(19) Europäisches Patentamt
European Patent Office
Office européen des brevets

(11) **EP 3 368 572 B9**

(12) **CORRECTED EUROPEAN PATENT SPECIFICATION**

(15) Correction information:
Corrected version no 1 (W1 B1)
Corrections, see
Description Paragraph(s) 7, 8, 10, 12, 13, 14, 17, 18, 19, 21, 29, 34, 38, 81, 85, 86, 88

(48) Corrigendum issued on:
26.04.2023 Bulletin 2023/17

(45) Date of publication and mention of the grant of the patent:
25.05.2022 Bulletin 2022/21

(21) Application number: **16774691.6**

(22) Date of filing: **30.09.2016**

(51) International Patent Classification (IPC):
C07K 16/28 *(2006.01)* ***A61K 39/00*** *(2006.01)*
A61P 35/00 *(2006.01)*

(52) Cooperative Patent Classification (CPC):
C07K 16/2818; A61P 35/00; A61K 2039/505; C07K 2317/24; C07K 2317/34; C07K 2317/55; C07K 2317/56; C07K 2317/565; C07K 2317/76; C07K 2317/92

(86) International application number:
PCT/EP2016/073421

(87) International publication number:
WO 2017/055547 (06.04.2017 Gazette 2017/14)

(54) ANTI-PD-1 ANTIBODIES AND COMPOSITIONS

ANTI-PD-1-ANTIKÖRPER ZUSAMMENSETZUNGEN

ANTICORPS ANTI-PD-1 ET COMPOSITIONS

(84) Designated Contracting States:
AL AT BE BG CH CY CZ DE DK EE ES FI FR GB GR HR HU IE IS IT LI LT LU LV MC MK MT NL NO PL PT RO RS SE SI SK SM TR

(30) Priority: **02.10.2015 US 201562236341 P**

(43) Date of publication of application:
05.09.2018 Bulletin 2018/36

(60) Divisional application:
22174837.9 / 4 105 235

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Description

BACKGROUND OF THE INVENTION

[0001] PD-1, also known as Programmed Cell Death Protein 1 and CD279, is a 268 amino acid cell surface receptor that belongs to the immunoglobulin superfamily. PD-1 is a member of the CD28 family of T cell regulators and is expressed on T cells, B cells and macrophages. It binds ligands PD-L1 (also known as B7 homolog) and PD-L2 (also known as B7-DC).

[0002] PD-1 is a type I membrane protein whose structure includes an extracellular IgV domain, a transmembrane region and an intracellular tail containing two phosphorylation sites. Known as an immune checkpoint protein, PD-1 functions as an inducible immune modulatory receptor, playing a role in, e.g., negative regulation of T cell responses to antigen stimulation.

[0003] PD-L1 is the predominant ligand for PD-1. Binding of PD-L1 to PD-1 inhibits T cell activity, reducing cytokine production and suppressing T cell proliferation. Cancer cells that express PD-L1 are able to exploit this mechanism to inactivate the anti-tumor activity of T cells via binding of PD-L1 to the PD-1 receptor.

[0004] In view of its immune response regulatory properties, PD-1 has been investigated as a potential target for immunotherapy, including treatment of cancer and autoimmune diseases. Two anti-PD-1 antibodies, pembrolizumab and nivolumab, have been approved in the United States and Europe for treating certain cancers. A-A

[0005] In view of the critical role of PD-1 as an immune modulator, there is a need for new and improved immune therapies that target PD-1 to treat cancers and certain disorders of the immune system.

SUMMARY OF THE INVENTION

[0006] The present invention is directed to novel recombinant antibodies targeting PD-1 as defined in the claims, as well as pharmaceutical compositions comprising one or more of these antibodies, and use of the antibodies and pharmaceutical compositions for enhancing immunity in a patient, and for treatment of cancers originating from tissues such as skin, lung, intestine, ovary, brain, prostate, kidney, soft tissues, the hematopoietic system, head & neck, liver, bladder, breast, stomach, uterus and pancreas. Compared to currently available treatments for such cancers, including antibody treatments, it is contemplated that the antibodies of the invention may provide a superior clinical response either alone or in combination with another cancer therapeutic, such as an antibody targeting another immune checkpoint protein.

[0007] In one embodiment, the present invention provides an anti-PD-1 antibody or an antigen-binding portion thereof, wherein the antibody binds to the same epitope of human PD-1 as, 12819.15384.

[0008] In some embodiments, the anti-PD-1 antibody comprises H-CDR1-3 comprising the H-CDR1-3 sequences, respectively, of antibody 12819.15384.

[0009] In some embodiments, the anti-PD-1 antibody has a heavy chain variable domain (V_H) that is at least 90% (e.g., at least 92%, at least 95%, at least 98%, or at least 99%) identical in amino acid sequence to the V_H domain of antibody 12819.15384, A- Wang et al., (Cancer Immunology Research, vol. 2, no. 9, pp. 846-856) discloses an in vivo characterization of the anti-PD-1 antibody nivolumab and in vivo toxicology in non-human primates. -A

[0010] In some embodiments, the anti-PD-1 antibody has a V_H that comprises the V_H amino acid sequence of antibody 12819.15384.

[0011] In some embodiments, the anti-PD-1 antibody has a heavy chain (HC) that comprises the V_H amino acid sequence of antibody 12819.15384, and the heavy chain constant region amino acid sequence of SEQ ID NO: 67.

[0012] In some embodiments, the anti-PD-1 antibody comprises L-CDR1-3 comprising the L-CDR1-3 sequences, respectively, of antibody 12819.15384.

[0013] In some embodiments, the anti-PD-1 antibody has a light chain variable domain (V_L) that is at least 90% (e.g., at least 92%, at least 95%, at least 98%, or at least 99%) identical in amino acid sequence to the V_L domain of antibody 12819.15384.

[0014] In some embodiments, the anti-PD-1 antibody has a V_L that comprises the V_L amino acid sequence of antibody 12819.15384.

[0015] In some embodiments, the anti-PD-1 antibody has a light chain (LC) that comprises the V_L amino acid sequence of antibody 12819.15384, and the light chain constant region amino acid sequence of SEQ ID NO: 68.

[0016] In some embodiments, the anti-PD-1 antibody comprises any of the above-described heavy chain sequences and any of the above light chain sequences.

[0017] In some embodiments, the anti-PD-1 antibody comprises the H-CDR3 and L-CDR3 amino acid sequences of antibody 12819.15384.

[0018] In some embodiments, the anti-PD-1 antibody comprises the H-CDR1-3 and L-CDR1-3 amino acid sequences of antibody 12819.15384.

[0019] In some embodiments, the anti-PD-1 antibody has a V_H and a V_L that are at least 90% (e.g., at least 92%, at

least 95%, at least 98%, or at least 99%) identical in amino acid sequence to the V_H and V_L, respectively, of antibody 12819.15384.

[0020] In some embodiments, the anti-PD-1 antibody has a V_H and V_L that comprise or consist of the V_H and V_L amino acid sequences, respectively, of antibody 12819.15384

[0021] In some embodiments, the anti-PD-1 antibody has an HC and an LC that comprise or consist of the HC and LC amino acid sequences, respectively, of antibody 12819.15384.

[0022] In some embodiments, the anti-PD-1 antibody has (1) an HC that comprises the V_H amino acid sequence of antibody 12819.15384 and the heavy chain constant region amino acid sequence of SEQ ID NO: 67; and (2) an LC that comprises the V_L amino acid sequence of that antibody and the light chain constant region amino acid sequence of SEQ ID NO: 68.

[0023] Disclosed herein is an anti-PD-1 antibody or antigen-binding portion comprising the H-CDR1-3 and L-CDR1-3 amino acid sequences of:

a) SEQ ID NOs: 24, 25, 26, 27, 28, and 29, respectively;
b) SEQ ID NOs: 30, 31, 32, 33, 34, and 35, respectively;
c) SEQ ID NOs: 36, 37, 38, 39, 40, and 41, respectively;
d) SEQ ID NOs: 42, 43, 44, 45, 46, and 47, respectively;
e) SEQ ID NOs: 48, 49, 50, 51, 52, and 53, respectively;
f) SEQ ID NOs: 54, 55, 56, 57, 58, and 59, respectively; or
g) SEQ ID NOs: 60, 61, 62, 63, 64, and 65, respectively.

[0024] Disclosed herein is an anti-PD-1 antibody or antigen-binding portion comprising a heavy chain variable domain and a light chain variable domain having the amino acid sequences of:

a) SEQ ID NOs: 4 and 5, respectively;
b) SEQ ID NOs: 4 and 66, respectively;
c) SEQ ID NOs: 6 and 7, respectively;
d) SEQ ID NOs: 8 and 9, respectively;
e) SEQ ID NOs: 10 and 11, respectively;
f) SEQ ID NOs: 12 and 13, respectively;
g) SEQ ID NOs: 14 and 15, respectively; or
h) SEQ ID NOs: 16 and 17, respectively.

[0025] Disclosed herein is an anti-PD-1 antibody comprising

a) an HC comprising the amino acid sequences of SEQ ID NOs: 4 and 67 and an LC comprising the amino acid sequences of SEQ ID NOs: 5 and 68;
b) an HC comprising the amino acid sequences of SEQ ID NOs: 4 and 67 and an LC comprising the amino acid sequences of SEQ ID NOs: 66 and 68;
c) an HC comprising the amino acid sequences of SEQ ID NOs: 6 and 67 and an LC comprising the amino acid sequences of SEQ ID NOs: 7 and 68;
d) an HC comprising the amino acid sequences of SEQ ID NOs: 8 and 67 and an LC comprising the amino acid sequences of SEQ ID NOs: 9 and 68;
e) an HC comprising the amino acid sequences of SEQ ID NOs: 10 and 67 and an LC comprising the amino acid sequences of SEQ ID NOs: 11 and 68;
f) an HC comprising the amino acid sequences of SEQ ID NOs: 12 and 67 and an LC comprising the amino acid sequences of SEQ ID NOs: 13 and 68;
g) an HC comprising the amino acid sequences of SEQ ID NOs: 14 and 67 and an LC comprising the amino acid sequences of SEQ ID NOs: 15 and 68; or
h) an HC comprising the amino acid sequences of SEQ ID NOs: 16 and 67 and an LC comprising the amino acid sequences of SEQ ID NOs: 17 and 68.

[0026] In some embodiments, the antibody or antigen-binding portion of the invention comprises H-CDR1-3 and L-CDR1-3 comprising the amino acid sequences of SEQ ID NOs: 18-20 and SEQ ID NOs: 21-23, respectively. In certain embodiments, the anti-PD-1 antibody comprises a V_H comprising the amino acid sequence of SEQ ID NO: 2 and a V_L comprising the amino acid sequence of SEQ ID NO: 3. In particular embodiments, the anti-PD-1 antibody comprises a heavy chain comprising the amino acid sequences of SEQ ID NOs: 2 and 67 and a light chain comprising the amino acid sequences of SEQ ID NOs: 3 and 68.

[0027] The invention also provides an anti-PD-1 antibody or an antigen-binding portion thereof that binds to an epitope of PD-1 comprising amino acid residue K131, P130, A132, V64 and L128 (e.g., a 12819 antibody). Disclosed herein is an anti-PD-1 antibody or antigen-binding portion thereof that binds to an epitope of PD-1 comprising amino acid residue K131 and E136 (e.g., a 12865 antibody).

[0028] Disclosed herein is an anti-PD-1 antibody or an antigen-binding portion thereof that binds to an epitope of PD-1 comprising amino acid residues V44 and T145 of SEQ ID NO: 1 (e.g., a 13112 antibody such as those listed in Tables 1, 4-7, 9, and 11-14).

[0029] In particular embodiments, the antibody or portion binds to an epitope of PD-1 comprising amino acid residues V64, L128, P130, K131, and A132 of SEQ ID NO: 1 (e.g., a 12819 antibody).

[0030] Disclosed in context of the present invention is a monoclonal antibody or an antigen-binding portion thereof that binds to an epitope of PD-1 comprising amino acid residues 69-90 and 122-140 of SEQ ID NO: 1 (e.g., a 12819 or 12865 antibody). In certain embodiments, the monoclonal antibody or antigen-binding portion binds to an epitope of PD-1 comprising amino acid residues 56-64, 69-90, and 122-140 of SEQ ID NO: 1 (e.g., a 12819 antibody). Disclosed herein is an antibody or portion binding residues 69-75 (or a fragment thereof) of SEQ ID NO: 1 (e.g., a 12819 or 12865 antibody). Disclosed herein is an antibody or portion binding residues 136-140 (or a fragment thereof) of SEQ ID NO: 1 (e.g., a 12819 or 12865 antibody). Disclosed herein is an antibody or portion binding residues 69-75 (or a fragment thereof) and residues 136-140 (or a fragment thereof) of SEQ ID NO: 1 (e.g., a 12819 or 12865 antibody).

[0031] In some embodiments, the anti-PD-1 antibody or antigen-binding portion of the invention has at least one of the following properties:

a) binds to human PD-1 with a K_D of 750 pM or less;
b) binds to cynomolgus PD-1 with a K_D of 7 nM or less;
c) binds to mouse PD-1 with a K_D of 1 nM or less;
d) does not bind to rat PD-1;
e) increases IL-2 secretion in an SEB whole blood assay;
f) increases IFN-γ secretion in a one-way mixed lymphocyte reaction assay;
g) inhibits the interaction of PD-1 with PD-L1 by at least 60% at a concentration of 10 μg/ml in a flow cytometric competition assay;
h) blocks binding of PD-L1 and PD-L2 to PD-1 by at least 90% at a concentration of 10 μg/ml as determined by Bio-Layer Interferometry analysis; and
i) inhibits tumor growth *in vivo.*

An example of such an antibody is, without limitation, antibody 12819 (having properties a-i). Antibodies disclosed herein that have some of the properties are 12748, 12892, and 12777 antibodies (having at least properties a, b, and eh); 12865 and 12796 antibodies (having at least properties a, b, e, f, and h), and 12760 and 13112 antibodies (having at least properties a, b, e, and f). In some embodiments, the anti-PD-1 antibody or antigen-binding portion of the invention has all of said properties. In some embodiments, the anti-PD-1 antibody or antigen-binding portion has at least properties a, b, and e-h. In some embodiments, the anti-PD-1 antibody or antigen-binding portion has at least properties a, b, e, f, and h. In some embodiments, the anti-PD-1 antibody or antigen-binding portion has at least properties a, b, e, and f.

[0032] Unless otherwise indicated, 12819, 12748, 12865, 12892, 12796, 12777, 12760 and 13112 each refers to a group of antibodies that have the same six CDRs and that share the first five digits in their ten-digit numerical designations. For example, 12748 includes antibody variants 12748.15381 and 12748.16124, which have the same six CDRs (as shown in Table 2). Each group of antibodies is expected to share the same or substantially the same biological properties.

[0033] In some embodiments, the anti-PD-1 antibody or antigen-binding portion of the invention does not compete for binding to PD-1 with pembrolizumab or nivolumab. In some embodiments, the anti-PD-1 antibody or antigen-binding portion of the invention does not bind to the same epitope as pembrolizumab or nivolumab; for example, the antibody or portion of the invention binds to one or more residues on PD-1 that are not bound by pembrolizumab or nivolumab.

[0034] In another aspect, the present invention provides pharmaceutical compositions comprising at least one anti-PD-1 antibody of the invention or antigen-binding portion thereof and a pharmaceutically acceptable excipient.

[0035] The present invention further provides isolated nucleic acid molecules comprising a nucleotide sequence that encodes the heavy chain or an antigen-binding portion thereof, a nucleotide sequence that encodes the light chain or an antigen-binding portion thereof, or both, of an anti-PD-1 antibody of the invention.

[0036] The present invention also provides vectors comprising such an isolated nucleic acid molecule, wherein said vector further comprises an expression control sequence.

[0037] The present invention also provide host cells comprising a nucleotide sequence that encodes the heavy chain or an antigen-binding portion thereof, a nucleotide sequence that encodes the light chain or an antigen-binding portion thereof, or both, of an anti-PD-1 antibody of the invention.

[0038] The present invention also provides a method for producing an antibody of the invention or antigen-binding

portion thereof, comprising providing a host cell that comprises a nucleotide sequence that encodes the heavy chain or an antigen-binding portion thereof and a nucleotide sequence that encodes the light chain or an antigen binding portion thereof of the anti-PD-1 antibody, cultivating said host cell under conditions suitable for expression of the antibody or portion, and isolating the resulting antibody or portion.

[0039] The present invention also provides a bispecific binding molecule having the binding specificity of an anti-PD-1 antibody of the invention and the binding specificity of another anti-PD-1 antibody (e.g., another anti-PD-1 antibody described herein) or an antibody that targets a different protein, such as another immune checkpoint protein, a cancer antigen, or another cell surface molecule whose activity mediates a disease condition such as cancer.

[0040] The present invention also provides antibodies or antigen-binding portions of the present invention for use in a method for enhancing immunity in a patient (e.g., a human patient) in need thereof, comprising administering to said patient an anti-PD-1 antibody or an antigen-binding portion thereof, a pharmaceutical composition, or a bispecific binding molecule of the invention.

[0041] The present invention further provides antibodies or antigen-binding portions of the present invention for use in a method for treating cancer in a patient (e.g., a human patient), comprising administering to said patient an anti-PD-1 antibody or an antigen-binding portion thereof, a pharmaceutical composition, or a bispecific binding molecule of the invention. In some embodiments, the cancer originates in a tissue selected from the group consisting of skin, lung, intestine, ovary, brain, prostate, kidney, soft tissues, hematopoietic system, head & neck, liver, bladder, breast, stomach, uterus and pancreas. The cancer may be, e.g., advanced or metastatic melanoma, non-small cell lung cancer, head and neck squamous cell cancer, renal cell carcinoma, or Hodgkin's lymphoma. In some embodiments, the method further comprises administering a chemotherapeutic agent, an anti-neoplastic agent, an anti-angiogenic agent, a tyrosine kinase inhibitor, or a PD-1 pathway inhibitor.

[0042] The present invention further provides antibodies or antigen-binding portions of the present invention for use in the aforementioned treatments, i.e., treatment of a human in need thereof to enhance his/her immune system, and treatment of a human with cancer, such as one of the aforementioned cancers.

BRIEF DESCRIPTION OF THE DRAWINGS

[0043]

Figure 1 shows a PCR product containing the V_H and V_L regions of the anti-PD-1 antibody AAS-12819 (shown in black) cloned in-frame with the corresponding human heavy chain IgG1-CH1-CH2-CH3 and human light chain lambda constant fragments, respectively. Restriction sites for this cloning are ApaI and AvrII. Restriction sites AscI and NheI are shown between the V_H and V_L 5'-ends. The plasmid origin of replication is depicted as pUC ori and the gene conferring ampicillin-resistance is depicted as AmpR.

Figure 2 shows an expression construct with a double CMV promoter inserted between 5'-ends of V_H and V_L using AscI and NheI restriction sites. V_H and V_L sequences are depicted in black, other annotated genetic elements are depicted in white.

Figures 3A-3C show representative flow cytometry dot plots for (A) an antibody clone that specifically binds to human PD-1-transfected cells, (B) a clone that non-specifically binds to CHO-S cells, and (C) a clone that does not bind either of the cell populations used in the screening.

Figure 4 shows the frequency of lymphocytes expressing PD-1 in six donors (D1-D6) before and after stimulation with SEB (*Staphylococcus* Enterotoxin B).

Figures 5A-I show titration of candidate anti-PD-1 antibodies in an SEB assay.

Figures 6A-H shows titration of candidate anti-PD-1 antibodies in a one-way MLR assay.

Figures 7A-B show PD-L1 binding to PD-1-expressing cells in the presence of anti-PD-1 antibodies.

Figure 8 shows an overview of the identified epitope groups (epitope bins) for tested anti-PD-1 antibodies 12866.13188, 12807.13177, 12819.17149, 12865.17150, 12892.13195, 12777.15382, 12760.13169, 13112.15380, and nivolumab and pembrolizumab analogues. Antibodies connected by black lines indicate cross blocking activity. Antibodies are grouped according to competition patterns with other anti-PD-1 antibodies. Nivo: nivolumab analogue; Pembro: pembrolizumab analogue.

Figure 9 (panels A-G) show the location of antibody epitopes on the structure of human PD-1 (PDB 4ZQK and 2M2D). A) Cartoon of human PD-1 extracellular domain (ECD) (residues 33-150). The location of the GFCC' and the ABED β-sheet and the C'-D loop are illustrated. B) Cartoon of the human PD-1 :human PD-L1 complex at same viewing angles as in (A). C) Molecular model of the pembrolizumab epitope shown as a density map with darker areas representing regions mediating stronger binding. Black areas represent contact residues found by alanine scanning. D) Molecular model of the nivolumab epitope represented as in (C). E) Molecular model of the 12819 antibody epitope represented as in (C). F) Molecular model of the 12865 antibody epitope represented as in (C). G) Molecular model of the non-ligand blocking 13112 antibody epitope represented as in (C).

Figure 10 (panels A-D) shows the effect of treatment with anti-PD-1 antibody 12819, 17149 or a vehicle on tumor growth in four syngeneic tumor models. A) CT26 (colon cancer). B) C38 (colon cancer). C) ASB-XIV (lung cancer). D) Sa1N (fibrosarcoma). The grey area denotes the treatment period. Data are presented as means ± SEM. * P<0.001.

Figure 11 shows the effect of treatment with anti-PD-1 antibody 12819.17149, pembrolizumab (Keytruda®), or vehicle on tumor growth of a semi-humanized xenograft tumor model, where the human melanoma cell line A375 was mixed with purified human CD8+ and CD4+ T cells prior to inoculation. The grey area denotes the treatment period. Data are presented as means ± SEM. * P<0.001.

DETAILED DESCRIPTION OF THE INVENTION

[0044] The present invention provides new anti-human PD-1 antibodies as defined in the claims that can be used to enhance the immune system in a human patient, such as a cancer patient. Unless otherwise stated, as used herein, "PD-1" refers to human PD-1. A human PD-1 polypeptide sequence is available under Uniprot Accession No. Q15116 (PDCD1_HUMAN), shown here as SEQ ID NO: 1.

[0045] The term "antibody" (Ab) or "immunoglobulin" (Ig), as used herein, refers to a tetramer comprising two heavy (H) chains (about 50-70 kDa) and two light (L) chains (about 25 kDa) inter-connected by disulfide bonds. Each heavy chain is comprised of a heavy chain variable domain (V_H) and a heavy chain constant region (CH). Each light chain is composed of a light chain variable domain (V_L) and a light chain constant region (CL). The V_H and V_L domains can be subdivided further into regions of hypervariability, termed "complementarity determining regions" (CDRs), interspersed with regions that are more conserved, termed "framework regions" (FRs). Each V_H and V_L is composed of three CDRs (H-CDR herein designates a CDR from the heavy chain; and L-CDR herein designates a CDR from the light chain) and four FRs, arranged from amino-terminus to carboxyl-terminus in the following order: FR1, CDR1, FR2, CDR2, FR3, CDR3, FR4. The assignment of amino acid numbers in the heavy or light chain may be in accordance with IMGT® definitions (Lefranc et al., Dev Comp Immunol 27(1):55-77 (2003)); or the definitions of Kabat, Sequences of Proteins of Immunological Interest (National Institutes of Health, Bethesda, MD (1987 and 1991)); Chothia & Lesk, J. Mol. Biol. 196:901-917 (1987); or Chothia et al., Nature 342:878-883 (1989).

[0046] The term "recombinant antibody" refers to an antibody that is expressed from a cell or cell line comprising the nucleotide sequence(s) that encode the antibody, wherein said nucleotide sequence(s) are not naturally associated with the cell.

[0047] The term "isolated protein", "isolated polypeptide" or "isolated antibody" refers to a protein, polypeptide or antibody that by virtue of its origin or source of derivation (1) is not associated with naturally associated components that accompany it in its native state, (2) is free of other proteins from the same species, (3) is expressed by a cell from a different species, and/or (4) does not occur in nature. Thus, a polypeptide that is chemically synthesized or synthesized in a cellular system different from the cell from which it naturally originates will be "isolated" from its naturally associated components. A protein may also be rendered substantially free of naturally associated components by isolation, using protein purification techniques well known in the art.

[0048] As used herein, the term "germline" refers to the nucleotide and amino acid sequences of antibody genes and gene segments as they are passed from parents to offspring via germ cells. Germline sequences are distinguished from the nucleotide sequences encoding antibodies in mature B cells, which have been altered by recombination and hypermutation events during the course of B cell maturation. An antibody that "utilizes" a particular germline sequence has a nucleotide or amino acid sequence that aligns with that germline nucleotide sequence or with the amino acid sequence that it specifies more closely than with any other germline nucleotide or amino acid sequence.

[0049] The term "affinity" refers to a measure of the attraction between an antigen and an antibody. The intrinsic attractiveness of the antibody for the antigen is typically expressed as the binding affinity equilibrium constant (K_D) of a particular antibody-antigen interaction. An antibody is said to specifically bind to an antigen when the K_D is ≤ 1 mM, preferably ≤ 100 nM. A K_D binding affinity constant can be measured, e.g., by surface plasmon resonance (BIAcore™) or Bio-Layer Interferometry, for example using the ProteOn™ XPR36 SPR system from Bio-Rad or the Octet™ system.

[0050] The term "k_{off}" refers to the dissociation rate constant of a particular antibody-antigen interaction. A k_{off} dissociation rate constant can be measured by Bio-Layer Interferometry, for example using the Octet™ system.

[0051] The term "epitope" as used herein refers to a portion (determinant) of an antigen that specifically binds to an antibody or a related molecule such as a bispecific binding molecule. Epitopic determinants generally consist of chemically active surface groupings of molecules such as amino acids or carbohydrate or sugar side chains and generally have specific three-dimensional structural characteristics, as well as specific charge characteristics. An epitope may be "linear" or "conformational." In a linear epitope, all of the points of interaction between a protein (e.g., an antigen) and an interacting molecule (such as an antibody) occur linearly along the primary amino acid sequence of the protein. In a conformational epitope, the points of interaction occur across amino acid residues on the protein that are separated from one another in the primary amino acid sequence. Once a desired epitope on an antigen is determined, it is possible

to generate antibodies to that epitope using techniques well known in the art. For example, an antibody to a linear epitope may be generated, e.g., by immunizing an animal with a peptide having the amino acid residues of the linear epitope. An antibody to a conformational epitope may be generated, e.g., by immunizing an animal with a mini-domain containing the relevant amino acid residues of the conformational epitope. An antibody to a particular epitope can also be generated, e.g., by immunizing an animal with the target molecule of interest or a relevant portion thereof (e.g., the ECD of PD-1), then screening for binding to the epitope.

[0052] One can determine whether an antibody binds to the same epitope as or competes for binding with an anti-PD-1 antibody of the invention by using methods known in the art, including, without limitation, competition assays, epitope binning, and alanine The scanning. The test antibody and an anti-PD-1 antibody of the invention may bind to at least one common residue (e.g., at least two, three, four, or five common residues) on PD-1. The contact residues on PD-1 can be completely identical between the test antibody and the anti-PD-1 antibody of the invention. One may allow the anti-PD-1 antibody of the invention to bind to PD-1 under saturating conditions and then measures the ability of the test antibody to bind to PD-1. If the test antibody is able to bind to PD-1 at the same time as the reference anti-PD-1 antibody, then the test antibody binds to a different epitope than the reference anti-PD-1 antibody. However, if the test antibody is not able to bind to PD-1 at the same time, then the test antibody binds to the same epitope, an overlapping epitope, or an epitope that is in close proximity to the epitope bound by the anti-PD-1 antibody of the invention. This experiment can be performed using ELISA, RIA, BIACORE™, Bio-Layer Interferometry or flow cytometry. To test whether an anti-PD-1 antibody cross-competes with another anti-PD-1 antibody, one may use the competition method described above in two directions, i.e., determining if the known antibody blocks the test antibody and vice versa. Such cross-competition experiments may be performed, e.g., using an IBIS MX96 SPR instrument or the Octet™ system.

[0053] The term "chimeric antibody" refers in its broadest sense to an antibody that contains one or more regions from one antibody and one or more regions from one or more other antibodies, typically an antibody that is partially of human origin and partially of non-human origin, i.e., derived in part from a non-human animal, for example a mouse, rat or other rodent, or an avian such as a chicken. Chimeric antibodies are preferred over non-human antibodies in order to reduce the risk of a human anti-antibody response, e.g., a human anti-mouse antibody response in the case of a murine antibody. An example of a typical chimeric antibody is one in which the variable domain sequences are murine while the constant region sequences are human. In the case of a chimeric antibody, the non-human parts may be subjected to further alteration in order to humanize the antibody. The chimeric antibodies described herein have chicken variable domain sequences and human constant region sequences.

[0054] The term "humanize" refers to the fact that where an antibody is wholly or partially of non-human origin (for example, a murine or chicken antibody obtained from immunization of mice or chickens, respectively, with an antigen of interest, or a chimeric antibody based on such a murine or chicken antibody), it is possible to replace certain amino acids, in particular in the framework regions and constant regions of the heavy and light chains, in order to avoid or minimize an immune response in humans. Although it is not possible to precisely predict the immunogenicity and thereby the human anti-antibody response of a particular antibody, non-human antibodies tend to be more immunogenic in humans than human antibodies. Chimeric antibodies, where the foreign (e.g., rodent or avian) constant regions have been replaced with sequences of human origin, have been shown to be generally less immunogenic than antibodies of fully foreign origin, and the trend in therapeutic antibodies is towards humanized or fully human antibodies. Chimeric antibodies or other antibodies of non-human origin thus can be humanized to reduce the risk of a human anti-antibody response.

[0055] For chimeric antibodies, humanization typically involves modification of the framework regions of the variable domain sequences. Amino acid residues that are part of complementarity determining regions (CDRs) most often will not be altered in connection with humanization, although in certain cases it may be desirable to alter individual CDR amino acid residues, for example to remove a glycosylation site, a deamidation site, an aspartate isomerization site or an undesired cysteine or methionine residue. N-linked glycosylation occurs by attachment of an oligosaccharide chain to an asparagine residue in the tripeptide sequence Asn-X-Ser or Asn-X-Thr, where X may be any amino acid except Pro. Removal of an N-glycosylation site may be achieved by mutating either the Asn or the Ser/Thr residue to a different residue, preferably by way of conservative substitution. Deamidation of asparagine and glutamine residues can occur depending on factors such as pH and surface exposure. Asparagine residues are particularly susceptible to deamidation, primarily when present in the sequence Asn-Gly, and to a lesser extent in other dipeptide sequences such as Asn-Ala. When such a deamidation site, in particular Asn-Gly, is present in a CDR sequence, it may therefore be desirable to remove the site, typically by conservative substitution to remove one of the implicated residues.

[0056] Numerous methods for humanization of an antibody sequence are known in the art; see, e.g., the review by Almagro & Fransson, Front Biosci. 13:1619-1633 (2008). One commonly used method is CDR grafting, which for, e.g., a murine-derived chimeric antibody involves identification of human germline gene counterparts to the murine variable domain genes and grafting of the murine CDR sequences into this framework. The specificity of an antibody's interaction with a target antigen resides primarily in the amino acid residues located in the six CDRs of the heavy and light chain. The amino acid sequences within CDRs are therefore much more variable between individual antibodies than sequences

outside of CDRs. Because CDR sequences are responsible for most antibody-antigen interactions, it is possible to express recombinant antibodies that mimic the properties of a specific naturally occurring antibody, or more generally any specific antibody with a given amino acid sequence, e.g., by constructing expression vectors that express CDR sequences from the specific antibody grafted into framework sequences from a different antibody. As a result, it is possible to "humanize" a non-human antibody and still substantially maintain the binding specificity and affinity of the original antibody. CDR grafting may be based on the Kabat CDR definitions, although a more recent publication (Magdelaine-Beuzelin et al., Crit Rev.Oncol Hematol. 64:210-225 (2007)) has suggested that the IMGT® definition (the international ImMunoGeneTics information system®, www.imgt.org) may improve the result of the humanization (see Lefranc et al., Dev. Comp Immunol. 27:55-77 (2003)).

[0057] In some cases, CDR grafting may reduce the binding specificity and affinity, and thus the biological activity, of a CDR-grafted non-human antibody as compared to the parent antibody from which the CDRs are obtained. Back mutations (sometimes referred to as "framework repair") may be introduced at selected positions of the CDR-grafted antibody, typically in the framework regions, in order to reestablish the binding specificity and affinity of the parent antibody. Identification of positions for possible back mutations can be performed using information available in the literature and in antibody databases. Amino acid residues that are candidates for back mutations are typically those that are located at the surface of an antibody molecule, while residues that are buried or that have a low degree of surface exposure will not normally be altered.

[0058] An alternative humanization technique to CDR grafting and back mutation is resurfacing, in which non-surface exposed residues of non-human origin are retained, while surface residues are altered to human residues.

[0059] In certain cases, it may also be desirable to alter one or more CDR amino acid residues in order to improve binding affinity to the target epitope. This is known as "affinity maturation" and may optionally be performed in connection with humanization, for example in situations where humanization of an antibody leads to reduced binding specificity or affinity and it is not possible to sufficiently improve the binding specificity or affinity by back mutations alone. Various affinity maturation methods are known in the art, for example the *in vitro* scanning saturation mutagenesis method described by Burks et al., Proc Natl Acad Sci USA, 94:412-417 (1997), and the stepwise *in vitro* affinity maturation method of Wu et al., Proc Natl Acad Sci USA 95:6037-6042 (1998).

[0060] The term "antigen-binding portion" of an antibody (or simply "antibody portion"), as used herein, refers to one or more portions or fragments of an antibody that retain the ability to specifically bind to an antigen (e.g., human PD-1, or a portion thereof). It has been shown that certain fragments of a full-length antibody can perform the antigen-binding function of the antibody. Examples of binding fragments encompassed within the term "antigen-binding portion" include (i) a Fab fragment: a monovalent fragment consisting of the V_L, V_H, C_L and C_H1 domains (for example, the 12819.17149 and 12865.17150 Fab fragments described below); (ii) a $F(ab')_2$ fragment: a bivalent fragment comprising two Fab fragments linked by a disulfide bridge at the hinge region; (iii) an Fd fragment consisting of the V_H and C_H1 domains; (iv) a Fv fragment consisting of the V_L and V_H domains of a single arm of an antibody, (v) a dAb fragment, which consists of a V_H domain; and (vi) an isolated complementarity determining region (CDR) capable of specifically binding to an antigen. Furthermore, although the two domains of the Fv fragment, V_L and V_H, are encoded by separate genes, they can be joined, using recombinant methods, by a synthetic linker that enables them to be made as a single protein chain in which the V_L and V_H domains pair to form monovalent molecules (known as single chain Fv (scFv)). Also within the invention are antigen-binding molecules comprising a V_H and/or a V_L. In the case of a V_H, the molecule may also comprise one or more of a CH1, hinge, CH2, or CH3 region. Such single chain antibodies are also intended to be encompassed within the term "antigen-binding portion" of an antibody. Other forms of single chain antibodies, such as diabodies, are also encompassed. Diabodies are bivalent, bispecific antibodies in which V_H and V_L domains are expressed on a single polypeptide chain, but using a linker that is too short to allow for pairing between the two domains on the same chain, thereby forcing the domains to pair with complementary domains of another chain and creating two antigen-binding sites.

[0061] Antibody portions, such as Fab and $F(ab')_2$ fragments, can be prepared from whole antibodies using conventional techniques, such as papain or pepsin digestion of whole antibodies. Moreover, antibodies, antibody portions and immunoadhesion molecules can be obtained using standard recombinant DNA techniques, e.g., as described herein.

[0062] The class (isotype) and subclass of anti-PD-1 antibodies may be determined by any method known in the art. In general, the class and subclass of an antibody may be determined using antibodies that are specific for a particular class and subclass of antibody. Such antibodies are available commercially. The class and subclass can be determined by ELISA, Western Blot as well as other techniques. Alternatively, the class and subclass may be determined by sequencing all or a portion of the constant regions of the heavy and/or light chains of the antibodies, comparing their amino acid sequences to the known amino acid sequences of various classes and subclasses of immunoglobulins, and determining the class and subclass of the antibodies.

[0063] When referring to particular amino acid residues in a given position of an antibody sequence, an indication of, e.g., "35S" refers to the position and residue, i.e., in this case indicating that a serine residue (S) is present in position 35 of the sequence. Similarly, an indication of, e.g., "13Q+35S" refers to the two residues in the respective positions.

[0064] Unless otherwise indicated, all antibody amino acid residue numbers referred to in this disclosure are those

under the IMGT® numbering scheme.

Anti-PD-1 Antibodies

[0065] The present invention provides antibodies directed against PD-1 as defined in the claims, and antigen-binding portions thereof. The antibodies may be chimeric, with variable domains derived from chickens, and human constant regions, or may be humanized. The antibodies disclosed herein are in particular humanized antibodies.

[0066] The V_H and V_L amino acid sequences (SEQ ID NOs: 2 to 17) of eight selected humanized anti-PD-1 antibodies are shown further below in Table 4 (Example 4). For reference, the SEQ ID NOs. are provided below in Table 1.

[0067] The anti-PD-1 antibodies disclosed herein may be referred to by either a 5-digit number, e.g., "12819," or by a 10-digit number, e.g., "12819.15384." As used herein, the 5-digit number refers to all antibodies having the heavy and light chain CDR1-3 sequences shown for that number in Table 2, whereas the use of a 10-digit number refers to a particular humanized variant. For example, 12819.15384 is a particular humanized variant having the CDR sequences of a 12819 antibody as shown in Table 2. The 5-digit number encompasses, for example, antibodies that are identical to the 10-digit variants shown below in Table 1 except for some changes in the FRs (e.g., lacking residues SY at the N-terminus of the mature light chain, or having residues SS in lieu of SY). These modifications do not change the functional (e.g., antigen-binding) properties of the antibodies.

Table 1 SEQ ID NOs for the amino acid sequences of the heavy and light chain variable domains of humanized anti-PD-1 antibodies

Antibody	V_H	V_L
12819.15384	2	3
12748.15381	4	5
12748.16124	4	66
12865.15377	6	7
12892.15378	8	9
12796.15376	10	11
12777.15382	12	13
12760.15375	14	15
13112.15380	16	17

[0068] Table 2 below provides the SEQ ID NOs for the heavy and light chain CDR amino acid sequences of the antibodies.

Table 2 SEQ ID NOs for the CDR amino acid sequences of anti-PD-1 antibodies

Antibody	H-CDR1	H-CDR2	H-CDR3	L-CDR1	L-CDR2	L-CDR3
12819	18	19	20	21	22	23
12748	24	25	26	27	28	29
12865	30	31	32	33	34	35
12892	36	37	38	39	40	41
12796	42	43	44	45	46	47
12777	48	49	50	51	52	53
12760	54	55	56	57	58	59
13112	60	61	62	63	64	65

[0069] In some embodiments, the anti-PD-1 antibody is selected from the group consisting of:

a) an antibody whose H-CDR1-3 comprise the amino acid sequences of SEQ ID NOs: 18-20, respectively;

b) an antibody whose heavy chain variable domain (V_H) is at least 90% identical in sequence to the amino acid sequence of SEQ ID NO: 2;
c) an antibody whose V_H comprises the amino acid sequence of SEQ ID NO: 2;
d) an antibody whose heavy chain (HC) comprises the amino acid sequences of SEQ ID NOs: 2 and 67;
e) an antibody whose L-CDR1-3 comprise the amino acid sequences of SEQ ID NOs: 21-23, respectively;
f) an antibody whose light chain variable domain (V_L) is at least 90% identical in sequence to the amino acid sequence of SEQ ID NO: 3;
g) an antibody whose V_L comprises the amino acid sequence of SEQ ID NO: 3;
h) an antibody whose light chain (LC) comprises the amino acid sequences of SEQ ID NOs: 3 and 68;
i) an antibody whose H-CDR1-3 and L-CDR1-3 comprise the amino acid sequences of SEQ ID NOs: 18-23, respectively;
j) an antibody whose V_H is at least 90% identical in sequence to the amino acid sequence of SEQ ID NO: 2 and whose V_L is at least 90% identical in sequence to the amino acid sequence of SEQ ID NO: 3;
k) an antibody whose V_H comprises the amino acid sequence of SEQ ID NO: 2 and whose V_L comprises the amino acid sequence of SEQ ID NO: 3; and
l) an antibody whose HC comprises the amino acid sequences of SEQ ID NOs: 2 and 67 and whose LC comprises the amino acid sequences of SEQ ID NOs: 3 and 68.

[0070] An anti-PD-1 antibody disclosed herein can be selected from the group consisting of:

a) an antibody whose H-CDR1-3 comprise the amino acid sequences of SEQ ID NOs: 24-26, respectively;
b) an antibody whose heavy chain variable domain (V_H) is at least 90% identical in sequence to the amino acid sequence of SEQ ID NO: 4;
c) an antibody whose V_H comprises the amino acid sequence of SEQ ID NO: 4;
d) an antibody whose heavy chain (HC) comprises the amino acid sequences of SEQ ID NOs: 4 and 67;
e) an antibody whose L-CDR1-3 comprise the amino acid sequences of SEQ ID NOs: 27-29, respectively;
f) an antibody whose light chain variable domain (V_L) is at least 90% identical in sequence to the amino acid sequence of SEQ ID NO: 5 or 66;
g) an antibody whose V_L comprises the amino acid sequence of SEQ ID NO: 5 or 66;
h) an antibody whose light chain (LC) comprises the amino acid sequences of SEQ ID NO: 5 or 66 and the amino acid sequence of SEQ ID NO: 68;
i) an antibody whose H-CDR1-3 and L-CDR1-3 comprise the amino acid sequences of SEQ ID NOs: 24-29, respectively;
j) an antibody whose V_H is at least 90% identical in sequence to the amino acid sequence of SEQ ID NO: 4 and whose V_L is at least 90% identical in sequence to the amino acid sequence of SEQ ID NO: 5 or 66;
k) an antibody whose V_H comprises the amino acid sequence of SEQ ID NO: 4 and whose V_L comprises the amino acid sequence of SEQ ID NO: 5 or 66; and
l) an antibody whose HC comprises the amino acid sequences of SEQ ID NOs: 4 and 67 and whose LC comprises the amino acid sequences of SEQ ID NO: 5 or 66 and SEQ ID NO: 68.

[0071] An anti-PD-1 antibody disclosed herein can be selected from the group consisting of:

a) an antibody whose H-CDR1-3 comprise the amino acid sequences of SEQ ID NOs: 30-32, respectively;
b) an antibody whose heavy chain variable domain (V_H) is at least 90% identical in sequence to the amino acid sequence of SEQ ID NO: 6;
c) an antibody whose V_H comprises the amino acid sequence of SEQ ID NO: 6;
d) an antibody whose heavy chain (HC) comprises the amino acid sequences of SEQ ID NOs: 6 and 67;
e) an antibody whose L-CDR1-3 comprise the amino acid sequences of SEQ ID NOs: 33-35, respectively;
f) an antibody whose light chain variable domain (V_L) is at least 90% identical in sequence to the amino acid sequence of SEQ ID NO: 7;
g) an antibody whose V_L comprises the amino acid sequence of SEQ ID NO: 7;
h) an antibody whose light chain (LC) comprises the amino acid sequences of SEQ ID NO: 7 and 68;
i) an antibody whose H-CDR1-3 and L-CDR1-3 comprise the amino acid sequences of SEQ ID NOs: 30-35, respectively;
j) an antibody whose V_H is at least 90% identical in sequence to the amino acid sequence of SEQ ID NO: 6 and whose V_L is at least 90% identical in sequence to the amino acid sequence of SEQ ID NO: 7;
k) an antibody whose V_H comprises the amino acid sequence of SEQ ID NO: 6 and whose V_L comprises the amino acid sequence of SEQ ID NO: 7; and

l) an antibody whose HC comprises the amino acid sequences of SEQ ID NOs: 6 and 67 and whose LC comprises the amino acid sequences of SEQ ID NOs: 7 and 68.

[0072] An anti-PD-1 antibody disclosed herein can be selected from the group consisting of:

a) an antibody whose H-CDR1-3 comprise the amino acid sequences of SEQ ID NOs: 36-38, respectively;
b) an antibody whose heavy chain variable domain (V_H) is at least 90% identical in sequence to the amino acid sequence of SEQ ID NO: 8;
c) an antibody whose V_H comprises the amino acid sequence of SEQ ID NO: 8;
d) an antibody whose heavy chain (HC) comprises the amino acid sequences of SEQ ID NOs: 8 and 67;
e) an antibody whose L-CDR1-3 comprise the amino acid sequences of SEQ ID NOs: 39-41, respectively;
f) an antibody whose light chain variable domain (V_L) is at least 90% identical in sequence to the amino acid sequence of SEQ ID NO: 9;
g) an antibody whose V_L comprises the amino acid sequence of SEQ ID NO: 9;
h) an antibody whose light chain (LC) comprises the amino acid sequences of SEQ ID NOs: 9 and 68;
i) an antibody whose H-CDR1-3 and L-CDR1-3 comprise the amino acid sequences of SEQ ID NOs: 36-41, respectively;
j) an antibody whose V_H is at least 90% identical in sequence to the amino acid sequence of SEQ ID NO: 8 and whose V_L is at least 90% identical in sequence to the amino acid sequence of SEQ ID NO: 9;
k) an antibody whose V_H comprises the amino acid sequence of SEQ ID NO: 8 and whose V_L comprises the amino acid sequence of SEQ ID NO: 9; and
l) an antibody whose HC comprises the amino acid sequences of SEQ ID NOs: 8 and 67 and whose LC comprises the amino acid sequences of SEQ ID NOs: 9 and 68.

[0073] An anti-PD-1 antibody disclosed herein can be selected from the group consisting of:

a) an antibody whose H-CDR1-3 comprise the amino acid sequences of SEQ ID NOs:42-44, respectively;
b) an antibody whose heavy chain variable domain (V_H) is at least 90% identical in sequence to the amino acid sequence of SEQ ID NO: 10;
c) an antibody whose V_H comprises the amino acid sequence of SEQ ID NO: 10;
d) an antibody whose heavy chain (HC) comprises the amino acid sequences of SEQ ID NOs: 10 and 67;
e) an antibody whose L-CDR1-3 comprise the amino acid sequences of SEQ ID NOs: 45-47, respectively;
f) an antibody whose light chain variable domain (V_L) is at least 90% identical in sequence to the amino acid sequence of SEQ ID NO: 11;
g) an antibody whose V_L comprises the amino acid sequence of SEQ ID NO: 11;
h) an antibody whose light chain (LC) comprises the amino acid sequences of SEQ ID NOs: 11 and 68;
i) an antibody whose H-CDR1-3 and L-CDR1-3 comprise the amino acid sequences of SEQ ID NOs: 42-47, respectively;
j) an antibody whose V_H is at least 90% identical in sequence to the amino acid sequence of SEQ ID NO: 10 and whose V_L is at least 90% identical in sequence to the amino acid sequence of SEQ ID NO: 11;
k) an antibody whose V_H comprises the amino acid sequence of SEQ ID NO: 10 and whose V_L comprises the amino acid sequence of SEQ ID NO: 11; and
l) an antibody whose HC comprises the amino acid sequences of SEQ ID NOs: 10 and 67 and whose LC comprises the amino acid sequences of SEQ ID NOs: 11 and 68.

[0074] An anti-PD-1 antibody disclosed herein can be selected from the group consisting of:

a) an antibody whose H-CDR1-3 comprise the amino acid sequences of SEQ ID NOs: 48-50, respectively;
b) an antibody whose heavy chain variable domain (V_H) is at least 90% identical in sequence to the amino acid sequence of SEQ ID NO: 12;
c) an antibody whose V_H comprises the amino acid sequence of SEQ ID NO: 12;
d) an antibody whose heavy chain (HC) comprises the amino acid sequences of SEQ ID NOs: 12 and 67;
e) an antibody whose L-CDR1-3 comprise the amino acid sequences of SEQ ID NOs: 51-53, respectively;
f) an antibody whose light chain variable domain (V_L) is at least 90% identical in sequence to the amino acid sequence of SEQ ID NO: 13;
g) an antibody whose V_L comprises the amino acid sequence of SEQ ID NO: 13;
h) an antibody whose light chain (LC) comprises the amino acid sequences of SEQ ID NOs: 13 and 68;
i) an antibody whose H-CDR1-3 and L-CDR1-3 comprise the amino acid sequences of SEQ ID NOs: 48-53, respec-

tively;
j) an antibody whose V_H is at least 90% identical in sequence to the amino acid sequence of SEQ ID NO: 12 and whose V_L is at least 90% identical in sequence to the amino acid sequence of SEQ ID NO: 13;
k) an antibody whose V_H comprises the amino acid sequence of SEQ ID NO: 12 and whose V_L comprises the amino acid sequence of SEQ ID NO: 13; and
l) an antibody whose HC comprises the amino acid sequences of SEQ ID NOs: 12 and 67 and whose LC comprises the amino acid sequences of SEQ ID NOs: 13 and 68.

[0075] An anti-PD-1 antibody disclosed herein can be selected from the group consisting of:

a) an antibody whose H-CDR1-3 comprise the amino acid sequences of SEQ ID NOs: 54-56, respectively;
b) an antibody whose heavy chain variable domain (V_H) is at least 90% identical in sequence to the amino acid sequence of SEQ ID NO: 14;
c) an antibody whose V_H comprises the amino acid sequence of SEQ ID NO: 14;
d) an antibody whose heavy chain (HC) comprises the amino acid sequences of SEQ ID NOs: 14 and 67;
e) an antibody whose L-CDR1-3 comprise the amino acid sequences of SEQ ID NOs: 57-59, respectively;
f) an antibody whose light chain variable domain (V_L) is at least 90% identical in sequence to the amino acid sequence of SEQ ID NO: 15;
g) an antibody whose V_L comprises the amino acid sequence of SEQ ID NO: 15;
h) an antibody whose light chain (LC) comprises the amino acid sequences of SEQ ID NOs: 15 and 68;
i) an antibody whose H-CDR1-3 and L-CDR1-3 comprise the amino acid sequences of SEQ ID NOs: 54-59, respectively;
j) an antibody whose V_H is at least 90% identical in sequence to the amino acid sequence of SEQ ID NO: 14 and whose V_L is at least 90% identical in sequence to the amino acid sequence of SEQ ID NO: 15;
k) an antibody whose V_H comprises the amino acid sequence of SEQ ID NO: 14 and whose V_L comprises the amino acid sequence of SEQ ID NO: 15; and
l) an antibody whose HC comprises the amino acid sequences of SEQ ID NOs: 14 and 67 and whose LC comprises the amino acid sequences of SEQ ID NOs: 15 and 68.

[0076] An anti-PD-1 antibody disclosed herein can be selected from the group consisting of:

a) an antibody whose H-CDR1-3 comprise the amino acid sequences of SEQ ID NOs: 60-62, respectively;
b) an antibody whose heavy chain variable domain (V_H) is at least 90% identical in sequence to the amino acid sequence of SEQ ID NO: 16;
c) an antibody whose V_H comprises the amino acid sequence of SEQ ID NO: 16;
d) an antibody whose heavy chain (HC) comprises the amino acid sequences of SEQ ID NOs: 16 and 67;
e) an antibody whose L-CDR1-3 comprise the amino acid sequences of SEQ ID NOs: 63-65, respectively;
f) an antibody whose light chain variable domain (V_L) is at least 90% identical in sequence to the amino acid sequence of SEQ ID NO: 17;
g) an antibody whose V_L comprises the amino acid sequence of SEQ ID NO: 17;
h) an antibody whose light chain (LC) comprises the amino acid sequences of SEQ ID NOs: 17 and 68;
i) an antibody whose H-CDR1-3 and L-CDR1-3 comprise the amino acid sequences of SEQ ID NOs: 60-65, respectively;
j) an antibody whose V_H is at least 90% identical in sequence to the amino acid sequence of SEQ ID NO: 16 and whose V_L is at least 90% identical in sequence to the amino acid sequence of SEQ ID NO: 17;
k) an antibody whose V_H comprises the amino acid sequence of SEQ ID NO: 16 and whose V_L comprises the amino acid sequence of SEQ ID NO: 17;
l) an antibody whose HC comprises the amino acid sequences of SEQ ID NOs: 16 and 67 and whose LC comprises the amino acid sequences of SEQ ID NOs: 17 and 68.

[0077] In some embodiments, the anti-PD-1 antibody or an antigen-binding portion thereof comprises the H-CDR1-3 and L-CDR1-3 amino acid sequences of a 12819 antibody disclosed herein can be (e.g., antibody 12819.15384).
[0078] An anti-PD-1 antibody disclosed herein or an antigen-binding portion thereof comprises the H-CDR1-3 and L-CDR1-3 amino acid sequences of a 12748 antibody disclosed herein can be (e.g., antibody 12748.15381 or antibody 12748.16124).
[0079] An anti-PD-1 antibody disclosed herein or an antigen-binding portion thereof comprises the H-CDR1-3 and L-CDR1-3 amino acid sequences of a 12865 antibody (e.g., antibody 12865.15377).
[0080] An anti-PD-1 antibody disclosed herein or an antigen-binding portion thereof comprises the H-CDR1-3 and L-

CDR1-3 amino acid sequences of a 12892 antibody (e.g., antibody 12892.15378).

[0081] An anti-PD-1 antibody disclosed herein or an antigen-binding portion thereof comprises the H-CDR1-3 and L-CDR1-3 amino acid sequences of a 12796 antibody disclosed herein can be (e.g., antibody 12796.15376).

[0082] An anti-PD-1 antibody disclosed herein or an antigen-binding portion thereof comprises the H-CDR1-3 and L-CDR1-3 amino acid sequences of a 12777 antibody (e.g., antibody 12777.15382).

[0083] An anti-PD-1 antibody disclosed herein or an antigen-binding portion thereof comprises the H-CDR1-3 and L-CDR1-3 amino acid sequences of a 12760 antibody (e.g., antibody 12760.15375).

[0084] An anti-PD-1 antibody disclosed herein or an antigen-binding portion thereof comprises the H-CDR1-3 and L-CDR1-3 amino acid sequences of a 13112 antibody (e.g., antibody 13112.15380).

[0085] In another embodiment, the anti-PD-1 antibody or an antigen-binding portion thereof has a V_H and a V_L that are at least 90% (e.g., at least 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, or 99%) identical in amino acid sequence to the V_H and V_L, respectively, of antibody of antibody 12819.15384.

[0086] In some embodiments, the anti-PD-1 antibody or an antigen-binding portion thereof has a V_H and a V_L that comprise the V_H and V_L amino acid sequences, respectively, of antibody of antibody 12819.15384.

[0087] In some embodiments, the anti-PD-1 antibody or an antigen-binding portion thereof comprises the H-CDR1-3 and L-CDR1-3 amino acid sequences of SEQ ID NOs: 18, 19, 20, 21, 22, and 23, respectively. An anti-PD-1 antibody disclosed herein or an antigen-binding portion thereof comprises the H-CDR1-3 and L-CDR1-3 amino acid sequences of:

a) SEQ ID NOs: 24, 25, 26, 27, 28, and 29, respctively,
b) SEQ ID NOs: 30, 31, 32, 33, 34, and 35, respectively;
c) SEQ ID NOs: 36, 37, 38, 39, 40, and 41, respectively;
d) SEQ ID NOs: 42, 43, 44, 45, 46, and 47, respectively;
e) SEQ ID NOs: 48, 49, 50, 51, 52, and 53, respectively;
f) SEQ ID NOs: 54, 55, 56, 57, 58, and 59, respectively; or
g) SEQ ID NOs: 60, 61, 62, 63, 64, and 65, respectively.

[0088] In some embodiments, the anti-PD-1 antibody or an antigen-binding portion thereof comprises a V_H that is 80%, 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, or 99% identical, and a V_L that is 80%, 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, or 99% identical, to the amino acid sequences of SEQ ID NOs: 2 and 3, respectively. An anti-PD-1 antibody disclosed herein or an antigen-biding portion thereof comprises a V_H that is 80%, 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, or 99% identical, and a V_L that is 80%, 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, or 99% identical, to the amino acid sequences of:

a) "SEQ-ID-NOs: 4 and 5, respectively;
b) SEQ ID NOs: 4 and 66, respectively
c) SEQ ID NOs: 6 and 7, respectively;
d) SEQ ID NOs: 8 and 9, respectively;
e) SEQ ID NOs: 10 and 11, respectively;
f) SEQ ID NOs: 12 and 13, respectively;
g) SEQ ID NOs: 14 and 15, respectively; or
h) SEQ ID NOs: 16 and 17, respectively.

[0089] In some embodiments, the anti-PD-1 antibody or an antigen-binding portion thereof comprises a V_H and a V_L that are the amino acid sequences of SEQ ID NOs: 2 and 3, respectively. An anti-PD-1 antibody disclosed herein or an antigen-binding portion thereof comprises a V_H and a V_L that are the amino acid sequences of:

a) SEQ ID NOs: 4 and 5,
b) SEQ ID NOs: 4 and 66, respectively;
c) SEQ ID NOs: 6 and 7, respectively;
d) SEQ ID NOs: 8 and 9, respectively;
e) SEQ ID NOs: 10 and 11, respectively;
f) SEQ ID NOs: 12 and 13, respectively;
g) SEQ ID NOs: 14 and 15, respectively; or
h) SEQ ID NOs: 16 and 17, respectively.

[0090] In some embodiments, the anti-PD-1 antibody comprises

an HC comprising the amino acid sequences of SEQ ID NOs: 2 and 67 and

an LC comprising the amino acid sequences of SEQ ID NOs: 3 and 68. An anti-PD-1 antibody disclosed herein comprises:

a) an HC comprising the amino acid sequences of SEQ ID NOs: 4 and 67 and an LC comprising the amino acid sequences of SEQ ID NOs: 5 and 68;
b) an HC comprising the amino acid sequences of SEQ ID NOs: 4 and 67 and an LC comprising the amino acid sequences of SEQ ID NOs: 66 and 68;
c) an HC comprising the amino acid sequences of SEQ ID NOs: 6 and 67 and an LC comprising the amino acid sequences of SEQ ID NOs: 7 and 68;
d) an HC comprising the amino acid sequences of SEQ ID NOs: 8 and 67 and an LC comprising the amino acid sequences of SEQ ID NOs: 9 and 68;
e) an HC comprising the amino acid sequences of SEQ ID NOs: 10 and 67 and an LC comprising the amino acid sequences of SEQ ID NOs: 11 and 68;
f) an HC comprising the amino acid sequences of SEQ ID NOs: 12 and 67 and an LC comprising the amino acid sequences of SEQ ID NOs: 13 and 68;
g) an HC comprising the amino acid sequences of SEQ ID NOs: 14 and 67 and an LC comprising the amino acid sequences of SEQ ID NOs: 15 and 68; or
h) an HC comprising the amino acid sequences of SEQ ID NOs: 16 and 67 and an LC comprising the amino acid sequences of SEQ ID NOs: 17 and 68.

[0091] In some embodiments, the anti-PD-1 antibody comprises

an HC consisting of the amino acid sequences of SEQ ID NOs: 2 and 67 and
an LC consisting of the amino acid sequences of SEQ ID NOs: 3 and 68. An anti-PD-1 antibody disclosed herein comprises:

a) an HC consisting of the amino acid sequences of SEQ ID NOs: 4 and 67 and an LC consisting of the amino acid sequences of SEQ ID NOs: 5 and 68;
b) an HC consisting of the amino acid sequences of SEQ ID NOs: 4 and 67 and an LC consisting of the amino acid sequences of SEQ ID NOs: 66 and 68;
c) an HC consisting of the amino acid sequences of SEQ ID NOs: 6 and 67 and an LC consisting of the amino acid sequences of SEQ ID NOs: 7 and 68;
d) an HC consisting of the amino acid sequences of SEQ ID NOs: 8 and 67 and an LC consisting of the amino acid sequences of SEQ ID NOs: 9 and 68;
e) an HC consisting of the amino acid sequences of SEQ ID NOs: 10 and 67 and an LC consisting of the amino acid sequences of SEQ ID NOs: 11 and 68;
f) an HC consisting of the amino acid sequences of SEQ ID NOs: 12 and 67 and an LC consisting of the amino acid sequences of SEQ ID NOs: 13 and 68;
g) an HC consisting of the amino acid sequences of SEQ ID NOs: 14 and 67 and an LC consisting of the amino acid sequences of SEQ ID NOs: 15 and 68; or
h) an HC consisting of the amino acid sequences of SEQ ID NOs: 16 and 67 and an LC consisting of the amino acid sequences of SEQ ID NOs: 17 and 68.

[0092] In some embodiments, any of the anti-PD-1 antibodies or antigen-binding portions of the invention may have at least one of the following properties:

a) binds to human PD-1 with a K_D of 750 pM or less;
b) binds to cynomolgus PD-1 with a K_D of 7 nM or less;
c) binds to mouse PD-1 with a K_D of 1 nM or less;
d) does not bind to rat PD-1;
e) increases IL-2 secretion in an SEB whole blood assay;
f) increases IFN-γ secretion in a one-way mixed lymphocyte reaction assay;
g) inhibits the interaction of PD-1 with PD-L1 by at least 60% at a concentration of 10 μg/ml in a flow cytometric competition assay;
h) blocks binding of PD-L1 and PD-L2 to PD-1 by at least 90% at a concentration of 10 μg/ml as determined by Bio-Layer Interferometry analysis; and
i) inhibits tumor growth *in vivo*.

[0093] In some embodiments, any of the anti-PD-1 antibodies or antigen-binding portions of the invention may bind to human PD-1 with a K_D of at least 900, at least 850, at least 800, at least 750, at least 700, at least 650, at least 600, at least 550, at least 500, at least 450, at least 400, at least 350, at least 300, at least 250, at least 200, at least 150, at least 100, at least 50, at least 40, at least 30, or at least 20 pM. In certain embodiments, the K_D is determined using surface plasmon resonance. In particular embodiments, the anti-PD-1 antibodies or antigen-binding portions bind to human PD-1 with a higher affinity than nivolumab, pembrolizumab, or both.

[0094] In some embodiments, any of the anti-PD-1 antibodies or antigen-binding portions of the invention may bind to cynomolgus PD-1 (SEQ ID NO: 89) with a K_D of at least 9000, at least 8000, at least 7000, at least 6000, at least 5000, at least 4000, at least 3000, at least 2500, at least 2000, at least 1500, at least 1000, at least 900, at least 800, at least 700, at least 600, at least 500, at least 400, at least 300, at least 200, at least 100, at least 75, at least 50, at least 25, at least 20, at least 15, at least 10, or at least 5 pM. In certain embodiments, the K_D is determined using surface plasmon resonance.

[0095] In some embodiments, any of the anti-PD-1 antibodies or antigen-binding portions of the invention may bind to mouse PD-1 (SEQ ID NO: 91) with a K_D of at least 1000, at least 950, at least 900, or at least 850 pM. In certain embodiments, the K_D is determined using surface plasmon resonance.

[0096] In some embodiments, any of the anti-PD-1 antibodies or antigen-binding portions of the invention may inhibit the interaction of PD-1 with PD-L1 by at least 60%, at least 65%, at least 70%, at least 75%, 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% at a concentration of 10 μg/ml in a flow cytometric competition assay. In certain embodiments, the anti-PD-1 antibodies or antigen-binding portions may inhibit the interaction of PD-1 with PD-L1 by at least 83%.

[0097] In some embodiments, any of the anti-PD-1 antibodies or antigen-binding portions of the invention may block binding of PD-L1 and PD-L2 to PD-1 by at least 20%, at least 30%, at least 40%, at least 50%, at least 60%, at least 70%, at least 80%, at least 85%, 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% at a concentration of 10 μg/ml as determined by Bio-Layer Interferometry analysis. In certain embodiments, the anti-PD-1 antibodies or antigen-binding portions block binding of PD-L1 and PD-L2 to PD-1 by at least 90%.

[0098] Any of the anti-PD-1 antibodies or antigen-binding portions described herein may compete or cross-compete for binding to PD-1 with 12865, 12892, and 12777 antibodies (e.g., antibodies 12865.15377, 12892.15378, and 12777.15382). Any of the anti-PD-1 antibodies or antigen-binding portions described herein may compete or cross-compete for binding to PD-1 with a 12819 antibody (e.g., antibody 12819.15384). Any of the anti-PD-1 antibodies or antigen-binding portions described herein may compete or cross-compete for binding to PD-1 with 12760 and 13112 antibodies (e.g., antibodies 12760.15375 and 13112.15380).

[0099] An anti-PD-1 antibody disclosed herein, or an antigen-binding portion thereof, binds to an epitope of PD-1 that includes at least one (e.g., at least one, at least two, at least three, at least four, or at least five) of the following residues of SEQ ID NO: 1: V44, V64, L128, P130, K131, A132, E136, and T145. The antibody of the invention or antigen-binding portion binds to an epitope of PD-1 that includes residues V64, L128, P130, K131, and A132 of SEQ ID NO: 1 (such as a 12819 antibody, e.g., antibody 12819.15384). An antibody disclosed herein or antigen-binding portion binds to an epitope of PD-1 that includes residues K131 and E136 of SEQ ID NO: 1 (such as a 12865 antibody, e.g., antibody 12865.15377). An antibody disclosed herein or antigen-binding portion binds to an epitope of PD-1 that includes residues V44 and T145 of SEQ ID NO: 1 (such as a 13112 antibody, e.g., antibody 13112.15380).

[0100] In some embodiments, an anti-PD-1 antibody of the invention, or an antigen-binding portion thereof, binds to an epitope of PD-1 that comprises residues 56-64, 69-90, and 122-140 of SEQ ID NO: 1. An antibody disclosed herein or antigen-binding portion binds to an epitope of PD-1 that comprises residues 69-90 and 122-140 of SEQ ID NO: 1 (such as 12865 antibodies, e.g., antibody 12865.15377). In certain embodiments, the antibody or antigen-binding portion binds to an epitope of PD-1 that comprises residues 56-64, 69-90, and 122-140 of SEQ ID NO: 1 (e.g., a 12819 antibody). An antibody disclosed herein or antigen-binding portion binds to an epitope of PD-1 that comprises residues 69-90 and 122-140 of SEQ ID NO: 1 (e.g., a 12865 antibody). An antibody disclosed herein or portion binds to residues 69-75 (or a fragment thereof, such as a one, two, three, four, five, or six residue fragment), of SEQ ID NO: 1 (such as 12865 antibodies, e.g., antibody 12865.15377). An antibody disclosed herein or portion binds to residues 136-140 (or a fragment thereof, such as a one, two, three, or four residue fragment) of SEQ ID NO: 1 (such as 12865 antibodies, e.g., antibody 12865.15377). An antibody discclosed herein or portion binds to residues 69-75 (or a fragment thereof) and residues 136-140 (or a fragment thereof) of SEQ ID NO: 1, (such as 12865 antibodies, e.g., antibody 12865.15377). An epitope with any combination of the above residues is also contemplated.

[0101] An amino acid sequence comprising a PD-1 epitope as described herein can be used as an immunogen (e.g., administered to an animal or as an antigen for screening antibody libraries) to generate or identify anti-PD-1 antibodies or antigen-binding portions thereof that bind to said epitope.

[0102] The class of an anti-PD-1 antibody obtained by the methods described herein may be changed or switched

with another class or subclass. In one aspect of the invention, a nucleic acid molecule encoding V_L or V_H is isolated using methods well-known in the art such that it does not include nucleic acid sequences encoding C_L or C_H. The nucleic acid molecules encoding V_L or V_H then are operatively linked to a nucleic acid sequence encoding a C_L or C_H, respectively, from a different class of immunoglobulin molecule. This may be achieved using a vector or nucleic acid molecule that comprises a C_L or C_H chain, as described above. For example, an anti-PD-1 antibody that was originally IgM may be class switched to IgG. Further, the class switching may be used to convert one IgG subclass to another, e.g., from IgG_1 to IgG_2. A κ light chain constant region can be changed to a λ light chain constant region. A preferred method for producing an antibody of the invention with a desired Ig isotype comprises the steps of isolating a nucleic acid molecule encoding the heavy chain of an anti-PD-1 antibody and a nucleic acid molecule encoding the light chain of an anti-PD-1 antibody, obtaining the variable domain of the heavy chain, ligating the variable domain of the heavy chain with the constant region of a heavy chain of the desired isotype, expressing the light chain and the ligated heavy chain in a cell, and collecting the anti-PD-1 antibody with the desired isotype.

[0103] The anti-PD-1 antibody of the invention can be an IgG, an IgM, an IgE, an IgA, or an IgD molecule, but is typically of the IgG isotype, e.g., of IgG subclass IgG_1, IgG_{2a} or IgG_{2b}, IgG_3 or IgG_4. In one embodiment, the antibody is an IgG_1. In another embodiment, the antibody is an IgG_4.

[0104] In one embodiment, the anti-PD-1 antibody may comprise at least one mutation in the Fc region. A number of different Fc mutations are known, where these mutations provide altered effector function. For example, in many cases it will be desirable to reduce or eliminate effector function, e.g., where ligand/receptor interactions are undesired or in the case of antibody-drug conjugates.

[0105] In one embodiment, the anti-PD-1 antibody comprises at least one mutation in the Fc region that reduces effector function. Fc region amino acid positions that may be advantageous to mutate in order to reduce effector function include one or more of positions 228, 233, 234 and 235, where amino acid positions are numbered according to the IMGT® numbering scheme.

[0106] In one embodiment, one or both of the amino acid residues at positions 234 and 235 may be mutated, for example from Leu to Ala (L234A/L235A). These mutations reduce effector function of the Fc region of IgG_1 antibodies. Additionally or alternatively, the amino acid residue at position 228 may be mutated, for example to Pro. In another embodiment, the amino acid residue at position 233 may be mutated, e.g., to Pro, the amino acid residue at position 234 may be mutated, e.g., to Val, and/or the amino acid residue at position 235 may be mutated, e.g., to Ala. The amino acid positions are numbered according to the IMGT® numbering scheme.

[0107] In another embodiment, where the antibody is of the IgG_4 subclass, it may comprise the mutation S228P, i.e., having a proline in position 228, where the amino acid position is numbered according to the IMGT® numbering scheme. This mutation is known to reduce undesired Fab arm exchange.

[0108] In certain embodiments, an antibody or antigen-binding portion thereof of the invention may be part of a larger immunoadhesion molecule, formed by covalent or noncovalent association of the antibody or antibody portion with one or more other proteins or peptides. Examples of such immunoadhesion molecules include use of the streptavidin core region to make a tetrameric scFv molecule (Kipriyanov et al., Human Antibodies and Hybridomas 6:93-101 (1995)) and use of a cysteine residue, a marker peptide and a C-terminal polyhistidine tag to make bivalent and biotinylated scFv molecules (Kipriyanov et al., Mol. Immunol. 31:1047-1058 (1994)). Other examples include where one or more CDRs from an antibody are incorporated into a molecule either covalently or noncovalently to make it an immunoadhesin that specifically binds to an antigen of interest. The CDR(s) may be incorporated as part of a larger polypeptide chain, may be covalently linked to another polypeptide chain, or may be incorporated noncovalently.

[0109] In another embodiment, a fusion antibody or immunoadhesin may be made that comprises all or a portion of an anti-PD-1 antibody of the invention linked to another polypeptide. In certain embodiments, only the variable domains of the anti-PD-1 antibody are linked to the polypeptide. In certain embodiments, the V_H domain of an anti-PD-1 antibody is linked to a first polypeptide, while the V_L domain of an anti-PD-1 antibody is linked to a second polypeptide that associates with the first polypeptide in a manner such that the V_H and V_L domains can interact with one another to form an antigen-binding site. In another preferred embodiment, the V_H domain is separated from the V_L domain by a linker such that the V_H and V_L domains can interact with one another (e.g., single-chain antibodies). The V_H-linker-V_L antibody is then linked to the polypeptide of interest. In addition, fusion antibodies can be created in which two (or more) single-chain antibodies are linked to one another. This is useful if one wants to create a divalent or polyvalent antibody on a single polypeptide chain, or if one wants to create a bispecific antibody.

[0110] To create a single chain antibody (scFv), the V_H- and V_L-encoding DNA fragments are operatively linked to another fragment encoding a flexible linker, e.g., encoding the amino acid sequence (Gly4 -Ser)3, such that the V_H and V_L sequences can be expressed as a contiguous single-chain protein, with the V_L and V_H domains joined by the flexible linker. See, e.g., Bird et al., Science 242:423-426 (1988); Huston et al., Proc. Natl. Acad. Sci. USA 85:5879-5883 (1988); and McCafferty et al., Nature 348:552-554 (1990). The single chain antibody may be monovalent, if only a single V_H and V_L are used; bivalent, if two V_H and V_L are used; or polyvalent, if more than two V_H and V_L are used. Bispecific or polyvalent antibodies may be generated that bind specifically to human PD-1 and to another molecule, for instance.

[0111] In other embodiments, other modified antibodies may be prepared using anti-PD-1 antibody-encoding nucleic acid molecules. For instance, "kappa bodies" (III et al., Protein Eng. 10:949-57 (1997)), "minibodies" (Martin et al., EMBO J. 13:5303-9 (1994)), "diabodies" (Holliger et al., Proc. Natl. Acad. Sci. USA 90:6444-6448 (1993)), or "Janusins" (Traunecker et al., EMBO J. 10:3655-3659 (1991) and Traunecker et al., Int. J. Cancer (Suppl.) 7:51-52 (1992)) may be prepared using standard molecular biological techniques following the teachings of the specification.

[0112] An anti-PD-1 antibody or antigen-binding portion of the invention can be derivatized or linked to another molecule (e.g., another peptide or protein). In general, the antibodies or portions thereof are derivatized such that PD-1 binding is not affected adversely by the derivatization or labeling. Accordingly, the antibodies and antibody portions of the invention are intended to include both intact and modified forms of the human anti-PD-1 antibodies. For example, an antibody or antibody portion of the invention can be functionally linked (by chemical coupling, genetic fusion, noncovalent association or otherwise) to one or more other molecular entities, such as another antibody (e.g., a bispecific antibody or a diabody), a detection agent, a pharmaceutical agent, and/or a protein or peptide that can mediate association of the antibody or antibody portion with another molecule (such as a streptavidin core region or a polyhistidine tag).

[0113] One type of derivatized antibody is produced by crosslinking two or more antibodies (of the same type or of different types, e.g., to create bispecific antibodies). Suitable crosslinkers include those that are heterobifunctional, having two distinctly reactive groups separated by an appropriate spacer (e.g., m-maleimidobenzoyl-N-hydroxysuccinimide ester) or homobifunctional (e.g., disuccinimidyl suberate). Such linkers are available, e.g., from Pierce Chemical Company, Rockford, II.

[0114] An anti-PD-1 antibody can also be derivatized with a chemical group such as polyethylene glycol (PEG), a methyl or ethyl group, or a carbohydrate group. These groups may be useful to improve the biological characteristics of the antibody, e.g., to increase serum half-life.

[0115] An antibody according to the present invention may also be labeled. As used herein, the terms "label" or "labeled" refer to incorporation of another molecule in the antibody. In one embodiment, the label is a detectable marker, e.g., incorporation of a radiolabeled amino acid or attachment to a polypeptide of biotinyl moieties that can be detected by marked avidin (e.g., streptavidin containing a fluorescent marker or enzymatic activity that can be detected by optical or colorimetric methods). In another embodiment, the label or marker can be therapeutic, e.g., a drug conjugate or toxin. Various methods of labeling polypeptides and glycoproteins are known in the art and may be used. Examples of labels for polypeptides include, but are not limited to, the following: radioisotopes or radionuclides (e.g., 3H, 14C, 15N, 35S, 90Y, 99Tc, 111In, 1251, 131I), fluorescent labels (e.g., FITC, rhodamine, lanthanide phosphors), enzymatic labels (e.g., horseradish peroxidase, β-gaiactosidase, luciferase, alkaline phosphatase), chemiluminescent markers, biotinyl groups, predetermined polypeptide epitopes recognized by a secondary reporter (e.g., leucine zipper pair sequences, binding sites for secondary antibodies, metal binding domains, epitope tags), magnetic agents, such as gadolinium chelates, toxins such as pertussis toxin, taxol, cytochalasin B, gramicidin D, ethidium bromide, emetine, mitomycin, etoposide, tenoposide, vincristine, vinblastine, colchicine, doxorubicin, daunorubicin, dihydroxy anthracin dione, mitoxantrone, mithramycin, actinomycin D, 1-dehydrotestosterone, glucocorticoids, procaine, tetracaine, lidocaine, propranolol, and puromycin and analogs or homologs thereof. In some embodiments, labels are attached by spacer arms of various lengths to reduce potential steric hindrance.

[0116] In certain embodiments, the antibodies of the invention may be present in a neutral form (including zwitter ionic forms) or as a positively or negatively-charged species. In some embodiments, the antibodies may be complexed with a counterion to form a pharmaceutically acceptable salt.

[0117] The term "pharmaceutically acceptable salt" refers to a complex comprising one or more antibodies and one or more counterions, wherein the counterions are derived from pharmaceutically acceptable inorganic and organic acids and bases.

Bispecific Binding Molecules

[0118] In a further aspect, the invention provides a bispecific binding molecule having the of the invention binding specificity of an anti-PD-1 antibody of the invention herein and the binding specificity of another anti-PD-1 antibody (e.g., another anti-PD-1 antibody described herein) or an antibody that targets a different protein, such as another immune checkpoint protein, a cancer antigen, or another cell surface molecule whose activity mediates a disease condition such as cancer. Such bispecific binding molecules are known in the art, and examples of different types of bispecific binding molecules are given elsewhere herein.

Nucleic Acid Molecules and Vectors

[0119] The present invention also provides nucleic acid molecules and sequences of the invention encoding anti-PD-1 antibodies or antigen-binding portions thereof of the invention. In some embodiments, different nucleic acid molecules encode the heavy chain and light chain amino acid sequences of the anti-PD-1 antibody or an antigen-binding portion

thereof. In other embodiments, the same nucleic acid molecule encodes the heavy chain and light chain amino acid sequences of the anti-PD-1 antibody or an antigen-binding portion thereof.

[0120] A reference to a nucleotide sequence encompasses its complement unless otherwise specified. Thus, a reference to a nucleic acid having a particular sequence should be understood to encompass its complementary strand, with its complementary sequence. The term "polynucleotide" as referred to herein means a polymeric form of nucleotides of at least 10 bases in length, either ribonucleotides or deoxynucleotides or a modified form of either type of nucleotide. The term includes single and double stranded forms. Also disclosed herein are

[0121] Also disclosed herein are nucleotide sequences that are at least 70%, 75%, 80%, 85%, 90%, 95%, 97%, 98% or 99% identical to one or more nucleotide sequences recited herein, e.g., to a nucleotide sequence selected from the group consisting of SEQ ID NOs: 69-88. The term "percent sequence identity" in the context of nucleic acid sequences refers to the residues in two sequences that are the same when aligned for maximum correspondence. The length of sequence identity comparison may be over a stretch of at least about nine nucleotides, usually at least about 18 nucleotides, more usually at least about 24 nucleotides, typically at least about 28 nucleotides, more typically at least about 32 nucleotides, and preferably at least about 36, 48 or more nucleotides. There are a number of different algorithms known in the art which can be used to measure nucleotide sequence identity. For instance, polynucleotide sequences can be compared using FASTA, Gap or Bestfit, which are programs in Wisconsin Package Version 10.0, Genetics Computer Group (GCG), Madison, Wisconsin. FASTA, which includes, e.g., the programs FASTA2 and FASTA3, provides alignments and percent sequence identity of the regions of the best overlap between the query and search sequences (see, e.g., Pearson, Methods Enzymol. 183:63-98 (1990); Pearson, Methods Mol. Biol. 132:185-219 (2000); Pearson, Methods Enzymol. 266:227-258 (1996); and Pearson, J. Mol. Biol. 276:71-84 (1998); Unless otherwise specified, default parameters for a particular program or algorithm are used. For instance, percent sequence identity between nucleic acid sequences can be determined using FASTA with its default parameters (a word size of 6 and the NOPAM factor for the scoring matrix) or using Gap with its default parameters as provided in GCG Version 6.1.

[0122] In one aspect, the invention provides a nucleic acid molecule comprising a nucleotide sequence selected from the group consisting of SEQ ID NOs: 69 and 70.

[0123] In any of the above embodiments, the nucleic acid molecules may be isolated.

[0124] In a further aspect, the present invention provides a vector suitable for expressing one of the chains of an antibody of the invention or antigen-binding portion thereof. The term "vector", as used herein, means a nucleic acid molecule capable of transporting another nucleic acid to which it has been linked. In some embodiments, the vector is a plasmid, i.e., a circular double stranded piece of DNA into which additional DNA segments may be ligated. In some embodiments, the vector is a viral vector, wherein additional DNA segments may be ligated into the viral genome. In some embodiments, the vectors are capable of autonomous replication in a host cell into which they are introduced (e.g., bacterial vectors having a bacterial origin of replication and episomal mammalian vectors). In other embodiments, the vectors (e.g., non-episomal mammalian vectors) can be integrated into the genome of a host cell upon introduction into the host cell, and thereby are replicated along with the host genome. Moreover, certain vectors are capable of directing the expression of genes to which they are operatively linked. Such vectors are referred to herein as "recombinant expression vectors" (or simply, "expression vectors").

[0125] The invention provides vectors comprising nucleic acid molecules that encode the heavy chain of an anti-PD-1 antibody of the invention or an antigen-binding portion thereof, the light chain of an anti-PD-1 antibody of the invention or an antigen-binding portion thereof, or both the heavy and light chains of an anti-PD-1 antibody of the invention or an antigen-binding portion thereof. The invention further provides vectors comprising nucleic acid molecules encoding fusion proteins, modified antibodies, antibody fragments, and probes thereof.

[0126] A nucleic acid molecule encoding the heavy and/or light chain of an anti-PD-1 antibody or antigen-binding portion thereof of the invention can be isolated from any The source that produces such an antibody or portion. The nucleic acid molecules can be isolated from B cells that express an anti-PD-1 antibody isolated from an animal immunized with a human PD-1 antigen, or from an immortalized cell produced from such a B cell. Methods of isolating nucleic acids encoding an antibody are well-known in the art. mRNA may be isolated and used to produce cDNA for use in polymerase chain reaction (PCR) or cDNA cloning of antibody genes. In certain embodiments, a nucleic acid molecule of the invention can be synthesized rather than isolated.

[0127] In some embodiments, a nucleic acid molecule of the invention can comprise a nucleotide sequence encoding a V_H domain from an anti-PD-1 antibody or antigen-binding portion of the invention joined in-frame to a nucleotide sequence encoding a heavy chain constant region from any source. Similarly, a nucleic acid molecule of the invention can comprise a nucleotide sequence encoding a V_L domain from an anti-PD-1 antibody or antigen-binding portion of the invention joined in-frame to a nucleotide sequence encoding a light chain constant region from any source.

[0128] In a further aspect of the invention, nucleic acid molecules encoding the variable domain of the heavy (V_H) and/or light (V_L) chains may be "converted" to full-length antibody genes. In one embodiment, nucleic acid molecules encoding the V_H or V_L domains are converted to full-length antibody genes by insertion into an expression vector already encoding heavy chain constant (CH) or light chain constant (CL) domains, respectively, such that the V_H segment is

operatively linked to the CH segment(s) within the vector, and/or the V_L segment is operatively linked to the CL segment within the vector. In another embodiment, nucleic acid molecules encoding the V_H and/or V_L domains are converted into full-length antibody genes by linking, e.g., ligating, a nucleic acid molecule encoding a V_H and/or V_L domains to a nucleic acid molecule encoding a CH and/or CL domain using standard molecular biological techniques. Nucleic acid molecules encoding the full-length heavy and/or light chains may then be expressed from a cell into which they have been introduced and the anti-PD-1 antibody isolated.

[0129] The nucleic acid molecules may be used to recombinantly express large quantities of anti-PD-1 antibodies. The nucleic acid molecules also may be used to produce chimeric antibodies, bispecific antibodies, single chain antibodies, immunoadhesins, diabodies, mutated antibodies and antibody derivatives, as described herein.

[0130] In another embodiment, a nucleic acid molecule of the invention is used as a probe or PCR primer for a specific antibody sequence. For instance, the nucleic acid can be used as a probe in diagnostic methods or as a PCR primer to amplify regions of DNA that could be used, inter alia, to isolate additional nucleic acid molecules encoding variable domains of anti-PD-1 antibodies. In some embodiments, the nucleic acid molecules are oligonucleotides. Oligonucleotides disclosed herein can be from highly variable domains of the heavy and light chains of the antibody of interest. Oligonucleotides disclosed herein may encode all or a part of one or more of the CDRs of the anti-PD-1 antibodies or antigen-binding portions thereof of the invention as described herein.

[0131] In another embodiment, the nucleic acid molecules and vectors may be used to make mutated anti-PD-1 antibodies. The antibodies may be mutated in the variable domains of the heavy and/or light chains, e.g., to alter a binding property of the antibody. For example, a mutation may be made in one or more of the CDRs to increase or decrease the K_D of the anti-PD-1 antibody, to increase or decrease k_{off}, or to alter the binding specificity of the antibody. In another embodiment, one or more mutations are made at an amino acid residue that is known to be changed compared to the germline in a monoclonal antibody of the invention. The mutations may be made in a CDR or framework region of a variable domain, or in a constant region. In a preferred embodiment, the mutations are made in a variable domain. In some embodiments, one or more mutations are made at an amino acid residue that is known to be changed compared to the germline in a CDR or framework region of a variable domain of an antibody or antigen-binding portion thereof of the invention.

[0132] In another embodiment, the framework region(s) are mutated so that the resulting framework region(s) have the amino acid sequence of the corresponding germline gene. A mutation may be made in a framework region or constant region to increase the half-life of the anti-PD-1 antibody. See, e.g., PCT Publication WO 00/09560. A mutation in a framework region or constant region also can be made to alter the immunogenicity of the antibody, and/or to provide a site for covalent or non-covalent binding to another molecule. According to the invention, a single antibody may have mutations in any one or more of the CDRs or framework regions of the variable domain or in the constant region.

[0133] In some embodiments, the anti-PD-1 antibodies of the invention or antigen-binding portions thereof are expressed by inserting DNAs encoding partial or full-length light and heavy chains, obtained as described above, into expression vectors such that the genes are operatively linked to necessary expression control sequences such as transcriptional and translational control sequences. Expression vectors include plasmids, retroviruses, adenoviruses, adeno-associated viruses (AAV), plant viruses such as cauliflower mosaic virus, tobacco mosaic virus, cosmids, YACs, EBV derived episomes. The antibody coding sequence may be ligated into a vector such that transcriptional and translational control sequences within the vector serve their intended function of regulating the transcription and translation of the antibody coding sequence. The expression vector and expression control sequences may be chosen to be compatible with the expression host cell used. The antibody light chain coding sequence and the antibody heavy chain coding sequence can be inserted into separate vectors, and may be operatively linked to the same or different expression control sequences (e.g., promoters). In one embodiment, both coding sequences are inserted into the same expression vector, and may be operatively linked to the same expression control sequences (e.g., a common promoter), to separate identical expression control sequences (e.g., promoters), or to different expression control sequences (e.g., promoters). The antibody coding sequences may be inserted into the expression vector by standard methods (e.g., ligation of complementary restriction sites on the antibody gene fragment and vector, or blunt end ligation if no restriction sites are present).

[0134] A convenient vector is one that encodes a functionally complete human CH or CL immunoglobulin sequence, with appropriate restriction sites engineered so that any V_H or V_L sequence can easily be inserted and expressed, as described above. The HC- and LCencoding genes in such vectors may contain intron sequences that will result in enhanced overall antibody protein yields by stabilizing the related mRNA. The intron sequences are flanked by splice donor and splice acceptor sites, which determine where RNA splicing will occur. Location of intron sequences can be either in variable or constant regions of the antibody chains, or in both variable and constant regions when multiple introns are used. Polyadenylation and transcription termination may occur at native chromosomal sites downstream of the coding regions. The recombinant expression vector also can encode a signal peptide that facilitates secretion of the antibody chain from a host cell. The antibody chain gene may be cloned into the vector such that the signal peptide is linked in-frame to the amino terminus of the immunoglobulin chain. The signal peptide can be an immunoglobulin signal

peptide or a heterologous signal peptide (i.e., a signal peptide from a non-immunoglobulin protein).

[0135] In addition to the antibody chain genes, the recombinant expression vectors of the invention may carry regulatory sequences that control the expression of the antibody chain genes in a host cell. It will be appreciated by those skilled in the art that the design of the expression vector, including the selection of regulatory sequences, may depend on such factors as the choice of the host cell to be transformed, the level of expression of protein desired. Preferred regulatory sequences for mammalian host cell expression include viral elements that direct high levels of protein expression in mammalian cells, such as promoters and/or enhancers derived from retroviral LTRs, cytomegalovirus (CMV) (such as the CMV promoter/enhancer), Simian Virus 40 (SV40) (such as the SV40 promoter/enhancer), adenovirus, (e.g., the adenovirus major late promoter (AdMLP)), polyoma and strong mammalian promoters such as native immunoglobulin and actin promoters. For further description of viral regulatory elements, and sequences thereof, see e.g., US Patents 5,168,062, 4,510,245 and 4,968,615. Methods for expressing antibodies in plants, including a description of promoters and vectors, as well as transformation of plants, are known in the art. See, e.g., US Patent 6,517,529. Methods of expressing polypeptides in bacterial cells or fungal cells, e.g., yeast cells, are also well known in the art.

[0136] In addition to the antibody chain genes and regulatory sequences, the recombinant expression vectors of the invention may carry additional sequences, such as sequences that regulate replication of the vector in host cells (e.g., origins of replication) and selectable marker genes. The selectable marker gene facilitates selection of host cells into which the vector has been introduced (see e.g., US Patents 4,399,216, 4,634,665 and 5,179,017). For example, typically the selectable marker gene confers resistance to drugs, such as G418, hygromycin or methotrexate, on a host cell into which the vector has been introduced. For example, selectable marker genes include the dihydrofolate reductase (DHFR) gene (for use in dhfr-host cells with methotrexate selection/amplification), the neo gene (for G418 selection), and the glutamate synthetase gene.

[0137] The term "expression control sequence" as used herein means polynucleotide sequences that are necessary to effect the expression and processing of coding sequences to which they are ligated. Expression control sequences include appropriate transcription initiation, termination, promoter and enhancer sequences; efficient RNA processing signals such as splicing and polyadenylation signals; sequences that stabilize cytoplasmic mRNA; sequences that enhance translation efficiency (i.e., Kozak consensus sequence); sequences that enhance protein stability; and when desired, sequences that enhance protein secretion. The nature of such control sequences differs depending upon the host organism; in prokaryotes, such control sequences generally include promoter, ribosomal binding site, and transcription termination sequence; in eukaryotes, generally, such control sequences include promoters and transcription termination sequence. The term "control sequences" is intended to include, at a minimum, all components whose presence is essential for expression and processing, and can also include additional components whose presence is advantageous, for example, leader sequences and fusion partner sequences.

Host Cells and Methods of Antibody and Antibody Composition Production

[0138] An additional aspect of the invention relates to methods for producing the antibody compositions and antibodies and antigen-binding portions thereof of the invention. One embodiment of this aspect of the invention relates to a method for producing an antibody of the invention, comprising providing a recombinant host cell capable of expressing the antibody, cultivating said host cell under conditions suitable for expression of the antibody, and isolating the resulting antibody. Antibodies produced by such expression in such recombinant host cells are referred to herein as "recombinant antibodies". The invention also provides progeny cells of such host cells, and antibodies produced by same.

[0139] The term "recombinant host cell" (or simply "host cell"), as used herein, means a cell into which a recombinant expression vector has been introduced. The invention provides host cells that comprise a vector according to the invention described above. The invention also provides host cells that comprise a nucleotide sequence encoding the heavy chain or an antigen-binding portion thereof, a nucleotide sequence encoding the light chain or an antigen-binding portion thereof, or both, of an anti-PD-1 antibody or antigen-binding portion thereof of the invention. It should be understood that "recombinant host cell" and "host cell" mean not only the particular subject cell but also the progeny of such a cell. Because certain modifications may occur in succeeding generations due to either mutation or environmental influences, such progeny may not, in fact, be identical to the parent cell, but are still included within the scope of the term "host cell" as used herein.

[0140] Nucleic acid molecules encoding anti-PD-1 antibodies and vectors comprising these nucleic acid molecules can be used for transfection of a suitable mammalian, plant, bacterial or yeast host cell. Transformation can be by any known method for introducing polynucleotides into a host cell. Methods for introduction of heterologous polynucleotides into mammalian cells are well known in the art and include dextran-mediated transfection, calcium phosphate precipitation, polybrene-mediated transfection, protoplast fusion, electroporation, encapsulation of the polynucleotide(s) in liposomes, and direct microinjection of the DNA into nuclei. In addition, nucleic acid molecules may be introduced into mammalian cells by viral vectors. Methods of transforming cells are well known in the art. See, e.g., US Patents 4,399,216, 4,912,040, 4,740,461, and 4,959,455. Methods of transforming plant cells are well known in the art, including, e.g.,

Agrobacterium-mediated transformation, biolistic transformation, direct injection, electroporation and viral transformation. Methods of transforming bacterial and yeast cells are also well known in the art.

[0141] Mammalian cell lines available as hosts for expression are well known in the art and include many immortalized cell lines available from the American Type Culture Collection (ATCC). These include, *inter alia,* Chinese hamster ovary (CHO) cells, NS0 cells, SP2 cells, HEK-293T cells, 293 Freestyle cells (Invitrogen), NIH-3T3 cells, HeLa cells, baby hamster kidney (BHK) cells, African green monkey kidney cells (COS), human hepatocellular carcinoma cells (e.g., Hep G2), A549 cells, and a number of other cell lines. Cell lines of particular preference are selected by determining which cell lines have high expression levels. Other cell lines that may be used are insect cell lines, such as Sf9 or Sf21 cells. When recombinant expression vectors encoding antibody genes are introduced into mammalian host cells, the antibodies are produced by culturing the host cells for a period of time sufficient to allow for expression of the antibody in the host cells or, more preferably, secretion of the antibody into the culture medium in which the host cells are grown. Antibodies can be recovered from the culture medium using standard protein purification methods. Plant host cells include, e.g., *Nicotiana, Arabidopsis,* duckweed, corn, wheat, potato. Bacterial host cells include *E. coli and Streptomyces* species. Yeast host cells include *Schizosaccharomyces pombe, Saccharomyces cerevisiae* and *Pichia pastoris.*

[0142] Further, expression of antibodies of the invention or antigen-binding portions thereof from production cell lines can be enhanced using a number of known techniques. For example, the glutamine synthetase gene expression system (the GS system) is a common approach for enhancing expression under certain conditions. The GS system is discussed in whole or part in connection with EP Patents 0 216 846, 0 256 055, 0 323 997 and 0 338 841.

[0143] It is likely that antibodies expressed by different cell lines or in transgenic animals will have different glycosylation patterns from each other. However, all antibodies encoded by the nucleic acid molecules provided herein, or comprising the amino acid sequences provided herein are part of the instant invention, regardless of the glycosylation state of the antibodies, and more generally, regardless of the presence or absence of posttranslational modification(s).

Pharmaceutical Compositions

[0144] Another aspect of the invention is a pharmaceutical composition comprising as an active ingredient (or as the sole active ingredient) an anti-PD-1 antibody or antigen-binding portion thereof or anti-PD-1 antibody composition of the invention. The pharmaceutical composition may comprise any anti-PD-1 antibody composition or antibody or antigen-binding portion thereof of the invention. In some embodiments, the compositions are intended for amelioration, prevention, and/or treatment of a PD-1-related disorder (e.g., a disorder characterized by overexpression or overactivity of PD-1) and/or cancer. In some embodiments, the compositions are intended for activation of the immune system. In certain embodiments, the compositions are intended for amelioration, prevention, and/or treatment of cancer originating in tissues such as skin, lung, intestine, ovary, brain, prostate, kidney, soft tissues, the hematopoietic system, head & neck, liver, bladder, breast, stomach, uterus and pancreas.

[0145] Generally, the antibodies of the invention or antigen-binding portions thereof are suitable to be administered as a formulation in association with one or more pharmaceutically acceptable excipient(s), e.g., as described below.

[0146] Pharmaceutical compositions of the invention will comprise one or more anti-PD-1 antibodies or binding portions of the invention, e.g., one or two anti-PD-1 antibodies or binding portions. In one embodiment, the composition comprises a single anti-PD-1 antibody of the invention or binding portion thereof.

[0147] In another embodiment, the pharmaceutical composition may comprise at least one anti-PD-1 antibody or antigen-binding portion thereof, e.g., one anti-PD-1 antibody or portion, and one or more additional antibodies that target one or more relevant cell surface receptors, e.g., one or more cancer-relevant receptors.

[0148] The term "excipient" is used herein to describe any ingredient other than the compound(s) of the invention. The choice of excipient(s) will to a large extent depend on factors such as the particular mode of administration, the effect of the excipient on solubility and stability, and the nature of the dosage form. As used herein, "pharmaceutically acceptable excipient" includes any and all solvents, dispersion media, coatings, antibacterial and antifungal agents, isotonic and absorption delaying agents that are physiologically compatible. Some examples of pharmaceutically acceptable excipients are water, saline, phosphate buffered saline, dextrose, glycerol, ethanol, as well as combinations thereof. In many cases, it will be preferable to include isotonic agents, for example, sugars, polyalcohols such as mannitol, sorbitol, or sodium chloride in the composition. Additional examples of pharmaceutically acceptable substances are wetting agents or minor amounts of auxiliary substances such as wetting or emulsifying agents, preservatives or buffers, which enhance the shelf life or effectiveness of the antibody.

[0149] Pharmaceutical compositions of the present invention and methods for their preparation will be readily apparent to those skilled in the art. Such compositions and methods for their preparation may be found, for example, in Remington's Pharmaceutical Sciences, 19th Edition (Mack Publishing Company, 1995). Pharmaceutical compositions are preferably manufactured under GMP (good manufacturing practices) conditions.

[0150] A pharmaceutical composition of the invention may be prepared, packaged, or sold in bulk, as a single unit dose, or as a plurality of single unit doses. As used herein, a "unit dose" is a discrete amount of the pharmaceutical

composition comprising a predetermined amount of the active ingredient. The amount of the active ingredient is generally equal to the dosage of the active ingredient which would be administered to a subject or a convenient fraction of such a dosage such as, for example, one-half or onethird of such a dosage.

[0151] Any method for administering peptides, proteins or antibodies accepted in the art may suitably be employed for the antibodies and antigen-binding portions of the invention.

[0152] The pharmaceutical compositions of the invention are typically suitable for parenteral administration. As used herein, "parenteral administration" of a pharmaceutical composition includes any route of administration characterized by physical breaching of a tissue of a subject and administration of the pharmaceutical composition through the breach in the tissue, thus generally resulting in the direct administration into the blood stream, into muscle, or into an internal organ. Parenteral administration thus includes administration of a pharmaceutical composition by injection of the composition, by application of the composition through a surgical incision, by application of the composition through a tissue-penetrating non-surgical wound. In particular, parenteral administration is contemplated to include subcutaneous, intraperitoneal, intramuscular, intrasternal, intravenous, intraarterial, intrathecal, intraventricular, intraurethral, intracranial, and intrasynovial injection or infusions; and kidney dialytic infusion techniques. Regional perfusion is also contemplated. Preferred embodiments include the intravenous and the subcutaneous routes.

[0153] Formulations of a pharmaceutical composition suitable for parenteral administration typically comprise the active ingredient combined with a pharmaceutically acceptable carrier, such as sterile water or sterile isotonic saline. Such formulations may be prepared, packaged, or sold in a form suitable for bolus administration or for continuous administration. Injectable formulations may be prepared, packaged, or sold in unit dosage form, such as in ampoules or in multi-dose containers containing a preservative. Formulations for parenteral administration include suspensions, solutions, emulsions in oily or aqueous vehicles, pastes. Such formulations may further comprise one or more additional ingredients including suspending, stabilizing, or dispersing agents. In one embodiment of a formulation for parenteral administration, the active ingredient is provided in dry (i.e., powder or granular) form for reconstitution with a suitable vehicle (e.g., sterile pyrogen-free water) prior to parenteral administration of the reconstituted composition. Parenteral formulations also include aqueous solutions which may contain excipients such as salts, carbohydrates and buffering agents (preferably to a pH of from 3 to 9), but, for some applications, they may be more suitably formulated as a sterile non-aqueous solution or as a dried form to be used in conjunction with a suitable vehicle such as sterile, pyrogen-free water. Exemplary parenteral administration forms include solutions or suspensions in sterile aqueous solutions, for example, aqueous propylene glycol or dextrose solutions. Such dosage forms can be suitably buffered, if desired. Other parentally-administrable formulations which are useful include those which comprise the active ingredient in microcrystalline form, or in a liposomal preparation. Formulations for parenteral administration may be formulated to be immediate and/or modified release. Modified release formulations include delayed-, sustained-, pulsed-, controlled-, targeted and programmed release.

[0154] For example, in one aspect, sterile injectable solutions can be prepared by incorporating the anti-PD-1 antibody or antigen-binding portion thereof or anti-PD-1 antibody composition in the required amount in an appropriate solvent with one or a combination of ingredients enumerated above, as required, followed by filtered sterilization. Generally, dispersions are prepared by incorporating the active compound into a sterile vehicle that contains a basic dispersion medium and the required other ingredients from those enumerated above. In the case of sterile powders for the preparation of sterile injectable solutions, the preferred methods of preparation are vacuum drying and freeze-drying that yields a powder of the active ingredient plus any additional desired ingredient from a previously sterile-filtered solution thereof. The proper fluidity of a solution can be maintained, for example, by the use of a coating such as lecithin, by the maintenance of the required particle size in the case of dispersion and by the use of surfactants. Prolonged absorption of injectable compositions can be brought about by including in the composition an agent that delays absorption, for example, monostearate salts and gelatin, and/or by using modified-release coatings (e.g., slowrelease coatings).

[0155] The antibodies of the invention can also be administered intranasally or by inhalation, typically in the form of a dry powder (either alone, as a mixture, or as a mixed component particle, for example, mixed with a suitable pharmaceutically acceptable excipient) from a dry powder inhaler, as an aerosol spray from a pressurised container, pump, spray, atomiser (preferably an atomiser using electrohydrodynamics to produce a fine mist), or nebuliser, with or without the use of a suitable propellant, or as nasal drops.

[0156] The pressurised container, pump, spray, atomizer, or nebuliser generally contains a solution or suspension of an antibody of the invention comprising, for example, a suitable agent for dispersing, solubilising, or extending release of the active, a propellant(s) as solvent.

[0157] Prior to use in a dry powder or suspension formulation, the drug product is generally micronised to a size suitable for delivery by inhalation (typically less than 5 microns). This may be achieved by any appropriate comminuting method, such as spiral jet milling, fluid bed jet milling, supercritical fluid processing to form nanoparticles, high pressure homogenisation, or spray drying.

[0158] Capsules, blisters and cartridges for use in an inhaler or insufflator may be formulated to contain a powder mix of the compound of the invention, a suitable powder base and a performance modifier.

[0159] A suitable solution formulation for use in an atomiser using electrohydrodynamics to produce a fine mist may contain a suitable dose of the antibody of the invention per actuation and the actuation volume may for example vary from 1 μL to 100 μL.

[0160] Suitable flavours, such as menthol and levomenthol, or sweeteners, such as saccharin or saccharin sodium, may be added to those formulations of the invention intended for inhaled/intranasal administration.

[0161] Formulations for inhaled/intranasal administration may be formulated to be immediate and/or modified release. Modified release formulations include delayed-, sustained-, pulsed-, controlled-, targeted and programmed release.

[0162] In the case of dry powder inhalers and aerosols, the dosage unit is determined by means of a valve which delivers a metered amount. Units in accordance with the invention are typically arranged to administer a metered dose or "puff" of an antibody of the invention. The overall daily dose will typically be administered in a single dose or, more usually, as divided doses throughout the day.

[0163] The antibodies and antibody portions of the invention may also be formulated for an oral route administration. Oral administration may involve swallowing, so that the compound enters the gastrointestinal tract, and/or buccal, lingual, or sublingual administration by which the compound enters the blood stream directly from the mouth.

[0164] Formulations suitable for oral administration include solid, semi-solid and liquid systems such as tablets; soft or hard capsules containing multi- or nano-particulates, liquids, or powders; lozenges (including liquid-filled); chews; gels; fast dispersing dosage forms; films; ovules; sprays; and buccal/mucoadhesive patches.

[0165] Liquid formulations include suspensions, solutions, syrups and elixirs. Such formulations may be employed as fillers in soft or hard capsules (made, for example, from gelatin or hydroxypropylmethylcellulose) and typically comprise a carrier, for example, water, ethanol, polyethylene glycol, propylene glycol, methylcellulose, or a suitable oil, and one or more emulsifying agents and/or suspending agents. Liquid formulations may also be prepared by the reconstitution of a solid, for example, from a sachet.

Therapeutic uses of antibodies and compositions of the invention

[0166] In one aspect, the anti-PD-1 antibodies and antigen-binding portions thereof, anti-PD-1 compositions, and bi-specific binding molecules of the invention are used to enhance or activate the immune system in a human in need thereof. In some embodiments, the patient has a condition characterized by overexpression or overactivity of PD-1. In some embodiments, the patient is immune-suppressed. In certain embodiments, the antibody or antigen-binding portion thereof, composition, or bi-specific binding molecule pharmaceutical composition is for use in the treatment of cancer, e.g., cancers that originate in tissues such as skin, lung, intestine, ovary, brain, prostate, kidney, soft tissues, hematopoietic system, head & neck, liver, bladder, breast, stomach, uterus and pancreas, and any cancers or other conditions which rely on PD-1 activity or in which the patient expresses or overexpresses PD-L1, PD-L2, or both. Cancers treated by the anti-PD-1 antibodies, antigen-binding portions thereof, anti-PD-1 antibody compositions, and/or bispecific binding molecules of the invention may include, e.g., melanoma (such as advanced melanoma, or unresectable or metastatic melanoma), non-small cell lung cancer, bladder cancer, head and neck squamous cell carcinoma, ovarian cancer, colorectal cancer, Hodgkin's lymphoma, and renal cell carcinoma (RCC).

[0167] In some embodiments, cancers treated by the anti-PD-1 antibodies, antigen-binding portions, anti-PD-1 compositions, and/or bi-specific binding molecules of the invention may include, e.g., melanoma (e.g., advanced or metastatic melanoma), non-small cell lung cancer, head and neck squamous cell cancer, renal cell carcinoma, Hodgkin's lymphoma, non-Hodgkin's lymphoma, glioblastoma, glioma, squamous cell lung cancer, small-cell lung cancer, hepatocellular carcinoma, bladder cancer, upper urinary tract cancer, esophageal cancer, gastroesophageal junction cancer, gastric cancer, liver cancer, colon cancer, colorectal carcinoma, multiple myeloma, sarcomas, acute myeloid leukemia, chronic myeloid leukemia, myelodysplastic syndrome, nasopharyngeal cancer, chronic lymphocytic leukemia, acute lymphoblastic leukemia, small lymphocytic lymphoma, ovarian cancer, gastrointestinal cancer, primary peritoneal cancer, fallopian tube cancer, urothelial cancer, HTLV-associated T-cell leukemia/lymphoma, prostate cancer, genitourinary cancer, meningioma, adrenocortical cancer, gliosarcoma, fibrosarcoma, kidney cancer, breast cancer, pancreatic cancer, endometrial cancer, skin basal cell cancer, cancer of the appendix, biliary tract cancer, salivary gland cancer, advanced Merkel cell cancer, diffuse large B cell lymphoma, follicular lymphoma, mesothelioma, and solid tumors.

[0168] "Treat", "treating" and "treatment" refer to a method of alleviating or abrogating a biological disorder and/or at least one of its attendant symptoms. As used herein, to "alleviate" a disease, disorder or condition means reducing the severity and/or occurrence frequency of the symptoms of the disease, disorder, or condition. Further, references herein to "treatment" include references to curative, palliative and prophylactic treatment.

[0169] "Therapeutically effective amount" refers to the amount of the therapeutic agent being administered that will relieve to some extent one or more of the symptoms of the disorder being treated. A therapeutically effective amount of an anti-cancer therapeutic may result in tumor shrinkage, increased survival, elimination of cancer cells, decreased disease progression, reversal of metastasis, or other clinical endpoints desired by healthcare professionals.

[0170] The antibody compositions or antibodies or antigen-binding portions thereof of the invention may be adminis-

tered alone or in combination with one or more other drugs or antibodies (or as any combination thereof). The pharmaceutical compositions, methods and uses of the invention thus also encompass embodiments of combinations (co-administration) with other active agents, as detailed below.

[0171] As used herein, the terms "co-administration", "co-administered" and "in combination with," referring to the antibody compositions and antibodies and antigen-binding portions thereof of the invention with one or more other therapeutic agents, is intended to mean, and does refer to and include the following:

- simultaneous administration of such combination of antibody composition / antibody / antigen-binding portion of the invention and therapeutic agent(s) to a patient in need of treatment, when such components are formulated together into a single dosage form which releases said components at substantially the same time to said patient,
- substantially simultaneous administration of such combination of antibody composition / antibody / antigen-binding portion of the invention and therapeutic agent(s) to a patient in need of treatment, when such components are formulated apart from each other into separate dosage forms which are taken at substantially the same time by said patient, whereupon said components are released at substantially the same time to said patient,
- sequential administration of such combination of antibody composition / antibody / antigen-binding portion of the invention and therapeutic agent(s) to a patient in need of treatment, when such components are formulated apart from each other into separate dosage forms which are taken at consecutive times by said patient with a significant time interval between each administration, whereupon said components are released at substantially different times to said patient; and
- sequential administration of such combination of antibody composition / antibody / antigen-binding portion of the invention and therapeutic agent(s) to a patient in need of treatment, when such components are formulated together into a single dosage form which releases said components in a controlled manner whereupon they are concurrently, consecutively, and/or overlappingly released at the same and/or different times to said patient,

where each part may be administered by either the same or a different route.

[0172] The antibody compositions and antibodies and antigen-binding portions thereof of the invention may be administered without additional therapeutic treatments, i.e., as a stand-alone therapy. Alternatively, treatment with the antibody compositions and antibodies and antigen-binding portions thereof of the invention may include at least one additional therapeutic treatment (combination therapy). In some embodiments, the antibody composition or antibody or antigen-binding portion thereof may be co-administered or formulated with another medication/drug for the treatment of cancer. The additional therapeutic treatment may comprise, e.g., a chemotherapeutic, anti-neoplastic, or anti-angiogenic agent, a different anti-cancer antibody, and/or radiation therapy.

[0173] By combining the antibody compositions, antibodies, or antigen-binding portions of the invention with agents known to induce terminal differentiation of cancer cells, the effect may be improved further. Such compounds may, for example, be selected from the group consisting of retinoic acid, trans-retinoic acids, cis-retinoic acids, phenylbutyrate, nerve growth factor, dimethyl sulfoxide, active form vitamin D3, peroxisome proliferatoractivated receptor gamma, 12-O-tetradecanoylphorbol 13-acetate, hexamethylene-bis-acetamide, transforming growth factor-beta, butyric acid, cyclic AMP, and vesnarinone. In some embodiments, the compound is selected from the group consisting of retinoic acid, phenylbutyrate, all-trans-retinoic acid and active form vitamin D.

[0174] Pharmaceutical articles comprising an anti-PD-1 antibody composition or anti-PD-1 antibody or antigen-binding portion thereof of the invention and at least one other agent (e.g., a chemotherapeutic, anti-neoplastic, or anti-angiogenic agent) may be used as a combination treatment for simultaneous, separate or successive administration in cancer therapy. The other agent may by any agent suitable for treatment of the particular cancer in question, for example, an agent selected from the group consisting of alkylating agents, e.g., platinum derivatives such as cisplatin, carboplatin and/or oxaliplatin; plant alkoids, e.g., paclitaxel, docetaxel and/or irinotecan; antitumor antibiotics, e.g., doxorubicin (adriamycin), daunorubicin, epirubicin, idarubicin mitoxantrone, dactinomycin, bleomycin, actinomycin, luteomycin, and/or mitomycin; topoisomerase inhibitors such as topotecan; and/or antimetabolites, e.g., fluorouracil and/or other fluoropyrimidines.

[0175] An anti-PD-1 antibody or antigen-binding portion thereof or anti-PD-1 antibody composition of the invention may also be used in combination with other anti-cancer therapies such as vaccines, cytokines, enzyme inhibitors and T cell therapies. In the case of a vaccine, it may, e.g., be a protein, peptide or DNA vaccine containing one or more antigens which are relevant for the cancer being treated, or a vaccine comprising dendritic cells along with an antigen. Suitable cytokines include, for example, IL-2, IFN-gamma and GM-CSF. An example of a type of enzyme inhibitor that has anti-cancer activity is an indoleamine-2,3-dioxygenase (IDO) inhibitor, for example 1-methyl-D-tryptophan (1-D-MT). Adoptive T cell therapy refers to various immunotherapy techniques that involve expanding or engineering patients' own T cells to recognize and attack their tumors.

[0176] It is also contemplated that an anti-PD-1 antibody or antigen-binding portion thereof or anti-PD-1 antibody composition of the invention may be used in adjunctive therapy in connection with tyrosine kinase inhibitors. These are

synthetic, mainly quinazoline-derived, low molecular weight molecules that interact with the intracellular tyrosine kinase domain of receptors and inhibiting ligand-induced receptor phosphorylation by competing for the intracellular Mg-ATP binding site.

[0177] In some embodiments, the antibody composition or antibody or antigen-binding portion thereof may be used in combination with another medication/drug that mediates immune system activation, including, but not limited to, an agent that mediates the expression or activity of A2AR, BLTA, B7-H3, B7-H4, CTLA-4, CD27, CD28, CD40, CD55, CD73, CD122, CD137, CD160, CGEN-15049, CHK1, CHK2, CTLA-3, CEACAM (e.g., CEACAM-1 and/or CEACAM-5), GAL9, GITR, HVEM, ICOS, IDO, KIR, LAIR1, LAG-3, OX40, TIGIT, TIM-3, TGFR-beta, VISTA and/or 2B4. In certain embodiments, the agent is an antibody or an antigen-binding fragment thereof that binds to one of the above molecules. In certain embodiments, the antibody composition or antibody or antigen-binding portion thereof of the invention may be administered in combination with a CTLA-4 inhibitor (e.g., an anti-CTLA-4 antibody such as tremelimumab or ipilimumab). In one embodiment, the antibody composition or antibody or antigen-binding portion thereof of the invention may be administered in combination with ipilimumab.

[0178] In certain aspects, the antibodies and antigen-binding portions of the invention may be administered in combination with another inhibitor of the PD-1 pathway, which may target PD-1 or one or more of its ligands. Examples of such inhibitors include other anti-PD-1 antibodies, anti-PD-L1 antibodies, and anti-PD-L2 antibodies. In some embodiments, an antibody composition, antibody, and/or antigen-binding portion of the invention may be administered in combination with pembrolizumab and/or nivolumab.

[0179] It is understood that the antibody compositions and antibodies and antigen-binding portions thereof of the invention may be used in a method of treatment as described herein, may be for use in a treatment as described herein, and/or may be for use in the manufacture of a medicament for a treatment as described herein,

Dose and Route of Administration

[0180] The antibody compositions of the invention will be administered in an effective amount for treatment of the condition in question, i.e., at dosages and for periods of time necessary to achieve a desired result. A therapeutically effective amount may vary according to factors such as the particular condition being treated, the age, sex and weight of the patient, and whether the antibodies are being administered as a stand-alone treatment or in combination with one or more additional anti-cancer treatments.

[0181] Dosage regimens may be adjusted to provide the optimum desired response. For example, a single bolus may be administered, several divided doses may be administered over time or the dose may be proportionally reduced or increased as indicated by the exigencies of the therapeutic situation. It is especially advantageous to formulate parenteral compositions in dosage unit form for ease of administration and uniformity of dosage. Dosage unit form, as used herein, refers to physically discrete units suited as unitary dosages for the patients/subjects to be treated; each unit containing a predetermined quantity of active compound calculated to produce the desired therapeutic effect in association with the required pharmaceutical carrier. The specification for the dosage unit forms of the invention are generally dictated by and directly dependent on (a) the unique characteristics of the chemotherapeutic agent and the particular therapeutic or prophylactic effect to be achieved, and (b) the limitations inherent in the art of compounding such an active compound for the treatment of sensitivity in individuals.

[0182] Thus, the skilled artisan would appreciate, based upon the disclosure provided herein, that the dose and dosing regimen are adjusted in accordance with methods well-known in the therapeutic arts. That is, the maximum tolerable dose can be readily established, and the effective amount providing a detectable therapeutic benefit to a patient may also be determined, as can the temporal requirements for administering each agent to provide a detectable therapeutic benefit to the patient. Accordingly, while certain dose and administration regimens are exemplified herein, these examples in no way limit the dose and administration regimen that may be provided to a patient in practicing the present invention.

[0183] It is to be noted that dosage values may vary with the type and severity of the condition to be alleviated, and may include single or multiple doses. It is to be further understood that for any particular subject, specific dosage regimens should be adjusted over time according to the individual need and the professional judgment of the person administering or supervising the administration of the compositions, and that dosage ranges set forth herein are exemplary only and are not intended to limit the scope or practice of the embodied composition. Further, the dosage regimen with the compositions of this invention may be based on a variety of factors, including the type of disease, the age, weight, sex, medical condition of the patient, the severity of the condition, the route of administration, and the particular antibody employed. Thus, the dosage regimen can vary widely, but can be determined routinely using standard methods. For example, doses may be adjusted based on pharmacokinetic or pharmacodynamic parameters, which may include clinical effects such as toxic effects and/or laboratory values. Thus, envisaged is an intra-patient dose-escalation as determined by the skilled artisan. Determining appropriate dosages and regimens are well-known in the relevant art.

[0184] It is contemplated that a suitable dose of an antibody composition of the invention will be in the range of 0.1-100 mg/kg, such as about 0.5-50 mg/kg, e.g., about 1-20 mg/kg. The antibody composition may for example be administered

in a dosage of at least 0.25 mg/kg, e.g., at least 0.5 mg/kg, such as at least 1 mg/kg, e.g., at least 1.5 mg/kg, such as at least 2 mg/kg, e.g., at least 3 mg/kg, such as at least 4 mg/kg, e.g., at least 5 mg/kg; and e.g., up to at most 50 mg/kg, such as up to at the most 30 mg/kg, e.g., up to at the most 20 mg/kg, such as up to at the most 15 mg/kg. Administration will normally be repeated at suitable intervals, e.g., once every week, once every two weeks, once every three weeks, or once every four weeks, and for as long as deemed appropriate by the responsible doctor, who may optionally increase or decrease the dosage as necessary.

[0185] An effective amount for tumor therapy may be measured by its ability to stabilize disease progression and/or ameliorate symptoms in a patient, and preferably to reverse disease progression, e.g., by reducing tumor size. The ability of an antibody or composition of the invention to inhibit cancer may be evaluated by in vitro assays, e.g., as described in the examples, as well as in suitable animal models that are predictive of the efficacy in human tumors. Suitable dosage regimens will be selected in order to provide an optimum therapeutic response in each particular situation, for example, administered as a single bolus or as a continuous infusion, and with possible adjustment of the dosage as indicated by the exigencies of each case.

Diagnostic Uses and Compositions

[0186] The antibodies of the present invention also are useful in diagnostic processes (e.g., in vitro, ex vivo). For example, the antibodies can be used to detect and/or measure the level of PD-1 in a sample from a patient (e.g., a tissue sample, or a body fluid sample such as an inflammatory exudate, blood, serum, bowel fluid, saliva, or urine). Suitable detection and measurement methods include immunological methods such as flow cytometry, enzyme-linked immunosorbent assays (ELISA), chemiluminescence assays, radioimmunoassay, and immunohistology. Also disclosed herein are kits (e.g., diagnostic kits) comprising the antibodies described herein.

[0187] Unless otherwise defined herein, scientific and technical terms used in connection with the present invention shall have the meanings that are commonly understood by those of ordinary skill in the art. Exemplary methods and materials are described below.

[0188] In case of conflict, the present specification, including definitions, will control.

[0189] Generally, nomenclature used in connection with, and techniques of, cell and tissue culture, molecular biology, immunology, microbiology, genetics, analytical chemistry, synthetic organic chemistry, medicinal and pharmaceutical chemistry, and protein and nucleic acid chemistry and hybridization described herein are those well-known and commonly used in the art. Enzymatic reactions and purification techniques are performed according to manufacturer's specifications, as commonly accomplished in the art or as described herein.

[0190] Further, unless otherwise required by context, singular terms shall include pluralities and plural terms shall include the singular. Throughout this specification and embodiments, the words "have" and "comprise," or variations such as "has," "having," "comprises," or "comprising," will be understood to imply the inclusion of a stated integer or group of integers but not the exclusion of any other integer or group of integers.

[0191] Although a number of documents are cited herein, this citation does not constitute an admission that any of these documents forms part of the common general knowledge in the art.

[0192] In order that this invention may be better understood, the following examples are set forth.

EXAMPLES

Example 1: Cloning of anti-PD-1 antibodies from chicken B cells

[0193] Cloning of chicken-derived antibody genes from antibody-secreting B cells (ASC) was performed by means of the Symplex™ antibody discovery technology. Briefly, ASC were isolated from lymphoid organs of chickens that had been immunized with PD-1 antigen, as soluble protein antigen and/or in its native cell membrane-bound form displayed on eukaryotic cells. Staining of the ASC with fluorescently labelled antibodies allowed discrimination of ASC from other cells (e.g., T cells, naïve B cells, monocytes, etc.) prior to sorting into PCR vessels. Single ASC sorting was performed by flow cytometry. Subsequently, the Symplex™ procedure was conducted to generate PCR products containing cognate V_H and V_L pairs for each sorted B cell as described hereafter.

[0194] Linkage of V_H and V_L coding sequences was performed on the sorted ASC, facilitating cognate pairing of the sequences. The process utilized a two-step PCR procedure based on a one-step multiplex overlap-extension RT-PCR followed by nested PCR. The principle for linkage of cognate V_H and V_L sequences using the Symplex™ technology is described in detail in WO 2005/042774; WO 2008/104184; WO 2010/022738, and Meijer et al., J Mol Biol 358(3):764-72 (2006). Briefly, cognate V_H and V_L amplified fragments are joined by overlap-extension PCR in a so-called nested PCR step. In the subsequent process, PCR products are pooled prior to cloning into a plasmid vector. This is done in such a way that the cloned DNA fragments encoding the variable domains of the chicken antibody can be expressed as a full chimeric antibody from a single plasmid expression construct in transfected mammalian cells. Consequently, it is possible

to screen cell supernatants for chimeric antibodies exhibiting specific binding to the PD-1 antigen.

Materials and Methods

[0195] The Symplex™ technology as described in the publications listed above was modified to amplify V_L and V_H from sorted chicken B-cells. Cloning of a functional expression construct was done in two steps, as described below.

[0196] Step 1. The amplified PCR products containing the paired V_H and V_L fragments were amplified in a nested PCR reaction. This allowed for addition of flanking restriction enzyme recognition sites for ApaI and AvrII at each end. Since the cognate V_H and V_L sequences were paired in a single PCR product from each sorted ASC, cloning of the PCR products was performed after pooling all the PCR fragments. The plasmid pML392 was constructed to receive the Symplex™ PCR products by digestion of the corresponding restriction sites ApaI and AvrII. The resulting ligation of pooled PCR products and pML392 is shown in Figure 1. Here the insertion of the PCR product placed the V_H and V_L sequences in front of human CH1-CH2-CH3 and lambda constant cDNA regions, respectively, so that full length heavy and light chain reading frames were obtained.

[0197] Step 2. In the initial constructs, the two reading frames encoding heavy and light chain sequences were placed head-to-head and separated by a DNA sequence that contained restriction enzyme recognition sites for AscI and NheI. By insertion of a corresponding AscI/NheI-digested double CMV promoter DNA fragment including 5'-UTRs and signal peptides between the two 5'-ends of the heavy and light chain genes, a complete expression construct was obtained as depicted in Figure 2.

Example 2: Cloning of anti-PD-1 reference antibody analogues

[0198] This example briefly explains how reference analogues of the anti-PD-1 antibodies nivolumab and pembrolizumab were generated.

[0199] Amino acid sequences encoding the variable heavy and light chain domains of antibody analogues of nivolumab and pembrolizumab were obtained from the IMGT® website imgt.org/mAb-DB/; see Table 3 below. The protein sequences were reverse translated to DNA sequences with human codon usage. The corresponding DNA sequences were then gene synthesized and cloned into expression vectors containing constant human IgG_4 heavy chain or kappa light chain domains, resulting in expression of full-length antibodies. To prevent Fab arm exchange, the serine residue at position 228 was substituted with proline (Angal et al., Mol. Immunol. 30:105-108 (1993)). CHO cells were transfected with the corresponding expression plasmids using a standard protein expression system. The corresponding antibody supernatants were purified using standard protein A purification column chromatography.

Table 3 Gene-synthesized antibody analogues

Antibody	Research code	Antibody format	Reference Website
Pembrolizumab / KEYTRUDA®	MK-3475	Recombinant IgG_4, S228P	imgt.org/mAb-DB/mAbcard?Abld=472
Nivolumab / OPDIVO®	BMS-936558, MDX-1106, ONO-4538	Recombinant IgG_4, S228P	imgt.org/mAb-DB/mAbcard?Abld=424

Example 3: Screening of antibody repertoires for binding to cell surface-expressed PD-1

[0200] Cloned antibodies of the anti-PD-1 repertoire were individually transfected and expressed in HEK293 cells using 293fectin™ Transfection reagent (Invitrogen, Cat. No. 12347-019) in 384-well format, and antibody-containing supernatants were collected on day 6 after transfection.

[0201] For cell-based antibody screening, CHO-S cells were transfected in 384-well format to express full-length human PD-1 using the Freestyle™ MAX reagent (Invitrogen, Cat. No. 16447-100), and non-transfected cells were used as negative control. In order to allow a multiplexed screening setup, non-transfected cells were labeled using CFSE and mixed with non-labeled PD-1-transfected cells at a ratio of 1 to 1, and a density of 1E6 cells per ml, each. In 384-well plates, 40 μl of this cell mix was mixed with 10 μl of antibody-containing supernatant, and cell-bound antibody was revealed by addition of goat anti-human IgG (H+L) AF647 secondary antibody (Molecular Probes, Cat. No. A21445) in a non-wash setup. Samples were acquired using high throughput flow cytometry (iQue® Screener, Intellicyt) and data was analyzed using ForeCyt® software by plotting CFSE vs. human IgG binding (AF647). PD-1-specific primary hits were identified as antibody clones binding only to human PD-1-transfected cells (CSFE negative), but not to control cells (CFSE positive), and plate numbers and plate coordinates were collected for hit picking and subsequent sequence analysis.

[0202] Figures 3A-3C show representative flow cytometry dot plots for (A) an antibody clone that specifically binds to human PD-1-transfected cells, (B) a clone that nonspecifically binds to CHO-S cells, and (C) a clone that does not bind either of the cell populations used in the screening.

Example 4: Humanization of anti-PD-1 antibodies

[0203] Humanization of the framework regions of the chicken anti-PD-1 antibodies was performed in order to produce antibody molecules having minimal immunogenicity when administered to humans, while substantially retaining the specificity and affinity of the parental chicken antibodies.

Materials and Methods

[0204] Humanization of the chicken-derived antibodies was performed using the "CDR grafting" approach, a method originally described by Jones et al., Nature 321:522-525 (1986). First, the variable heavy (V_H) and variable light (V_L) domains of the antibodies were blasted against human IgG databases in order to find the closest human germline genes. This identified the human IGHV3-23*01 (M99660) and IGLV3-19*01 (X56178) genes as being closest to the chicken V_H and V_L genes, respectively. Similarly, the selected human amino acid sequences for J-gene region humanization were derived from IGHJ1*01 (J00256) and IGLJ6*01 (M18338) for V_H and V_L, respectively. Furthermore, the antibody V_H and V_L genes were aligned against chicken immunoglobulin germline genes to identify somatic mutations in the framework regions that may play a role in antibody function and/or structure. Such residues may be included in the final humanized antibody genes as so-called "back mutation" residues. Finally, some amino acid positions, so-called "Vernier residues" (Foote and Winter, J Mol Biol. 224(2):487-99 (1992)), that are known to play an important role in antibody structure, stability and function, were considered to generate alternative humanized antibody variants including either human or chicken residues from the corresponding germlines.

[0205] The CDR sequences herein were determined according to the IMGT® definitions for CDR1 and CDR2. For heavy and light chain CDR3, the definitions herein include one extra amino acid residue upstream of the IMGT-CDR3 (Cys) and one extra amino acid residue downstream (Trp for V_H CDR3, Phe for V_L CDR3).

[0206] Assembly of the chicken CDR and human framework regions was performed by overlap extension PCR. The resulting humanized V_H and V_L PCR products were cloned into expression vectors (plasmids) harboring human heavy and light chain constant regions. To increase correct cleavage of the signal peptide upstream of the lambda chain, the second amino acid (Ser) of the lambda gene IGLV3.19 was replaced by another amino acid (Tyr) which is present in other human germlines, for example IGLV3.25. The heavy chain sequence contains the two "LALA" mutations (L234A/L235A) known to reduce effector function of the Fc region of IgG1 antibodies (Armour et al., Eur J Immunol. 29(8):2613-24 (1999); and Armour et al., Mol Immunol. 40(9):585-93 (2003)). The expression vector also contained the necessary regulatory sequences, allowing simultaneous expression of light and heavy chains that are assembled into full-length antibodies after transfection of mammalian cells.

Results

[0207] The final humanized antibody sequences are shown below in Table 4, and the CDR sequences are shown separately in Table 5. The CDR sequences are defined in the Tables in accordance with the IMGT® numbering scheme.

Table 4 V_H and V_L sequences of humanized anti-PD-1 antibodies*

Humanized Antibody	V_H Amino Acid Sequence	V_L Amino Acid Sequence
[12819.15384]	EVQLLESGGGLVQPGGSLRLSCAAS***GFTFTRYD***MVWVRQAPGKGLEWVAG***IGDSNKMT***RYAPAVKGRATISRDNSK NTLYLQMNSLRAEDTAVYY***CAKGSCI ACWDEAGRIDAW***GQGTLVTVSS (SEQ ID NO: 2)	SYELTQDPAVSVALGQTVRITCSGG***GSYD GSSY***YGWYQQKPGQAPVTVIY***NNN***NRP SDIPDRFSGSSSGNTASLTITGAQAEDEAD YY***CGSYDRPETNSDYVGMF***GSGTKVTVL (SEQ ID NO: 3)
[12748.15381]	EVQLLESGGGLVQPGGSLRLSCAAS***GFTFSDYA***MNWVRQAPGKGLEWVA G***IGNDGSYT***NYGAAVKGRATISRDN SKNTLYLQMNSLRAEDTAVYY***CASDI RSRNDCSYFLGGCSSGFIDVW***GQGT LVTVSS (SEQ ID NO: 4)	SYELTQDPAVSVALGQTVRITCSGG***SSYS***Y GWFQQKPGQAPVTVIY***ESN***NRPSDIPDR FSGSSSGNTASLTITGAQAEDEADYY***CGN ADSSSGIF***GSGTKVTVL (SEQ ID NO: 5)
[12865.15377]	EVQLLESGGGLVQPGGSLRLSCAAS***GFDFSDHG***MQWVRQAPGKGLEYVG V***IDTTGRYT***YYAPAVKGRATISRDNS KNTLYLQMNSLRAEDTAVYY***CAKTT CVGGYLCNTVGSIDAW***GQGTLVTV SS (SEQ ID NO: 6)	SYELTQDPAVSVALGQTVRITCSGG***GSSS Y***YGWYQQKPGQAPVTVIY***DDT***NRPSGIP DRFSGSSSGNTASLTITGAQAEDEADYY***C GGYEGSSHAGIF***GSGTKVTVL (SEQ ID NO: 7)
[12892.15378]	EVQLLESGGGLVQPGGSLRLSCAAS***GFDFSSYT***MQWVRQAPGKGLEWVG V***ISSTGGST***GYGPAVKGRATISRDNS KNTLYLQMNSLRAEDTAVYY***CVKSIS GDAWSVDGLDAW***GQGTLVTVSS (SEQ ID NO: 8)	SYELTQDPAVSVALGQTVRITCSGG***GSA***Y GWYQQKPGQAPVTVIY***YNN***QRPSGIPD RFSGSSSGNTASLTITGAQAEDEADYY***CG SYDSSAVGIF***GSGTKVTVL (SEQ ID NO: 9)

(continued)

Humanized Antibody	V_H Amino Acid Sequence	V_L Amino Acid Sequence
[12796.15376]	EVQLLESGGGLVQPGGSLRLSCAAS***GFDFSSYT***MQWVRQAPGKGLEWVG V***ISSTGGST***GYGPAVKGRATISRDNS KNTLYLQMNSLRAEDTAVYY***CVKSV SGDAWSVDGLDAW***GQGTLVTVSS (SEQ ID NO: 10)	SYELTQDPAVSVALGQTVRITCSGG***GSA***Y GWYQQKPGQAPVTVIY***YNN***QRPSDIPD RFSGSSSGNTASLTITGAQAEDEADYY***CG SYDSSAVGIF***GSGTKVTVL (SEQ ID NO: 11)
[12777.15382]	EVQLLESGGGLVQPGGSLRLSCAAS***GFDFSSYG***MQWVRQAPGKGLEWVG V***ISGSGITT***LYAPAVKGRATISRDNSK NTVYLQMNSLRAEDTAVYY***CTRSPSI TDGWTYGGAWIDAW***GQGTLVTVS S (SEQ ID NO: 12)	SYELTQDPAVSVALGQTVRITCSGG***DGS***Y GWFQQKPGQAPVTVIY***DND***NRPSDIPD RFSGSSSGNTASLTITGAQAEDEADYY***CG NADLSGGIF***GSGTKVTVL (SEQ ID NO: 13)
[12760.15375]	EVQLLESGGGLVQPGGSLRLSCAAS***GFTFSTFN***MVWVRQAPGKGLEYVAE***I SSDGSFT***WYATAVKGRATISRDNSK NTVYLQMNSLRAEDTAVYY***CAKSDC SSSYYGYSCIGIIDAW***GQGTLVTVSS (SEQ ID NO: 14)	SYELTQDPAVSVALGQTVRITCSGG***ISDD GSYY***YGWFQQKPGQAPVTVIY***IND***RRPS NIPDRFSGSSSGNTASLTITGAQAEDEAD YY***CGSYDSSAGVGIF***GSGTKVTVL (SEQ ID NO: 15)
[13112.15380]	EVQLLESGGGLVQPGGSLRLSCAAS***GFTFSSYN***MFWVRQAPGKGLEFVAE***I SGSNTGSRT***WYAPAVKGRATISRDN SKNTLYLQMNSLRAEDTAVYY***CAKSI YGGYCAGGYSCGVGLIDAW***GQGTL VTVSS (SEQ ID NO: 16)	SYELTQDPAVSVALGQTVRITCSGG***SSDY*** YGWFQQKPGQAPVTVIY***YNN***KRPSDIPD RFSGSSSGNTASLTITGAQAEDEADYY***CG NADSSVGVF***GSGTKVTVL (SEQ ID NO: 17)
*CDR regions are italicized, underlined, and in boldface.		

Table 5 H- and L-CDR sequences of humanized anti-PD-1 antibodies

Humanized antibody	HCDR1	HCDR2	HCDR3	LCDR1	LCDR2	LCDR3
[12819.15384]	GFTFTRYD	IGDSNKMT	CAKGSCIACW DEAGRIDAW	GSYDGSSY	NNN	CGSYDRPETNSDY VGMF
SEQ ID NO:	18	19	20	21	22	23
[12748.15381]	GFTFSDYA	IGNDGSYT	CASDIRSRND CSYFLGGCSSG FIDVW	SSYS	ESN	CGNADSSSGIF
SEQ ID NO:	24	25	26	27	28	29
[12865.15377]	GFDFSDHG	IDTTGRYT	CAKTTCVGGY LCNTVGSIDA W	GSSSY	DDT	CGGYEGSSHAGIF
SEQ ID NO:	30	31	32	33	34	35
[12892.15378]	GFDFSSYT	ISSTGGST	CVKSISGDAW SVDGLDAW	GSA	YNN	CGSYDSSAVGIF
SEQ ID NO:	36	37	38	39	40	41
[12796.15376]	GFDFSSYT	ISSTGGST	CVKSVSGDA WSVDGLDAW	GSA	YNN	CGSYDSSAVGIF
SEQ ID NO:	42	43	44	45	46	47
[12777.15382]	GFDFSSYG	ISGSGITT	CTRSPSITDG WTYGGAWID AW	DGS	DND	CGNADLSGGIF
SEQ ID NO:	48	49	50	51	52	53
[12760.15375]	GFTFSTFN	ISSDGSFT	CAKSDCSSSYY GYSCIGIIDAW	ISDDGSYY	IND	CGSYDSSAGVGIF
SEQ ID NO:	54	55	56	57	58	59
[13112.15380]	GFTFSSYN	ISGSNTGSRT	CAKSIYGGYCA GGYSCGVGLI DAW	SSDY	YNN	CGNADSSVGVF
SEQ ID NO:	60	61	62	63	64	65

[0208] All of the humanized antibodies comprised the IgG1 "LALA" variant heavy chain constant region and light chain constant region amino acid sequences shown below.

Heavy chain constant region (SEQ ID NO: 67):

ASTKGPSVFPLAPSSKSTSGGTAALGCLVKDYFPEPVTVSWNSGALTSGVHTFPAVLQS
SGLYSLSSVVTVPSSSLGTQTYICNVNHKPSNTKVDKRVEPKSCDKTHTCPPCPAPE**AA**
GGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSHEDPEVKFNWYVDGVEVHNAKTKPREE
QYNSTYRVVSVLTVLHQDWLNGKEYKCKVSNKALPAPIEKTISKAKGQPREPQVYTLPPS
REEMTKNQVSLTCLVKGFYPSDIAVEWESNGQPENNYKTTPPVLDSDGSFFLYSKLTVD
KSRWQQGNVFSCSVMHEALHNHYTQKSLSLSPGK

Light chain constant region (SEQ ID NO: 68):

GQPKANPTVTLFPPSSEELQANKATLVCLISDFYPGAVTVAWKADGSPVKAGVETTKPSK
QSNNKYAASSYLSLTPEQWKSHRSYSCQVTHEGSTVEKTVAPTECS

Example 5: Screening of anti-PD-1 antibody candidates

[0209] PD-1 is mainly expressed on the surface of activated T-lymphocytes, where it negatively regulates T-cell activity. In order to select the most functional anti-PD-1 antibody candidates, two different *in vitro* screening systems were established, a *Staphylococcus* Enterotoxin B (SEB) whole blood assay and a one-way mixed lymphocyte reaction assay.

Materials and Methods

[0210] A repertoire of 69 unique humanized mAbs in the IgG1-LALA scaffold format, i.e., having the "LALA" mutations described in Example 4, and cloned and humanized as described above, were initially screened for functional activity in the SEB whole blood assay. SEB is a super-antigen that binds to MHC class II molecules and specific Vβ regions of T cell receptors (TCR) and drives non-specific stimulation of T-cells. This results in polyclonal T cell activation/proliferation and release of cytokines including IL-2 and IFN-γ.

[0211] In order to investigate the relevance of the SEB assay for screening of anti-PD-1 activity, the expression level of PD-1 was investigated for different donors before and after SEB stimulation. PBMCs from six different donors were tested for PD-1 expression by flow cytometry at day 0 and day 3 after SEB stimulation. A relevant lymphocyte gate was set for further analysis.

[0212] Based on screening in the SEB whole blood assays, using blood from at least three different donors, the top 10 anti-PD-1 antibody lead candidates were identified. The anti-PD-1 antibody lead candidates were then further titrated to obtain dose-response curves for each individual antibody in comparison with the positive controls, reference analogues of the anti-PD-1 antibodies pembrolizumab (Merck) and nivolumab (Bristol-Myers Squibb); see Example 2.

[0213] The functionality of the top 10 selected anti-PD-1 antibodies was validated in an alternative *in vitro* assay, the one-way mixed lymphocyte reaction (MLR) assay. In this assay, dendritic cells (DCs) from one donor were co-cultured with $CD4^+$ T-cells from another donor to obtain alloantigen specific stimulation, induced in 10-15% of all T-cells, leading to T-cell activation/proliferation and cytokine secretion.

[0214] Due to a protein stability issue for one of the candidates (12748.15381), alternative germline sequences for this specific antibody were used. One of the resulting antibodies, 12748.16124, is referred to below. This variant has a different V_L sequence, but the same V_H sequence as 12748.15381 (Table 1, *supra*).

Results

[0215] The data in Figure 4 clearly shows that the frequency of lymphocytes expressing PD-1 is increased in all tested donors after SEB stimulation. These observations confirm the relevance of this assay for anti-PD-1 antibody screening.

[0216] Titration of the most functional anti-PD-1 antibodies in the SEB assay, shown in Figures 5A-I, identified anti-PD-1 lead candidates with functionality similar or superior to the positive control antibody analogues pembrolizumab and nivolumab. In this assay, whole blood was stimulated with SEB for 48 h in the presence of the indicated antibodies, and IL-2 secretion after 48 hours was measured by ELISA. Each data point represents an average of six replicates, with the bars indicating the SEM.

[0217] Figures 5A-H shows the results obtained with the humanized anti-PD-1 antibodies. Due to aggregation above 5% for one of the antibodies [12748.15381], an alternative framework for this antibody was tested. Data in Figure 5I shows similar functionality of the original humanized antibody [12748.15381] and its germline (framework) variant [12748.16124].

[0218] The functionality of the anti-PD-1 antibodies was validated in a one-way MLR assay. In this assay, dendritic cells and $CD4^+$ T cells (ratio 1:10) from two different donors were co-cultured, and IFN-γ secretion was measured by MesoScale after 5 days. Each data point represents an average of six replicates, with the bars indicating the SEM. The data obtained from the one-way MLR assay and illustrated in Figures 6A-H show the same functionality and ranking of the anti-PD-1 antibodies as the data obtained from the SEB assay. This consistency in data between different assays provides further confirmation that the selected antibodies are functional.

[0219] The selected antibodies originate from two different main epitope bins, indicating that they bind to two different non-overlapping epitopes. All of the anti-PD-1 antibodies shown belong to Bin 1, except for 12760 and 13112 antibodies, which belong to Bin 2. It was found that the anti-PD-1 antibodies from Bin 1 show the highest functionality in these *in vitro* assays.

Example 6: Flow cytometric analysis of anti-PD-1 antibodies for PD-L1 ligand blocking activity

[0220] This example illustrates how the panel of anti-PD-1 antibodies was tested for PD-L1 ligand blocking activity by performing a flow cytometric competition assay using cell surface-expressed PD-1 and fluorochrome-labeled soluble PD-L1.

Materials and Methods

[0221] PD-L1 ligand blocking activity was investigated in a multiplex cellular assay, in which human and cynomolgus PD-1 were recombinantly expressed on CHO-S cells and binding of R-PE (R-phycoerythrin) labeled human PD-L1-Fc chimera protein was analyzed by flow cytometry. Commercially available recombinant PD-L1-Fc chimera protein (R&D Systems, USA) was conjugated to R-PE using the Lightning-Link® R-Phycoerythrin Conjugation Kit (Innova Biosciences, UK). CHO-S cells transiently transfected to express human PD-1 were mixed with CFSE-stained CHO-S cells transiently expressing cynomolgus PD-1. This cell mixture was then incubated with 50 µl anti-PD-1 antibody at 20 µg/ml on ice, followed by addition of 50 µl R-PE-labeled PD-L1-Fc at approx. 3.4 µg/ml (16.4 nM final concentration) and further incubation for an additional 20 min (final anti-PD-1 antibody concentration: 10 µg/ml). Bound antibody was detected using APC (allophycocyanin) conjugated anti-human IgG light chain antibody. Binding of PD-L1 and anti-PD-1 antibody was quantified by flow cytometry detecting R-PE and APC fluorescence, respectively.

Results

[0222] The results of the competition experiment are presented in Figures 7A-B and are summarized in Table 6 below. All anti-PD-1 antibodies were tested at a final antibody concentration of 10 µg/ml (see above). Three of the tested antibodies were able to inhibit PD-L1 binding by 83% or more, similar to the anti-PD-1 reference antibody lambrolizumab (Merck), which is the same as pembrolizumab and was included as a positive control. One antibody (12777.13362) only partially inhibited binding by 69%. One antibody (13112.13208) did not block PD-1 binding. Binding of PD-L1 to PD-1 -expressing cells in the presence of the negative control anti-VEGFR2 antibody ramucirumab (Genentech) was set to 0%.

Table 6 PD-L1 binding inhibition in the presence of anti-PD-1 antibodies

Antibody	% PD-L1 binding inhibition
12819.13367	87%
12748.13354	86%
12892.13195	88%
12777.13362	69%
13112.13208	5%

(continued)

Antibody	% PD-L1 binding inhibition
Lambrolizumab (pos. control)	88%
Ramucirumab (neg. control)	set to 0%

[0223] The humanized variants shown in Table 6 have the same amino acid sequences as those in Table 1 sharing the first 5 digits in their names, except that the variants in Table 1 have amino acid residues "SY" at the N-terminus of the light chain. In some embodiments, the SY dipeptide improves signal peptide processing during expression of the antibody light chain. The variants in Tables 1 and 6 are expected to have identical functional properties.

Example 7: Measurement of PD-1 antibody affinities against human and cynomolgus PD-1 ECD antigen

[0224] This example demonstrates that the majority of anti-PD-1 antibodies show high picomolar (pM) affinity and good cross reactivity against both human and cynomolgus PD-1 extracellular domains (ECDs).

Materials and Methods

[0225] Kinetic binding analysis of the purified anti-PD-1 antibody repertoire was performed on an XPR-36 surface plasmon resonance (SPR) biosensor (Bio-Rad, USA). His-tagged human or cynomolgus PD-1 ECD antigens were purchased from Acro Biosystems, UK. Binding kinetics were measured under monovalent antigen conditions by immobilizing anti-PD-1 antibodies and keeping the monovalent PD-1 antigen in solution as described previously (Canziani et al., Anal Biochem 325(2):301-307 (2004)). The lowest possible anti-PD-1 antibody density was applied to prevent non-specific binding and mass transport limitation. For measuring antibody kinetics, anti-PD-1 antibodies were adjusted to a concentration of 1.0 μg/ml and captured on anti-human IgG Fc surfaces generated by immobilizing approximately 1000 RU of a monoclonal anti-human Fc antibody (Biacore, Denmark). Anti-PD-1 antibodies were tested for binding to human or cynomolgus PD-1 ECD in a 3-fold concentration range from 25 nM to 0.31 nM followed by regeneration of the surfaces with 3 M MgCl2 regeneration buffer (Biacore, Denmark). A high flow rate of 20 μl/min, an association time of 3.33 min and a dissociation time between 1.5 hours and 2.75 hours was employed. The recorded binding responses were fitted to a simple Langmuir 1:1 binding model for calculation of the on-rate (k_{on} or ka), off-rate (k_{off} or kd) and affinity (K_D) constants using double referencing.

Results

[0226] The binding kinetics are tabulated in Table 7 below, which illustrates that the panel of anti-PD-1 antibodies binds PD-1 with very high affinities in the pM range. All antibodies recognized human PD-1 with higher affinity than the nivolumab and pembrolizumab analogues. The highest affinity antibody [12819.15384] binds human PD-1 with a K_D of 20 pM.

Table 7 Binding kinetics of anti-PD-1 antibodies to human or cynomolgus PD-1 ECD as measured by Surface Plasmon Resonance (SPR)

Antibody	PD-1 ECD	k_{on} (M-1 s-1)		kon Error	k_{off} (s-1)		k_{off} Error	K_D (pM)
[12819.15384]	Human	1.1E+06	±	1.7E+03	2.3E-05	±	1.3E-07	20
[12819.15384]	Cynomolgus	9.7E+05	±	1.6E+03	4.5E-06	±	1.5E-07	5
[12748.15381]	Human	3.2E+06	±	1.0E+04	1.7E-04	±	7.1E-07	54
[12748.15381]	Cynomolgus	4.6E+06	±	1.6E+04	4.7E-04	±	9.1E-07	101
[12748.16124]	Human	3.4E+06	±	8.2E+03	1.6E-04	±	5.9E-07	47
[12748.16124]	Cynomolgus	4.8E+06	±	1.8E+04	3.9E-04	±	9.8E-07	81
[12865.15377]	Human	4.2E+05	±	2.2E+03	2.3E-04	±	5.5E-07	558
[12865.15377]	Cynomolgus	5.1E+05	±	2.2E+03	3.8E-04	±	7.3E-07	738

(continued)

Antibody	PD-1 ECD	kon(M-1 s-1)		k_{on} Error	k_{off} (s-1)		k_{off} Error	K_D (pM)
[12892.15378]	Human	4.6E+05	±	2.3E+03	3.4E-04	±	7.1E-07	737
[12892.15378]	Cynomolgus	2.9E+05	±	1.0E+10	6.9E-04	±	8.5E-01	2340
[12796.15376]	Human	7.1E+05	±	3.9E+03	3.8E-04	±	1.1E-06	542
[12796.15376]	Cynomolgus	3.2E+05	±	3.5E+03	7.0E-04	±	2.5E-06	2220
[12777.15382]	Human	2.4E+05	±	1.7E+03	8.0E-05	±	4.0E-06	337
[12777.15382]	Cynomolgus	2.5E+05	±	7.3E+03	1.7E-04	±	3.5E-06	681
[12760.15375]	Human	1.2E+06	±	3.4E+03	1.4E-04	±	6.5E-07	112
[12760.15375]	Cynomolgus	1.0E+06	±	1.7E+04	7.2E-03	±	5.8E-05	6940
[13112.15380]	Human	1.2E+06	±	4.8E+03	6.9E-05	±	7.4E-07	60
[13112.15380]	Cynomolgus	2.5E+06	±	1.5E+04	1.1E-03	±	3.9E-06	452
nivolumab analogue	Human	1.4E+06	±	9.2E+03	1.1E-03	±	4.1E-06	758
nivolumab analogue	Cynomolgus	1.4E+06	±	8.5E+03	7.7E-04	±	2.9E-06	542
pembrolizumab analogue	Human	2.4E+06	±	2.7E+04	2.1E-03	±	1.1E-05	852
pembrolizumab analogue	Cynomolgus	1.7E+06	±	1.0E+04	3.3E-04	±	9.5E-07	190

Example 8: Epitope binning of anti-PD-1 antibodies

[0227] This example illustrates how the PD-1 antibodies were grouped into epitope bins based on paired competition patterns. Antibodies belonging to different epitope bins recognize different epitopes on PD-1 ECD.

Methods

[0228] Investigation of paired antibody competition was performed by Surface Plasmon Resonance (SPR) analysis using a Continuous Flow Microspotter (CFM) (Wasatch Microfluidics, US) combined with an IBIS MX96 SPR instrument (IBIS Technologies, The Netherlands). Surface Plasmon Resonance imaging analysis was performed on E2S SensEye® SPR sensors (Ssens BV, The Netherlands). A total of ten anti-PD-1 antibodies (human, IgG1) were diluted to 10 μg/ml in 50 mM sodium acetate buffer, pH 4.5. Antibodies were spotted onto an E2S SensEye® and conjugated for 15 minutes using a Continuous Flow Microspotter. After spotting, the SensEye® was positioned in the IBIS MX96 biosensor and deactivated with 1 M ethanolamine, pH 8.5 for 10 minutes. After sensor preparation, antibody competition analysis was performed using a classical sandwich assay. Monovalent PD-1 ECD antigen (Sino Biological, China) was diluted in HBS-EP running buffer and injected at 50 nM concentration and captured by the conjugated array of anti-PD-1 antibodies. Next, individual injections of each of the ten PD-1 antibodies diluted to 100 nM in HBS-EP running buffer were performed to establish antibody competition patterns. After each competition cycle, the sensor surface was regenerated with 10 mM Glycine HCl buffer, pH 2.0.

Results

[0229] The competition pattern of ten anti-PD-1 antibodies is presented in Figure 8. 12866 and 12807 were not found to have functional activity in cell-based assays, but were included because they recognize distinct epitopes. The tested functional anti-PD-1 antibodies were found to bind two non-overlapping epitope bins. Functional antibodies belonging to epitope Bin 1 all cross blocked each other and included nivolumab analogue ("Nivo"), pembrolizumab analogue ("Pembro"), 12819, 12892, 12865, and 12777. These antibodies were found to significantly block PD-L1 and PD-L2 binding. 12760 and 13112 were found to bind a separate epitope Bin 2 because they cross blocked each other, but did not block the binding of any of the antibodies from epitope Bin 1. Consequently 12760 and 13112 likely bind to a different site on PD-1 that does not overlap with the PD-L1 and PD-L2 ligand binding site.

[0230] The cross blocking functional antibodies 12819, 12865, 12892, 12777, nivolumab and pembrolizumab belonging to epitope Bin 1 could be further subdivided into four subbins based on competition with 12866 and 12807 (Figure 8). 12819 (Bin 1C) was the only antibody that blocked the binding of both 12866 and 12807, while nivolumab (Bin 1D) only blocked 12866 and pembrolizumab (Bin 1F) only blocked 12807. The group of antibodies belonging to Bin 1E (12865, 12892 and 12777) was unique in that they did not block the binding of either 12866 or 12807.

[0231] Finally, 12866 (Bin 1A) and 12807 (Bin 1B) bound unique epitope bins. 12866 was blocked by 12819 and nivolumab but not by other anti-PD-1 antibodies, and 12807 was blocked by 12819 and pembrolizumab but not by other anti-PD-1 antibodies.

Example 9 : **Measurement of PD-1 antibody cross reactivity to mouse and rat PD-1 ECD antigen**

[0232] This example demonstrates that anti-PD-1 antibody 12819.15384 strongly crossreacts with mouse PD-1 but does not bind to rat PD-1.

Materials and Methods

[0233] His-tagged mouse and rat PD-1 ECD were purchased from Sino Biologicals. Kinetic binding analysis was conducted as described in Example 7.

Results

[0234] The binding kinetics are tabulated in Table 8 below. The anti-PD-1 antibody 12819.15384 binds mouse PD-1 with a K_D of 809 pM but does not recognized rat PD-1. The affinity to human PD-1 ECD was similar to that measured in Example 7. Antibody 12865.17150 did not bind mouse or rat PD-1. Neither of nivolumab and pembrolizumab reference analogues cross- reacted with mouse or rat PD-1 (data not shown).

Table 8 Binding kinetics of PD-1 antibody 12819.15384 to human, mouse or rat PD-1 ECD as measured by Surface Plasmon Resonance (SPR)

Antibody	PD-1 ECD	k_{on} (M-1 s-1)		k_{on} Error	k_{off} (s-1)		k_{off} Error	K_D (pM)
[12819.15384]	human	3.26E+05	±	3E+02	8.85E-06	±	5E-08	28
[12819.15384]	mouse	3,71E+04	±	5E+01	3,04E-05	±	7E-09	809
[12819.15384]	rat	N.B.*	±		N.B.	±		N.B.
*N.B: Not binding.								

Example 10: Analysis of PD-L1 and PD-L2 ligand blocking activity of PD-1 mAbs

[0235] This example illustrates how the panel of anti-PD-1 antibodies was analyzed for PD-L1 or PD-L2 ligand blocking activity by performing a competition assay using Bio-Layer Interferometry analysis.

Materials and Methods

[0236] Investigation of PD-L1 or PD-L2 ligand blocking activity was performed by Bio-Layer Interferometry (BLI) analysis using an Octet QK384 instrument (Fortebio, USA). Commercially available human PD-1 Fc fusion protein (Sino Biological) at 5 μg/ml concentration was captured on anti-human Fc sensor chips (Fortebio, USA) and residual anti-Fc sites blocked with Herceptin® negative control antibody. Next the antigen coated surface was saturated with anti-PD-1 antibody at a concentration of 10 μg/ml. After PD-1 saturation with anti-PD-1 antibody, ligand blocking activity of PD-L1 or PD-L2 was assessed by incubation with human PD-L1 or PD-L2 Fc fusion proteins (Sino Biological) tested at 5 μg/ml.

Results

[0237] The result of the competition analysis is presented in Table 9 below. All antibodies fully blocked both PD-L1 or PD-L2 ligand binding except for antibody 12760.13169, which showed no significant blocking of PD-L1 or PD-L2 (26% and 36%, respectively), and 13112.13208, which showed no blocking of PD-L1 and weak blocking of PD-L2 (27% and 53%, respectively). The results were in good agreement with the epitope binning analysis (Example 8) and epitope mapping analysis (Example 11), which showed that all antibodies except 12760 and 13112 bind to overlapping epitopes

that map to the PD-L1 and PD-L2 binding site on PD-1, while 12760 and 13112 antibodies bind to a separate PD-1 site and do not significantly cross compete with PD-L1 and PD-L2.

Table 9 PD-L1 and PD-L2 inhibition after anti-PD-1 antibody saturation

mAb	Ligand	% Blocking
12748.13354	PD-L1-Fc	97
12748.13354	PD-L2-Fc	96
12760.13169	PD-L1-Fc	44
12760.13169	PD-L2-Fc	26
12777.13362	PD-L1-Fc	93
12777.13362	PD-L2-Fc	90
12796.13173	PD-L1-Fc	99
12796.13173	PD-L2-Fc	92
12819.13367	PD-L1-Fc	94
12819.13367	PD-L2-Fc	94
12865.13185	PD-L1-Fc	98
12865.13185	PD-L2-Fc	94
12892.13195	PD-L1-Fc	88
12892.13195	PD-L2-Fc	77
13112.13208	PD-L1-Fc	53
13112.13208	PD-L2-Fc	27
nivolumab analogue	PD-L1-Fc	100
nivolumab analogue	PD-L2-Fc	98
pembrolizumab analogue	PD-L1-Fc	100
pembrolizumab analogue	PD-L2-Fc	99

	No significant ligand blocking
50 - 70	Intermediate ligand blocking
70 - 90	Intermediate ligand blocking
90 - 100	Full ligand blocking

Example 11: Epitope mapping of anti-PD-1 antibodies by PD-1 mutagenesis

[0238] Antibody epitopes can generally be characterized as linear epitopes (also termed continuous epitopes) or conformational epitopes (also termed discontinuous epitopes). While linear epitopes are defined based on a single continuous amino acid sequence, conformational epitopes may consist of many smaller discontinuous linear sequences or single contact residues. A collection of contact residues that cluster at the intermolecular protein interface between the antibody and the antigen is also termed a hot spot or core epitope (Moreira et al., Proteins 68(4):803-12 (2007)). It is now widely acknowledged that most B-cell epitopes are discontinuous in nature (Sivalingam and Shepherd, Mol Immunol. 51(3-4):304-92012 (2012), Kringelum et al., Mol Immunol. 53(1-2):24-34 (2013)) with the average epitope spanning 15-22 amino acid residues of which 2-5 amino acids contribute with most of the binding energy (Sivalingam and Shepherd, *supra*).

[0239] By ranking binding affinity to 111 different PD-1 mutants, this example illustrates how the binding epitopes of 12819 and 12865 antibodies can be divided into linear epitopes and hotspots that are distinct from the epitopes recognized by nivolumab and pembrolizumab.

Methods

[0240] The human PD-1 receptor consists of an extracellular domain of 268 amino acids (residues 21-288). The extracellular domain spans amino acids 21-170 followed by a transmembrane domain (residues 171-191) and a cytoplasmic domain (residues 192-288). PD-1 belongs to the immunoglobulin superfamily and is composed of a two layer β-sandwich made from interactions of 8 anti-parallel β-strands arranged into two β-sheets with GFCC' β-strands on one side and ABED β-strands on the opposing side. The two β-sheets are stabilized by a disulphide bond between residues C54 - C123. A crystal structure is available for the human PD-1:human PD-L1 complex (PDB 4ZQK), but the C'D loop between the C' and D β-strands was unstructured and is missing as well as some of the C-terminal sequence after residue 146 (PDB 4ZQK, Zak et al., Structure 23(12):2341-2348 (2015)). Recently a crystal structure of the human PD-1:pembrolizumab complex was published (PDB 5JXE, Na et al., Cell Res. 2016 [Epub ahead of print], PMID: 27325296). In this structure the C'D loop is much more ordered and the contact residues important for pembrolizumab binding were shown to cluster to a core epitope on this loop. No crystal structure of the human PD-1 :human PD-L2 complex is available. A NMR structure of human PD-1 in solution shows high structural similarity to the crystal structure PDB 4ZQK (PDB 2M2D, Cheng et al., J Biol Chem 288(17):11771-85 (2013)). Human PD-1 binds human PD-L1 or PD-L2 ligands in a 1:1 stoichiometry and binding mainly occurs at overlapping binding sites mediated by the GFCC' β-sheet (Cheng et al., J Biol Chem 288(17):11771-11785 (2013)) (Figure 9, panels A and B). Human PD-L1 binds human PD-1 through contact residues V64, N66, Y68 situated in the C β-strand and G124, 1126, L128, A132, 1134 and E136 located in the F and G β-strands (Zak et. al., Structure 23(12):2341-8 (2015)). Human PD-L1 and PD-L2 bind human PD-1 with K_Ds of 8 μM and 2 μM, respectively (Cheng et. al., *supra*).

[0241] The protein sequence of human PD-1 was downloaded from Uniprot (Accession No. Q15116; the amino acid sequence is represented in SEQ ID NO: 1). The full-length Macaca fascicularis protein sequence was downloaded from Uniprot (Accession No. B0LAJ3_MACFA (SEQ ID NO: 89)). The full-length protein sequences of Gallus galus, Mus musculus and Rattus norvegicus PD-1 were downloaded from NCBI (XP_422723. (SEQ ID NO: 90), NP_032824.1 (SEQ ID NO: 91) and XP_006245633.1 (SEQ ID NO: 92), respectively). The sequence identities of the different PD-1 extracellular amino acid sequences as compared to human PD-1 are shown in Table 10 below.

Table 10 PD-1 ECD sequence comparison among species

	Amino Acid Differences	% Sequence Identity
Macaca fascicularis PD-1 ECD	6	96.0
Rattus norvegicus PD-1 ECD	50	66.7
Mus musculus PD-1 ECD	57	62.0
Gallus gallus PD-1 ECD	73	51.3

[0242] A molecular model of human PD-1 was built by combining structural information from the crystal structure of human PD-1 :Human PD-L1 complex determined at 2.45 Å resolution (PDB 4ZQK) and a NMR structure of APO human PD-1 (PDB 2M2D). The structure PDB 4ZQK was used as the basis for the model with the missing C'D loop and c-terminal part of PD-1 provided from the NMR structure. Next, surface exposed amino acid residues were highlighted and 83 individual alanine substitutions were designed on surface exposed residues on human PD-1 ECD (alanine scanning) and 5 exposed residue positions that differed between human, mouse and rat PD-1 were back-mutated to rat PD-1 residues.

[0243] To map linear antibody epitopes in the context of the native human PD-1 structure, 23 chimeric proteins were generated where 10 amino acids in the human PD-1 ECD sequence were sequentially exchanged to chicken sequence in segments that overlapped by 5 amino acids. Sequence exchanges were performed in the extracellular domain of human PD-1 spanning amino acids 31-146, since the *Gallus gallus* protein sequence outside this segment did not align well with human PD-1 and was omitted.

[0244] The PD-1 cDNA coding for the extracellular domain of human PD-1 was synthesized and cloned into a vector containing CMV promoter and human IgG1 Fc sequence (residues P101 -K330) resulting in fusion of IgG1 Fc C-terminally to the cloned PD-1 ECD. The mutated human PD-1 Fc fusion constructs were generated by standard PCR and engineering techniques and protein was expressed transiently in 2 ml culture using an ExpiCHO™ expression system. The human PD-1 Fc fusion constructs were harvested after 9 days and supernatants were tested for binding affinity to anti-PD-1 Fabs by Surface Plasmon Resonance (SPR). Culture supernatants containing PD-1 fusion proteins were immobilized onto an G-a-hu-IgG Fc SensEye® (Ssens BV, The Netherlands) for 15 minutes using a Continuous Flow Microspotter (CFM, Wasatch Microfluidics, Salt Lake City, US). After spotting, the SensEye® was positioned in an IBIS MX96 biosensor and captured proteins were fixed to the surface using FixIT kit (Ssens BV, The Netherlands). Kinetic analysis was

performed by applying a so called kinetic titration series (Karlsson R. 2006), where monomeric Fab fragments of the antibodies of the invention were injected in increasing concentrations from 1 nM to 50 nM without application of surface regeneration steps after each antigen injection. Fab association was performed for 15 minutes and antigen dissociation was performed for 30 minutes. The recorded binding responses were fitted to a simple Langmuir 1:1 binding model with Scrubber 2 software for calculation of the on-rate (k_{on} or ka), off-rate (k_{off} or k_d) and affinity (K_D) constants.

Results

[0245] The binding affinities of anti-PD-1 Fabs 12819.17149 and 12865.17150 and reference analogs nivolumab and pembrolizumab were evaluated. 12819.17149 and 12865.17150 are identical in VH and VL amino acid sequence to 12819.15384 and 12865.15377, respectively, but are identified by different 10-digit numbers because the heavy and light chain sequences of each of the former two variants were co-expressed on the same plasmid rather than on separate plasmids in the host cells. The non-PD-L1 and PD-L2 ligand blocking Fab 13112.15380 and Herceptin® were included as controls.

[0246] All 111 tested PD-1 mutants expressed well. Only three chimeric constructs did not bind any of the tested antibodies, suggesting that the mutations introduced into these three constructs presumably resulted in major conformational perturbations that affected the binding of all of the tested PD-1 antibodies. The change in binding affinity of Fab antibodies binding to the mutated PD-1 constructs compared to wild-type were expressed as the ratio of K_D mutant / K_D wild-type (normalized binding affinity). An overview of the linear epitope scanning performed by inserting 10 amino acid *Gallus gallus* sequences into human PD-1 ECD is shown in Table 11 below. At least a 5 fold affinity reduction was employed as a cut-off criteria for detecting reduced binding affinity to mutated PD-1 constructs. In some instances, no binding could be detected to specific antibodies. These constructs were listed as N.B. (not binding).

[0247] Single contact residues were also mapped by performing 83 alanine substitutions or 5 rat back-mutations (Table 12 below).

[0248] An overview of the linear epitopes or contact residues identified for tested antibodies is presented in Table 13. An illustration of the mapped binding epitopes shown as density plots on the structure of human PD-1 ECD is shown in Figure 9.

[0249] The analysis showed that binding epitopes of 12819 and 12865 anti-PD-1 antibodies were clearly distinct compared to the reference antibodies nivolumab and pembrolizumab (Tables 11-13, Figure 9). The core epitope of pembrolizumab (Figure 9, panel C) was located on the C' β-strand and on the C'-D loop. Contact residues / linear epitopes were also found on the C and F β-strand where contact residues for PD-L1 also are present. The core epitope of nivolumab (Figure 9, panel D) was present on the end of the F β-strand and on the whole G β-strand covering some of the reported PD-1 contact residues utilized by human PD-L1. The core epitopes of 12819 and 12865 (Figure 9, panels E and F) were located on the F and G β-strands covering more area than nivolumab and overlapping with all reported contact residues for human PD-L1 in this region. 12865 was also very sensitive for mutations at residues 69-75. 12819 also shared one contact residue with pembrolizumab (V64) on the C β-strand that has also been reported to be a contact residue for human PD-L1. Both 12819 and 12865 shared linear epitopes that mapped to the C and C' β-strands and some of the C'D Loop. Apart from residue V64, no other contact residues were shared between tested antibodies. The non-ligand blocking antibody 13112 was shown by alanine scanning to map to a region distant from the PD-L1 and PD-L2 ligand blocking site (Figure 9, panel G).

[0250] In summary, this example illustrates that although 12819, 12865, nivolumab and pembrolizumab bind to overlapping epitopes on human PD-1 that can block the binding of PD-L1 and PD-L2 ligands, each antibody has a distinct binding epitope as evidenced from competition binding analysis (epitope binning, Example 8) and shown at a molecular level by mapping individual linear epitopes and contact residues with a panel of 111 PD-1 mutants as summarized in Table 13. 12819 is also the only antibody in the investigated anti-PD-1 panel that cross-reacts with mouse PD-1 ECD (K_D of 809 pM, Example 9), highlighting that the binding epitope of this antibody is unique compared to the other tested PD-1 antibodies.

Table 11 Binding affinity analysis for Fab antibodies binding chimeric PD-1 ECD constructs with inserted *Gallus gallus* sequence segments*

Chimeric construct #	Scanned Region hu PD-1	Mutated Region hu PD-1	Introduced Gallus gallus mutations	12819.17149	12865.17150	nivolumab	pembrolizumab	13112.15380
1	31-40	AA 37-38	F37L;S38F	1.2	0.6	0.4	0.9	0.4
2	36-45	AA 37-45	F37L;S38F;L41T;V43T;V44R;T45P	2.3	0.7	0.9	0.5	1.0
3	41-50	AA 41-49	L41T;V43T;V44R;T45P;E46A;D48S;N49S	1.3	0.6	0.5	0.6	0.7
4	46-55	AA 46-55	E46A;D48S;N49S;T53I;S55N	0.3	0.6	0.5	0.6	0.6
5	51-60	AA 53-59	T53I;S55N;S56I;T59S	1.2	0.9	0.8	0.9	2.5
6	56-65	AA 56-64	F56I;T59S;E61L;S62E;V64N	7.1	0.2	0.9	12.0	0.8
8	66-75	AA 69-75	R69Q;M70K;S71T;P72N;S73N;N74S;Q75N	6.4	N.B.	1.2	0.9	1.6
10	76-85	AA 76-85	T76P;D77Q;L79I;A81G;F82I;P83IE84R;D85N	33.7	13.8	0.8	N.B.	1.0
11	81-90	AA 81-90	A81G;F82I;P83IE84R;D85N;R86I;S87P;P89K; G90K	17.7	5.4	2.0	N.B.	0.8
12	86-95	AA 86-95	R86I;S87P;P89K;G90K;Q91M;D92E;R94K; F95Y	2.2	0.6	0.5	N.B.	0.6
15	101-110	AA 103-110	G103T;R104P;D105V;H107K;S109E;V110I	1.3	0.8	0.9	0.6	0.9
16	106-115	AA 107-115	H107K;S109E;V110I;V111L;R112N;A113L; R114H R115Q	3.6	0.4	0.6	0.7	0.7
17	111-120	AA 111-120	V111L;R112N;A113L;R114H R115Q;T120F	0.2	0.5	0.4	0.7	0.7
18	116-125	AA 120-125	T120F;L122Y;A125L	3.8	4.6	2.7	8.8	1.5
19	121-130	AA 122-130	L122Y;A125L;S127T;L128F;A129S;P130R	175.0	N.B.	0.8	2.3	0.8
20	126-135	AA 127-135	S127T;L128F;A129S;P130R;K131S;A132D; Q133K;I134VK135V	N.B.	N.B.	N.B.	3.7	0.6
21	131-140	AA 131-140	K131S;A132D;Q133K;I134VK135V;L138S; R139H;A140S	N.B.	N.B.	1.0	3.1	0.5

22	136-145	AA 138-143	L138S;R139H;A140S;E141Q;R143V	1.9	1.0	0.5	0.8	1.0
23	141-146	AA 141-143	E141Q;R143V	1.7	1.0	0.5	1.1	1.5
			KD Hu PD-1 ECD wild-type (nM)	2.68E-11	3.38E-09	5.67E-09	6.08E-09	1.24E-09

	< 5 fold K_D change Chimeric mutants
5-10	5-10 fold K_D change Chimeric mutants
10-50	10 - 50 fold K_D change Chimeric mutants
50-1000	50 - 1000 fold K_D change Chimeric mutants
N.B.	No binding of Chimeric mutants

*Normalized binding expressed as K_D mutant / K_D wild-type is listed.

Table 12 Fab antibody binding affinity to alanine-scanned human PD-1 ECD residues*

Mutation	12819.17149	12865.17150	nivolumab	pembrolizumab	13112.15380
P21A	2.1	1.0	0.5	0.6	1.1
G22A	1.0	0.9	0.6	0.8	1.0
D26A	1.1	0.8	1.7	0.7	1.2
S27A	0.8	0.8	2.7	0.8	1.1
D29A	1.0	0.9	1.7	0.7	1.0
R30A	1.2	1.2	1.9	1.3	1.0
P31A	1.1	1.0	2.6	1.0	1.1
N33A	1.0	1.1	0.8	0.8	1.0
T36A	1.0	1.0	0.9	0.9	0.9
L42A	1.3	0.5	0.3	0.2	2.2
V44A	1.4	0.8	0.5	0.3	9.9
G47A	1.6	0.5	0.2	0.2	0.6
D48A	1.0	0.6	0.6	0.4	2.4
N49A	1.1	0.7	0.5	0.5	1.0
A50G	1.4	0.6	0.5	0.4	1.7
F56A	1.5	0.5	1.7	0.6	0.8
S57A	1.2	1.0	0.9	0.9	1.1
N58A	1.3	0.7	1.9	0.7	1.0
T59A	0.9	1.0	1.3	0.3	0.9
S60A	1.8	0.6	1.5	0.7	1.0
E61A	1.2	1.1	0.5	0.3	1.0
N66A	2.2	2.1	0.8	201.3	1.0
Y68A	2.2	1.4	0.2	0.2	0.8
S71A	0.9	0.9	0.6	0.6	0.9
P72A	1.1	1.8	0.9	0.8	1.0
S73A	0.9	0.5	0.9	0.7	0.9
Q75A	1.0	0.5	1.0	0.8	0.9
T76A	1.4	0.3	1.2	0.9	1.2
D77A	2.8	0.3	1.0	134.2	1.0
K78A	2.6	0.7	1.0	268.5	1.1
A80G	1.2	0.4	1.1	0.8	1.0
P83A	1.5	0.8	0.9	N.B.	0.9
E84A	1.3	1.0	0.8	1.3	1.0
D85A	2.5	1.6	0.6	N.B.	0.9
R86A	1.1	0.7	0.4	0.3	0.9
S87A	1.3	0.9	0.8	107.4	1.0
Q88A	1.4	1.4	0.8	0.2	0.9
P89A	1.1	0.9	1.1	N.B.	0.9

Mutation	12819.17149	12865.17150	nivolumab	pembrolizumab	13112.15380
G90A	1.0	0.8	1.0	671.1	1.0
Q91A	0.8	1.0	1.0	0.7	1.0
D92A	1.2	0.8	1.0	335.6	0.9
C93A	1.2	0.9	1.1	1.2	1.1
R96A	1.0	0.8	0.9	0.5	1.0
T98A	1.1	0.9	0.8	0.6	1.0
P101A	1.1	1.0	0.6	0.5	0.9
N102A	1.5	0.6	0.2	0.3	0.7
G103A	1.1	0.8	0.5	0.5	0.9
R104A	1.0	0.8	0.6	0.4	0.7
R112A	0.7	0.9	0.8	0.6	0.9
R114A	0.8	0.8	0.7	0.6	0.9
N116A	1.4	0.8	0.6	0.6	0.9
G119A	1.0	0.4	0.5	0.5	1.0
G124A	0.5	4.0	0.3	0.6	1.0
L128A	17.1	2.1	1.5	335.6	0.9
P130A	105.1	0.5	2.6	0.6	0.9
K131A	N.B.	N.B.	1.0	1.6	0.9
A132G	53.4	1.4	0.9	0.5	1.0
Q133A	0.7	1.4	1.2	0.6	0.9
K135A	4.2	1.1	0.7	1.4	1.2
E136A	0.8	N.B.	0.9	0.9	1.0
L138A	1.0	1.1	1.0	1.0	1.2
R143A	0.9	0.7	0.7	0.5	1.0
T145A	1.1	0.7	0.9	0.5	82.4
E146A	1.2	1.0	1.0	0.9	1.0
R147A	0.7	1.0	0.8	0.7	0.7
R148A	0.3	1.0	0.8	0.8	1.1
A149G	0.8	1.0	0.8	0.8	1.0
E150A	1.0	1.0	0.7	0.7	1.0
P152A	1.4	0.9	0.6	0.6	1.0
T153A	0.9	1.0	0.9	0.9	1.0
A154G	0.8	1.0	1.0	1.0	1.0
H155A	1.1	1.0	1.3	1.2	1.0
P156A	1.0	1.1	2.2	1.2	1.5
S157A	0.9	1.0	2.2	1.8	1.0
P158A	1.2	0.9	0.9	0.9	1.0
S159A	1.1	0.8	0.7	0.7	0.9
P160A	1.0	0.8	0.9	0.7	1.0
R161A	0.9	1.1	1.0	1.1	1.1
P162A	1.3	1.1	1.0	1.0	1.1

Mutation	12819.17149	12865.17150	nivolumab	pembrolizumab	13112.15380
A163G	1.0	1.1	0.8	0.8	1.1
G164A	1.1	0.9	0.5	0.6	1.0
Q165A	1.1	1.1	0.9	1.0	1.1
Rat mutation Q167A	0.8	1.0	0.8	0.7	0.9
Rat mutation P28L	1.9	0.5	0.8	0.7	0.7
Rat mutation R30K	2.9	0.5	0.5	1.1	0.7
Rat mutation A40T	2.0	1.0	0.8	0.8	0.6
Rat mutation V64K	35.7	1.3	0.7	N.B.	1.0
Rat mutation S157R	1.6	0.9	0.9	0.8	1.0
K_D hu PD-1 ECD (nM)	2.68E-11	3.38E-09	5.67E-09	6.08E-09	1.24E-09

	< 5 fold K_D change Alanine mutants
5-10	5-10 fold K_D change Alanine mutants
10-50	10 - 50 fold K_D change Alanine mutants
50-1000	50 - 1000 fold K_D change Alanine mutants
N.B.	No binding of alanine mutants

*Normalized binding expressed as K_D mutant / K_D wild-type is listed.

Table 13 Anti-PD-1 antibody binding epitopes identified by using mutated PD-1 Fc fusion constructs

Antibody	Significant PD-L1/L2 blocking	Epitope Bin	Linear epitope	Contact Residues
12819.17149	Yes	1C	56-64, 69-90, 122-140	V64, L128, P130, K131, A132
12865.17150	Yes	1E	69-90, 122-140	K131, E136
nivolumab	Yes	1D	127-135	
pembrolizumab	Yes	1F	56-64, 76-95, 120-125	V64, N66, D77, K78, P83, D85, S87, P89, G90, D92, L128
13112.15380	No	2		V44, T145

Example 12: ***In vivo* efficacy of a 12819 antibody in four syngeneic murine tumor models**

[0251] This example demonstrates the *in vivo* efficacy of a 12819 antibody in four syngeneic murine tumor models.

Methods

[0252] 2×10^5 Sa1N (fibrosarcoma), 1×10^6 CT26 (colon carcinoma), 5×10^6 ASB-XIV (lung carcinoma), or 8×10^6 MC38 (colon carcinoma) cells were inoculated subcutaneously into the flank of 6-8 week old female A/J (Sa1N), BALB/cAnNRj (CT26 and ASB-XIV), or C57BL/6 (MC38) mice. Tumors were measured three times weekly by caliper in two dimensions and tumor volume in mm^3 was calculated according to the formula: $(width)^2 \times length \times 0.5$. At an average tumor size of 30-50 mm^3, the mice were randomized into two groups of ten animals and treatment was initiated. The mice were treated three times weekly with a total of six treatments by intraperitoneal injection of vehicle buffer or the monoclonal antibody 12819.17149 followed by an observation period. The antibody treatments were dosed at 10 mg/kg. Two-way ANOVA with Bonferroni's multiple comparisons test was applied to compare tumor volumes at each time-point between treatment groups. Statistical analyses were performed using GraphPad Prism version 5.0 (GraphPad Software, Inc.).

Results

[0253] The results show a profound tumor inhibitory effect of antibody 12819.17149 in all tested syngeneic tumor models ($P<0.001$ vs. vehicle) (Figure 10). Antibody 12819.17149 induced tumor growth regression in the Sa1N tumor model and resulted in tumor growth delay in the CT26, MC38 and ASB-XIV tumor models.

Example 13: *In vivo* efficacy of a 12819 antibody in a semi-humanized xenograft tumor model with a mixture of $CD8^+/CD4^+$ T cells and A375 melanoma cells

[0254] This example demonstrates the *in vivo* efficacy of a 12819 antibody in a semi-humanized xenograft tumor model, where the human melanoma cell line A375 was mixed with purified human $CD8^+$ and $CD4^+$ T cells.

Methods

[0255] 4.5×10^5 $CD8^+$ and $CD4^+$ T cells were isolated from a human PBMC donor and mixed with 2.05×10^6 A375 (human melanoma) cancer cells prior to subcutaneous inoculation into the flank of 6-8 week old female NOD*scid* mice. Treatment was initiated on the day of tumor inoculation and the mice were treated three times weekly for a total of six treatments by intraperitoneal injection of vehicle buffer, Keytruda® (pembrolizumab) (10 mg/kg), or the monoclonal antibody 12819.17149 (10 mg/kg) followed by an observation period. Tumors were measured three times weekly by caliper in two dimensions and tumor volume in mm^3 was calculated according to the formula: $(width)^2 \times length \times 0.5$. Two-way ANOVA with Bonferroni's multiple comparisons test was applied to compare tumor volumes at each time-point between treatment groups. Statistical analyses were performed using GraphPad Prism version 5.0 (GraphPad Software, Inc.).

Results

[0256] In the semi-humanized tumor model, treatment with antibody 12819.17149 resulted in significant tumor growth delay ($P<0.001$ vs. vehicle), whereas Keytruda® showed limited effect on tumor growth compared to the vehicle treated group (Figure 11).

Table 14 List of SEQ ID NOs

SEQ ID NO	Sequence
1	Human PD-1 amino acid sequence
2	Humanized [12819.15384] V_H amino acid sequence
3	Humanized [12819.15384] V_L amino acid sequence
4	Humanized [12748.15381] V_H amino acid sequence
5	Humanized [12748.15381] V_L amino acid sequence

(continued)

SEQ ID NO	Sequence
6	Humanized [12865.15377] V_H amino acid sequence
7	Humanized [12865.15377] V_L amino acid sequence
8	Humanized [12892.15378] V_H amino acid sequence
9	Humanized [12892.15378] V_L amino acid sequence
10	Humanized [12796.15376] V_H amino acid sequence
11	Humanized [12796.15376] V_L amino acid sequence
12	Humanized [12777.15382] V_H amino acid sequence
13	Humanized [12777.15382] V_L amino acid sequence
14	Humanized [12760.15375] V_H amino acid sequence
15	Humanized [12760.15375] V_L amino acid sequence
16	Humanized [13112.15380] V_H amino acid sequence
17	Humanized [13112.15380] V_L amino acid sequence
18-65	CDR sequences; see SEQ ID NOs in Table 2 and sequences in Table 5, as well as the List of Sequences below
66	Humanized [12748.16124] V_L amino acid sequence (alternative germline)
67	Heavy chain IgG1 constant region amino acid sequence (LALA variant)
68	Light chain constant region amino acid sequence
69	Humanized [12819.15384] V_H DNA sequence
70	Humanized [12819.15384] V_L DNA sequence
71	Humanized [12748.15381] V_H DNA sequence
72	Humanized [12748.15381] V_L DNA sequence
73	Humanized [12865.15377] V_H DNA sequence
74	Humanized [12865.15377] V_L DNA sequence
75	Humanized [12892.15378] V_H DNA sequence
76	Humanized [12892.15378] V_L DNA sequence
77	Humanized [12796.15376] V_H DNA sequence
78	Humanized [12796.15376] V_L DNA sequence
79	Humanized [12777.15382] V_H DNA sequence
80	Humanized [12777.15382] V_L DNA sequence
81	Humanized [12760.15375] V_H DNA sequence
82	Humanized [12760.15375] V_L DNA sequence
83	Humanized [13112.15380] V_H DNA sequence
84	Humanized [13112.15380] V_L DNA sequence
85	Humanized [12748.16124] V_L DNA sequence (alternative germline)
86	Heavy chain constant region genomic DNA sequence with introns included
87	Heavy chain constant region cDNA sequence
88	Light chain Lambda constant region DNA sequence
89	Macaca fascicularis PD-1 polypeptide, NCBI Accession B0LAJ3_MACFA

(continued)

SEQ ID NO	Sequence
90	Gallus Gallus PD-1 polypeptide, NCBI Accession No. XP_422723.3
91	Mus musculus PD-1 polypeptide, NCBI Accession No. NP_032824.1
92	Rattus norvegicus PD-1 polypeptide, NCBI Accession No. XP_006245633.1

List of Sequences

[0257] * Italics in DNA sequences indicates cloning sites

SEQ ID NO: 1 (Human PD-1 polypeptide, Uniprot Accession No. Q15116 (PDCD1_HUMAN))

MQIPQAPWPVVWAVLQLGWRPGWFLDSPDRPWNPPTFSPALLVVTEGDNATFTCSFSNTS
ESFVLNWYRMSPSNQTDKLAAFPEDRSQPGQDCRFRVTQLPNGRDFHMSVVRARRNDSGT
YLCGAISLAPKAQIKESLRAELRVTERRAEVPTAHPSPSPRPAGQFQTLVVGVVGGLLGS
LVLLVWVLAVICSRAARGTIGARRTGQPLKEDPSAVPVFSVDYGELDFQWREKTPEPPVP
CVPEQTEYATIVFPSGMGTSSPARRGSADGPRSAQPLRPEDGHCSWPL

SEQ ID NO: 2 (Humanized [12819.15384] V_H amino acid sequence)

EVQLLESGGGLVQPGGSLRLSCAASGFTFTRYDMVWVRQAPGKGLEWVAGIGDSNKMTRYAPAVKG
RATISRDNSKNTLYLQMNSLRAEDTAVYYCAKGSCIACWDEAGRIDAWGQGTLVTVSS

SEQ ID NO: 3 (Humanized [12819.15384] V_L amino acid sequence)

SYELTQDPAVSVALGQTVRITCSGGGSYDGSSYYGWYQQKPGQAPVTVIYNNNNRPSDIPDRFSGS
SSGNTASLTITGAQAEDEADYYCGSYDRPETNSDYVGMFGSGTKVTVL

SEQ ID NO: 4 (Humanized [12748.15381] and [12748.16124] V_H amino acid sequence)

EVQLLESGGGLVQPGGSLRLSCAASGFTFSDYAMNWVRQAPGKGLEWVAGIGNDGSYTNYGAAVKG
RATISRDNSKNTLYLQMNSLRAEDTAVYYCASDIRSRNDCSYFLGGCSSGFIDVWGQGTLVTVSS

SEQ ID NO: 5 (Humanized [12748.15381] V_L amino acid sequence)

SYELTQDPAVSVALGQTVRITCSGGSSYSYGWFQQKPGQAPVTVIYESNNRPSDIPDRFSGSSSGN
TASLTITGAQAEDEADYYCGNADSSSGIFGSGTKVTVL

SEQ ID NO: 6 (Humanized [12865.15377] V_H amino acid sequence)

EVQLLESGGGLVQPGGSLRLSCAASGFDFSDHGMQWVRQAPGKGLEYVGVIDTTGRYTYYAPAVKG
RATISRDNSKNTLYLQMNSLRAEDTAVYYCAKTTCVGGYLCNTVGSIDAWGQGTLVTVSS

SEQ ID NO: 7 (Humanized [12865.15377] V_L amino acid sequence)

SYELTQDPAVSVALGQTVRITCSGGGSSSYYGWYQQKPGQAPVTVIYDDTNRPSGIPDRFSGSSSG
NTASLTITGAQAEDEADYYCGGYEGSSHAGIFGSGTKVTVL

SEQ ID NO: 8 (Humanized [12892.15378] V_H amino acid sequence)

EVQLLESGGGLVQPGGSLRLSCAASGFDFSSYTMQWVRQAPGKGLEWVGVISSTGGSTGYGPAVKG
RATISRDNSKNTLYLQMNSLRAEDTAVYYCVKSISGDAWSVDGLDAWGQGTLVTVSS

SEQ ID NO: 9 (Humanized [12892.15378] V_L amino acid sequence)

SYELTQDPAVSVALGQTVRITCSGGGSAYGWYQQKPGQAPVTVIYYNNQRPSGIPDRFSGSSSGNT
ASLTITGAQAEDEADYYCGSYDSSAVGIFGSGTKVTVL

SEQ ID NO: 10 (Humanized [12796.15376] V_H amino acid sequence)

EVQLLESGGGLVQPGGSLRLSCAASGFDFSSYTMQWVRQAPGKGLEWVGVISSTGGSTGYGPAVKG
RATISRDNSKNTLYLQMNSLRAEDTAVYYCVKSVSGDAWSVDGLDAWGQGTLVTVSS

SEQ ID NO: 11 (Humanized [12796.15376] V_L amino acid sequence)

SYELTQDPAVSVALGQTVRITCSGGGSAYGWYQQKPGQAPVTVIYYNNQRPSDIPDRFSGSSSGNT
ASLTITGAQAEDEADYYCGSYDSSAVGIFGSGTKVTVL

SEQ ID NO: 12 (Humanized [12777.15382] V_H amino acid sequence)

EVQLLESGGGLVQPGGSLRLSCAASGFDFSSYGMQWVRQAPGKGLEWVGVISGSGITTLYAPAVKG
RATISRDNSKNTVYLQMNSLRAEDTAVYYCTRSPSITDGWTYGGAWIDAWGQGTLVTVSS

SEQ ID NO: 13 (Humanized [12777.15382] V_L amino acid sequence)

SYELTQDPAVSVALGQTVRITCSGGDGSYGWFQQKPGQAPVTVIYDNDNRPSDIPDRFSGSSSGNT
ASLTITGAQAEDEADYYCGNADLSGGIFGSGTKVTVL

SEQ ID NO: 14 (Humanized [12760.15375] V_H amino acid sequence)

EVQLLESGGGLVQPGGSLRLSCAASGFTFSTFNMVWVRQAPGKGLEYVAEISSDGSFTWYATAVKG
RATISRDNSKNTVYLQMNSLRAEDTAVYYCAKSDCSSSYYGYSCIGIIDAWGQGTLVTVSS

SEQ ID NO: 15 (Humanized [12760.15375] V_L amino acid sequence)

SYELTQDPAVSVALGQTVRITCSGGISDDGSYYYGWFQQKPGQAPVTVIYINDRRPSNIPDRFSGS
SSGNTASLTITGAQAEDEADYYCGSYDSSAGVGIFGSGTKVTVL

SEQ ID NO: 16 (Humanized [13112.15380] V_H amino acid sequence

EVQLLESGGGLVQPGGSLRLSCAASGFTFSSYNMFWVRQAPGKGLEFVAEISGSNTGSRTWYAPAV
KGRATISRDNSKNTLYLQMNSLRAEDTAVYYCAKSIYGGYCAGGYSCGVGLIDAWGQGTLVTVSS

SEQ ID NO: 17 (Humanized [13112.15380] V_L amino acid sequence)

SYELTQDPAVSVALGQTVRITCSGGSSDYYGWFQQKPGQAPVTVIYYNNKRPSDIPDRFSGSSSGN
TASLTITGAQAEDEADYYCGNADSSVGVFGSGTKVTVL

SEQ ID NO: 18 (12819 HCDR1 amino acid sequence)
GFTFTRYD

SEQ ID NO: 19 (12819 HCDR2 amino acid sequence)
IGDSNKMT

SEQ ID NO: 20 (12819 HCDR3 amino acid sequence)
CAKGSCIACWDEAGRIDAW

SEQ ID NO: 21 (12819 LCDR1 amino acid sequence)
GSYDGSSY

SEQ ID NO: 22 (12819 LCDR2 amino acid sequence)
NNN

SEQ ID NO: 23 (12819 LCDR3 amino acid sequence)
CGSYDRPETNSDYVGMF

SEQ ID NO: 24 (12748 HCDR1 amino acid sequence)
GFTFSDYA

SEQ ID NO: 25 (12748 HCDR2 amino acid sequence)
IGNDGSYT

SEQ ID NO: 26 (12748 HCDR3 amino acid sequence)
CASDIRSRNDCSYFLGGCSSGFIDVW

SEQ ID NO: 27 (12748 LCDR1 amino acid sequence)
SSYS

SEQ ID NO: 28 (12748 LCDR2 amino acid sequence)
ESN

SEQ ID NO: 29 (12748 LCDR3 amino acid sequence)
CGNADSSSGIF

SEQ ID NO: 30 (12865 HCDR1 amino acid sequence)
GFDFSDHG

SEQ ID NO: 31 (12865 HCDR2 amino acid sequence)
IDTTGRYT

SEQ ID NO: 32 (12865 HCDR3 amino acid sequence)
CAKTTCVGGYLCNTVGSIDAW

SEQ ID NO: 33 (12865 LCDR1 amino acid sequence)
GSSSY

SEQ ID NO: 34 (12865 LCDR2 amino acid sequence)
DDT

SEQ ID NO: 35 (12865 LCDR3 amino acid sequence)
CGGYEGSSHAGIF

SEQ ID NO: 36 (12892 HCDR1 amino acid sequence)
GFDFSSYT

SEQ ID NO: 37 (12892 HCDR2 amino acid sequence)
ISSTGGST

SEQ ID NO: 38 (12892 HCDR3 amino acid sequence)
CVKSISGDAWSVDGLDAW

SEQ ID NO: 39 (12892 LCDR1 amino acid sequence)
GSA

SEQ ID NO: 40 (12892 LCDR2 amino acid sequence)
YNN

SEQ ID NO: 41 (12892 LCDR3 amino acid sequence)
CGSYDSSAVGIF

SEQ ID NO: 42 (12796 HCDR1 amino acid sequence)
GFDFSSYT

SEQ ID NO: 43 (12796 HCDR2 amino acid sequence)
ISSTGGST

SEQ ID NO: 44 (12796 HCDR3 amino acid sequence)
CVKSVSGDAWSVDGLDAW

SEQ ID NO: 45 (12796 LCDR1 amino acid sequence)
GSA

SEQ ID NO: 46 (12796 LCDR2 amino acid sequence)
YNN

SEQ ID NO: 47 (12796 LCDR3 amino acid sequence)
CGSYDSSAVGIF

SEQ ID NO: 48 (12777 HCDR1 amino acid sequence)
GFDFSSYG

SEQ ID NO: 49 (12777 HCDR2 amino acid sequence)
ISGSGITT

SEQ ID NO: 50 (12777 HCDR3 amino acid sequence)
CTRSPSITDGWTYGGAWIDAW

SEQ ID NO: 51 (12777 LCDR1 amino acid sequence)
DGS

SEQ ID NO: 52 (12777 LCDR2 amino acid sequence)
DND

SEQ ID NO: 53 (12777 LCDR3 amino acid sequence)
CGNADLSGGIF

SEQ ID NO: 54 (12760 HCDR1 amino acid sequence)
GFTFSTFN

SEQ ID NO: 55 (12760 HCDR2 amino acid sequence)

ISSDGSFT

SEQ ID NO: 56 (12760 HCDR3 amino acid sequence)
CAKSDCSSSYYGYSCIGIIDAW

SEQ ID NO: 57 (12760 LCDR1 amino acid sequence)
ISDDGSYY

SEQ ID NO: 58 (12760 LCDR2 amino acid sequence)
IND

SEQ ID NO: 59 (12760 LCDR3 amino acid sequence)
CGSYDSSAGVGIF

SEQ ID NO: 60 (13112 HCDR1 amino acid sequence)
GFTFSSYN

SEQ ID NO: 61 (13112 HCDR2 amino acid sequence)
ISGSNTGSRT

SEQ ID NO: 62 (13112 HCDR3 amino acid sequence)
CAKSIYGGYCAGGYSCGVGLIDAW

SEQ ID NO: 63 (13112 LCDR1 amino acid sequence)
SSDY

SEQ ID NO: 64 (13112 LCDR2 amino acid sequence)
YNN

SEQ ID NO: 65 (13112 LCDR3 amino acid sequence)
CGNADSSVGVF

SEQ ID NO: 66 (Humanized [12748.16124] V_L amino acid sequence (alternative germline))

SYELTQPPSVSVSPGQTARITCSGGSSYSYGWFQQKPGQAPVTVIYESNNRPSDIPERFSGSSSGT
TVTLTISGVQAEDEADYYCGNADSSSGIFGSGTKVTVL

SEQ ID NO: 67 (Heavy chain constant region amino acid sequence)

ASTKGPSVFPLAPSSKSTSGGTAALGCLVKDYFPEPVTVSWNSGALTSGVHTFPAVLQSSGLYSLS
SVVTVPSSSLGTQTYICNVNHKPSNTKVDKRVEPKSCDKTHTCPPCPAPE**AA**GGPSVFLFPPKPKD
TLMISRTPEVTCVVVDVSHEDPEVKFNWYVDGVEVHNAKTKPREEQYNSTYRVVSVLTVLHQDWLN
GKEYKCKVSNKALPAPIEKTISKAKGQPREPQVYTLPPSREEMTKNQVSLTCLVKGFYPSDIAVEW
ESNGQPENNYKTTPPVLDSDGSFFLYSKLTVDKSRWQQGNVFSCSVMHEALHNHYTQKSLSLSPGK

SEQ ID NO: 68 (Light chain lambda constant region amino acid sequence)

GQPKANPTVTLFPPSSEELQANKATLVCLISDFYPGAVTVAWKADGSPVKAGVETTKPSKQSNNKY
AASSYLSLTPEQWKSHRSYSCQVTHEGSTVEKTVAPTECS

SEQ ID NO: 69 (Humanized [12819.15384] V_H DNA sequence)

*GGCGCGCC*GAGGTGCAGCTGCTGGAATCTGGAGGAGGACTGGTCCAGCCAGGTGGATCCCTGCGAC
TGAGCTGCGCCGCTTCTGGATTCACCTTTACAAGATACGACATGGTGTGGGTCCGCCAGGCACCAG
GAAAGGGACTGGAGTGGGTGGCTGGTATCGGCGATAGTAACAAGATGACCCGCTACGCACCTGCCG
TCAAAGGGAGGGCAACAATTAGTCGGGACAACTCAAAGAATACTCTGTATCTGCAGATGAATTCCC

TGCGAGCTGAGGATACAGCAGTGTACTATTGTGCCAAAGGTAGCTGCATCGCCTGTTGGGACGAAG
CTGGCCGTATTGATGCATGGGGACAGGGGACTCTGGTGACCGT*CTCGAG*

SEQ ID NO: 70 (Humanized [12819.15384] V_L DNA sequence)

*GCTAGC*CTCTTACGAGCTGACTCAGGACCCTGCAGTGAGTGTCGCCCTGGGCCAGACAGTGAGAAT
CACTTGCTCCGGCGGAGGGAGCTACGATGGTTCCAGCTACTATGGCTGGTATCAGCAGAAGCCAGG
ACAGGCACCTGTGACCGTCATCTATAACAATAACAATAGGCCATCTGACATTCCCGATCGGTTCAG
TGGATCTAGTTCAGGGAACACAGCTTCTCTGACCATTACAGGAGCCCAGGCTGAGGACGAAGCAGA
TTACTATTGTGGGTCATACGACAGGCCAGAAACAAATTCCGATTATGTGGGAATGTTTGGTAGCGG
CACTAAAGTCACCGT*CCTAGG*

SEQ ID NO: 71 (Humanized [12748.15381] and [12748.16124] V_H DNA sequence)

*GGCGCGCC*GAGGTGCAGCTGCTGGAAAGCGGAGGAGGACTGGTCCAGCCAGGTGGATCTCTGCGAC
TGAGTTGCGCCGCTTCAGGCTTCACATTTTCTGACTACGCCATGAACTGGGTGAGGCAGGCTCCTG
GCAAGGGACTGGAGTGGGTCGCAGGAATCGGGAACGATGGAAGTTACACTAATTATGGAGCAGCCG
TGAAGGGGAGAGCTACTATTTCCCGCGACAACAGCAAAAATACCCTGTACCTGCAGATGAACTCAC
TGAGAGCTGAAGATACCGCAGTGTACTATTGTGCCTCTGACATCAGGAGTCGGAATGATTGCTCCT
ATTTCCTGGGAGGGTGTTCCAGCGGCTTTATTGACGTGTGGGGTCAGGGCACCCTGGTCACAGT*CT*
CGAG

SEQ ID NO: 72 (Humanized [12748.15381] V_L DNA sequence)

*GCTAGC*CTCTTACGAGCTGACCCAGGACCCAGCAGTGTCCGTCGCCCTGGGCCAGACAGTGAGAAT
CACTTGCTCCGGCGGATCCAGCTACAGCTATGGGTGGTTCCAGCAGAAGCCCGGTCAGGCCCCTGT
GACCGTCATCTATGAAAGTAACAATAGGCCATCAGACATTCCCGATCGGTTTTCTGGCTCTAGTTC
AGGAAACACAGCTAGTCTGACCATCACAGGGGCCCAGGCTGAGGACGAAGCTGATTACTATTGTGG
CAATGCAGATTCCAGCTCTGGAATTTTCGGGTCCGGTACTAAAGTCACCGT*CCTAGG*

SEQ ID NO: 73 (Humanized [12865.15377] V_H DNA sequence

*GGCGCGCC*GAGGTGCAGCTGCTGGAATCCGGAGGAGGACTGGTCCAGCCAGGTGGATCCCTGCGAC
TGAGCTGCGCCGCTTCTGGATTCGACTTTAGCGATCACGGGATGCAGTGGGTGAGACAGGCACCAG
GCAAGGGACTGGAGTACGTGGGTGTCATCGACACCACAGGCCGCTATACATACTATGCACCTGCCG
TCAAGGGCAGGGCTACCATTAGTCGGGACAACTCAAAAAATACACTGTACCTGCAGATGAACTCTC
TGAGGGCTGAAGATACTGCAGTGTACTATTGCGCCAAAACTACCTGCGTGGGAGGGTACCTGTGCA
ATACCGTCGGAAGTATCGATGCTTGGGGACAGGGGACACTGGTGACTGT*CTCGAG*

SEQ ID NO: 74 (Humanized [12865.15377] V_L DNA sequence)

*GCTAGC*CTCCTACGAGCTGACTCAGGACCCAGCAGTGAGCGTCGCCCTGGGCCAGACAGTGAGAAT
CACTTGCTCTGGCGGAGGGTCCAGCTCTTACTATGGTTGGTACCAGCAGAAGCCCGGCCAGGCTCC
TGTGACCGTCATCTATGACGATACAAACAGGCCAAGTGGAATTCCCGATCGGTTCTCAGGTAGTTC
ATCCGGCAATACAGCTTCTCTGACCATCACAGGGGCCCAGGCTGAGGACGAAGCAGATTACTATTG
TGGTGGCTATGAAGGAAGCTCTCACGCCGGGATTTTTGGAAGTGGGACTAAAGTCACCGT*CCTAGG*

SEQ ID NO: 75 (Humanized [12892.15378] V_H DNA sequence)

*GGCGCGCC*GAGGTGCAGCTGCTGGAAAGTGGAGGAGGACTGGTCCAGCCAGGTGGAAGCCTGAGAC
TGTCTTGCGCCGCTAGTGGCTTCGACTTTTCCAGCTACACCATGCAGTGGGTGAGGCAGGCACCAG
GCAAGGGACTGGAGTGGGTGGGCGTCATCTCTAGTACTGGAGGGTCTACCGGATACGGGCCTGCTG
TGAAGGGAAGGGCAACAATTTCACGGGATAACTCCAAAAATACTCTGTATCTGCAGATGAACAGCC
TGAGGGCAGAAGACACAGCCGTGTACTATTGCGTGAAATCAATCTCCGGAGATGCCTGGTCTGTGG
ACGGGCTGGATGCTTGGGGTCAGGGCACCCTGGTCACAGT*CTCGAG*

SEQ ID NO: 76 (Humanized [12892.15378] V_L DNA sequence)

*GCTAGC*CTCATACGAGCTGACCCAGGACCCAGCAGTGTCCGTCGCCCTGGGACAGACAGTGAGAAT
CACTTGCTCCGGAGGAGGATCCGCCTACGGTTGGTATCAGCAGAAGCCCGGCCAGGCACCTGTGAC
CGTCATCTACTATAACAATCAGAGGCCATCTGGCATTCCCGACCGGTTCAGTGGATCCAGCTCTGG
GAACACAGCAAGTCTGACCATCACAGGCGCCCAGGCTGAGGACGAAGCCGATTACTATTGTGGAAG
CTATGATAGTTCAGCTGTGGGGATTTTTGGTTCTGGCACTAAAGTCACCGT*CCTAGG*

SEQ ID NO: 77 (Humanized [12796.15376] V_H DNA sequence)

*GGCGCGCC*GAGGTGCAGCTGCTGGAAAGTGGAGGAGGACTGGTCCAGCCAGGTGGAAGCCTGAGAC
TGTCTTGCGCCGCTAGTGGCTTCGACTTTTCCAGCTACACCATGCAGTGGGTGAGGCAGGCACCAG
GCAAGGGACTGGAGTGGGTGGGCGTCATCTCTAGTACTGGAGGGTCTACCGGATACGGGCCTGCTG
TGAAGGGAAGGGCAACAATTTCACGGGATAACTCCAAAAATACTCTGTATCTGCAGATGAACAGCC
TGAGGGCAGAAGACACAGCCGTGTACTATTGCGTGAAATCAGTCTCCGGAGATGCCTGGTCTGTGG
ACGGGCTGGATGCTTGGGGTCAGGGCACCCTGGTCACAGT*CTCGAG*

SEQ ID NO: 78 (Humanized [12796.15376] V_L DNA sequence)

*GCTAGC*CTCATACGAGCTGACCCAGGACCCAGCAGTGTCCGTCGCCCTGGGCCAGACAGTGAGAAT
CACTTGCTCCGGAGGAGGATCCGCCTACGGTTGGTATCAGCAGAAGCCCGGCCAGGCACCTGTGAC
CGTCATCTACTATAACAATCAGAGGCCATCTGACATTCCCGATCGGTTCAGTGGATCCAGCTCTGG
GAACACAGCAAGTCTGACCATCACAGGCGCCCAGGCTGAGGACGAAGCCGATTACTATTGTGGAAG
CTATGATAGTTCAGCTGTGGGGATTTTTGGTTCTGGCACTAAAGTCACCGT*CCTAGG*

SEQ ID NO: 79 (Humanized [12777.15382] V_H DNA sequence)

*GGCGCGCC*GAGGTGCAGCTGCTGGAATCCGGAGGAGGACTGGTCCAGCCAGGTGGAAGCCTGCGAC
TGTCTTGCGCCGCTAGTGGATTCGACTTTTCCAGCTACGGAATGCAGTGGGTGAGGCAGGCACCAG
GCAAGGGACTGGAGTGGGTGGGCGTCATCTCTGGAAGTGGGATTACCACACTGTACGCACCTGCCG
TCAAGGGAAGGGCTACTATCTCACGGGACAACTCTAAAAATACAGTGTATCTGCAGATGAACTCCC
TGAGAGCTGAAGATACCGCAGTCTACTATTGTACACGCTCACCCTCCATCACAGACGGCTGGACTT
ATGGAGGGGCCTGGATTGATGCTTGGGGTCAGGGCACTCTGGTGACCGT*CTCGAG*

SEQ ID NO: 80 (Humanized [12777.15382] V_L DNA sequence)

*GCTAGC*CAGCTACGAGCTGACCCAGGACCCAGCAGTGTCCGTCGCCCTGGGCCAGACAGTGAGAAT
CACTTGCAGTGGCGGAGATGGGTCATACGGTTGGTTCCAGCAGAAGCCCGGACAGGCCCCTGTGAC
CGTCATCTATGACAACGATAATAGGCCATCTGACATTCCCGATCGGTTTAGTGGCTCCAGCTCTGG
AAACACAGCTTCTCTGACCATCACAGGGGCCCAGGCTGAGGACGAAGCTGATTACTATTGTGGCAA
TGCAGACCTGTCCGGGGGTATTTTCGGCAGCGGAACTAAAGTCACCGT*CCTAGG*

SEQ ID NO: 81 (Humanized [12760.15375] V_H DNA sequence)

*GGCGCGCC*GAGGTGCAGCTGCTGGAATCTGGAGGAGGACTGGTCCAGCCAGGTGGATCCCTGAGAC
TGAGCTGCGCCGCTTCTGGATTCACCTTTAGTACATTCAACATGGTGTGGGTCAGGCAGGCACCTG
GAAAGGGACTGGAGTACGTGGCTGAAATCTCCAGCGACGGCTCTTTTACATGGTATGCAACTGCCG
TCAAGGGCAGGGCCACCATTAGTCGGGATAACTCAAAAAATACAGTGTACCTGCAGATGAATTCCC
TGAGGGCTGAGGACACCGCAGTCTACTATTGCGCAAAATCCGATTGTTCTAGTTCATACTATGGAT
ATAGCTGTATCGGGATCATTGACGCTTGGGGTCAGGGCACTCTGGTGACCGT*CTCGAG*

SEQ ID NO: 82 (Humanized [12760.15375] V_L DNA sequence)

*GCTAGC*CTCCTATGAGCTGACCCAGGACCCAGCAGTGAGCGTCGCCCTGGGCCAGACAGTGAGAAT
CACTTGCTCCGGCGGAATTAGCGACGATGGCTCTTACTATTACGGATGGTTCCAGCAGAAGCCCGG

ACAGGCCCCTGTGACCGTCATCTATATTAACGACAGGCGGCCAAGTAATATCCCCGATAGGTTTTC
AGGGTCCAGCTCTGGTAACACAGCTTCTCTGACCATTACAGGGGCCCAGGCTGAGGACGAAGCTGA
TTATTACTGTGGCTCTTACGATAGTTCAGCAGGGGTGGGTATCTTCGGCAGTGGAACTAAAGTCAC
CGT*CCTAGG*

SEQ ID NO: 83 (Humanized [13112.15380] V_H DNA sequence

*GGCGCGCC*GAGGTGCAGCTGCTGGAAAGTGGAGGAGGACTGGTCCAGCCAGGTGGATCACTGAGAC
TGTCCTGCGCCGCCTCCGGCTTCACCTTTTCCAGCTACAACATGTTCTGGGTGCGCCAGGCACCAG
GAAAGGGACTGGAGTTTGTCGCTGAAATCTCTGGTAGTAATACTGGAAGCCGAACCTGGTACGCAC
CTGCCGTGAAGGGCAGGGCTACAATTTCTCGGGACAACAGTAAAAATACTCTGTATCTGCAGATGA
ACTCTCTGAGGGCTGAGGATACAGCAGTGTACTATTGTGCAAAATCAATCTACGGAGGGTATTGCG
CCGGTGGCTATTCCTGTGGTGTGGGCCTGATTGACGCATGGGGACAGGGGACCCTGGTCACAGT*CT*
CGAG

SEQ ID NO: 84 (Humanized [13112.15380] V_L DNA sequence)

*GCTAGC*CTCATACGAGCTGACCCAGGACCCAGCAGTGTCCGTCGCCCTGGGCCAGACAGTGAGAAT
CACTTGCAGTGGCGGATCCAGCGATTACTATGGGTGGTTCCAGCAGAAGCCCGGTCAGGCCCCTGT
GACCGTCATCTACTATAACAACAAGAGGCCATCTGACATTCCCGATCGGTTTAGTGGCTCTAGTTC
AGGAAACACAGCCTCCCTGACCATTACAGGGGCCCAGGCTGAGGACGAAGCTGATTACTATTGTGG
CAATGCAGACTCCAGCGTGGGAGTCTTCGGGTCTGGTACTAAGGTGACCGT*CCTAGG*

SEQ ID NO: 85 (Humanized [12748.16124] V_L DNA sequence (alternative germline))

*GCTAGC*CTCTTACGAGCTGACTCAGCCACCTTCCGTGTCCGTGTCCCCAGGACAGACCGCAAGAAT
CACATGCAGTGGCGGATCCAGCTACTCATATGGGTGGTTCCAGCAGAAGCCTGGTCAGGCCCCCGT
GACAGTCATCTATGAGAGCAACAATAGGCCTTCTGACATTCCAGAACGGTTTAGTGGCTCTAGTTC
AGGAACCACAGTGACTCTGACCATCAGCGGGGTCCAGGCCGAGGACGAAGCTGATTACTATTGTGG
CAACGCTGATTCCAGCTCTGGAATTTTCGGGTCCGGTACAAAAGTGACTGT*CCTAGG*

SEQ ID NO: 86 (Heavy chain constant region genomic DNA sequence with introns included)

*CTCGAG*TGCCTCCACCAAGGGCCCATCGGTCTTCCCCCTGGCACCCTCCTCCAAGAGCACCTCTGG
GGGCACAGCGGCCCTGGGCTGCCTGGTCAAGGACTACTTCCCCGAACCGGTGACGGTGTCGTGGAA
CTCAGGCGCCCTGACCAGCGGCGTGCACACCTTCCCGGCTGTCCTACAGTCCTCAGGACTCTACTC
CCTCAGCAGCGTGGTGACCGTGCCCTCCAGCAGCTTGGGCACCCAGACCTACATCTGCAACGTGAA
TCACAAGCCCAGCAACACCAAGGTGGACAAGAGAGTTGGTGAGAGGCCAGCACAGGGAGGGAGGGT
GTCTGCTGGAAGCCAGGCTCAGCGCTCCTGCCTGGACGCATCCCGGCTATGCAGTCCCAGTCCAGG
GCAGCAAGGCAGGCCCCGTCTGCCTCTTCACCCGGAGGCCTCTGCCCGCCCCACTCATGCTCAGGG
AGAGGGTCTTCTGGCTTTTTCCCCAGGCTCTGGGCAGGCACAGGCTAGGTGCCCCTAACCCAGGCC
CTGCACACAAAGGGGCAGGTGCTGGGCTCAGACCTGCCAAGAGCCATATCCGGGAGGACCCTGCCC
CTGACCTAAGCCCACCCCAAAGGCCAAACTCTCCACTCCCTCAGCTCGGACACCTTCTCTCCTCCC
AGATTCCAGTAACTCCCAATCTTCTCTCTGCAGAGCCCAAATCTTGTGACAAAACTCACACATGCC
CACCGTGCCCAGGTAAGCCAGCCCAGGCCTCGCCCTCCAGCTCAAGGCGGGACAGGTGCCCTAGAG
TAGCCTGCATCCAGGGACAGGCCCCAGCCGGGTGCTGACACGTCCACCTCCATCTCTTCCTCAGCA
CCTGAAgccgccGGGGGACCGTCAGTCTTCCTCTTCCCCCCAAAACCCAAGGACACCCTCATGATC
TCCCGGACCCCTGAGGTCACATGCGTGGTGGTGGACGTGAGCCACGAAGACCCTGAGGTCAAGTTC
AACTGGTACGTGGACGGCGTGGAGGTGCATAATGCCAAGACAAAGCCGCGGGAGGAGCAGTACAAC
AGCACGTACCGTGTGGTCAGCGTCCTCACCGTCCTGCACCAGGACTGGCTGAATGGCAAGGAGTAC
AAGTGCAAGGTCTCCAACAAAGCCCTCCCAGCCCCCATCGAGAAAACCATCTCCAAAGCCAAAGGT
GGGACCCGTGGGGTGCGAGGGCCACATGGACAGAGGCCGGCTCGGCCCACCCTCTGCCCTGAGAGT

GACCGCTGTACCAACCTCTGTCCCTACAGGGCAGCCCCGAGAACCACAGGTGTACACCCTGCCCCC
ATCCCGGGAGGAGATGACCAAGAACCAGGTCAGCCTGACCTGCCTGGTCAAAGGCTTCTATCCCAG
CGACATCGCCGTGGAGTGGGAGAGCAATGGGCAGCCGGAGAACAACTACAAGACCACGCCTCCCGT
GCTGGACTCCGACGGCTCCTTCTTCCTCTATAGCAAGCTCACCGTGGACAAGAGCAGGTGGCAGCA
GGGGAACGTCTTCTCATGCTCCGTGATGCATGAGGCTCTGCACAACCACTACACGCAGAAGAGCCT
CTCCCTGTCCCCGGGTAAATGA

SEQ ID NO: 87 (Heavy chain constant region cDNA sequence)

*CTCGAG*TGCCTCCACCAAGGGCCCATCGGTCTTCCCCCTGGCACCCTCCTCCAAGAGCACCTCTGG
GGGCACAGCGGCCCTGGGCTGCCTGGTCAAGGACTACTTCCCCGAACCGGTGACGGTGTCGTGGAA
CTCAGGCGCCCTGACCAGCGGCGTGCACACCTTCCCGGCTGTCCTACAGTCCTCAGGACTCTACTC
CCTCAGCAGCGTGGTGACCGTGCCCTCCAGCAGCTTGGGCACCCAGACCTACATCTGCAACGTGAA
TCACAAGCCCAGCAACACCAAGGTGGACAAGAGAGTTGAGCCCAAATCTTGTGACAAAACTCACAC
ATGCCCACCGTGCCCAGCACCTGAAgccgccGGGGGACCGTCAGTCTTCCTCTTCCCCCCAAAACC
CAAGGACACCCTCATGATCTCCCGGACCCCTGAGGTCACATGCGTGGTGGTGGACGTGAGCCACGA
AGACCCTGAGGTCAAGTTCAACTGGTACGTGGACGGCGTGGAGGTGCATAATGCCAAGACAAAGCC
GCGGGAGGAGCAGTACAACAGCACGTACCGTGTGGTCAGCGTCCTCACCGTCCTGCACCAGGACTG
GCTGAATGGCAAGGAGTACAAGTGCAAGGTCTCCAACAAAGCCCTCCCAGCCCCCATCGAGAAAAC
CATCTCCAAAGCCAAAGGGCAGCCCCGAGAACCACAGGTGTACACCCTGCCCCCATCCCGGGAGGA
GATGACCAAGAACCAGGTCAGCCTGACCTGCCTGGTCAAAGGCTTCTATCCCAGCGACATCGCCGT
GGAGTGGGAGAGCAATGGGCAGCCGGAGAACAACTACAAGACCACGCCTCCCGTGCTGGACTCCGA
CGGCTCCTTCTTCCTCTATAGCAAGCTCACCGTGGACAAGAGCAGGTGGCAGCAGGGGAACGTCTT
CTCATGCTCCGTGATGCATGAGGCTCTGCACAACCACTACACGCAGAAGAGCCTCTCCCTGTCCCC
GGGTAAATGA

SEQ ID NO: 88 (Light chain lambda constant region DNA sequence)

*CCTAGG*TCAGCCCAAGGCCAACCCCACTGTCACTCTGTTCCCGCCCTCCTCTGAGGAGCTCCAAGC
CAACAAGGCCACACTAGTGTGTCTGATCAGTGACTTCTACCCGGGAGCTGTGACAGTGGCCTGGAA
GGCAGATGGCAGCCCCGTCAAGGCGGGAGTGGAGACCACCAAACCCTCCAAACAGAGCAACAACAA
GTACGCGGCCAGCAGCTACCTGAGCCTGACGCCCGAGCAGTGGAAGTCCCACAGAAGCTACAGCTG
CCAGGTCACGCATGAAGGGAGCACCGTGGAGAAGACAGTGGCCCCTACAGAATGTTCATAA

SEQ ID NO: 89 (*Macaca fascicularis* PD-1 polypeptide, NCBI Accession B0LAJ3_MACFA)

MQIPQAPWPV VWAVLQLGWR PGWFLESPDR PWNAPTFSPA LLLVTEGDNA TFTCSFSNAS
ESFVLNWYRM SPSNQTDKLA AFPEDRSQPG QDCRFRVTRL PNGRDFHMSV VRARRNDSGT
YLCGAISLAP KAQIKESLRA ELRVTERRAE VPTAHPSPSP RPAGQFQALV VGVVGGLLGS
LVLLVWVLAV ICSRAAQGTI EARRTGQPLK EDPSAVPVFS VDYGELDFQW REKTPEPPAP
CVPEQTEYAT IVFPSGLGTS SPARRGSADG PRSPRPLRPE DGHCSWPL

SEQ ID NO: 90 (*Gallus Gallus* PD-1 polypeptide, NCBI Accession No. XP_422723.3)

MGKEAPSGTG HRHRAQQGTR RPAMALGTSR TMWDSTEAAL VVLCVLLLCC NPPLAGCHQV
TLFPATLTRP AGSSATFICN ISMENSSLEF NLNWYQKTNN SNPQKIAGII RNIPQKKMEK
YRLFNNTPVF KMEILNLHQN DSGFYYCGLI TFSRSDKVVE SSHSQLVVTE APEKTNTIDE
PSEEESSPPD HIKAVLLGTL LLAGVIVLLL FGYIIINNRR ADVQKPSSGN TLAEVKPPVV
PVPTVDYGVL EFQRDPHSQV PLETCPAEQT EYATIVFPEE KPITPERGKR HKDERTWQLP
SQPC

SEQ ID NO: 91 (*Mus musculus* PD-1 polypeptide, NCBI Accession No. NP_032824.1)

MWVRQVPWSF TWAVLQLSWQ SGWLLEVPNG PWRSLTFYPA WLTVSEGANA TFTCSLSNWS

EDLMLNWNRL SPSNQTEKQA AFCNGLSQPV QDARFQIIQL PNRHDFHMNI LDTRRNDSGI
YLCGAISLHP KAKIEESPGA ELVVTERILE TSTRYPSPSP KPEGRFQGMV IGIMSALVGI
PVLLLLAWAL AVFCSTSMSE ARGAGSKDDT LKEEPSAAPV PSVAYEELDF QGREKTPELP
TACVHTEYAT IVFTEGLGAS AMGRRGSADG LQGPRPPRHE DGHCSWPL

SEQ ID NO: 92 *(Rattus norvegicus* PD-1 polypeptide, NCBI Accession No. XP_006245633.1)

MWVRQVPWSF TWAVLQLSWQ SGWLLEVPNG PWRSLTFYPA WLTVSEGANA TFTCSLSNWS
EDLMLNWNRL SPSNQTEKQA AFCNGLSQPV QDARFQIIQL PNRHDFHMNI LDTRRNDSGI
YLCGAISLHP KAKIEESPGA ELVVTERILE TSTRYPSPSP KPEGRFQGMV IGIMSALVGI
PVLLLLAWAL AVFCSTSMSE ARGAGSKDDT LKEEPSAAPV PSVAYEELDF QGREKTPELP
TACVHTEYAT IVFTEGLGAS AMGRRGSADG LQGPRPPRHE DGHCSWPL

Claims

1. An anti-PD-1 antibody or an antigen-binding portion thereof that binds to an epitope on human PD-1 comprising amino acid residues V64, L128, P130, K131, and A132 of SEQ ID NO: 1.

2. The anti-PD-1 antibody or antigen-binding portion of claim 1, wherein said antibody comprises H-CDR1-3 and L-CDR1-3 comprising the amino acid sequences of SEQ ID NOs: 18-20 and SEQ ID NOs: 21-23, respectively.

3. The anti-PD-1 antibody or antigen-binding portion of claim 1, wherein said antibody comprises a V_H comprising the amino acid sequence of SEQ ID NO: 2 and a V_L comprising the amino acid sequence of SEQ ID NO: 3.

4. The anti-PD-1 antibody of any one of claims 1-3, wherein the antibody is an IgG_1, optionally comprising a mutation in one or both of heavy chain amino acid positions 234 and 235, which are numbered according to the IMGT numbering scheme, optionally wherein one or both of the amino acid residues at positions 234 and 235 are mutated to Ala.

5. The anti-PD-1 antibody or antigen-binding portion of any one of claims 1-4, wherein the antibody or portion has at least one of the following properties:

   a) binds to human PD-1 with a K_D of 750 pM or less;
   b) binds to cynomolgus PD-1 with a K_D of 7 nM or less;
   c) binds to mouse PD-1 with a K_D of 1 nM or less;
   d) does not bind to rat PD-1;
   e) increases IL-2 secretion in an SEB whole blood assay;
   f) increases IFN-γ secretion in a one-way mixed lymphocyte reaction assay;
   g) inhibits the interaction of PD-1 with PD-L1 by at least 60% at a concentration of 10 μg/ml in a flow cytometric competition assay;
   h) blocks binding of PD-L1 and PD-L2 to PD-1 by at least 90% at a concentration of 10 μg/ml as determined by Bio-Layer Interferometry analysis; and
   i) inhibits tumor growth *in vivo*.

6. An anti-PD-1 antibody that comprises a heavy chain comprising the amino acid sequences of SEQ ID NOs: 2 and 67 and a light chain comprising the amino acid sequences of SEQ ID NOs: 3 and 68.

7. A pharmaceutical composition comprising an anti-PD-1 antibody or antigen-binding portion according to any one of claims 1-6 and a pharmaceutically acceptable excipient, optionally further comprising a chemotherapeutic agent, an anti-neoplastic agent, an anti-angiogenic agent, a tyrosine kinase inhibitor, or a PD-1 pathway inhibitor.

8. An isolated nucleic acid molecule comprising a nucleotide sequence that encodes the heavy chain or an antigen-binding portion thereof, or a nucleotide sequence that encodes the light chain or an antigen-binding portion thereof, or both, of the anti-PD-1 antibody of any one of claims 1-6.

9. A vector comprising the isolated nucleic acid molecule of claim 8, wherein said vector further comprises an expression control sequence.

10. A host cell comprising a nucleotide sequence that encodes the heavy chain or an antigen-binding portion thereof, and a nucleotide sequence that encodes the light chain or an antigen-binding portion thereof, of the anti-PD-1 antibody of any one of claims 1-6.

11. A method for producing an anti-PD-1 antibody or an antigen-binding portion thereof, comprising providing a host cell according to claim 10, cultivating said host cell under conditions suitable for expression of the antibody or portion, and isolating the resulting antibody or portion.

12. A bispecific binding molecule having the binding specificity of an anti-PD-1 antibody according to any one of claims 1-6 and the binding specificity of another, distinct antibody.

13. An anti-PD-1 antibody or antigen-binding portion according to any one of claims 1-6, a pharmaceutical composition according to claim 7, or a bispecific binding molecule according to claim 12, for use in enhancing immunity in a patient in need thereof.

14. An anti-PD-1 antibody or antigen-binding portion according to any one of claims 1-6, a pharmaceutical composition according to claim 7, or a bispecific binding molecule according to claim 12, for use in treating cancer in a patient.

15. The anti-PD-1 antibody or antigen-binding portion, pharmaceutical composition, or bispecific binding molecule for use of claim 14, wherein the cancer originates in a tissue selected from the group consisting of skin, lung, intestine, ovary, brain, prostate, kidney, soft tissues, hematopoietic system, head and neck, liver, bladder, breast, stomach, uterus and pancreas.

16. The anti-PD-1 antibody or antigen-binding portion, pharmaceutical composition, or bispecific binding molecule for use of claim 14, wherein the cancer is selected from the group consisting of advanced or metastatic melanoma, non-small cell lung cancer, head and neck squamous cell cancer, renal cell carcinoma, and Hodgkin's lymphoma.

17. The anti-PD-1 antibody or antigen-binding portion, pharmaceutical composition, or bispecific binding molecule for use of any one of claims 13-16, wherein said antibody or antigen-binding portion, pharmaceutical composition, or bispecific binding molecule is administered with a chemotherapeutic agent, an anti-neoplastic agent, an anti-angiogenic agent, a tyrosine kinase inhibitor, or a PD-1 pathway inhibitor.

Patentansprüche

1. Anti-PD-1-Antikörper oder antigenbindender Teil davon, der bzw. das an ein Epitop auf humanem PD-1 bindet, umfassend Aminosäurereste V64, L128, P130, K131 und A132 von SEQ ID NR: 1.

2. Anti-PD-1-Antikörper oder antigenbindender Teil nach Anspruch 1, wobei der Antikörper H-CDR1-3 und L-CDR1-3, umfassend die Aminosäuresequenzen von SEQ ID NR: 18-20 bzw. SEQ ID NR: 21-23, umfasst.

3. Anti-PD-1-Antikörper oder antigenbindender Teil nach Anspruch 1, wobei der Antikörper eine V_H, umfassend die Aminosäuresequenz von SEQ ID NR: 2, und eine V_L, umfassend die Aminosäuresequenz von SEQ ID NR: 3, umfasst.

4. Anti-PD-1-Antikörper nach einem der Ansprüche 1 bis 3, wobei der Antikörper ein IgG_1 ist, wahlweise umfassend eine Mutation in einer oder beiden der Aminosäurepositionen 234 und 235 der schweren Kette, die nach dem IMGT-Nummerierungsschema nummeriert sind, wobei wahlweise einer oder beide der Aminosäurereste an den Positionen 234 und 235 zu Ala mutiert sind.

5. Anti-PD-1-Antikörper oder antigenbindender Teil nach einem der Ansprüche 1 bis 4, wobei der Antikörper oder Teil mindestens eine der folgenden Eigenschaften aufweist:

a) bindet an humanes PD-1 mit einem K_D-Wert von 750 pM oder weniger;
b) bindet an Javaneraffen-PD-1 mit einem K_D-Wert von 7 nM oder weniger;
c) bindet an Maus-PD-1 mit einem K_D-Wert von 1 nM oder weniger;

d) bindet nicht an Ratten-PD-1;
e) erhöht die IL-2-Sekretion in einem SEB-Vollblut-Assay;
f) erhöht die IFN-γ-Sekretion in einem Einweg-Reaktionsassay mit gemischten Lymphozyten;
g) hemmt die Wechselwirkung von PD-1 mit PD-L1 bei einer Konzentration von 10 μg/ml in einem durchfluss-zytometrischen Kompetitionsassay um mindestens 60 %;
h) blockiert die Bindung von PD-L1 und PD-L2 an PD-1 bei einer Konzentration von 10 μg/ml um mindestens 90 %, bestimmt durch die Bio-Layer-Interferometrie-Analyse; und
i) hemmt das Tumorwachstum *in vivo*.

6. Anti-PD-1-Antikörper, der eine schwere Kette, umfassend die Aminosäuresequenzen von SEQ ID NR: 2 und 67, und eine leichte Kette, umfassend die Aminosäuresequenzen von SEQ ID NR: 3 und 68, umfasst.

7. Pharmazeutische Zusammensetzung, umfassend einen Anti-PD-1-Antikörper oder antigenbindenden Teil nach einem der Ansprüche 1 bis 6 und einen pharmazeutisch verträglichen Hilfsstoff, wahlweise ferner umfassend ein chemotherapeutisches Mittel, ein antineoplastisches Mittel, ein anti-angiogenes Mittel, einen Tyrosinkinase-Hemmer oder einen PD-1-Signalweg-Hemmer.

8. Isoliertes Nukleinsäuremolekül, umfassend eine Nukleotidsequenz, die die schwere Kette oder einen antigenbindenden Teil davon kodiert, oder eine Nukleotidsequenz, die die leichte Kette oder einen antigenbindenden Teil davon, oder beides, des Anti-PD-1-Antikörpers nach einem der Ansprüche 1 bis 6, kodiert.

9. Vektor, umfassend das isolierte Nukleinsäuremolekül nach Anspruch 8, wobei der Vektor ferner eine Expressionskontrollsequenz umfasst.

10. Wirtszelle, umfassend eine Nukleotidsequenz, die die schwere Kette oder einen antigenbindenden Teil davon, und eine Nukleotidsequenz, die die leichte Kette oder einen antigenbindenden Teil davon, des Anti-PD-1-Antikörpers nach einem der Ansprüche 1 bis 6, kodiert.

11. Verfahren zur Herstellung eines Anti-PD-1-Antikörpers oder eines antigenbindenden Anteils davon, umfassend das Bereitstellen einer Wirtszelle nach Anspruch 10, Kultivieren der Wirtszelle unter Bedingungen, die für die Expression des Antikörpers oder Teils geeignet sind, und Isolieren des resultierenden Antikörpers oder Teils.

12. Bispezifisches Bindungsmolekül, das die Bindungsspezifität eines Anti-PD-1-Antikörpers nach einem der Ansprüche 1 bis 6 und die Bindungsspezifität eines anderen, unterschiedlichen Antikörpers, aufweist.

13. Anti-PD-1-Antikörper oder antigenbindender Teil nach einem der Ansprüche 1 bis 6, eine pharmazeutische Zusammensetzung nach Anspruch 7 oder ein bispezifisches Bindungsmolekül nach Anspruch 12, zur Verwendung beim Verbessern der Immunität bei einem Patienten, der dessen bedarf.

14. Anti-PD-1-Antikörper oder antigenbindender Teil nach einem der Ansprüche 1 bis 6, eine pharmazeutische Zusammensetzung nach Anspruch 7 oder ein bispezifisches Bindungsmolekül nach Anspruch 12, zur Verwendung bei der Behandlung von Krebs bei einem Patienten.

15. Anti-PD-1-Antikörper oder antigenbindender Teil, pharmazeutische Zusammensetzung oder bispezifisches Bindungsmolekül zur Verwendung nach Anspruch 14, wobei der Krebs aus einem Gewebe, ausgewählt aus der Gruppe bestehend aus Haut, Lunge, Darm, Eierstock, Gehirn, Prostata, Niere, Weichgeweben, hämatopoetischem System, Kopf und Hals, Leber, Blase, Brust, Magen, Uterus und Pankreas, stammt.

16. Anti-PD-1-Antikörper oder antigenbindender Teil, pharmazeutische Zusammensetzung oder bispezifisches Bindungsmolekül zur Verwendung nach Anspruch 14, wobei der Krebs ausgewählt ist aus der Gruppe bestehend aus fortgeschrittenem oder metastasierendem Melanom, nicht-kleinzelligem Lungenkrebs, Kopf- und Hals-Plattenepithelzellkrebs, Nierenzellkarzinom und Hodgkin-Lymphom.

17. Anti-PD-1-Antikörper oder antigenbindender Teil, pharmazeutische Zusammensetzung oder bispezifisches Bindungsmolekül zur Verwendung nach einem der Ansprüche 13 bis 16, wobei der Antikörper oder antigenbindende Teil, die pharmazeutische Zusammensetzung oder das bispezifische Bindungsmolekül mit einem chemotherapeutischen Mittel, einem antineoplastischen Mittel, einem anti-angiogenen Mittel, einem Tyrosinkinase-Hemmer oder einem PD-1-Signalweg-Hemmer verabreicht wird.

Revendications

1. Anticorps ou partie de liaison à l'antigène de celui-ci qui se lie à un épitope de PD-1 humain comprenant des résidus d'acides aminés V64, L128, P130, K131, et A132 de SEQ ID NO: 1.

2. Anticorps anti-PD-1 ou partie de liaison à l'antigène selon la revendication 1, dans lequel ledit anticorps comprend H-CDR1-3 et L-CDR1-3 comprenant les séquences d'acides aminés de SEQ ID NO: 18 à 20 et SEQ ID NO: 21 à 23, respectivement.

3. Anticorps anti-PD-1 ou partie de liaison à l'antigène selon la revendication 1, dans lequel ledit anticorps comprend un V_H comprenant la séquence d'acides aminés de SEQ ID NO: 2 et un V_L comprenant la séquence d'acides aminés de SEQ ID NO: 3.

4. Anticorps anti-PD-1 selon l'une quelconque des revendications 1 à 3, dans lequel l'anticorps est une IgG_1, comprenant facultativement une mutation dans l'une et/ou l'autre des positions d'acide aminé de chaîne lourde 234 et 235, qui sont numérotées selon le schéma de numérotation IMGT, facultativement dans lequel l'un et/ou l'autre des résidus d'acides aminés aux positions 234 et 235 sont mutés en Ala.

5. Anticorps anti-PD-1 ou partie de liaison à l'antigène selon l'une quelconque des revendications 1 à 4, dans lequel l'anticorps ou la partie a au moins une des propriétés suivantes :

   a) se lie à du PD-1 humain avec une K_D de 750 pM ou moins ;
   b) se lie à du PD-1 de cynomolgus avec une K_D de 7 nM ou moins ;
   c) se lie à du PD-1 de souris avec une K_D de 1 nM ou moins ;
   d) ne se lie pas à du PD-1 de rat ;
   e) augmente la sécrétion d'IL-2 dans un dosage de sang total SEB ;
   f) augmente la sécrétion d'IFN-γ dans un dosage de réaction lymphocytaire mixte unidirectionnelle ;
   g) inhibe l'interaction de PD-1 avec PD-L1 d'au moins 60 % à une concentration de 10 μg/mL dans un dosage de compétition cytométrique en flux ;
   h) bloque la liaison de PD-L1 et de PD-L2 à PD-1 d'au moins 90 % à une concentration de 10 μg/mL comme déterminé par une analyse d'interférométrie de bio-couche ; et
   i) inhibe une croissance tumorale *in vivo*.

6. Anticorps anti-PD-1 qui comprend une chaîne lourde comprenant les séquences d'acides aminés de SEQ ID NO: 2 et 67 et une chaîne légère comprenant les séquences d'acides aminés de SEQ ID NO: 3 et 68.

7. Composition pharmaceutique comprenant un anticorps anti-PD-1 ou partie de liaison à l'antigène selon l'une quelconque des revendications 1 à 6 et un excipient acceptable sur le plan pharmaceutique, comprenant facultativement en outre un agent chimiothérapeutique, un agent anti-néoplasique, un agent antiangiogénique, un inhibiteur de tyrosine kinase, ou un inhibiteur de la voie de PD-1.

8. Molécule d'acide nucléique isolée comprenant une séquence de nucléotides qui code pour la chaîne lourde ou une partie de liaison à l'antigène de celle-ci, ou une séquence de nucléotides qui code pour la chaîne légère ou une partie de liaison à l'antigène de celle-ci, ou l'une et l'autre, de l'anticorps anti-PD-1 selon l'une quelconque des revendications 1 à 6.

9. Vecteur comprenant la molécule d'acide nucléique isolée selon la revendication 8, dans lequel ledit vecteur comprend en outre une séquence de contrôle d'expression.

10. Cellule hôte comprenant une séquence de nucléotides qui code pour la chaîne lourde ou une partie de liaison à l'antigène de celle-ci, et une séquence de nucléotides qui code pour la chaîne légère ou une partie de liaison à l'antigène de celle-ci, de l'anticorps anti-PD-1 selon l'une quelconque des revendications 1 à 6.

11. Procédé de production d'un anticorps anti-PD-1 ou d'une partie de liaison à l'antigène de celui-ci, comprenant la fourniture d'une cellule hôte selon la revendication 10, la culture de ladite cellule hôte dans des conditions appropriées pour une expression de l'anticorps ou de la partie, et l'isolement de l'anticorps ou de la partie résultant(e).

12. Molécule de liaison bispécifique ayant la spécificité de liaison d'un anticorps anti-PD-1 selon l'une quelconque des

revendications 1 à 6 et la spécificité de liaison d'un autre anticorps, distinct.

13. Anticorps anti-PD-1 ou partie de liaison à l'antigène selon l'une quelconque des revendications 1 à 6, composition pharmaceutique selon la revendication 7, ou molécule de liaison bispécifique selon la revendication 12, pour utilisation dans le renforcement d'immunité chez un patient qui en a besoin.

14. Anticorps anti-PD-1 ou partie de liaison à l'antigène selon l'une quelconque des revendications 1 à 6, composition pharmaceutique selon la revendication 7, ou molécule de liaison bispécifique selon la revendication 12, pour utilisation dans le traitement d'un cancer chez un patient.

15. Anticorps anti-PD-1 ou partie de liaison à l'antigène, composition pharmaceutique, ou molécule de liaison bispécifique pour utilisation selon la revendication 14, dans lequel le cancer provient d'un tissu choisi dans le groupe constitué de peau, poumon, intestin, ovaire, cerveau, prostate, rein, tissus mous, système hématopoïétique, tête et cou, foie, vessie, sein, estomac, utérus et pancréas.

16. Anticorps anti-PD-1 ou partie de liaison à l'antigène, composition pharmaceutique, ou molécule de liaison bispécifique pour utilisation selon la revendication 14, dans lequel le cancer est choisi dans le groupe constitué de mélanome avancé ou métastatique, cancer du poumon non à petites cellules, cancer spinocellulaire de la tête et du cou, carcinome de cellules rénales, et lymphome de Hodgkin.

17. Anticorps anti-PD-1 ou partie de liaison à l'antigène, composition pharmaceutique, ou molécule de liaison bispécifique pour utilisation selon l'une quelconque des revendications 13 à 16, dans lequel ledit anticorps ou ladite partie de liaison à l'antigène, composition pharmaceutique, ou molécule de liaison bispécifique est administré avec un agent chimiothérapeutique, un agent anti-néoplasique, un agent antiangiogénique, un inhibiteur de tyrosine kinase, ou un inhibiteur de la voie de PD-1.

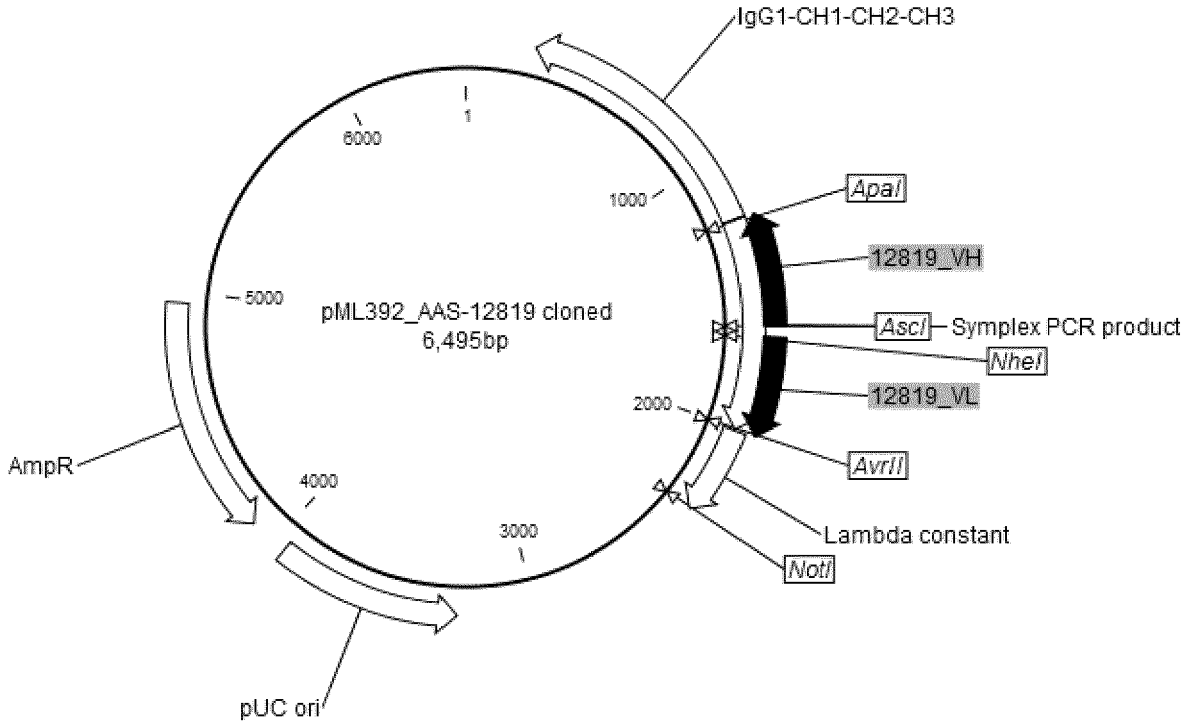


Figure 1

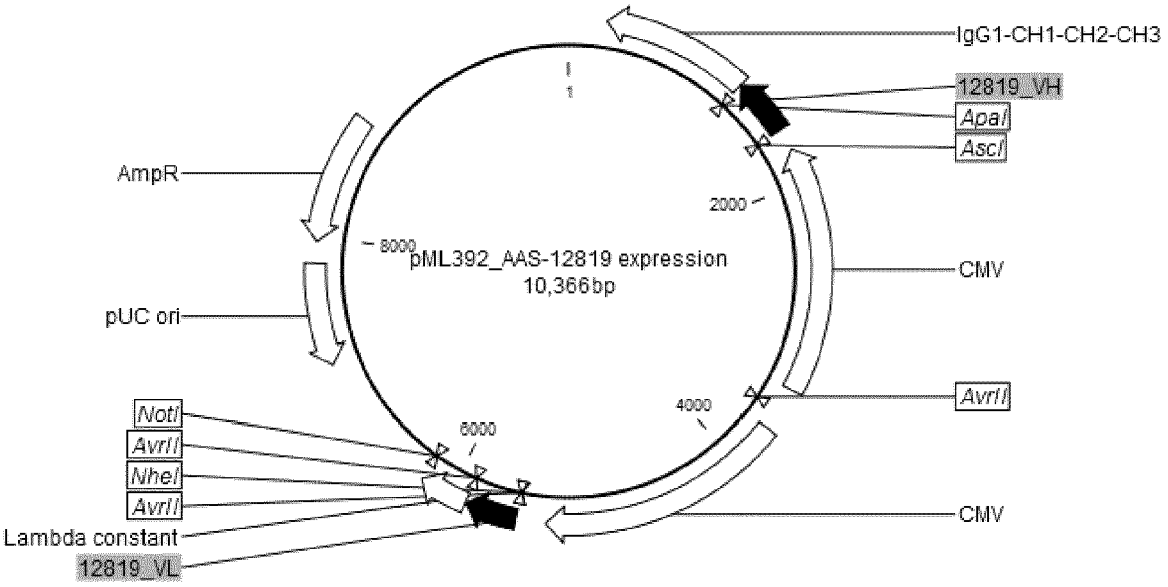


Figure 2

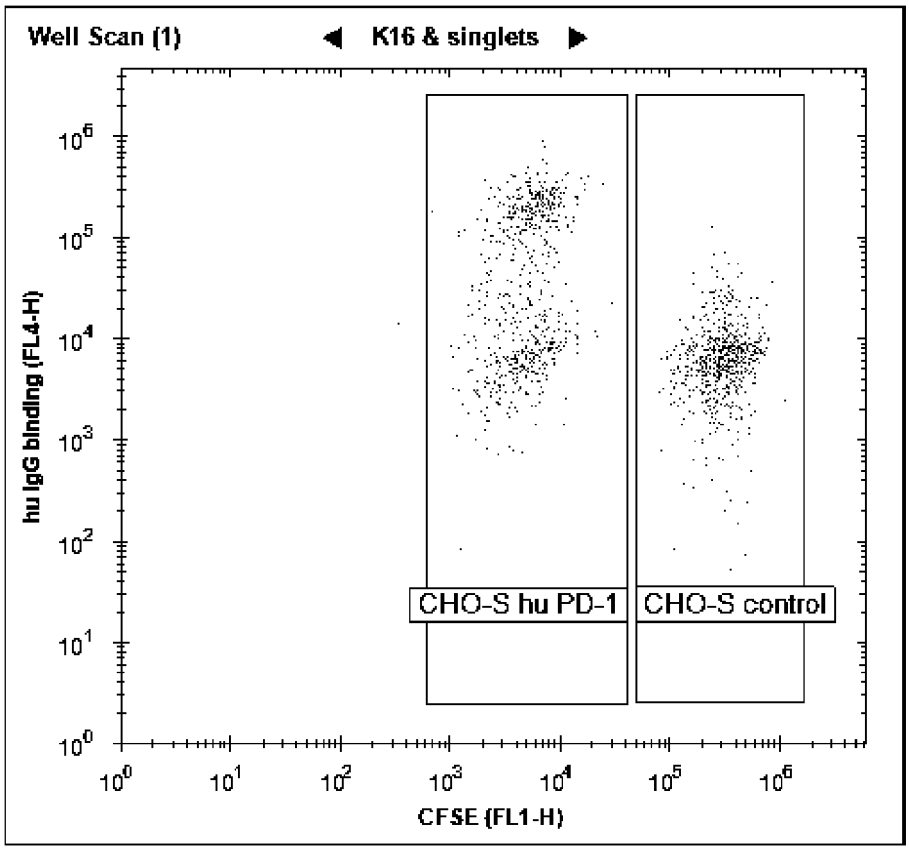


Figure 3A

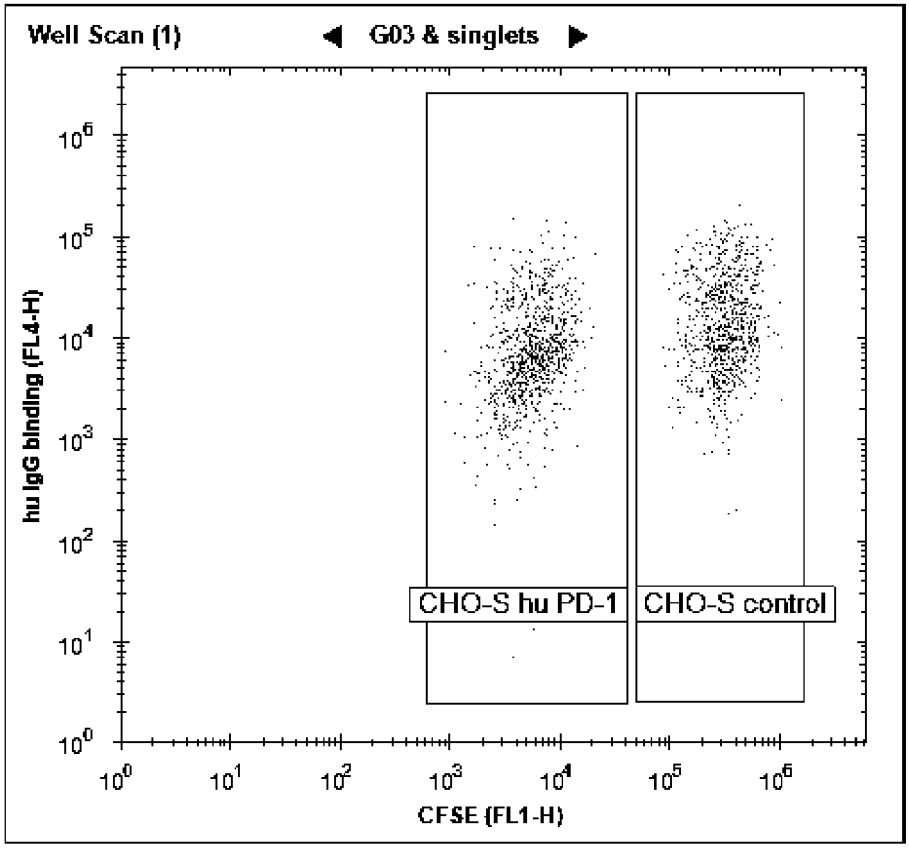


Figure 3B

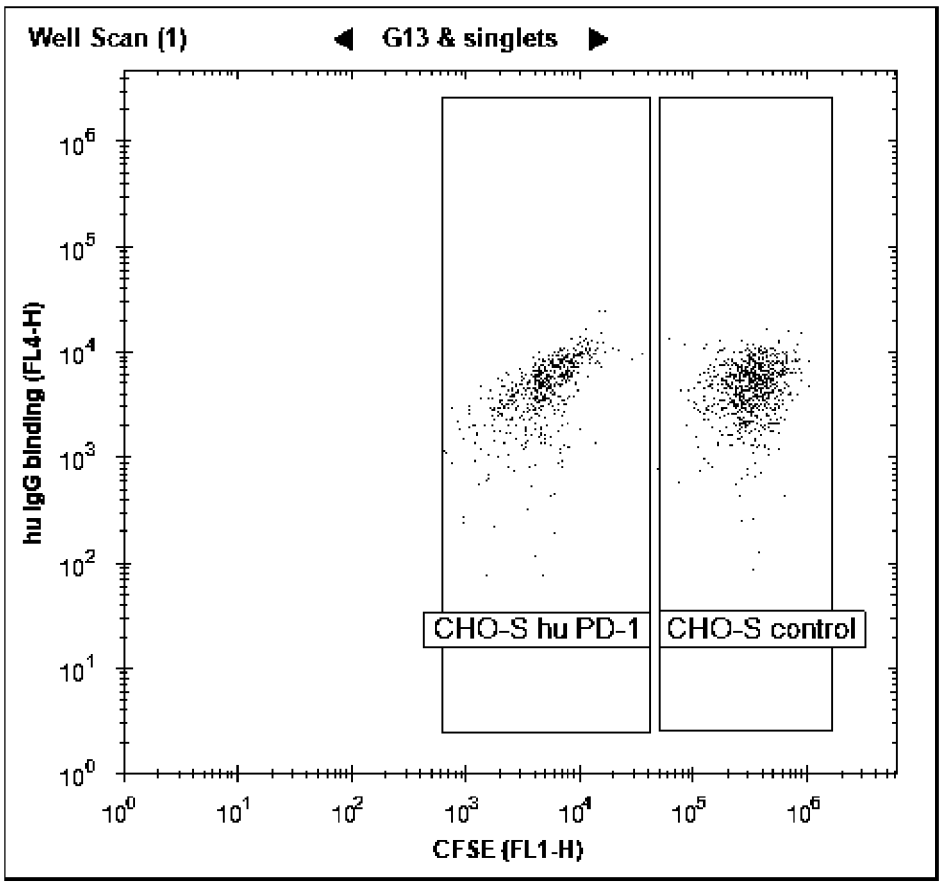

Well Scan (1)
G13 & singlets
hu IgG binding (FL4-H)
CFSE (FL1-H)
CHO-S hu PD-1
CHO-S control

Figure 3C

Figure 4

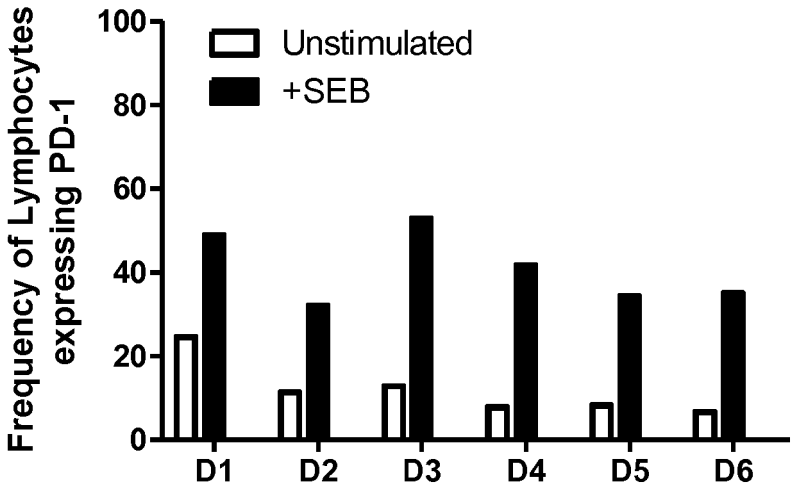

Frequency of Lymphocytes expressing PD-1
100
80
60
40
20
0
Unstimulated
+SEB
D1
D2
D3
D4
D5
D6

Figure 5A

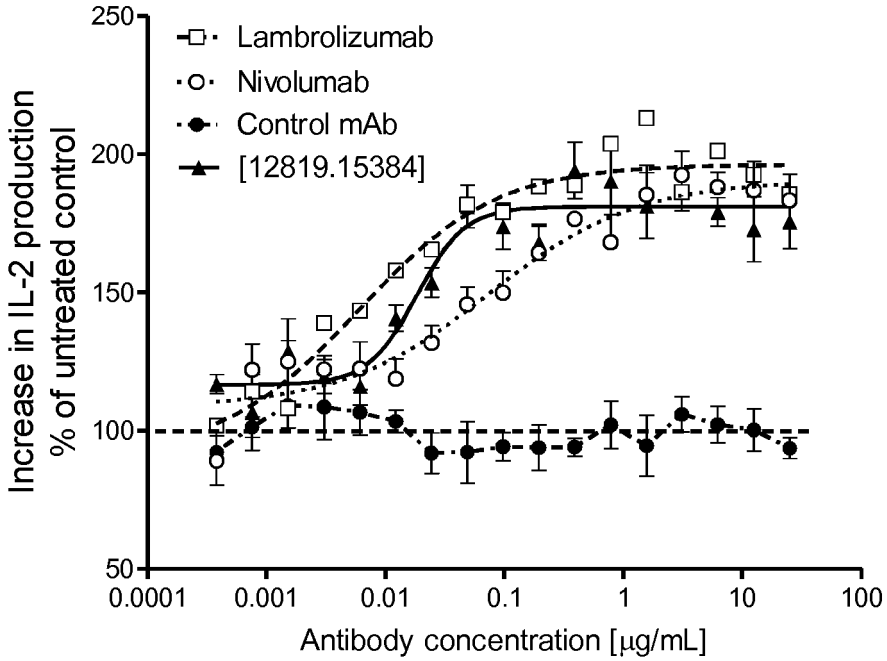


Figure 5B

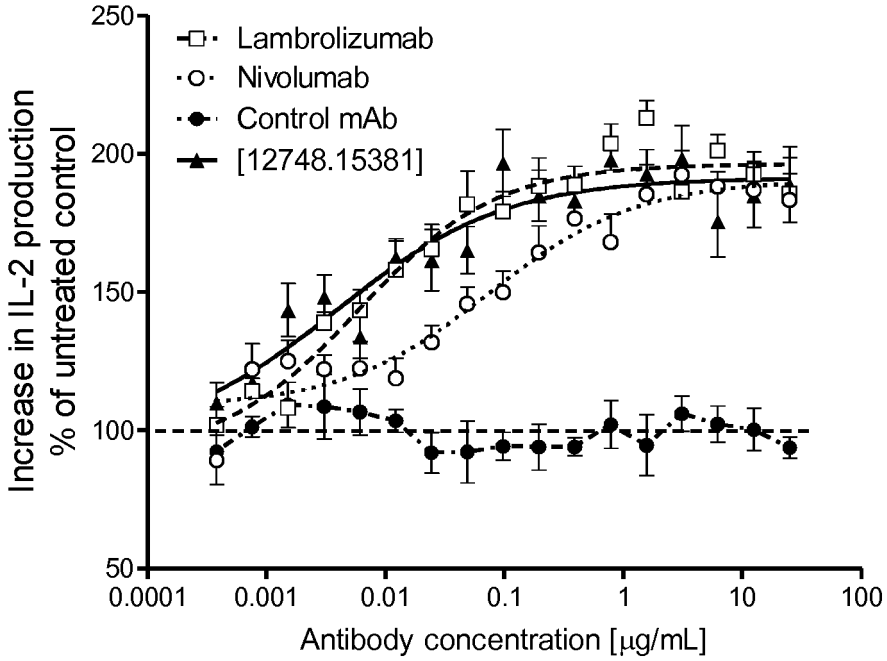


Figure 5C

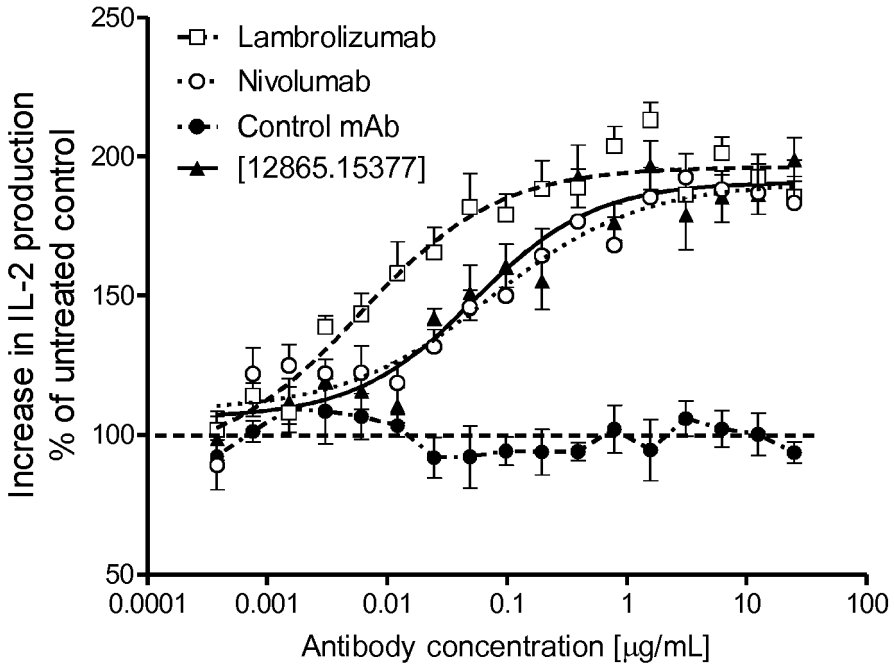


Figure 5D

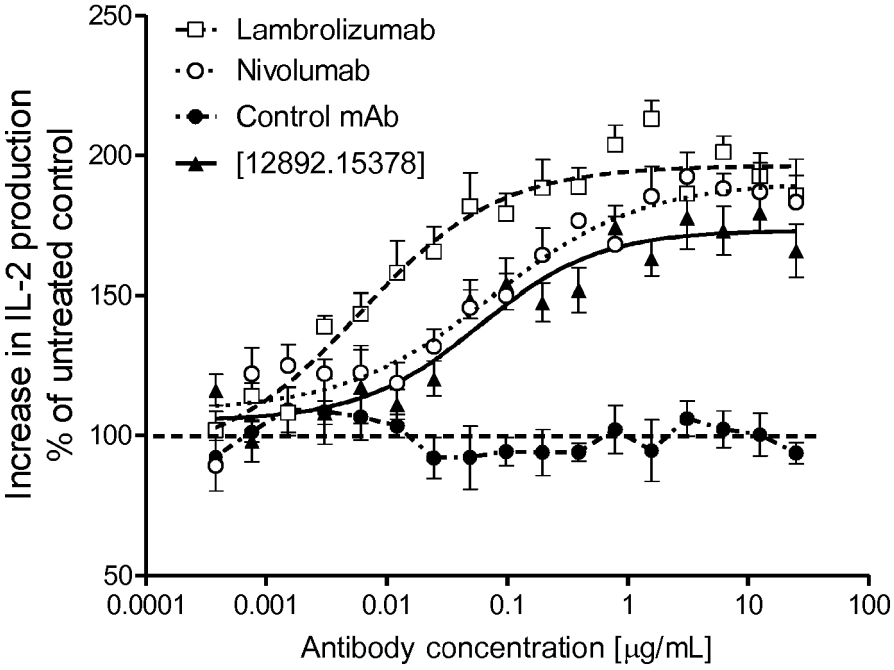


Figure 5E

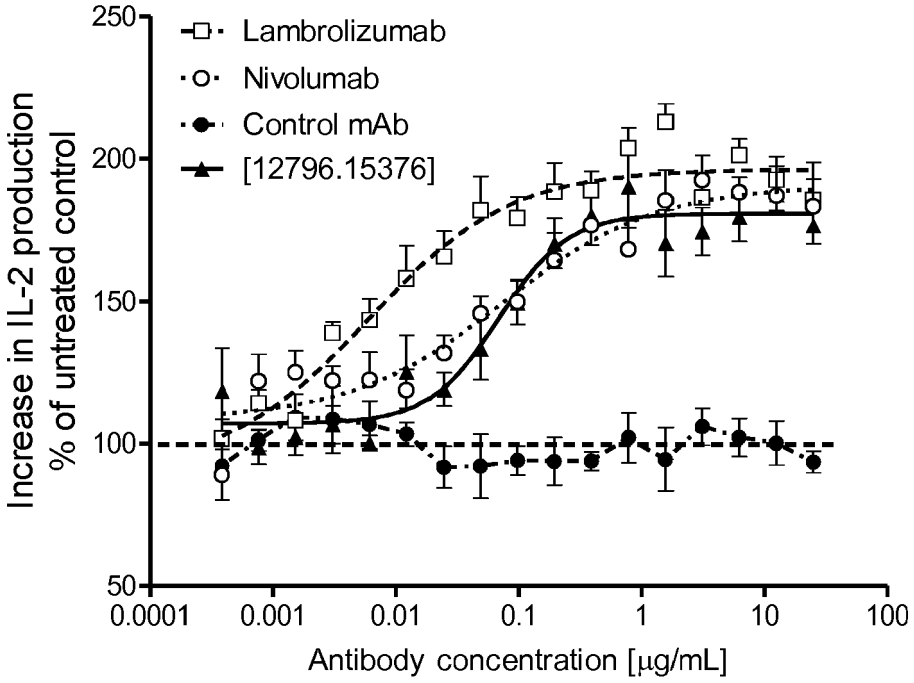


Figure 5F

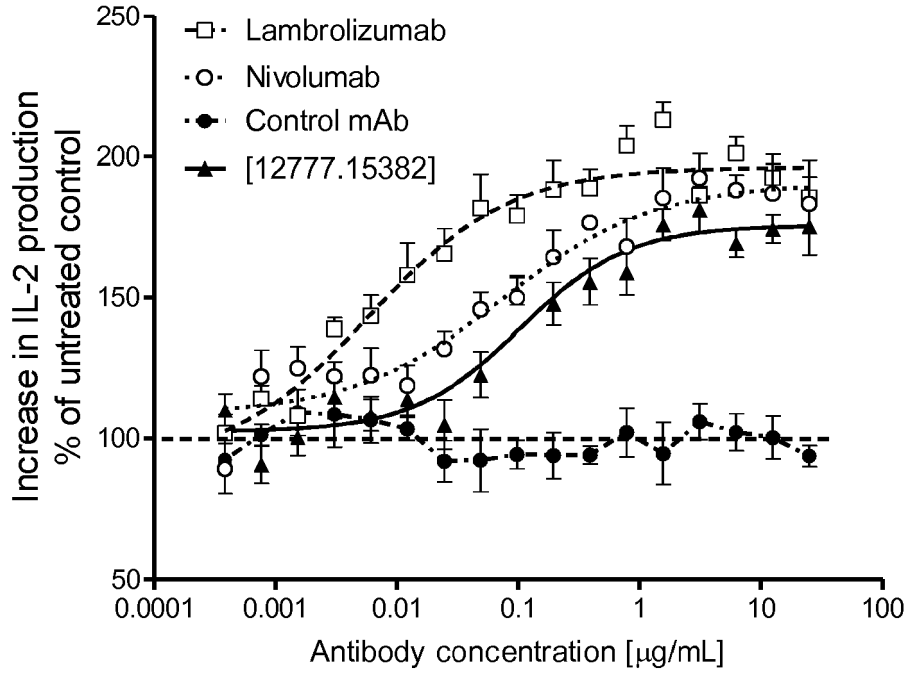


Figure 5G

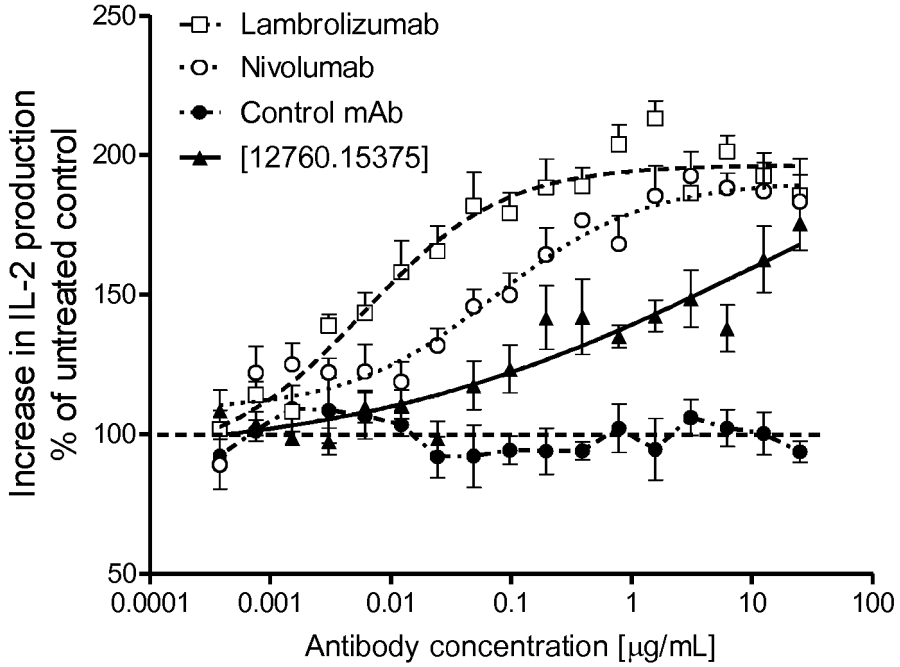


Figure 5H

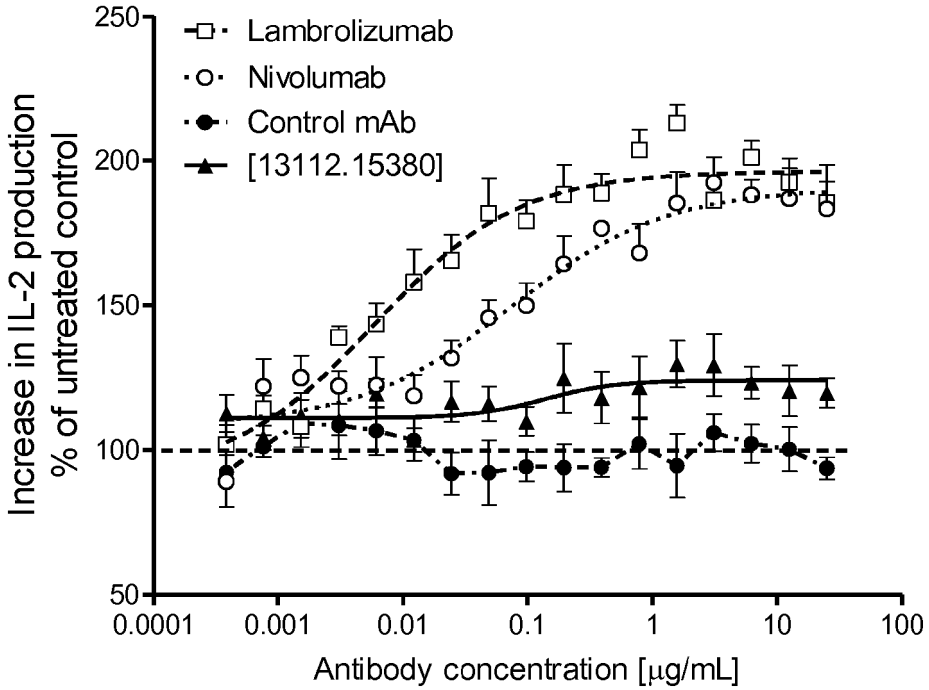


Figure 5l

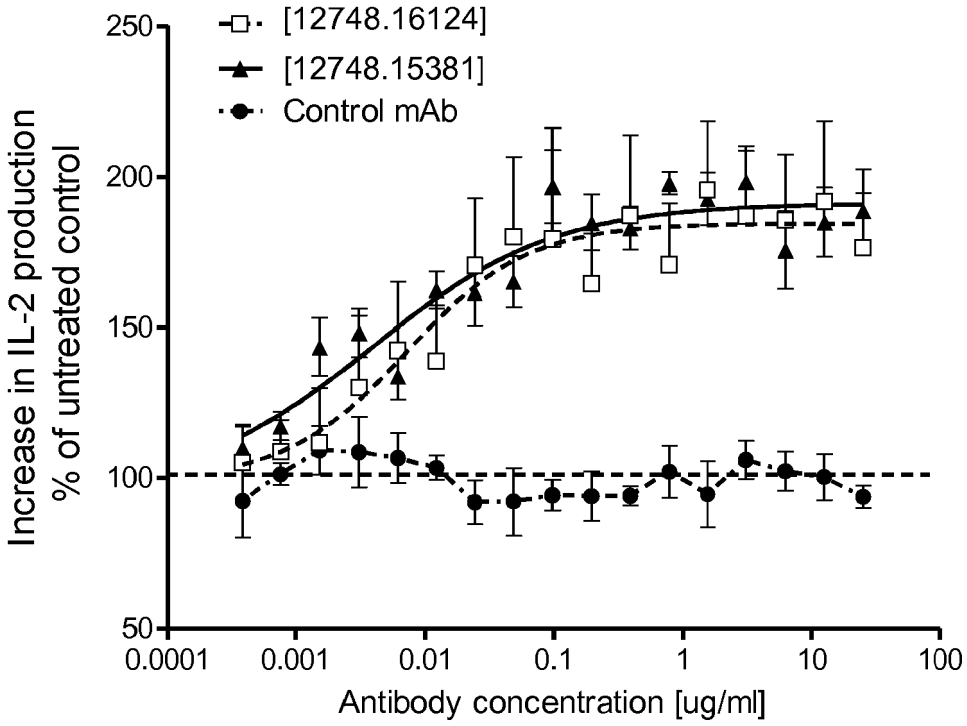

[12748.16124]
[12748.15381]
Control mAb
Increase in IL-2 production
% of untreated control
250
200
150
100
50
0.0001
0.001
0.01
0.1
1
10
100
Antibody concentration [ug/ml]

Figure 6A

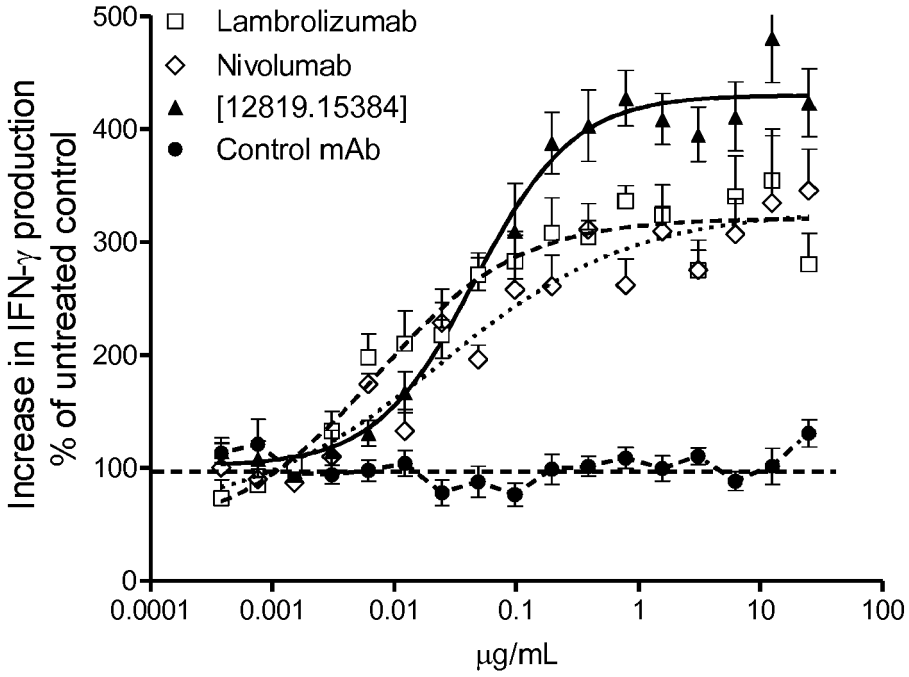


Figure 6B

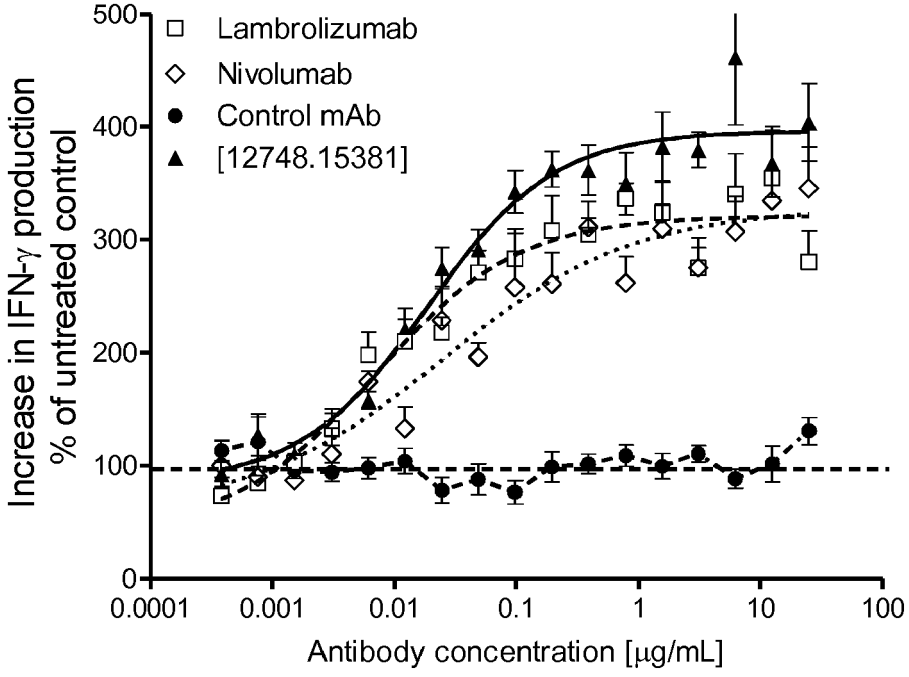


Figure 6C

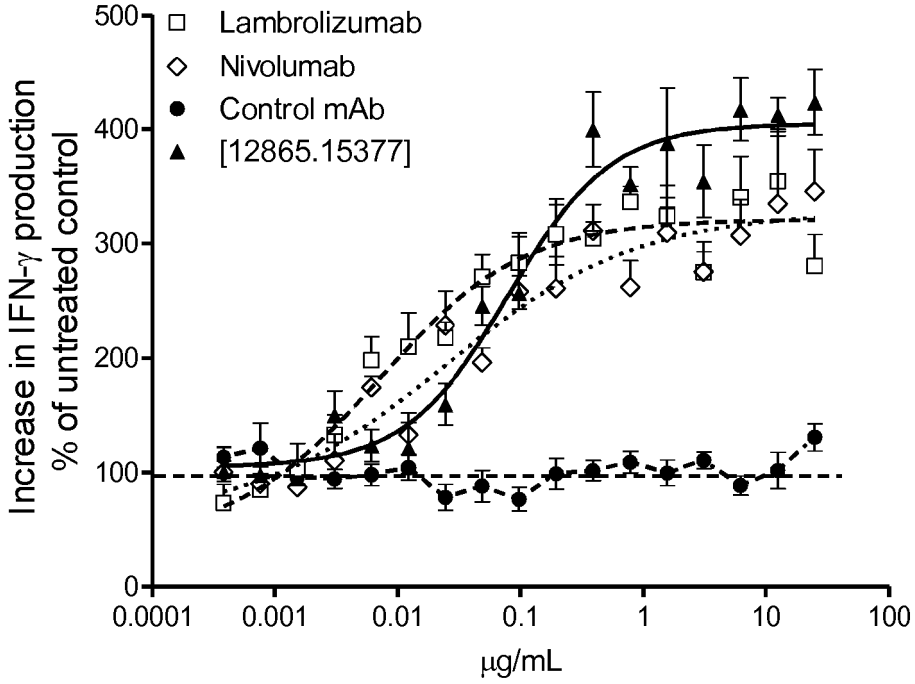


Figure 6D

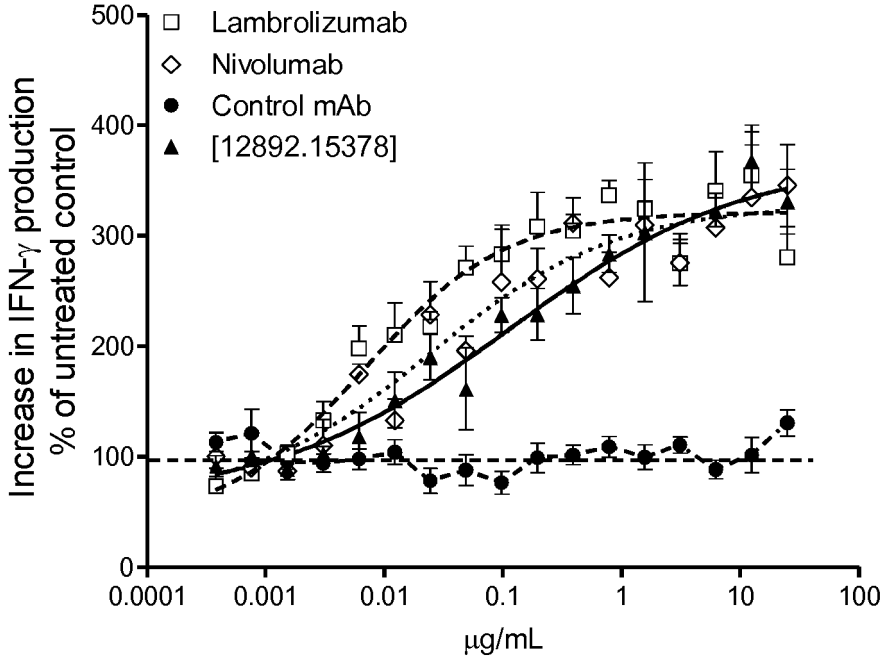


Figure 6E

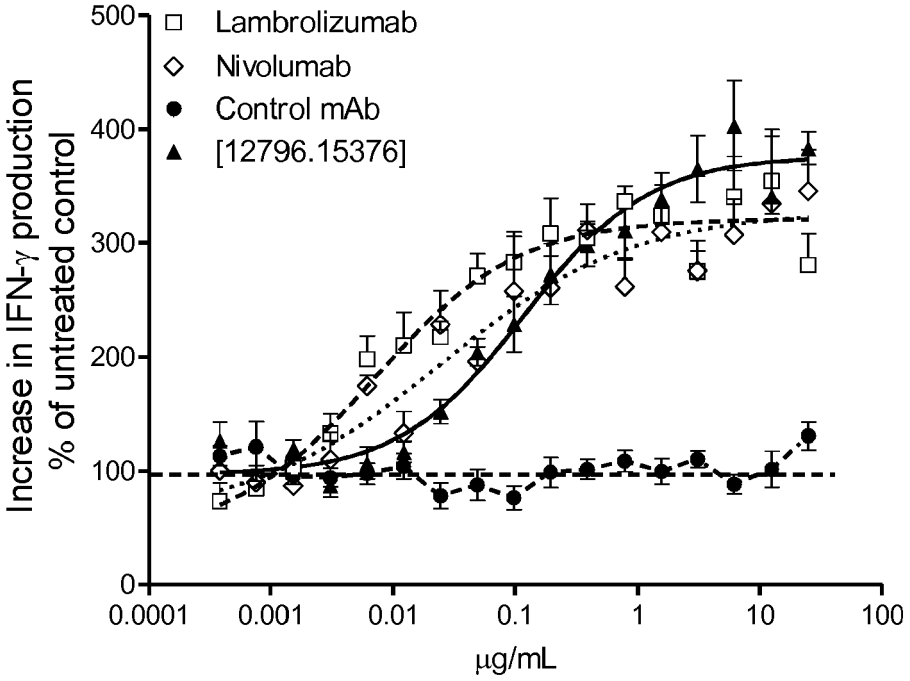


Figure 6F

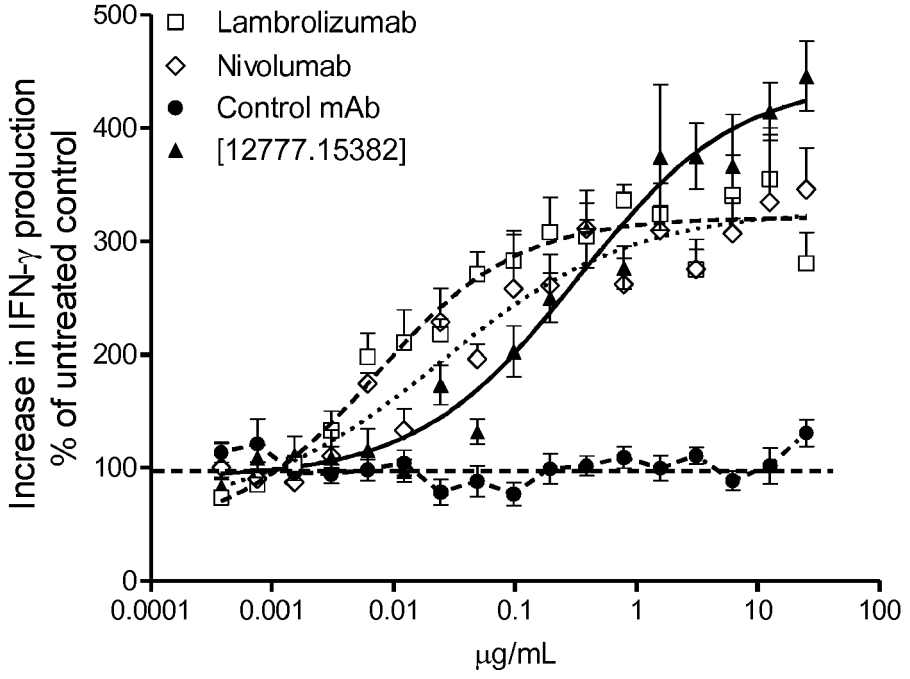


Figure 6G

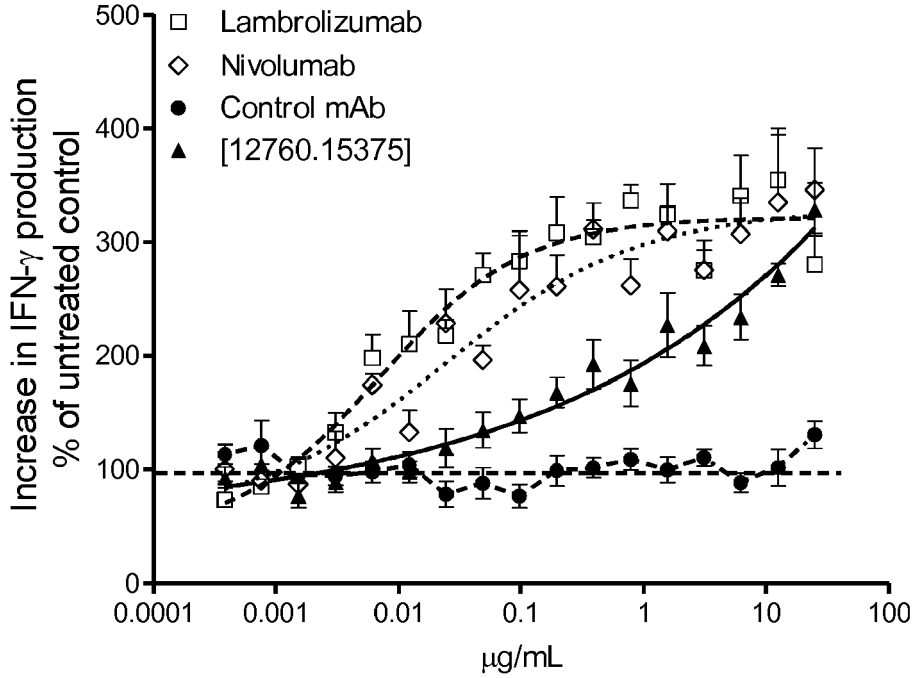


Figure 6H

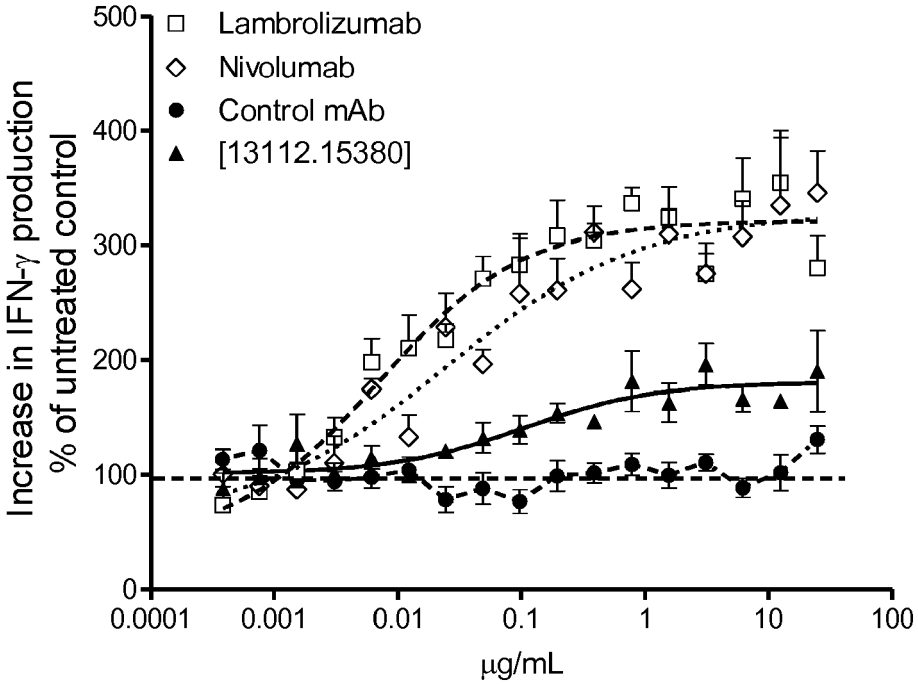


Figure 7A

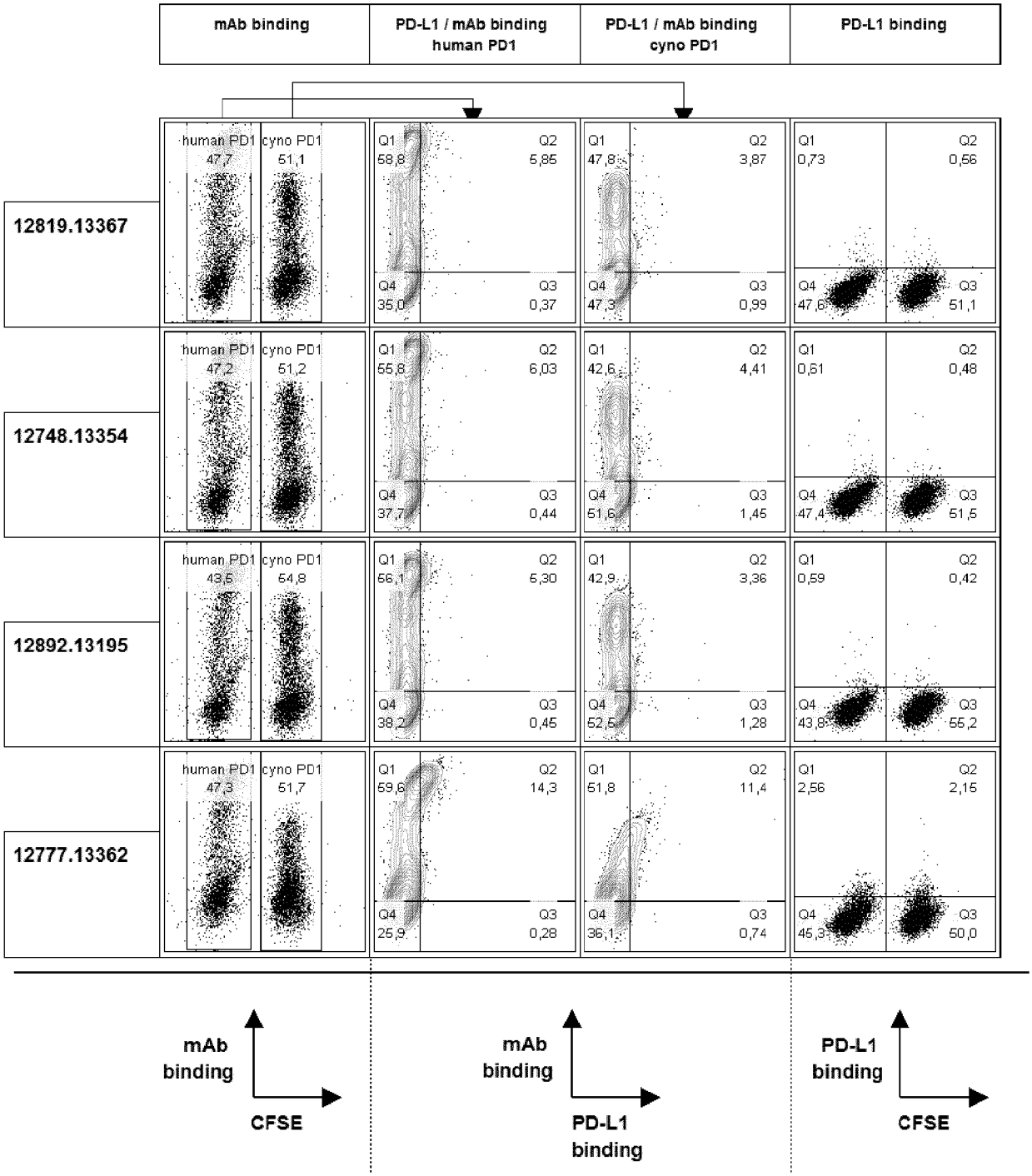

mAb binding
PD-L1 / mAb binding human PD1
PD-L1 / mAb binding cyno PD1
PD-L1 binding
12819.13367
12748.13354
12892.13195
12777.13362
human PD1
cyno PD1
47,7
51,1
Q1 58,8
Q2 5,85
Q4 35,0
Q3 0,37
Q1 47,8
Q2 3,87
Q4 47,3
Q3 0,99
Q1 0,73
Q2 0,56
Q4 47,6
Q3 51,1
47,2
51,2
Q1 55,8
Q2 6,03
Q4 37,7
Q3 0,44
Q1 42,6
Q2 4,41
Q4 51,6
Q3 1,45
Q1 0,61
Q2 0,48
Q4 47,4
Q3 51,5
43,5
54,8
Q1 56,1
Q2 5,30
Q4 38,2
Q3 0,45
Q1 42,9
Q2 3,36
Q4 52,5
Q3 1,28
Q1 0,59
Q2 0,42
Q4 43,8
Q3 55,2
47,3
51,7
Q1 59,6
Q2 14,3
Q4 25,9
Q3 0,28
Q1 51,8
Q2 11,4
Q4 36,1
Q3 0,74
Q1 2,56
Q2 2,15
Q4 45,3
Q3 50,0
mAb binding
CFSE
mAb binding
PD-L1 binding
PD-L1 binding
CFSE

Figure 7B

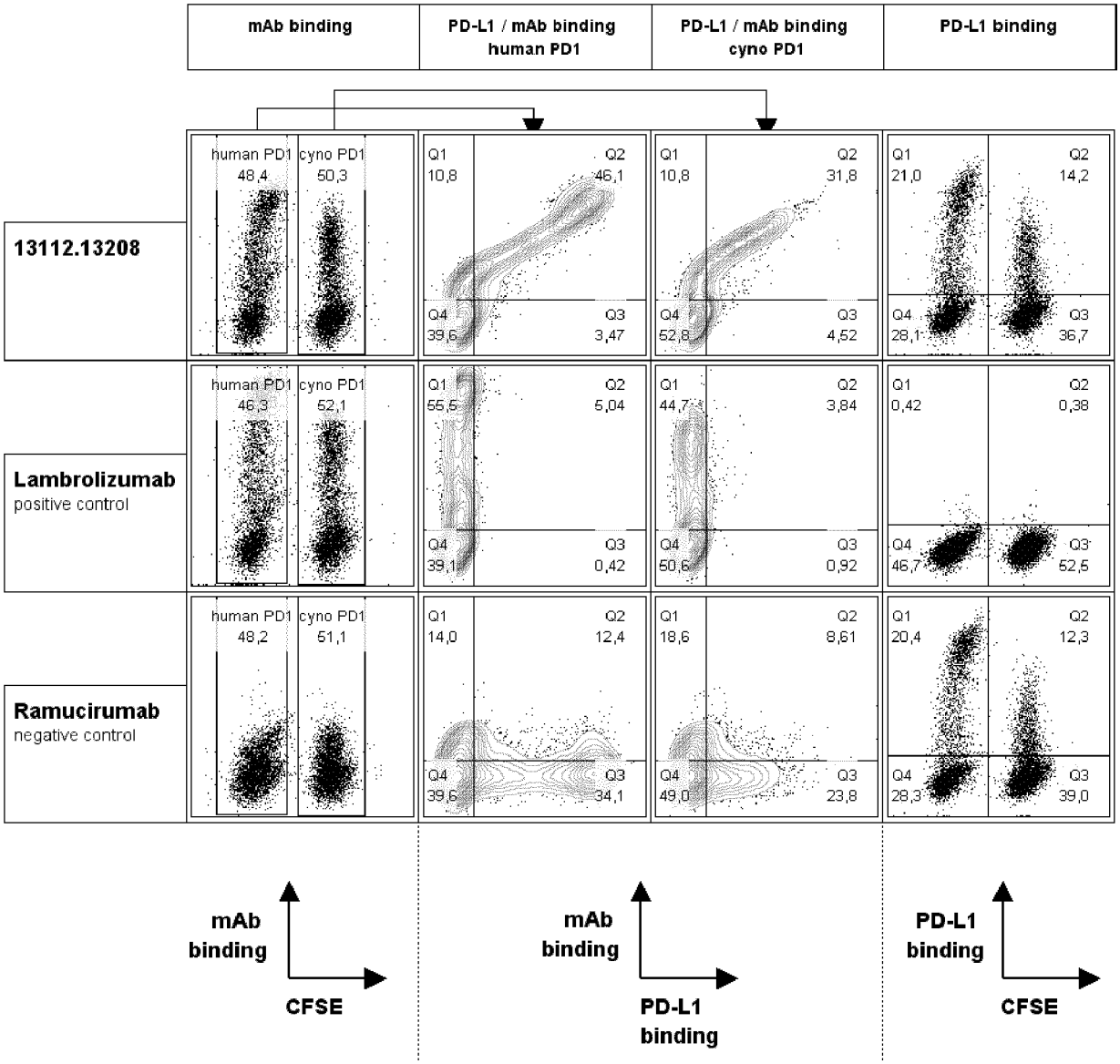

mAb binding
PD-L1 / mAb binding human PD1
PD-L1 / mAb binding cyno PD1
PD-L1 binding
13112.13208
Lambrolizumab
positive control
Ramucirumab
negative control
human PD1
cyno PD1
48,4
50,3
Q1 10,8
Q2 46,1
Q4 39,6
Q3 3,47
Q1 10,8
Q2 31,8
Q4 52,8
Q3 4,52
Q1 21,0
Q2 14,2
Q4 28,1
Q3 36,7
46,3
52,1
Q1 55,5
Q2 5,04
Q4 39,1
Q3 0,42
Q1 44,7
Q2 3,84
Q4 50,6
Q3 0,92
Q1 0,42
Q2 0,38
Q4 46,7
Q3 52,5
48,2
51,1
Q1 14,0
Q2 12,4
Q4 39,6
Q3 34,1
Q1 18,6
Q2 8,61
Q4 49,0
Q3 23,8
Q1 20,4
Q2 12,3
Q4 28,3
Q3 39,0
mAb binding
CFSE
mAb binding
PD-L1 binding
PD-L1 binding
CFSE

Figure 8

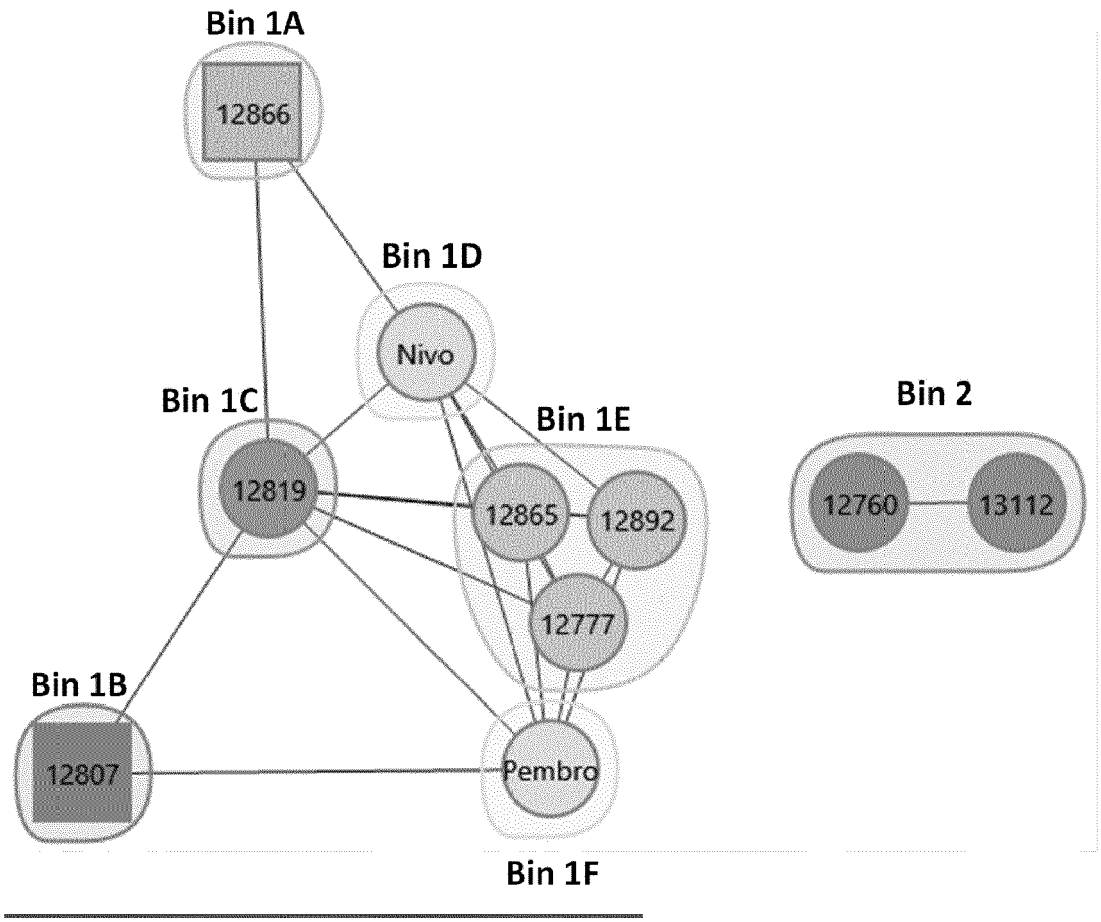

Bin 1A
12866
Bin 1D
Nivo
Bin 1C
Bin 1E
Bin 2
12819
12865
12892
12760
13112
12777
Bin 1B
12807
Pembro
Bin 1F

Figure 9

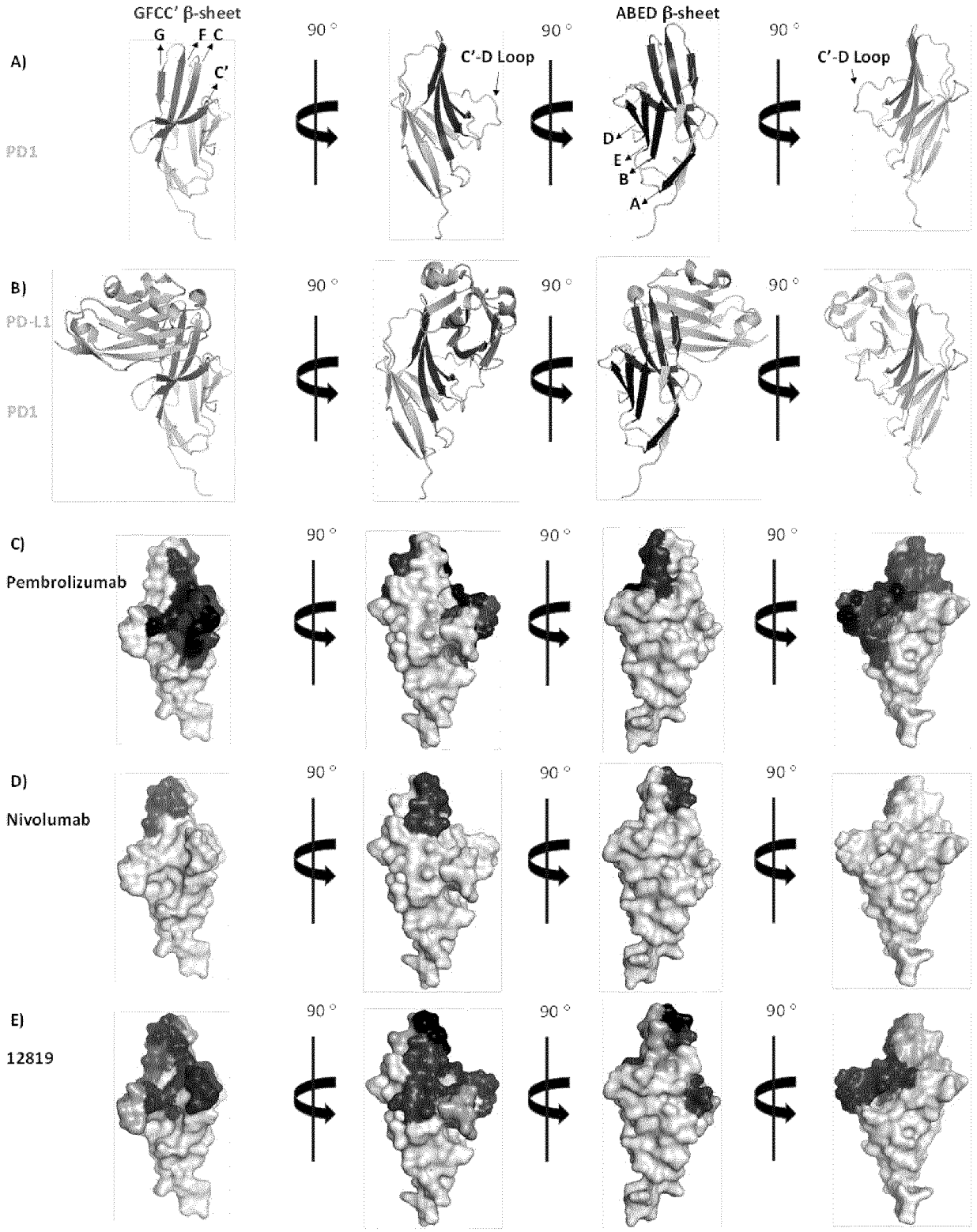

GFCC' β-sheet
G
F
C
C'
ABED β-sheet
A)
PD1
90 °
C'-D Loop
D
E
B
A
B)
PD-L1
PD1
C)
Pembrolizumab
D)
Nivolumab
E)
12819

Figure 9 (cont.)

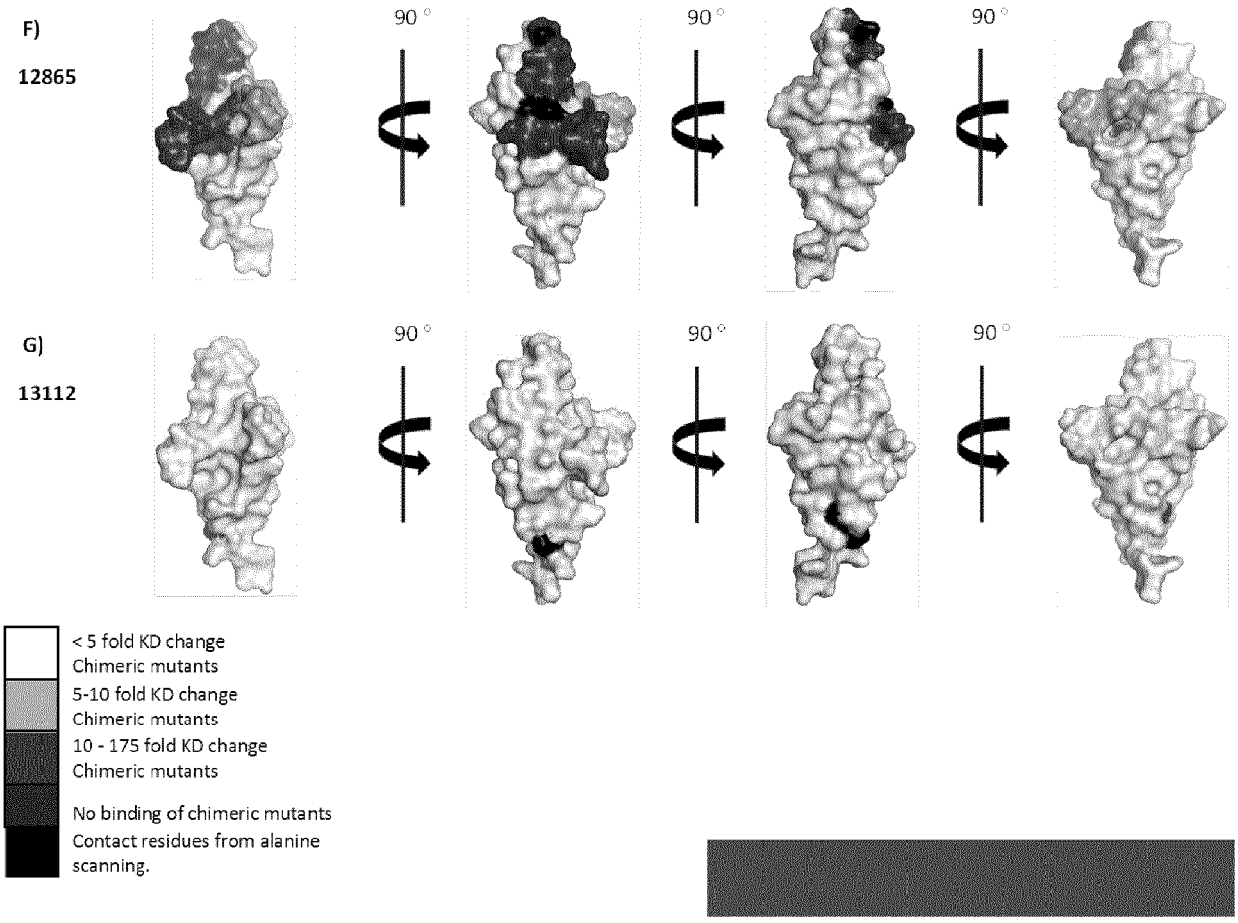


Figure 10

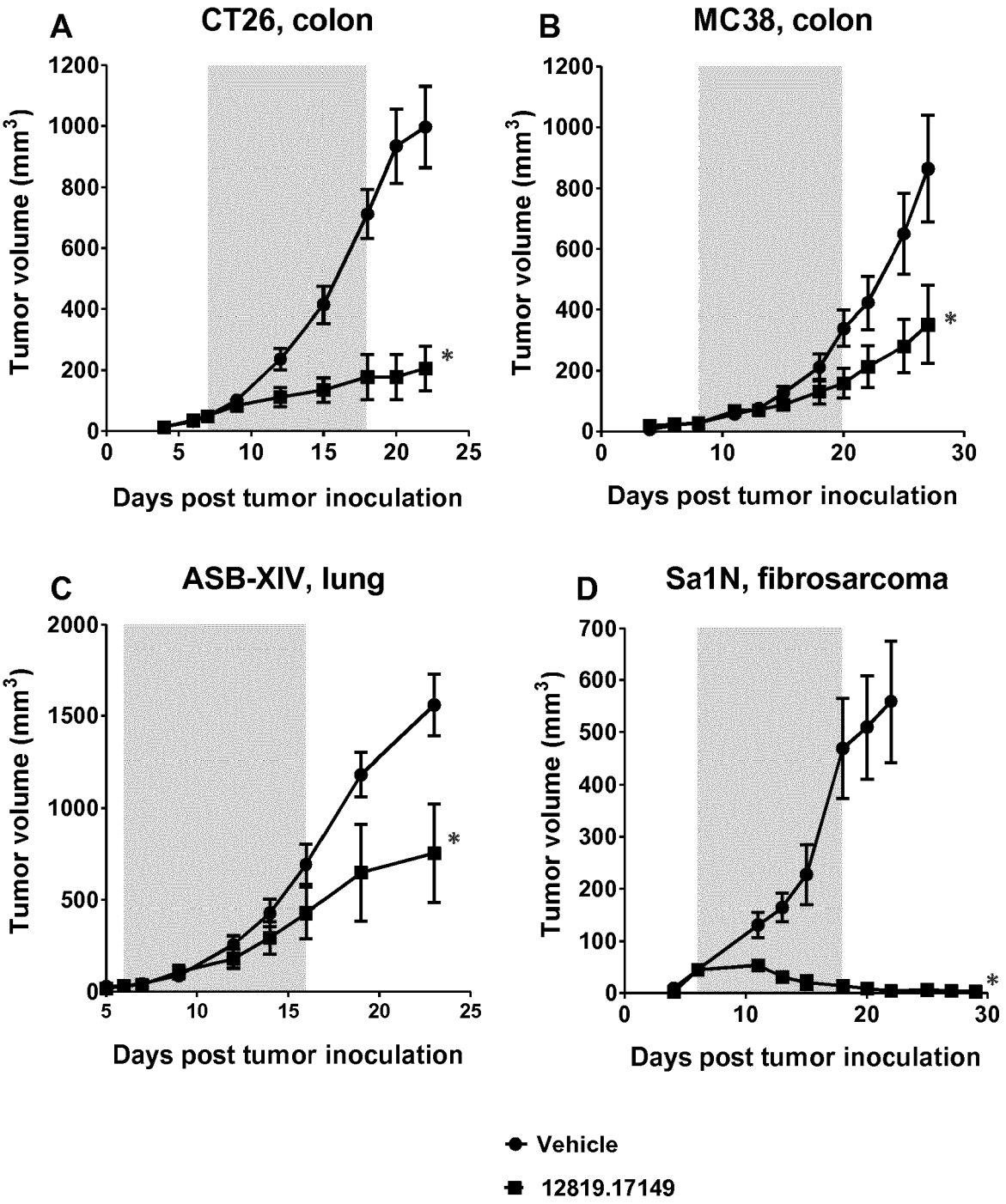
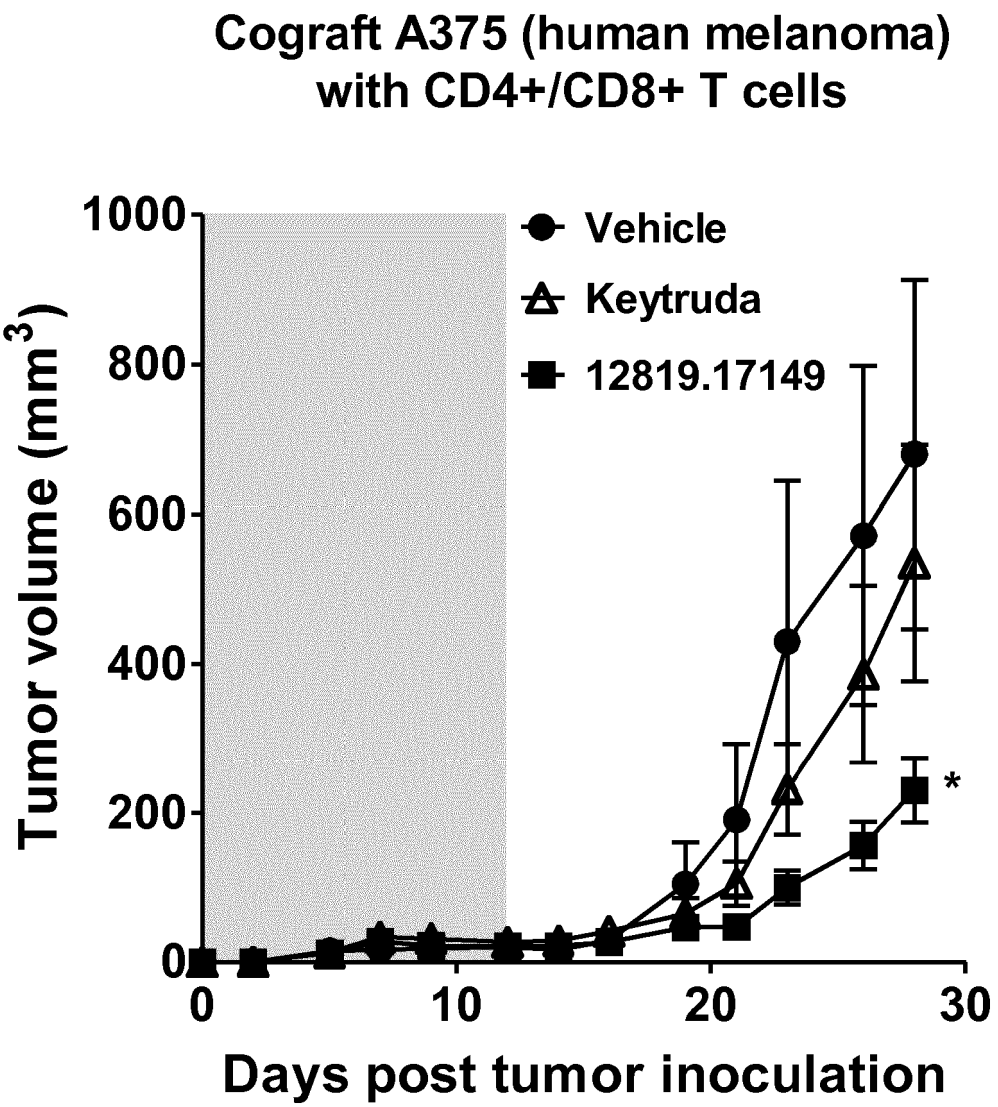

A
CT26, colon
Tumor volume (mm3)
Days post tumor inoculation
B
MC38, colon
Tumor volume (mm3)
Days post tumor inoculation
C
ASB-XIV, lung
Tumor volume (mm3)
Days post tumor inoculation
D
Sa1N, fibrosarcoma
Tumor volume (mm3)
Days post tumor inoculation
Vehicle
12819.17149

Figure 11


Cograft A375 (human melanoma)
with CD4+/CD8+ T cells
Vehicle
Keytruda
12819.17149
Tumor volume (mm3)
1000
800
600
400
200
0
0
10
20
30
Days post tumor inoculation
*

REFERENCES CITED IN THE DESCRIPTION

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Patent documents cited in the description

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