.09 ± 7.91	3
APE11958	437M5-112 N31D,Y32H,R71A,T73K,I75S,G99R/parental	49.09 ± 9.38	4
APE11960	437M5-112 N31D,Y32H,M34L,R71A,T73K,I75S,G99R,V100cA/parental	51.42 ± 13.29	3
APE12043	437M5-112; N31S,T33S,R71A,T73K,I75S,G99R/Y32F	83.14 ± 5.72	7

Figure
10B

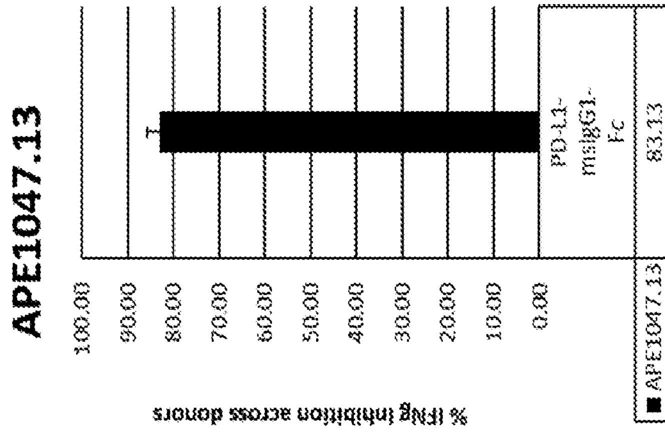


Figure 11B

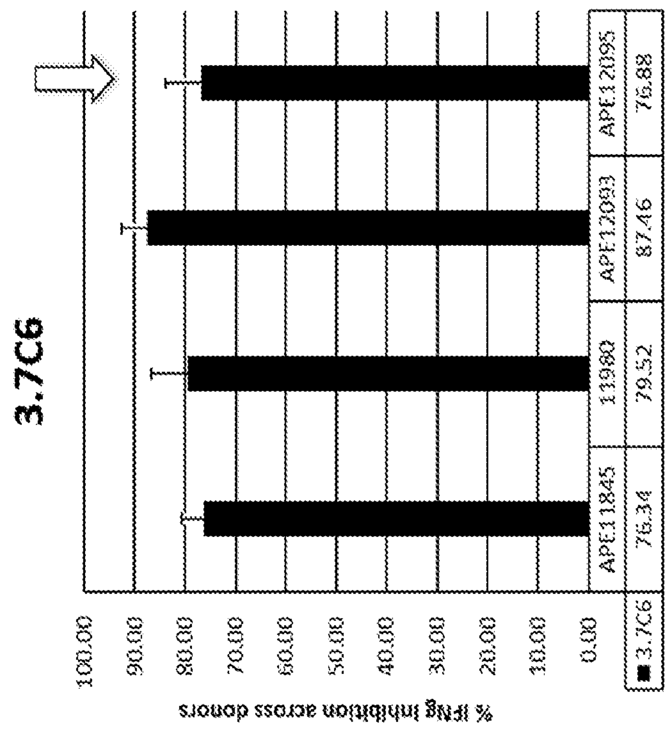


Figure 11A

3.7C6	Description	% IFNγ Inihibition Mean +/- SEM	Donors (N)
APE11845	CDR-grafted 3.7C6 V68A/Parental	76.34 ± 4.34	4
APE11980	3.7C6 V68A/G100Q,K103R,V104L	79.52 ± 7.09	4
APE12093	3.7C6 A52aV,D62Q,V68A/G100Q,K103R,V104L	87.46 ± 5.16	4
APE12095	3.7C6 A52aI,D62Q,V68Q/parental	76.88 ± 7.00	6
APE1047.13	PD-L1-msIgG1-Fc	83.13 ± 2.79	10

Figure
11C

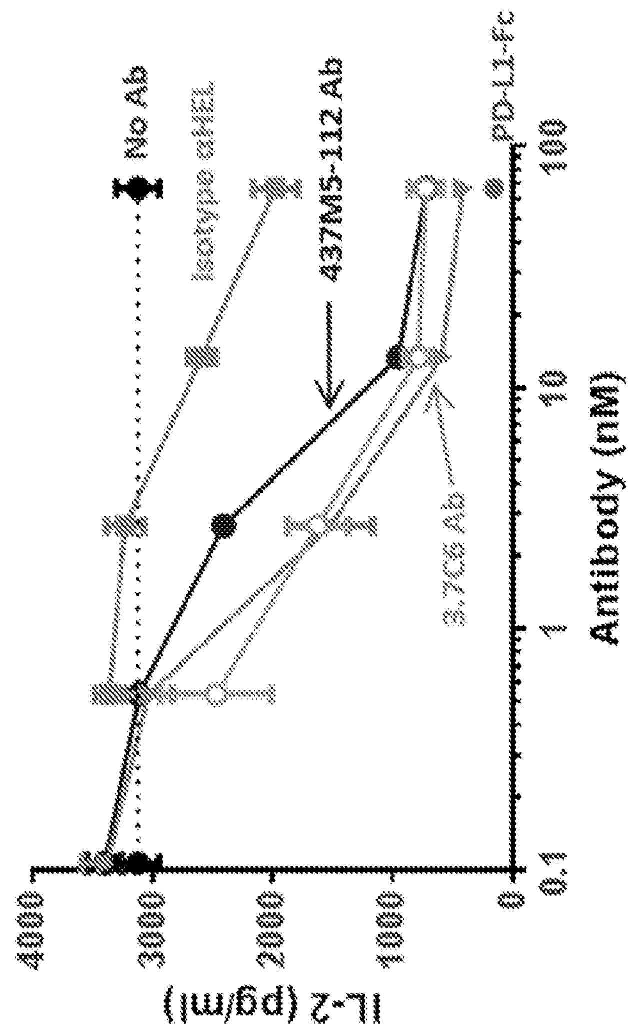


Figure
12A

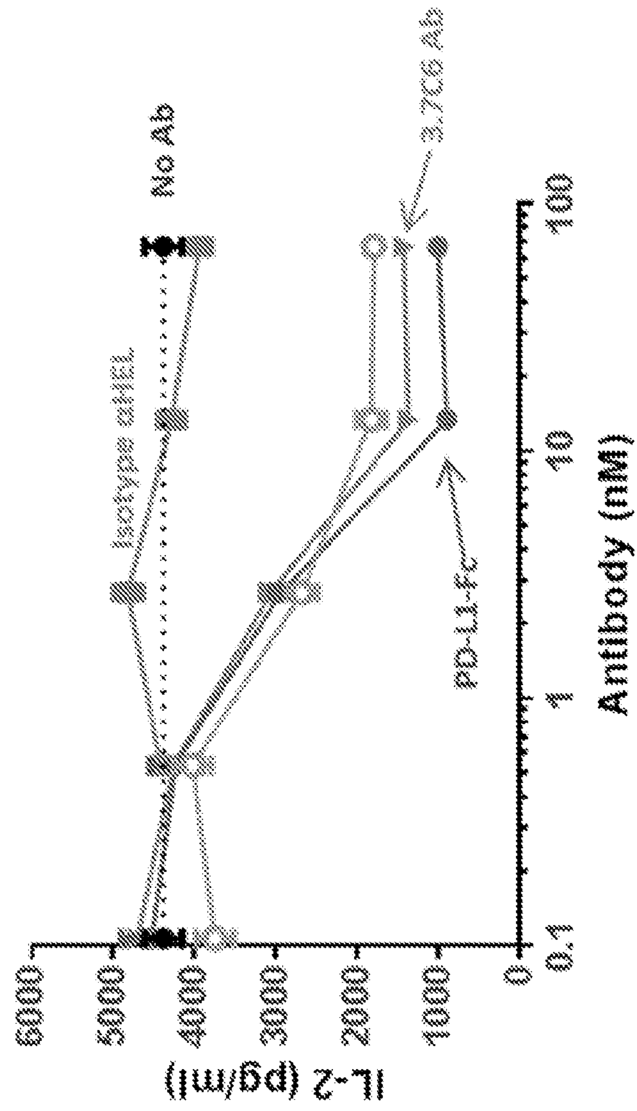


Figure 12B

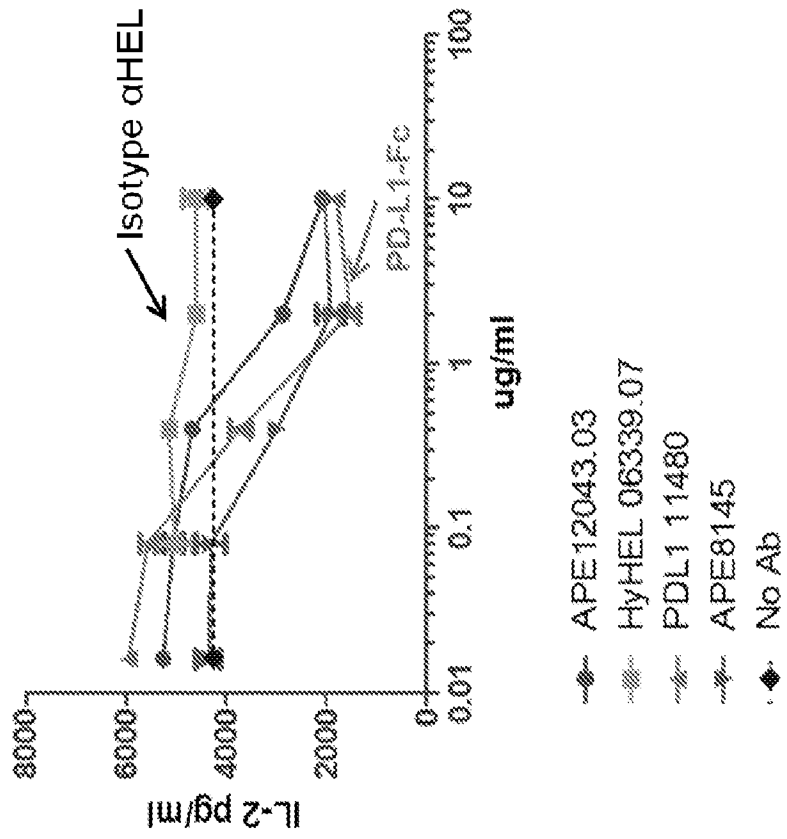


Figure 13A

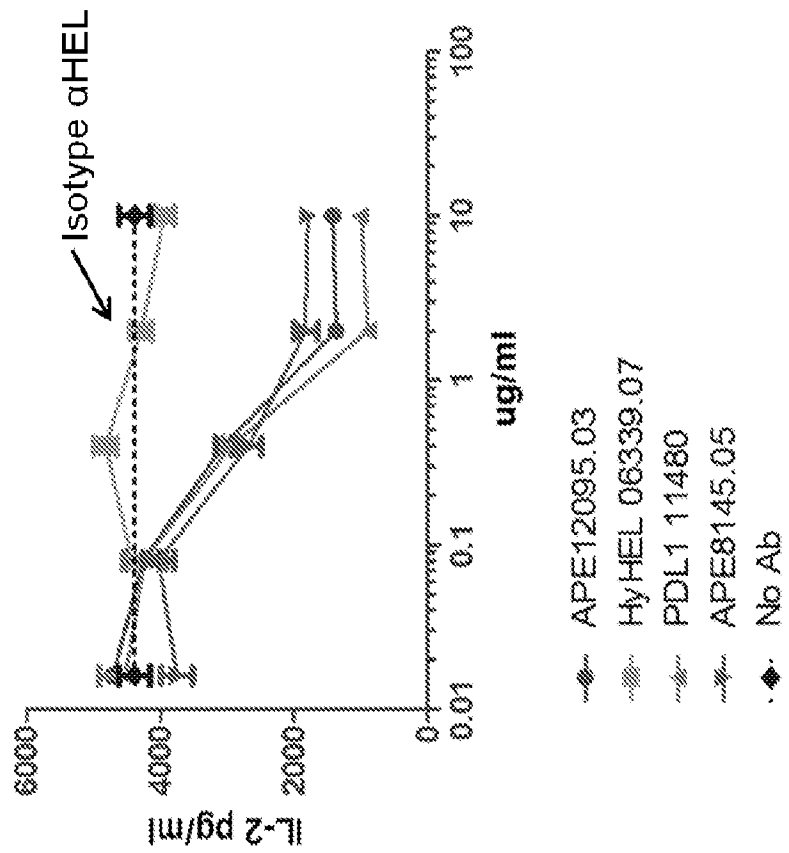


Figure 13B

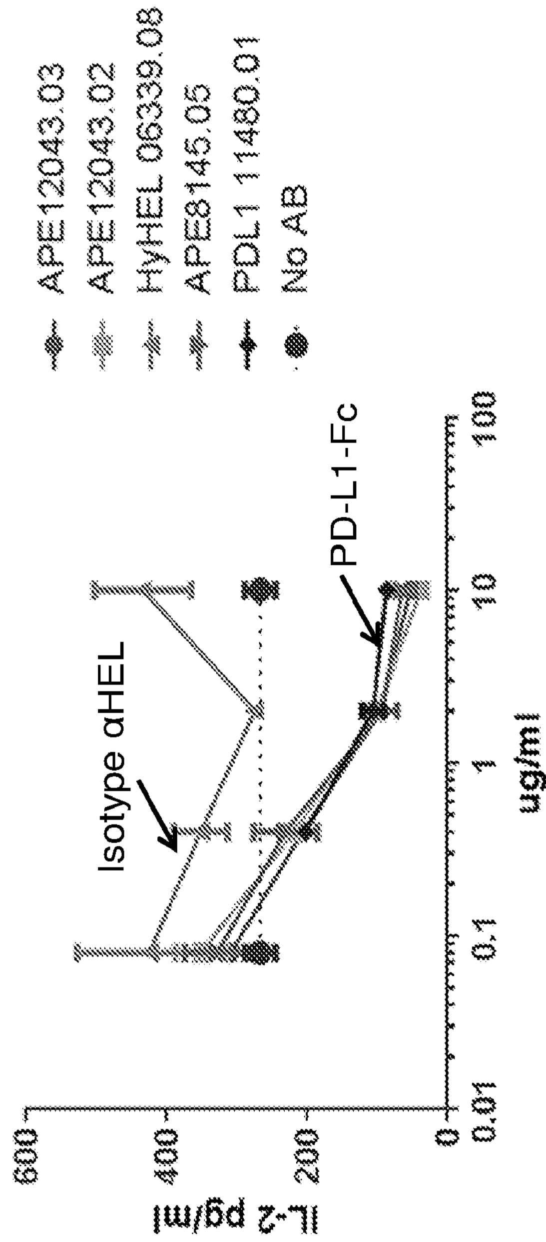


Figure 14A

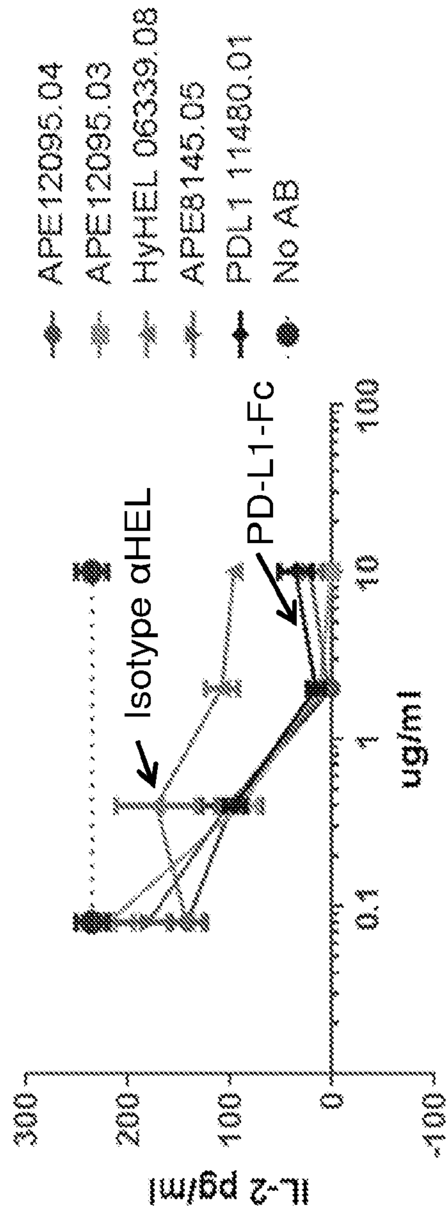


Figure 14B

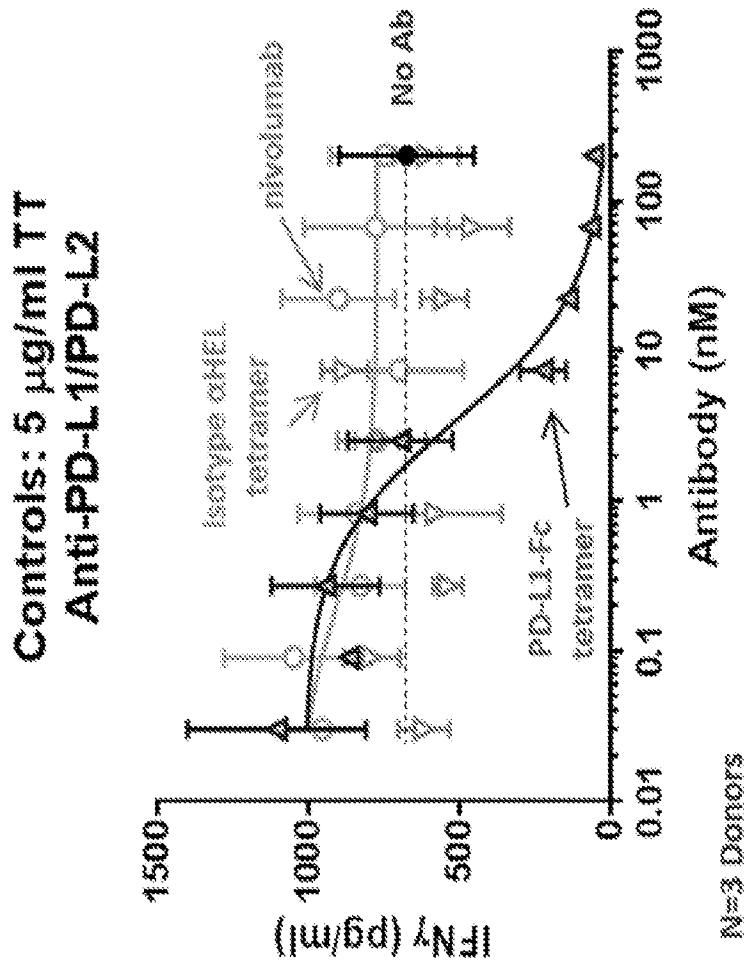


Figure 15A

PD-1 Agonist Abs: 5 μg/ml TT
Anti-PD-L1/PD-L2

IFNγ (pg/ml)

1500
1000
500
0

0.01 0.1 1 10 100 1000

Antibody (nM)

Isotype αHEL

437M5-112 Ab

3.7C6 Ab

Figure 15B

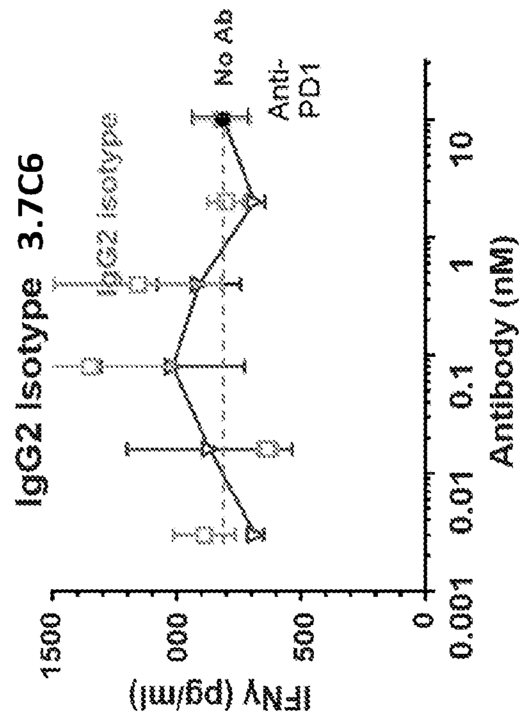


Figure 15D

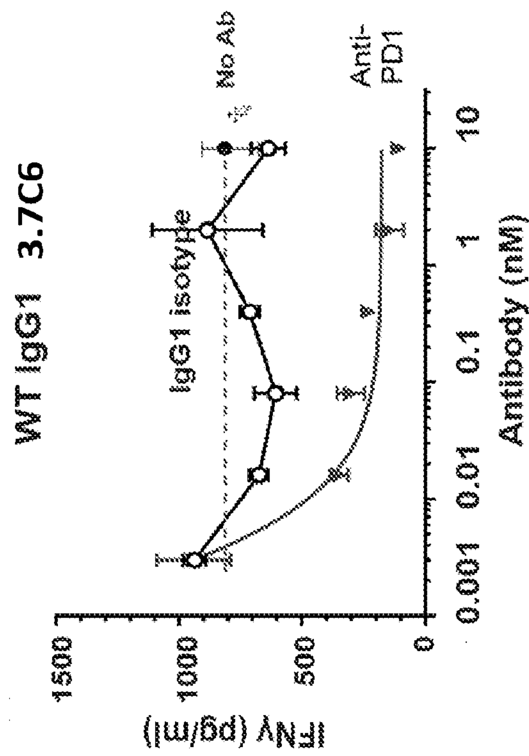


Figure 15C

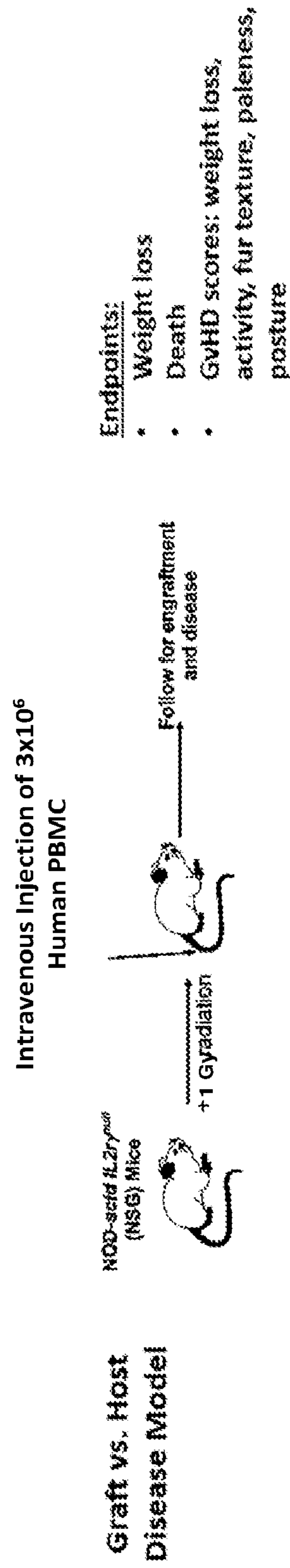


Figure 16A

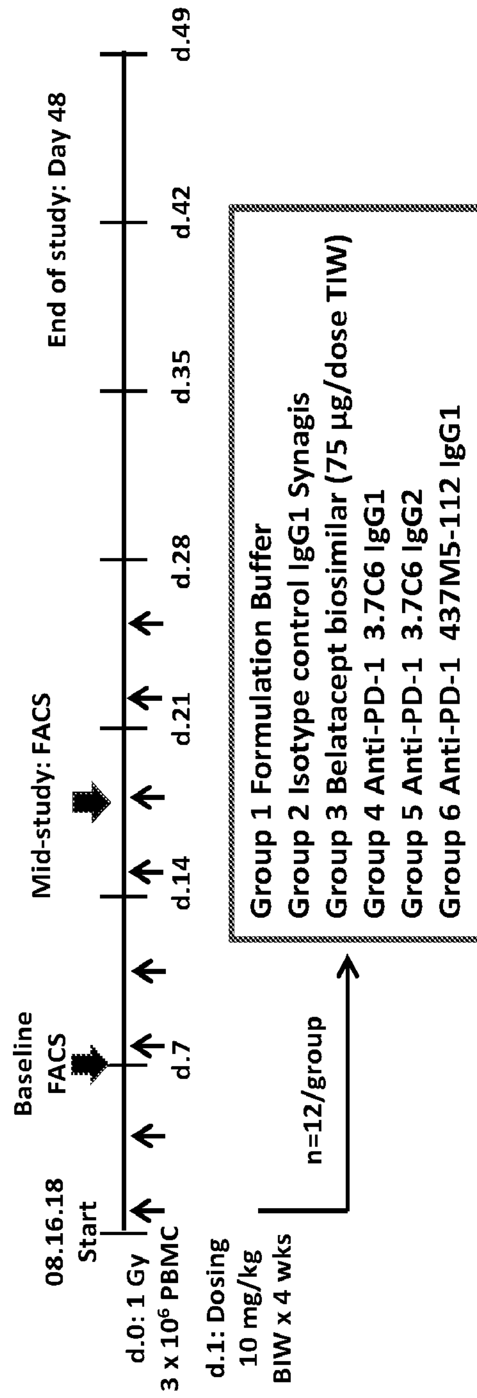
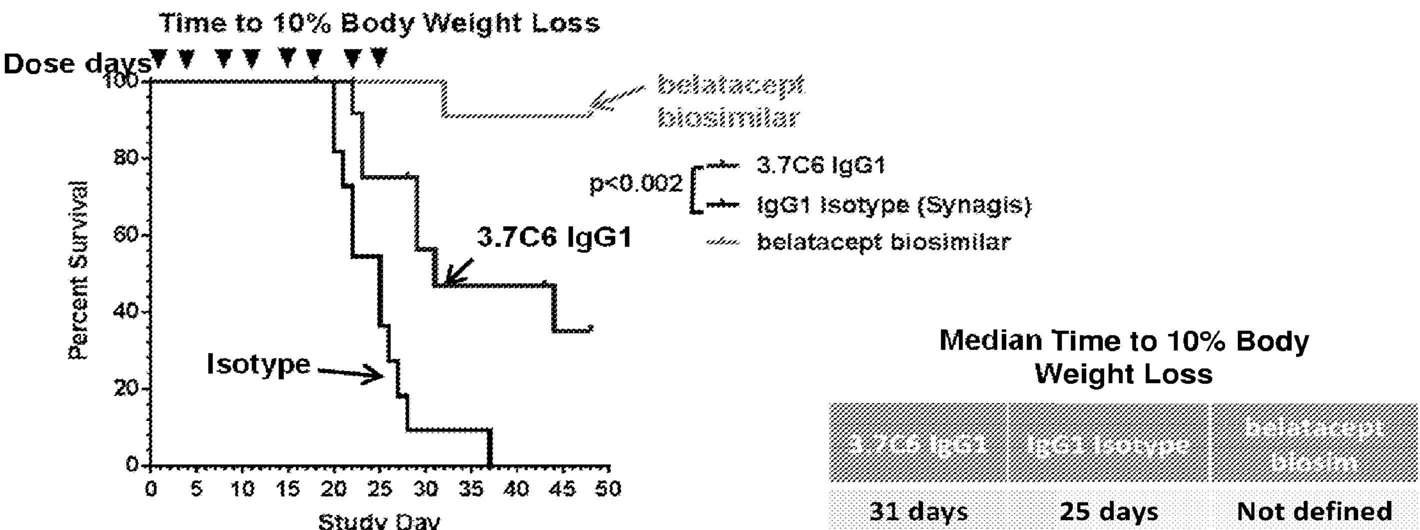


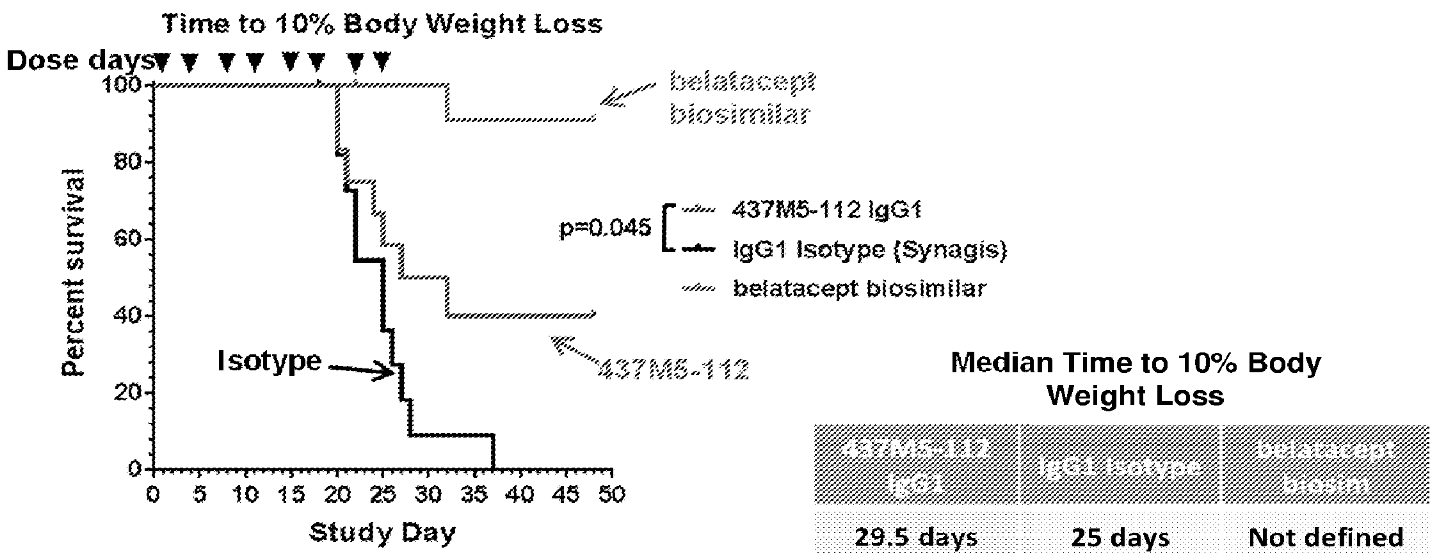
Figure 16B



Median Time to 10% Body Weight Loss

3.7C6 IgG1	IgG1 Isotype	belatacept biosim
31 days	25 days	Not defined

Figure 16C



437M5-112 IgG1	IgG1 Isotype	belatacept biosim
29.5 days	25 days	Not defined

Figure 16D

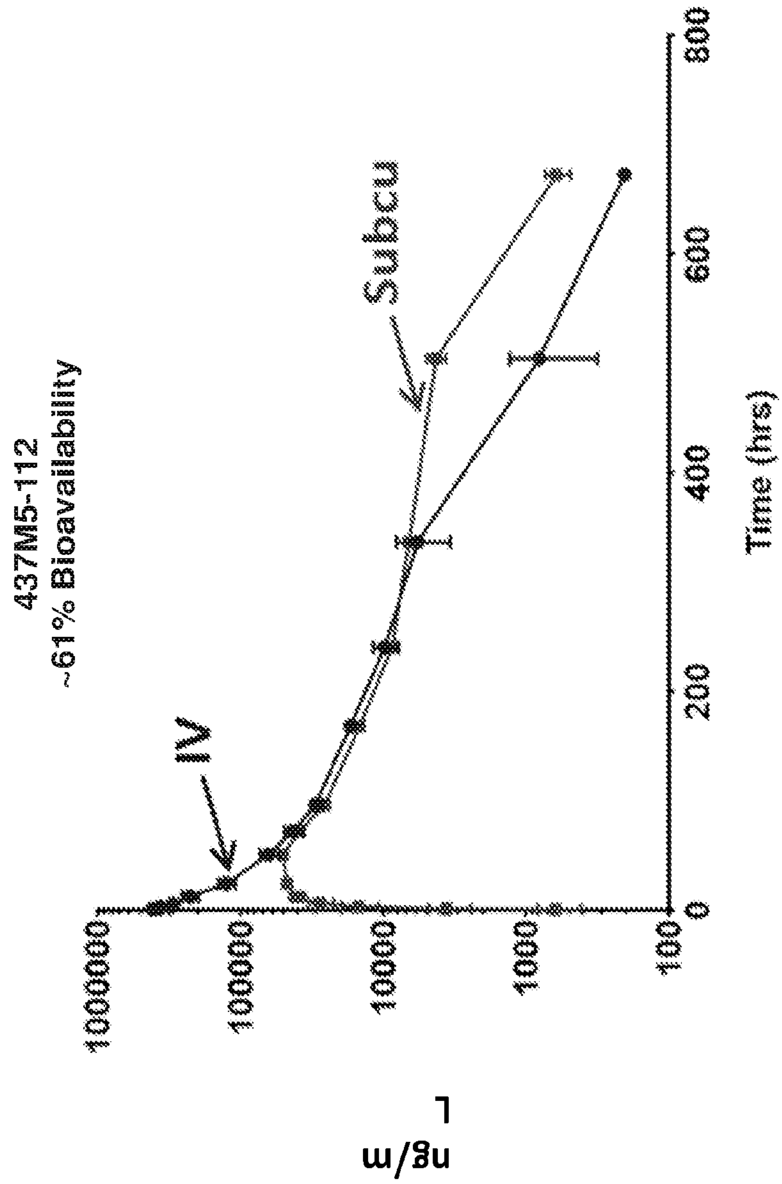


Figure 17A

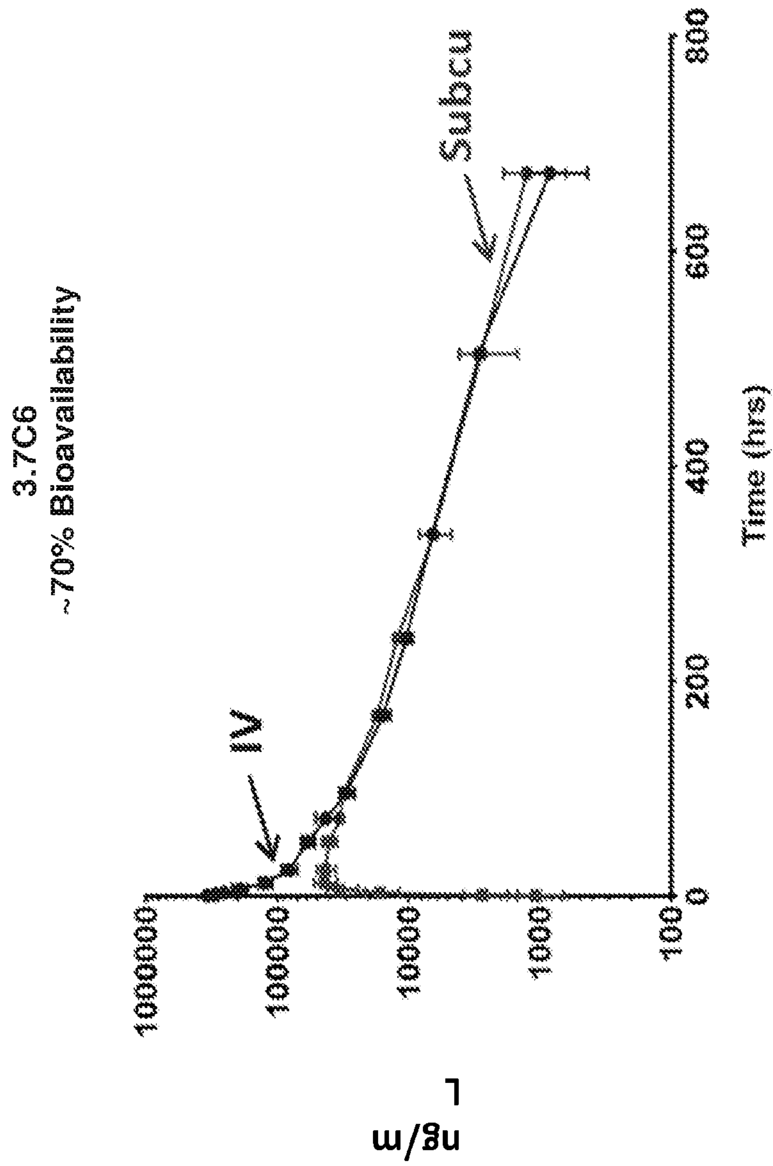


Figure 17B

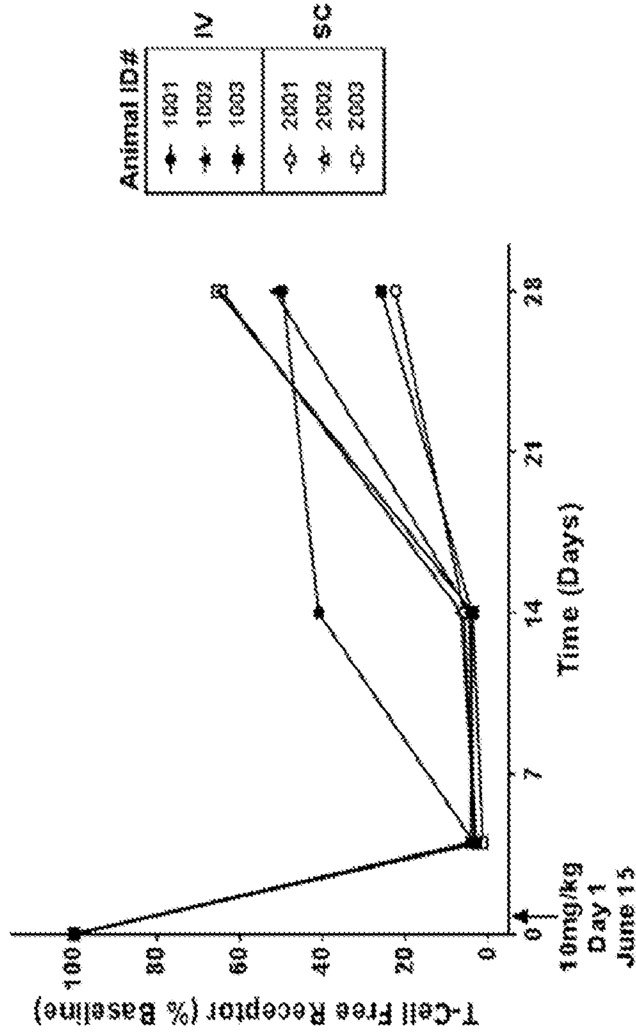


Figure 18A

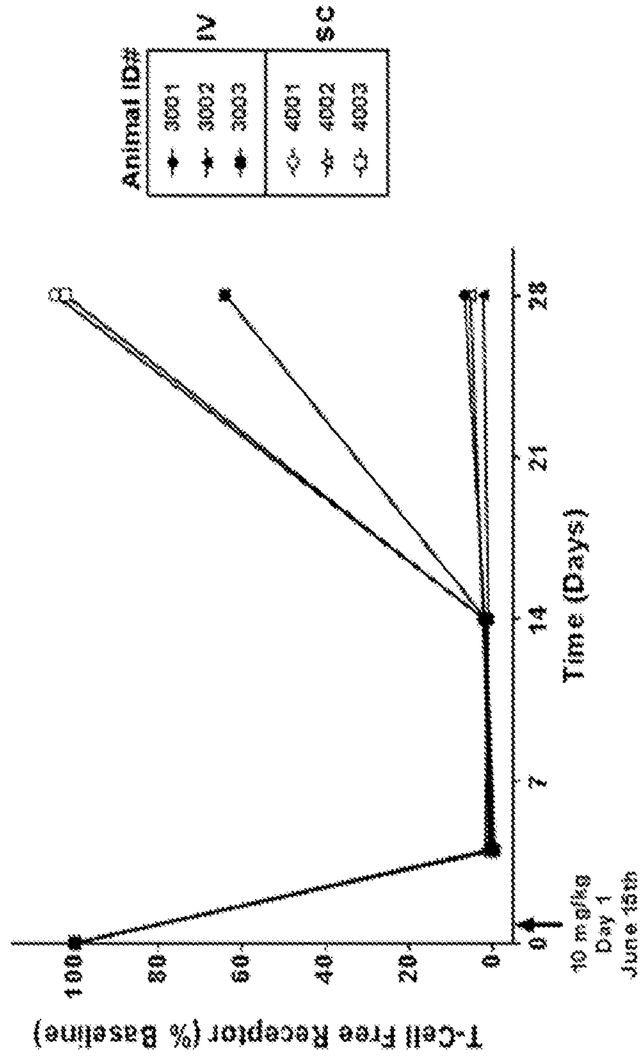


Figure 18B

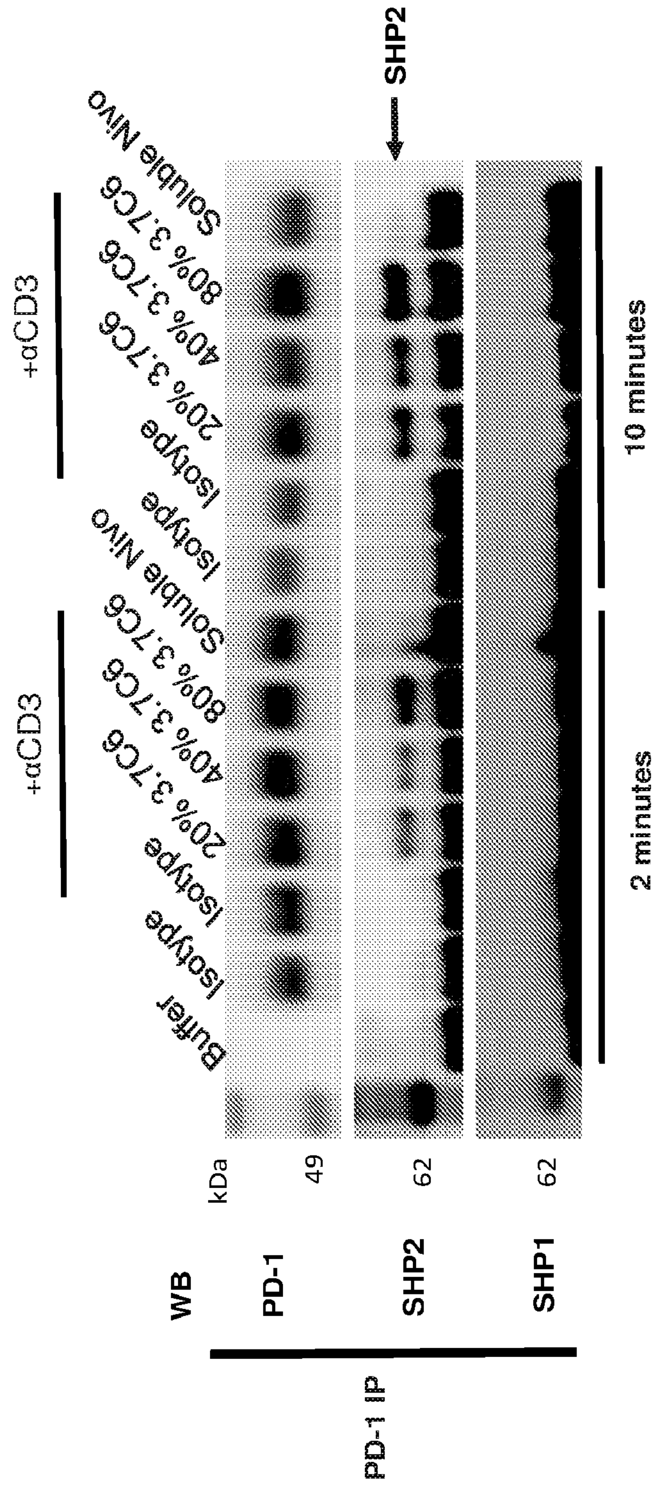


Figure 19A

3.7C6 Antibody Induces SHP2 but not SHP1 Recruitment to PD-1 after Activation of Jurkat PD-1 Cells

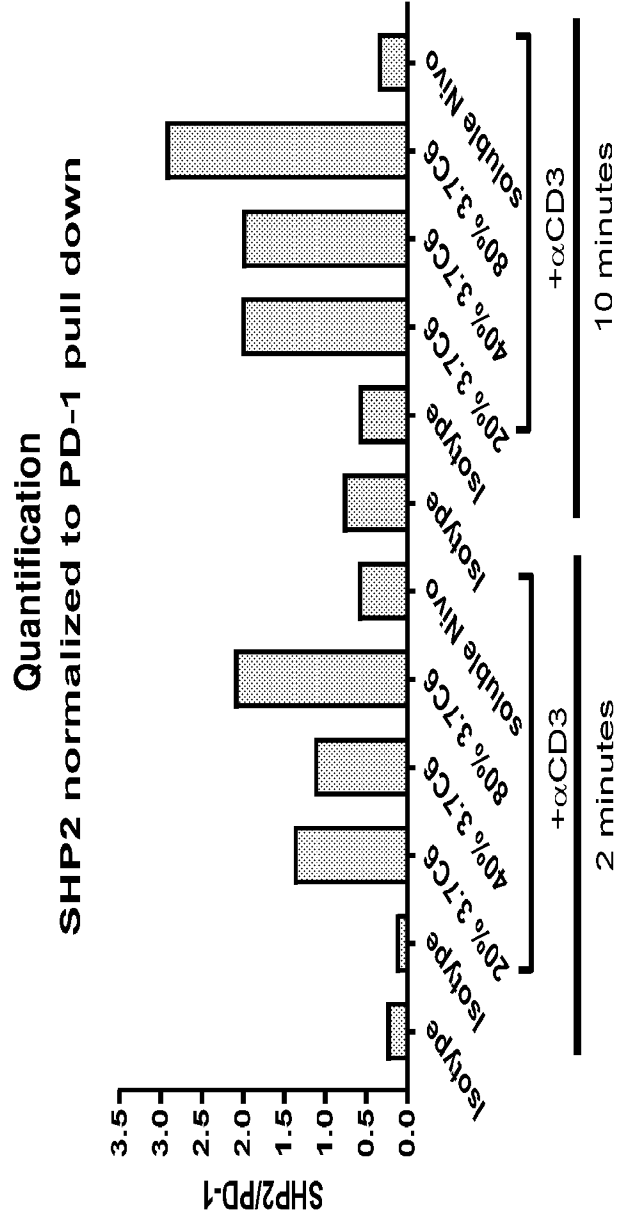


Figure 19B

Mapping of the Epitope on PD-1 Bound by the 3.7C6 Antibody by Hydrogen/Deuterium Exchange and PD-1 Mutational Analyses

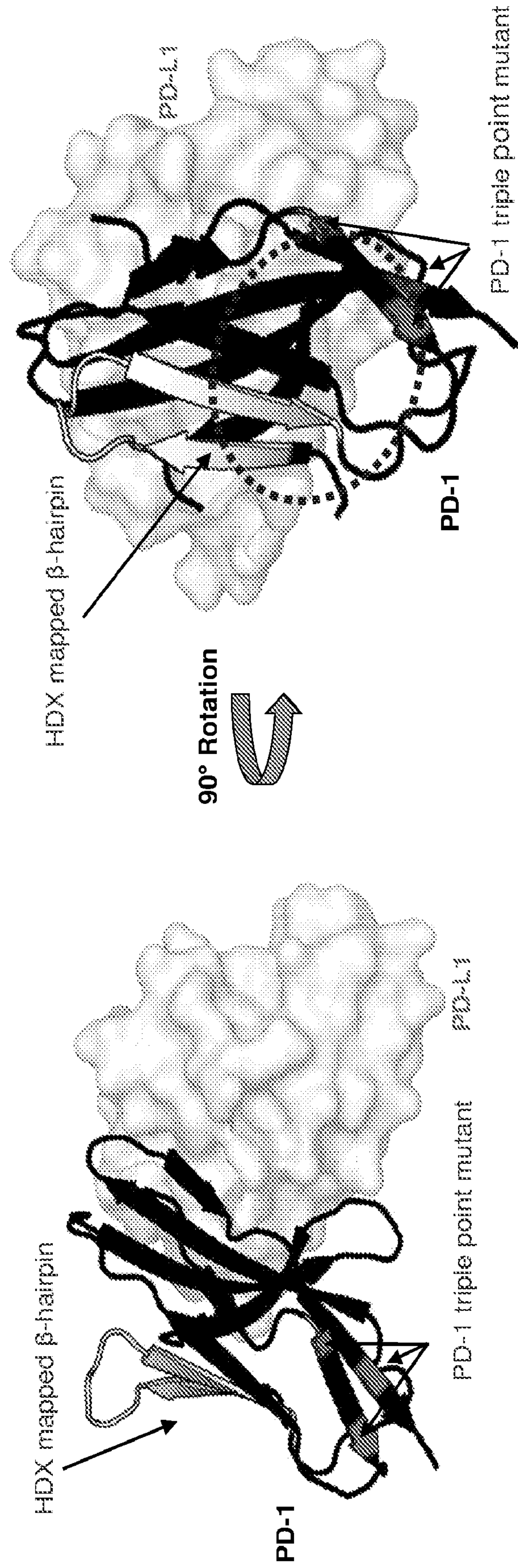


Figure 20A

Figure 20B

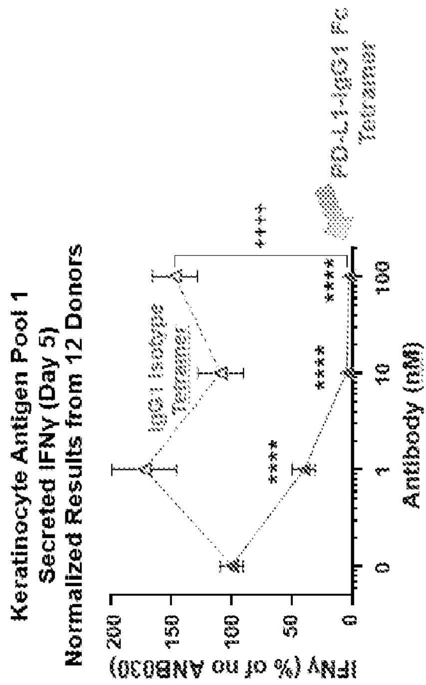


Figure 21B

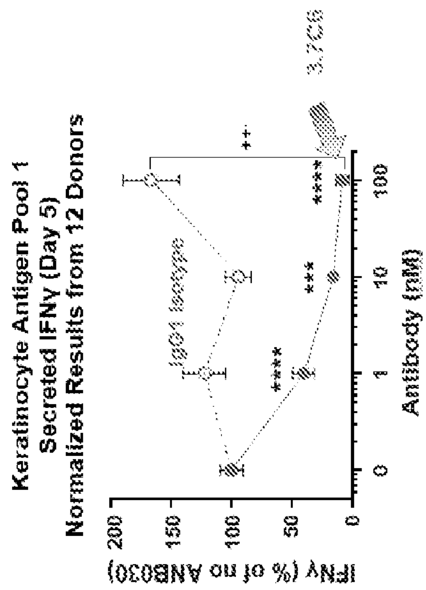


Figure 21A

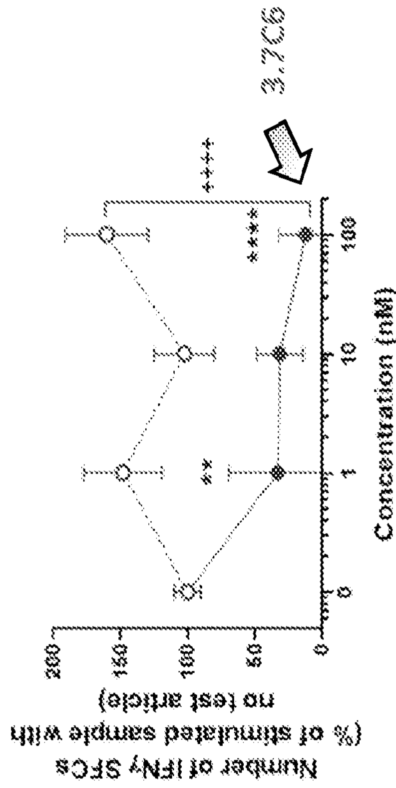


Figure 21C

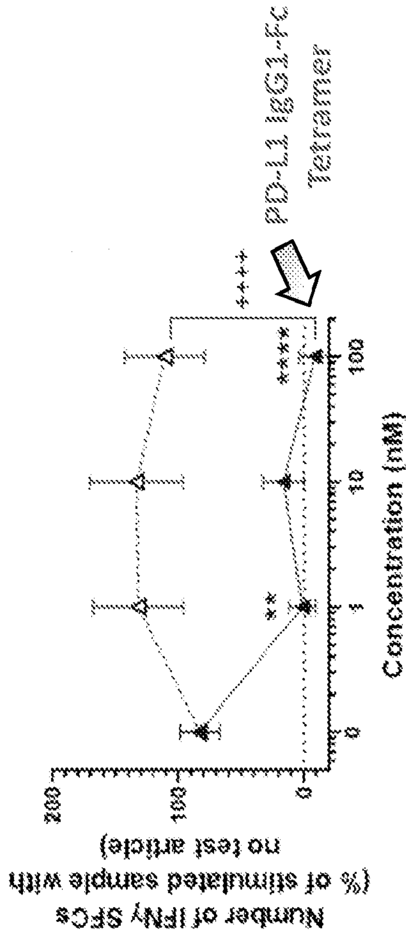


Figure 21D

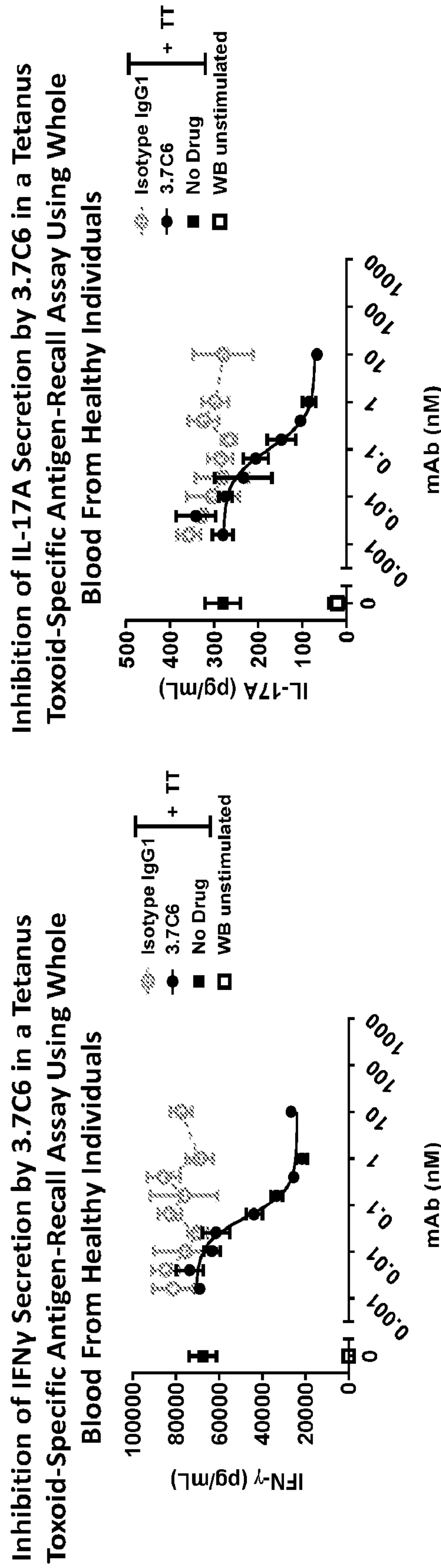


Figure 22A

Figure 22B

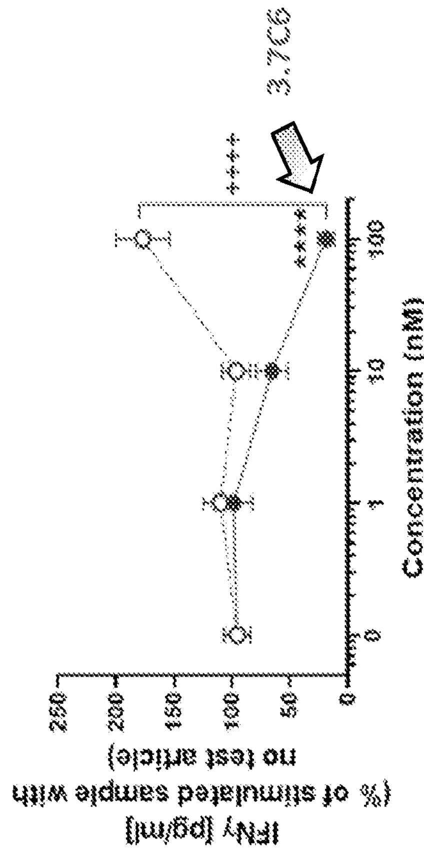


Figure 23A

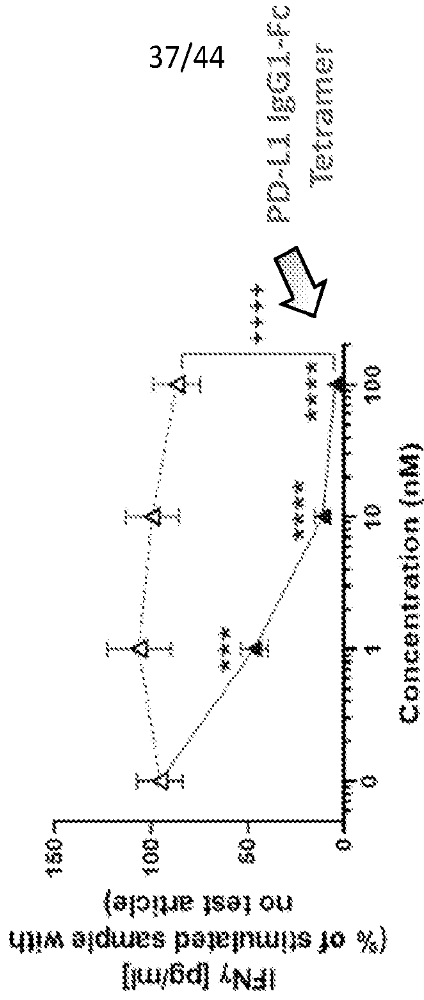


Figure 23B

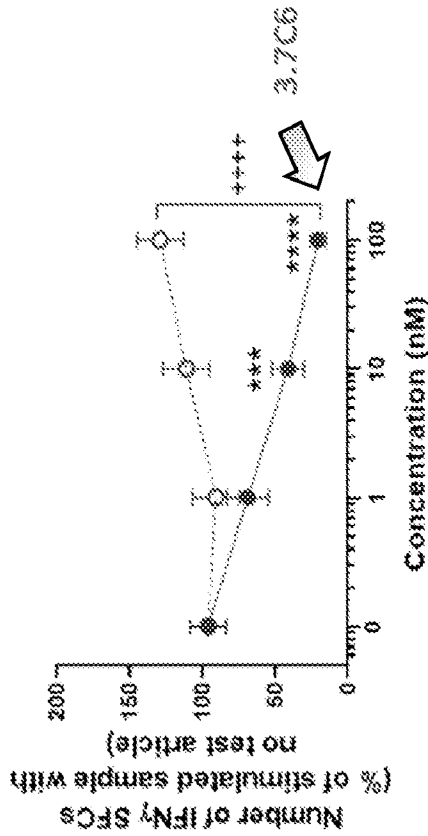


Figure 23C

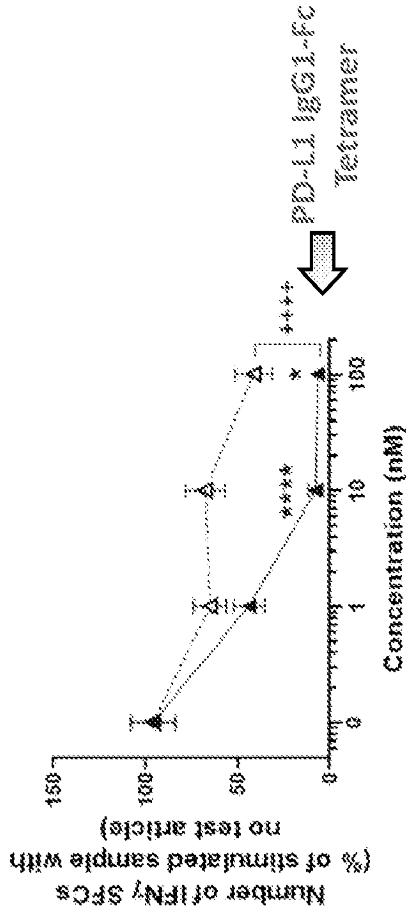


Figure 23D

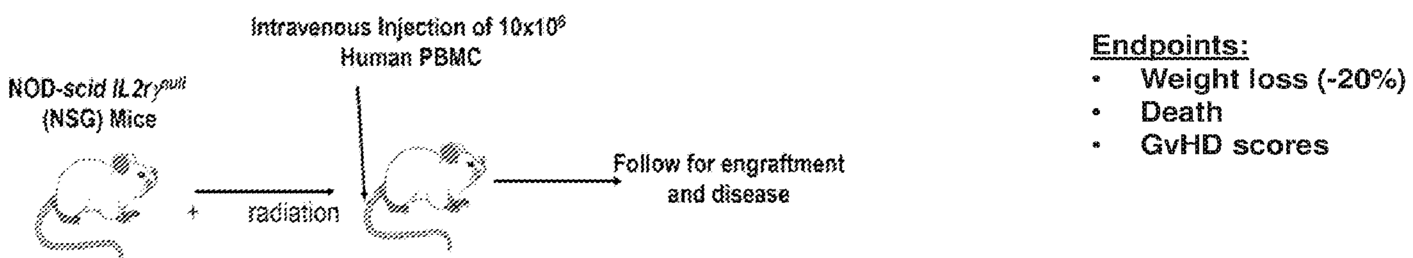


Figure 24A

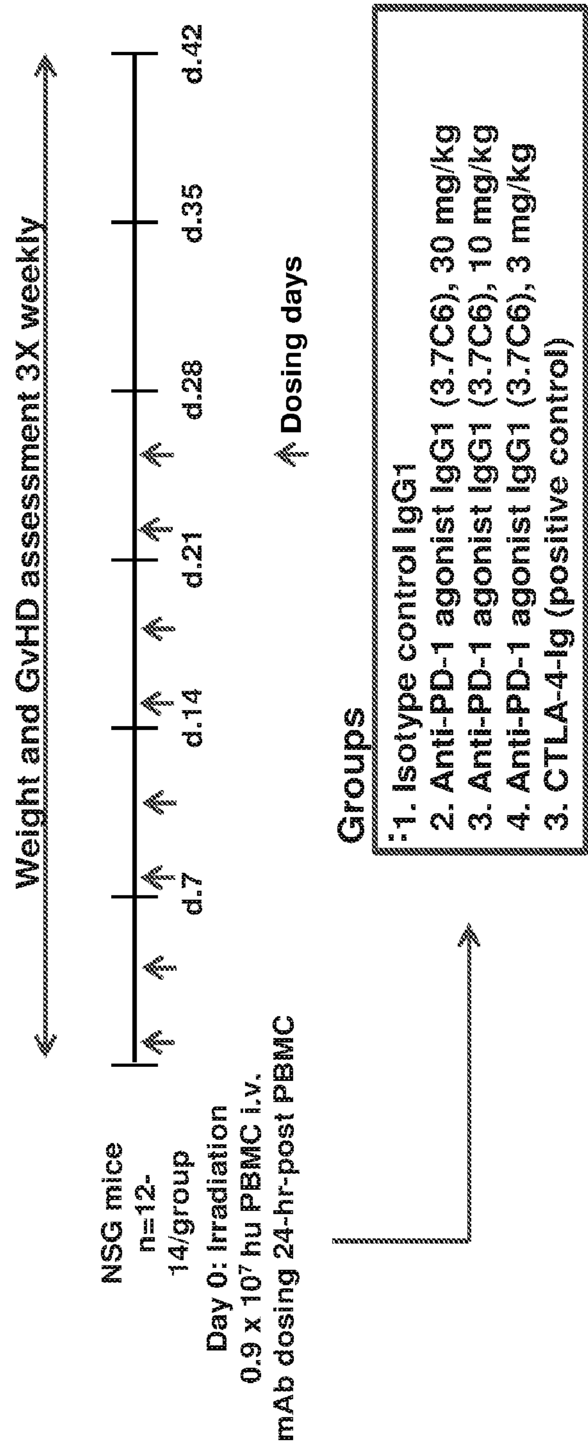
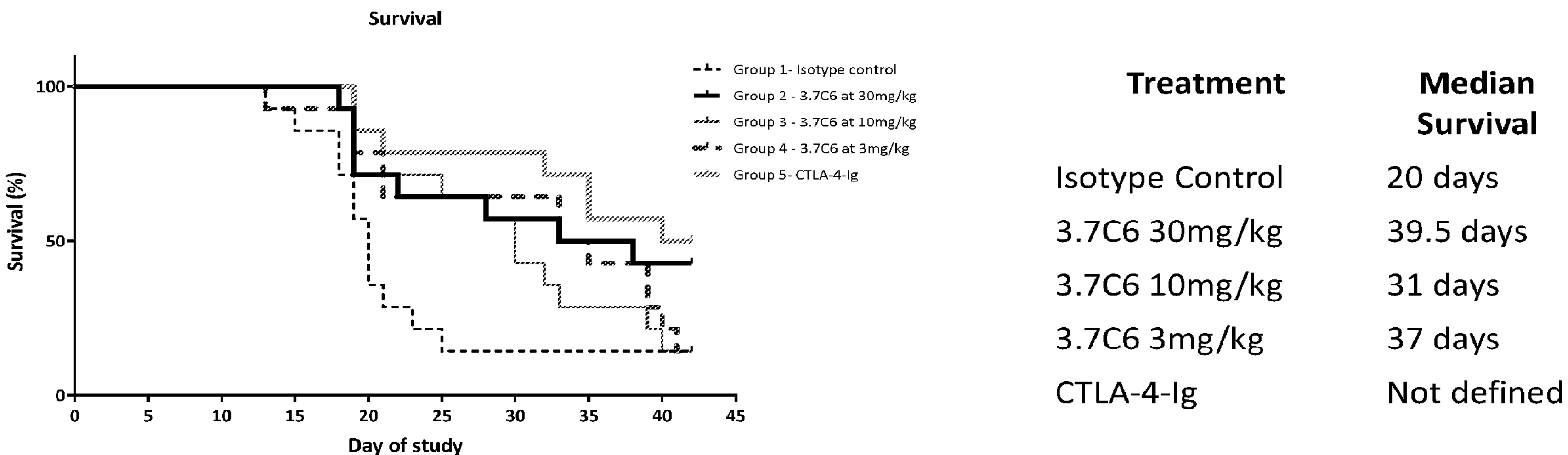


Figure 24B



Treatment	Median Survival
Isotype Control	20 days
3.7C6 30mg/kg	39.5 days
3.7C6 10mg/kg	31 days
3.7C6 3mg/kg	37 days
CTLA-4-Ig	Not defined

Figure 24C

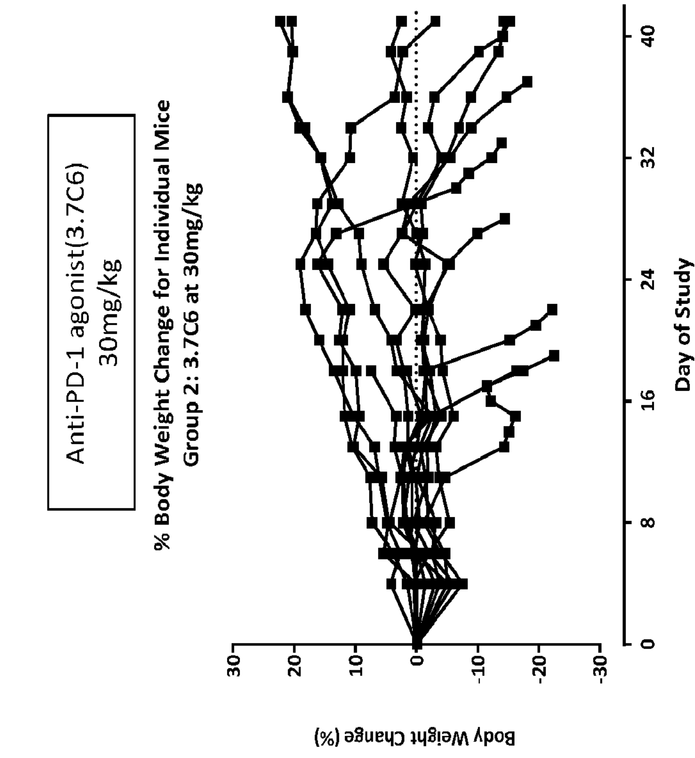


Figure 24E

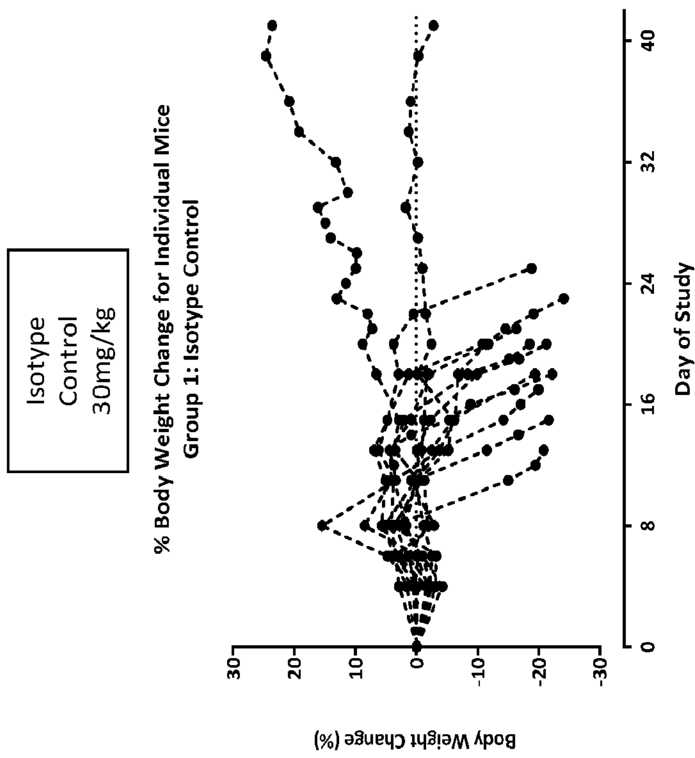


Figure 24D

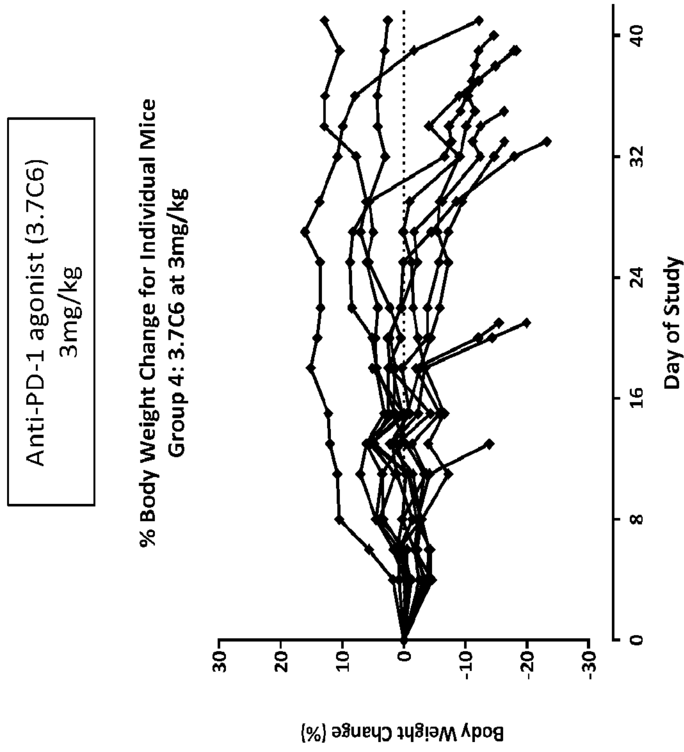


Figure 24G

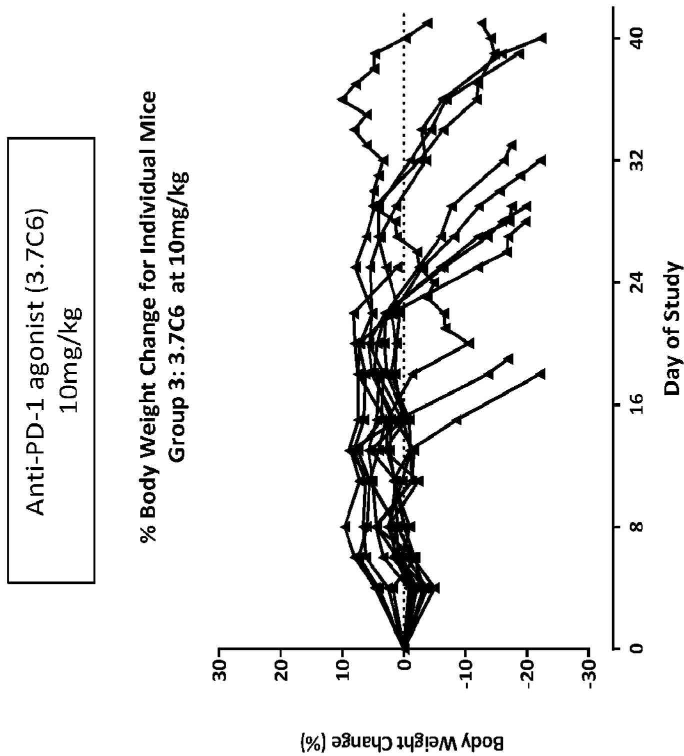


Figure 24F

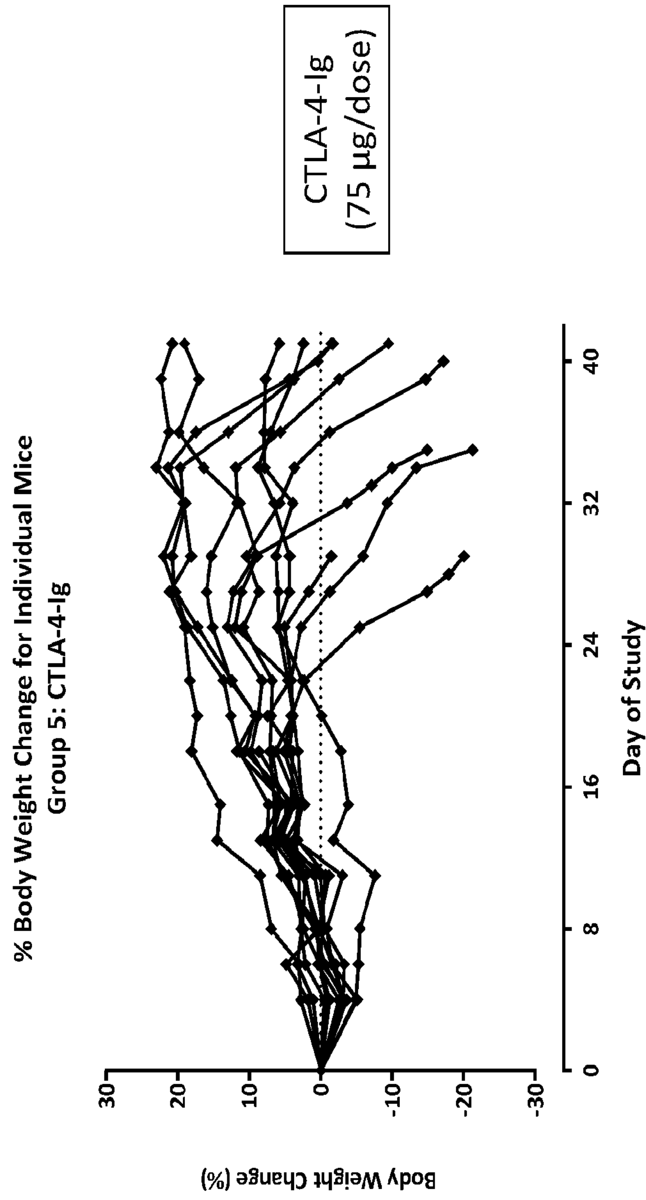


Figure 24H