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(71) Applicant(s)
Les Laboratoires Servier

(72) Inventor(s)
Lantto, Johan;Bouquin, Thomas;Koefoed, Klaus;Gjetting, Torben;Bhatia, Vikram Kjoller;Gad, Monika;Galler, Gunther Roland;Frohlich, Camilla

(74) Agent / Attorney
Spruson & Ferguson, GPO Box 3898, Sydney, NSW, 2001, AU

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(71) Applicant: **SYMPHOGEN A/S** [DK/DK]; Pederstrupvej 93, 2750 Ballerup (DK).

(72) Inventors: **LANTTO, Johan**; Bryggaregaten 4, 227 36 Lund (SE). **BOUQUIN, Thomas**; Kirkeltevej 44, 3450 Allerød (DK). **KOEFOED, Klaus**; Havneholmen 24, 1. tv, 1561 København V (DK). **GJETTING, Torben**; Vibevej 7, 4040 Jyllinge (DK). **BHATIA, Vikram Kjoller**; Hovmarksvej 31, 2920 Charlottenlund (DK). **GAD, Monika**; Gravenstensvej 8, 3450 Allerød (DK). **GALLER, Gunther Roland**; Solsikkevej 17, 4040 Jyllinge (DK). **FROHLICH, Camilla**; Nøjsomhedsvej 13, 2. th, 2100 København Ø (DK).

(74) Agent: **POHLMAN, Sandra**; Theatinerstraße 16, 80333 München (DE).

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(54) Title: ANTI-PD-1 ANTIBODIES AND COMPOSITIONS

(57) Abstract: This invention relates to anti-PD-1 antibodies and methods of using them in treating diseases and conditions related to PD-1activity, e.g.,cancer.

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ANTI-PD-1 ANTIBODIES AND COMPOSITIONS

CROSS-REFERENCE TO RELATED APPLICATION

[0001] This application claims priority from United States Provisional Patent Application 62/424,163, filed November 18, 2016, whose disclosure is incorporated by reference herein in its entirety.

SEQUENCE LISTING

[0002] The instant application contains a Sequence Listing which has been submitted electronically in ASCII format and is hereby incorporated by reference in its entirety. The electronic copy of the Sequence Listing, created on November 13, 2017, is named 022675_WO056_SL.txt and is 99,158 bytes in size.

BACKGROUND OF THE INVENTION

[0003] 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).

[0004] 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.

[0005] 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.

[0006] 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.

[0001] 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 receptor to treat cancers and certain disorders of the immune system.

SUMMARY OF THE INVENTION

[0002] In a first aspect, the present invention provides an anti-PD-1 antibody or an antigen-binding portion thereof, wherein said antibody comprises H-CDR1-3 and L-CDR1-3 amino acid sequences of SEQ ID NOs: 52, 53, 54, 38, 45, and 55, respectively.

[0003a] In a second aspect, the present invention provides an anti-PD-1 antibody or an antigen-binding portion thereof, wherein said antibody comprises a heavy chain variable domain (VH) and a light chain variable domain (VL) that comprise:

a) SEQ ID NOs: 9 and 10, respectively;
b) SEQ ID NOs: 78 and 10, respectively;
c) SEQ ID NOs: 99 and 10, respectively;
d) SEQ ID NOs: 9 and 88, respectively;
e) SEQ ID NOs: 78 and 88, respectively;
f) SEQ ID NOs: 99 and 88, respectively;
g) SEQ ID NOs: 19 and 20, respectively;
h) SEQ ID NOs: 82 and 20, respectively;
i) SEQ ID NOs: 103 and 20, respectively;
j) SEQ ID NOs: 19 and 93, respectively;
k) SEQ ID NOs: 82 and 93, respectively;
l) SEQ ID NOs: 103 and 93, respectively;
m) SEQ ID NOs: 19 and 112, respectively;
n) SEQ ID NOs: 82 and 112, respectively; or
o) SEQ ID NOs: 103 and 112, respectively.

[0004b] In a third aspect, the present invention provides an anti-PD-1 antibody that comprises:

a) a heavy chain (HC) with the amino acid sequences of SEQ ID NOs: 9 and 26 and a light chain (LC) with the amino acid sequences of SEQ ID NOs: 10 and 28;

b) an HC with the amino acid sequences of SEQ ID NOs: 78 and 26 and an LC with the amino acid sequences of SEQ ID NOs: 10 and 28;
c) an HC with the amino acid sequences of SEQ ID NOs: 99 and 26 and an LC with the amino acid sequences of SEQ ID NOs: 10 and 28;
d) an HC with the amino acid sequences of SEQ ID NOs: 9 and 26 and an LC with the amino acid sequences of SEQ ID NOs: 88 and 28;
e) an HC with the amino acid sequences of SEQ ID NOs: 78 and 26 and an LC with the amino acid sequences of SEQ ID NOs: 88 and 28; or
f) an HC with the amino acid sequences of SEQ ID NOs: 99 and 26 and an LC with the amino acid sequences of SEQ ID NOs: 88 and 28;
g) an HC with the amino acid sequences of SEQ ID NOs: 19 and 26 and an LC with the amino acid sequences of SEQ ID NOs: 20 and 28;
h) an HC with the amino acid sequences of SEQ ID NOs: 82 and 26 and an LC with the amino acid sequences of SEQ ID NOs: 20 and 28;
i) an HC with the amino acid sequences of SEQ ID NOs: 103 and 26 and an LC with the amino acid sequences of SEQ ID NOs: 20 and 28;
j) an HC with the amino acid sequences of SEQ ID NOs: 19 and 26 and an LC with the amino acid sequences of SEQ ID NOs: 93 and 28;
k) an HC with the amino acid sequences of SEQ ID NOs: 82 and 26 and an LC with the amino acid sequences of SEQ ID NOs: 93 and 28;
l) an HC with the amino acid sequences of SEQ ID NOs: 103 and 26 and an LC with the amino acid sequences of SEQ ID NOs: 93 and 28;
m) an HC with the amino acid sequences of SEQ ID NOs: 19 and 26 and an LC with the amino acid sequences of SEQ ID NOs: 112 and 28;
n) an HC with the amino acid sequences of SEQ ID NOs: 82 and 26 and an LC with the amino acid sequences of SEQ ID NOs: 112 and 28; or
o) an HC with the amino acid sequences of SEQ ID NOs: 103 and 26 and an LC with the amino acid sequences of SEQ ID NOs: 112 and 28.

[0008c] In a fourth aspect, the present invention provides a pharmaceutical composition comprising the anti-PD-1 antibody according to any one of the first to third aspects or antigen-binding portion according to the first or second aspect and a pharmaceutically acceptable excipient.

[0008d] In a fifth aspect, the present invention provides an isolated nucleic acid molecule(s) comprising a nucleotide sequence that encodes the heavy chain

sequence, and a nucleotide sequence that encodes the light chain sequence, of the anti-PD-1 antibody of any one of any one of the first to third aspects or antigen-binding portion of the first or second aspect.

[0008e] In a sixth aspect, the present invention provides vector(s) comprising the isolated nucleic acid molecule(s) of the fifth aspect, wherein said vector(s) further comprise an expression control sequence.

[0008f] In a seventh aspect, the present invention provides a host cell comprising a nucleotide sequence that encodes the heavy chain sequence, and a nucleotide sequence that encodes the light chain sequence, of the anti-PD-1 antibody of any one of the first to third aspects or antigen-binding portion of the first or second aspect.

[0008g] In an eighth aspect, the present invention provides a method for producing the antibody of any one of the first to third aspects or antigen-binding portion of the first or second aspect, comprising providing the host cell according to the seventh aspect, cultivating said host cell under conditions suitable for expression of the antibody or portion, and isolating the resulting antibody or portion.

[0008h] In a ninth aspect, the present invention provides a bi-specific binding molecule comprising the binding domain of the anti-PD-1 antibody of any one of the first to third aspects and the binding domain of another, distinct antibody.

[0008i] In a tenth aspect, the present invention provides a method for enhancing immunity in a patient in need thereof, comprising administering to said patient the anti-PD-1 antibody of any one of the first to third aspects or antigen-binding portion of the first or second aspects, the pharmaceutical composition of the fourth aspect, or the bi-specific binding molecule of the ninth aspect.

[0008j] In an eleventh aspect, the present invention provides a use of the anti-PD-1 antibody of any one of the first to third aspects or antigen-binding portion of the first or second aspects, the pharmaceutical composition of the fourth aspect, or the bi-specific binding molecule of the ninth aspect, for the manufacture of a medicament for enhancing immunity in a patient.

[0008k] In a twelfth aspect, the present invention provides a method for treating cancer in a patient, comprising administering to said patient the antibody of any one of the first to third aspects or antigen-binding portion of the first or second aspects, the pharmaceutical composition of the fourth aspect, or the bi-specific binding molecule of the ninth aspect.

[0008l] In a thirteenth aspect, the present invention provides use of the anti-PD-1 antibody of any one of the first to third aspects or antigen-binding portion of the first or second aspects, the pharmaceutical composition of the fourth aspect, or the bi-specific binding molecule of the ninth aspect, for the manufacture of a medicament for treating cancer in a patient.

[0008m] The present invention is directed to novel recombinant antibodies targeting PD-1, 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, colon, ovary, brain, prostate, kidney, soft tissues, the hematopoietic system, head and 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.

[0005] In one embodiment, the present invention provides an anti-PD-1 antibody or an antigen-binding portion thereof, wherein the antibody competes for binding to human PD-1 with, or binds to the same epitope of human PD-1 as, any one of antibodies 18040, 18049, 18098, 18113, 18201, 18247, 18250, 18325, 18366, 18400, 18413 and 18483.

[0006] In one embodiment, the anti-PD-1 antibody competes for binding to human PD-1 with an antibody whose heavy chain (H) CDR1-3 and light chain (L) CDR1-3 are the same as or derived from the H-CDR1-3 and L-CDR1-3 of antibody 18040, 18049, 18098, 18113, 18201, 18247, 18250, 18325, 18366, 18400, 18413, or 18483.

[0007] In one embodiment, the anti-PD-1 antibody binds to the same epitope of human PD-1 as an antibody whose heavy chain (H) CDR1-3 and light chain (L) CDR1-3 are the same as or derived from the H-CDR1-3 and L-CDR1-3 of antibody 18040, 18049, 18098, 18113, 18201, 18247, 18250, 18325, 18366, 18400, 18413, or 18483.

[0012] In one embodiment, the anti-PD-1 antibody comprises H-CDR1-3 comprising the H-CDR1-3 amino acid sequences, respectively, of antibody 18040, 18049, 18098, 18113, 18201, 18247, 18250, 18325, 18366, 18400, 18413, or 18483.

[0013] In one embodiment, the anti-PD-1 antibody has a heavy chain variable domain (VH) 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 VH domain of antibody 18040, 18049, 18098, 18113, 18201, 18247, 18250, 18325, 18366, 18400, 18413, or 18483.

[0014] In one embodiment, the anti-PD-1 antibody has a VH that comprises the VH amino acid sequence of antibody 18040, 18049, 18098, 18113, 18201, 18247, 18250, 18325, 18366, 18400, 18413, or 18483.

[0015] In one embodiment, the anti-PD-1 antibody has a heavy chain (HC) that comprises the VH amino acid sequence of antibody 18040, 18049, 18098, 18113, 18201, 18247, 18250, 18325, 18366, 18400, 18413, or 18483, and the heavy chain constant region amino acid sequence of SEQ ID NO: 26.

[0016] In one embodiment, the anti-PD-1 antibody comprises L-CDR1-3 comprising the L-CDR1-3 amino acid sequences, respectively, of antibody 18040, 18049, 18098, 18113, 18201, 18247, 18250, 18325, 18366, 18400, 18413, or 18483.

[0017] In one embodiment, the anti-PD-1 antibody has a light chain variable domain (VL) 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 VL domain of antibody 18040, 18049, 18098, 18113, 18201, 18247, 18250, 18325, 18366, 18400, 18413, or 18483.

[0018] In one embodiment, the anti-PD-1 antibody has a VL that comprises the VL amino acid sequence of antibody 18040, 18049, 18098, 18113, 18201, 18247, 18250, 18325, 18366, 18400, 18413, or 18483.

[0019] In one embodiment, the anti-PD-1 antibody has a light chain (LC) that comprises the VL amino acid sequence of antibody 18040, 18049, 18098, 18113, 18201, 18247, 18250, 18325, 18366, 18400, 18413, or 18483, and the light chain constant region amino acid sequence of SEQ ID NO: 28.

[0020] In one embodiment, the anti-PD-1 antibody comprises the H-CDR3 and L-CDR3 amino acid sequences of antibody 18040, 18049, 18098, 18113, 18201, 18247, 18250, 18325, 18366, 18400, 18413, or 18483.

[0021] In one embodiment, the anti-PD-1 antibody comprises the H-CDR1-3 and L-CDR1-3 amino acid sequences of antibody 18040, 18049, 18098, 18113, 18201, 18247, 18250, 18325, 18366, 18400, 18413, or 18483.

[0022] In one embodiment, the anti-PD-1 antibody has a VH and a VL 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 VH and VL, respectively, of antibody 18040, 18049, 18098, 18113, 18201, 18247, 18250, 18325, 18366, 18400, 18413, or 18483.

[0023] In one embodiment, the anti-PD-1 antibody has a VH and a VL that comprise or consist of the VH and VL amino acid sequences, respectively, of antibody 18040, 18049, 18098, 18113, 18201, 18247, 18250, 18325, 18366, 18400, 18413, or 18483.

[0024] In one embodiment, 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 18040, 18049, 18098, 18113, 18201, 18247, 18250, 18325, 18366, 18400, 18413, or 18483.

[0025] In one embodiment, the anti-PD-1 antibody has (1) an HC that comprises the VH amino acid sequence of an antibody selected from the group consisting of antibody 18040, 18049, 18098, 18113, 18201, 18247, 18250, 18325, 18366, 18400, 18413, and 18483, and the heavy chain constant region amino acid sequence of SEQ ID NO: 26; and (2) an LC that comprises the VL amino acid sequence of that selected antibody and the light chain constant region amino acid sequence of SEQ ID NO: 28.

[0026] In certain embodiments, the anti-PD-1 antibody or antigen-binding portion of the invention has at least one of the following properties:

a) binds to cynomolgus PD-1 with a K_D of, for example, 4×10^{-8} M or less;

b) binds to mouse PD-1 with a K_D of, for example, 2×10^{-8} M or less;

c) binds to human PD-1 with a K_D of 3×10^{-9} M or less;

d) inhibits the interaction of PD-1 with PD-L1 at a concentration of 10 μg/ml;

e) stimulates IL-2 production in an SEB whole blood assay; and

f) stimulates IFN-γ production in a one-way mixed lymphocyte reaction assay.

Examples of such an antibody include, without limitation, antibodies 18040, 18049, 18098, 18113, 18247, 18250, 18325, 18366, 18413, and 18483 (having properties a) and c)-f)) and antibodies 18201 and 18400 (having properties a)-f)). In some embodiments, the anti-PD-1 antibody or antigen-binding portion of the invention has all of said properties.

[0027] In some embodiments, the anti-PD-1 antibody or antigen-binding portion of the invention competes for binding to human PD-1 with antibody 18366, 18250,

18040, 18247, 18113, and/or 18483. In some embodiments, the anti-PD-1 antibody or antigen-binding portion of the invention competes for binding to human PD-1 with antibody 12760 and/or 13112.

[0028] In certain 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 certain embodiments, the anti-PD-1 antibody or antigen-binding portion of the invention does not bind to the same epitope as pembrolizumab or nivolumab.

[0029] In another aspect, the present invention provides pharmaceutical compositions comprising at least one anti-PD-1 antibody or antigen-binding portion thereof as described herein and a pharmaceutically acceptable excipient, optionally with an additional therapeutic, such as an anti-cancer antibody therapeutic.

[0030] 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 as described herein.

[0031] The present invention also provides vectors comprising such an isolated nucleic acid molecule, wherein said vector may further comprise an expression control sequence.

[0032] The present invention also provides 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 as described herein.

[0033] The present invention also provides a method for producing an antibody or antigen-binding portion thereof as described herein, 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 an anti-PD-1 antibody as described herein, culturing said host cell under conditions suitable for expression of the antibody or portion, and isolating the resulting antibody or portion.

[0034] The present invention also provides a multi-specific (e.g., bi-specific) binding molecule having the binding specificity of an anti-PD-1 antibody described herein and the binding specificity of another, distinct antibody such as another anti-

PD-1 antibody (e.g., as 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. In some embodiments, the multi-specific (e.g., bi-specific) binding molecule comprises the antigen-binding portions (e.g., the six CDRs) of the anti-PD-1 antibody and the other antibody.

[0035] The present invention also provides a method for enhancing immunity 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 bi-specific binding molecule as described herein.

[0036] The present invention further provides 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 bi-specific binding molecule as described herein. In some embodiments, the cancer originates in a tissue selected from skin, lung, intestine, colon, ovary, brain, prostate, kidney, soft tissues, hematopoietic system, head and 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, bladder cancer, gastric cancer, renal cell carcinoma, hepatocellular carcinoma, colorectal cancer, or Hodgkin's lymphoma.

[0037] Any of the above methods may further comprise administration of, e.g., a chemotherapeutic agent, an anti-neoplastic agent, an anti-angiogenic agent, a tyrosine kinase inhibitor, a PD-1 pathway inhibitor, or radiation therapy.

[0038] The present invention further provides the use of an antibody composition comprising an anti-PD-1 antibody or antigen-binding portion as described herein for the manufacture of a medicament for treating cancer in a patient and/or enhancing immunity in a patient.

[0039] The present invention further provides an anti-PD-1 antibody or antigen-binding portion as described herein for use in treating cancer in a patient and/or enhancing immunity in a patient, e.g., in a treatment method described herein.

[0040] The present invention further provides an article of manufacture comprising an anti-PD-1 antibody or antigen-binding portion as described herein, wherein said article of manufacture is suitable for treating cancer in a patient and/or enhancing immunity in a patient, e.g., in a treatment method described herein.

BRIEF DESCRIPTION OF THE DRAWINGS

[0041] Figures 1A-1D show representative flow cytometry dot plots for four anti-PD-1 antibody clones exhibiting different reactivity towards PD-1 orthologs.

[0042] Figures 2A-2H show blocking of PD-L1-binding to cell-expressed PD-1 for anti-PD-1 antibodies of the invention.

[0043] Figures 3A-3F show dose-response curves of twelve anti-PD-1 antibodies in the SEB whole blood assay.

[0044] Figures 4A-4F show dose-response curves of twelve anti-PD-1 antibodies in the MLR (one-way mixed lymphocyte reaction) assay.

[0045] Figure 5 shows an overview of the identified epitope groups (epitope bins) for tested anti-PD-1 antibodies 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.

DETAILED DESCRIPTION OF THE INVENTION

[0046] The present invention provides new anti-human PD-1 antibodies 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).

[0047] 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 (VH) and a heavy chain constant region (CH). Each light chain is composed of a light chain variable domain (VL) and a light chain constant region (CL). The VH and VL 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 VH and VL 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).

[0048] 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.

[0049] 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.

[0050] 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.

[0051] 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 (SPR) (BIAcore™) or Bio-Layer Interferometry, for example using the IBIS MX96 SPR system from IBIS

Technologies, the ProteOn™ XPR36 SPR system from Bio-Rad, or the Octet™ system from ForteBio.

[0052] 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 SPR or Bio-Layer Interferometry, for example using one of the systems listed above.

[0053] 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 bi-specific 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 (e.g., PD-1) or a relevant portion thereof, then screening for binding to the epitope.

[0054] 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 scanning. In some embodiments, the test antibody and an anti-PD-1 antibody of the invention bind to at least one common residue (e.g., at least two, three, four, five, or six common residues) on PD-1. In further embodiments, the contact residues on PD-1 are completely identical between the test antibody and the anti-PD-1 antibody of the invention. In one embodiment, one allows 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, e.g., ELISA, RIA, BIACORE™, SPR, 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.

[0055] 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).

[0056] In some embodiments, the antibodies of the invention may be chimeric, humanized, or fully human. Although it is not possible to precisely predict the immunogenicity of a particular antibody drug, 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. The trend in therapeutic antibodies is towards humanized or fully human antibodies.

[0057] The term "chimeric antibody" refers to an antibody that comprises sequences from two different animal species. For example, a chimeric antibody may contain the variable domains of a murine antibody (i.e., an antibody encoded by murine antibody genes such as an antibody obtained from an immunized mouse

using hybridoma technology) linked to the constant regions of an antibody from another species (e.g., human, rabbit, or rat). In the case of a chimeric antibody, the non-human parts may be subjected to further alteration in order to humanize the antibody.

[0058] The term “humanize” refers to modifying an antibody that 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), by replacing certain amino acid sequences, in particular in the framework regions (FR) and constant regions of the heavy and light chains, with corresponding human FR and constant region amino aicd sequences, in order to avoid or minimize an anti-drug antibody response in human patients. Antibodies of non-human origin thus can be humanized to reduce the risk of a human anti-drug antibody response.

[0059] The term "human antibody" refers to an antibody in which the variable domain and constant region sequences are derived from human sequences. The term encompasses antibodies with sequences that are derived from human genes but have been modified, e.g., to decrease immunogenicity, increase affinity, and/or increase stability. Further, the term encompasses antibodies produced recombinantly in nonhuman cells, which may impart glycosylation not typical of human cells. The term also encompasses antibodies produced in transgenic nonhuman organisms with human antibody genes (e.g., OmniRat® rats).

[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 VL, VH, CL and CH1 domains; (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 VH and CH1 domains; (iv) a Fv fragment consisting of the VL and VH domains of a single arm of an antibody, (v) a dAb fragment, which consists of a VH 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, VL and VH, 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 VL and VH domains pair to form monovalent molecules (known as single chain Fv (scFv)). Also within the invention are antigen-binding molecules comprising a VH and/or a VL. In the case of a VH, 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, bi-specific antibodies in which VH and VL 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. 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

[0064] The present invention provides antibodies directed against PD-1, and antigen-binding portions thereof. In a particular embodiment, the antibodies disclosed herein are human antibodies, e.g., generated from transgenic rats with human antibody genes. In certain embodiments, the human antibodies may contain certain mutations, e.g., to mutate primer-derived mutations back to the germline sequence (see, e.g., the "Symplex-corrected" variant sequences below) or to change mutations in framework regions back to the sequence of the closest V- or J-germline (see, e.g., the "germlined" variant sequences below).

[0065] In one embodiment, 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: 29-31, respectively;

b) an antibody whose heavy chain variable domain (VH) is at least 90% identical in sequence to the amino acid sequence of SEQ ID NO: 1;

c) an antibody whose VH comprises the amino acid sequence of SEQ ID NO: 1;

d) an antibody whose heavy chain (HC) comprises the amino acid sequences of SEQ ID NOs: 1 and 26;

e) an antibody whose L-CDR1-3 comprise the amino acid sequences of SEQ ID NOs: 32-34, respectively;

f) an antibody whose light chain variable domain (VL) is at least 90% identical in sequence to the amino acid sequence of SEQ ID NO: 2;

g) an antibody whose VL comprises the amino acid sequence of SEQ ID NO: 2;

h) an antibody whose light chain (LC) comprises the amino acid sequences of SEQ ID NOs: 2 and 28;

i) an antibody whose H-CDR1-3 and L-CDR1-3 comprise the amino acid sequences of SEQ ID NOs: 29-34, respectively;

j) an antibody whose VH is at least 90% identical in sequence to the amino acid sequence of SEQ ID NO: 1 and whose VL is at least 90% identical in sequence to the amino acid sequence of SEQ ID NO: 2;

k) an antibody whose VH comprises the amino acid sequence of SEQ ID NO: 1 and whose VL comprises the amino acid sequence of SEQ ID NO: 2; and

l) an antibody whose HC comprises the amino acid sequences of SEQ ID NOs: 1 and 26 and whose LC comprises the amino acid sequences of SEQ ID NOs: 2 and 28.

[0066] In one embodiment, 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: 35-37, respectively;

b) an antibody whose heavy chain variable domain (VH) is at least 90% identical in sequence to the amino acid sequence of SEQ ID NO: 3;

c) an antibody whose VH comprises the amino acid sequence of SEQ ID NO: 3;

d) an antibody whose heavy chain (HC) comprises the amino acid sequences of SEQ ID NOs: 3 and 26;

e) an antibody whose L-CDR1-3 comprise the amino acid sequences of SEQ ID NOs: 38-40, respectively;

f) an antibody whose light chain variable domain (VL) is at least 90% identical in sequence to the amino acid sequence of SEQ ID NO: 4;

g) an antibody whose VL comprises the amino acid sequence of SEQ ID NO: 4;

h) an antibody whose light chain (LC) comprises the amino acid sequences of SEQ ID NOs: 4 and 28;

i) an antibody whose H-CDR1-3 and L-CDR1-3 comprise the amino acid sequences of SEQ ID NOs: 35-40, respectively;

j) an antibody whose VH is at least 90% identical in sequence to the amino acid sequence of SEQ ID NO: 3 and whose VL is at least 90% identical in sequence to the amino acid sequence of SEQ ID NO: 4;

k) an antibody whose VH comprises the amino acid sequence of SEQ ID NO: 3 and whose VL comprises the amino acid sequence of SEQ ID NO: 4; and

l) an antibody whose HC comprises the amino acid sequences of SEQ ID NOs: 3 and 26 and whose LC comprises the amino acid sequences of SEQ ID NOs: 4 and 28.

[0067] In one embodiment, 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: 41-43, respectively;

b) an antibody whose heavy chain variable domain (VH) is at least 90% identical in sequence to the amino acid sequence of SEQ ID NO: 5;

c) an antibody whose VH comprises the amino acid sequence of SEQ ID NO: 5;

d) an antibody whose heavy chain (HC) comprises the amino acid sequences of SEQ ID NOs: 5 and 26;

e) an antibody whose L-CDR1-3 comprise the amino acid sequences of SEQ ID NOs: 44-46, respectively;

f) an antibody whose light chain variable domain (VL) is at least 90% identical in sequence to the amino acid sequence of SEQ ID NO: 6;

g) an antibody whose VL comprises the amino acid sequence of SEQ ID NO: 6;

h) an antibody whose light chain (LC) comprises the amino acid sequences of SEQ ID NOs: 6 and 28;

i) an antibody whose H-CDR1-3 and L-CDR1-3 comprise the amino acid sequences of SEQ ID NOs: 41-46, respectively;

j) an antibody whose VH is at least 90% identical in sequence to the amino acid sequence of SEQ ID NO: 5 and whose VL is at least 90% identical in sequence to the amino acid sequence of SEQ ID NO: 6;

k) an antibody whose VH comprises the amino acid sequence of SEQ ID NO: 5 and whose VL comprises the amino acid sequence of SEQ ID NO: 6; and

l) an antibody whose HC comprises the amino acid sequences of SEQ ID NOs: 5 and 26 and whose LC comprises the amino acid sequences of SEQ ID NOs: 6 and 28.

[0068] In one embodiment, 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: 29, 47 and 48, respectively;

b) an antibody whose heavy chain variable domain (VH) is at least 90% identical in sequence to the amino acid sequence of SEQ ID NO: 7;

c) an antibody whose VH comprises the amino acid sequence of SEQ ID NO: 7;

d) an antibody whose heavy chain (HC) comprises the amino acid sequences of SEQ ID NOs: 7 and 26;

e) an antibody whose L-CDR1-3 comprise the amino acid sequences of SEQ ID NOs: 49-51, respectively;

f) an antibody whose light chain variable domain (VL) is at least 90% identical in sequence to the amino acid sequence of SEQ ID NO: 8;

g) an antibody whose VL comprises the amino acid sequence of SEQ ID NO: 8;

h) an antibody whose light chain (LC) comprises the amino acid sequences of SEQ ID NOs: 8 and 28;

i) an antibody whose H-CDR1-3 and L-CDR1-3 comprise the amino acid sequences of SEQ ID NOs: 29, 47, 48 and 49-51, respectively;

j) an antibody whose VH is at least 90% identical in sequence to the amino acid sequence of SEQ ID NO: 7 and whose VL is at least 90% identical in sequence to the amino acid sequence of SEQ ID NO: 8;

k) an antibody whose VH comprises the amino acid sequence of SEQ ID NO: 7 and whose VL comprises the amino acid sequence of SEQ ID NO: 8; and

l) an antibody whose HC comprises the amino acid sequences of SEQ ID NOs: 7 and 26 and whose LC comprises the amino acid sequences of SEQ ID NOs: 8 and 28.

[0069] In one embodiment, 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: 52-54, respectively;

b) an antibody whose heavy chain variable domain (VH) is at least 90% identical in sequence to the amino acid sequence of SEQ ID NO: 9;

c) an antibody whose VH comprises the amino acid sequence of SEQ ID NO: 9;

d) an antibody whose heavy chain (HC) comprises the amino acid sequences of SEQ ID NOs: 9 and 26;

e) an antibody whose L-CDR1-3 comprise the amino acid sequences of SEQ ID NOs: 38, 45 and 55, respectively;

f) an antibody whose light chain variable domain (VL) is at least 90% identical in sequence to the amino acid sequence of SEQ ID NO: 10;

g) an antibody whose VL comprises the amino acid sequence of SEQ ID NO: 10;

h) an antibody whose light chain (LC) comprises the amino acid sequences of SEQ ID NOs: 10 and 28;

i) an antibody whose H-CDR1-3 and L-CDR1-3 comprise the amino acid sequences of SEQ ID NOs: 52-54 and 38, 45 and 55, respectively;

j) an antibody whose VH is at least 90% identical in sequence to the amino acid sequence of SEQ ID NO: 9 and whose VL is at least 90% identical in sequence to the amino acid sequence of SEQ ID NO: 10;

k) an antibody whose VH comprises the amino acid sequence of SEQ ID NO: 9 and whose VL comprises the amino acid sequence of SEQ ID NO: 10; and

l) an antibody whose HC comprises the amino acid sequences of SEQ ID NOs: 9 and 26 and whose LC comprises the amino acid sequences of SEQ ID NOs: 10 and 28.

[0070] In one embodiment, 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: 29, 56 and 48, respectively;

b) an antibody whose heavy chain variable domain (VH) is at least 90% identical in sequence to the amino acid sequence of SEQ ID NO: 1;

c) an antibody whose VH comprises the amino acid sequence of SEQ ID NO: 11;

d) an antibody whose heavy chain (HC) comprises the amino acid sequences of SEQ ID NOs: 11 and 26;

e) an antibody whose L-CDR1-3 comprise the amino acid sequences of SEQ ID NOs: 57, 33 and 51, respectively;

f) an antibody whose light chain variable domain (VL) is at least 90% identical in sequence to the amino acid sequence of SEQ ID NO: 12;

g) an antibody whose VL comprises the amino acid sequence of SEQ ID NO: 12;

h) an antibody whose light chain (LC) comprises the amino acid sequences of SEQ ID NOs: 12 and 28;

i) an antibody whose H-CDR1-3 and L-CDR1-3 comprise the amino acid sequences of SEQ ID NOs: 29, 56 and 48 and 57, 33 and 51, respectively;

j) an antibody whose VH is at least 90% identical in sequence to the amino acid sequence of SEQ ID NO: 11 and whose VL is at least 90% identical in sequence to the amino acid sequence of SEQ ID NO: 12;

k) an antibody whose VH comprises the amino acid sequence of SEQ ID NO: 11 and whose VL comprises the amino acid sequence of SEQ ID NO: 12; and

l) an antibody whose HC comprises the amino acid sequences of SEQ ID NOs: 11 and 26 and whose LC comprises the amino acid sequences of SEQ ID NOs: 12 and 28.

[0071] In one embodiment, 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: 58-60, respectively;

b) an antibody whose heavy chain variable domain (VH) is at least 90% identical in sequence to the amino acid sequence of SEQ ID NO: 13;

c) an antibody whose VH comprises the amino acid sequence of SEQ ID NO: 13;

d) an antibody whose heavy chain (HC) comprises the amino acid sequences of SEQ ID NOs: 13 and 26;

e) an antibody whose L-CDR1-3 comprise the amino acid sequences of SEQ ID NOs: 57, 33 and 34, respectively;

f) an antibody whose light chain variable domain (VL) is at least 90% identical in sequence to the amino acid sequence of SEQ ID NO: 14;

g) an antibody whose VL comprises the amino acid sequence of SEQ ID NO: 14;

h) an antibody whose light chain (LC) comprises the amino acid sequences of SEQ ID NOs: 14 and 28;

i) an antibody whose H-CDR1-3 and L-CDR1-3 comprise the amino acid sequences of SEQ ID NOs: 58-60 and 57, 33 and 34, respectively;

j) an antibody whose VH is at least 90% identical in sequence to the amino acid sequence of SEQ ID NO: 13 and whose VL is at least 90% identical in sequence to the amino acid sequence of SEQ ID NO: 14;

k) an antibody whose VH comprises the amino acid sequence of SEQ ID NO: 13 and whose VL comprises the amino acid sequence of SEQ ID NO: 14; and

l) an antibody whose HC comprises the amino acid sequences of SEQ ID NOs: 13 and 26 and whose LC comprises the amino acid sequences of SEQ ID NOs: 14 and 28.

[0072] In one embodiment, 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: 61, 62 and 43, respectively;

b) an antibody whose heavy chain variable domain (VH) is at least 90% identical in sequence to the amino acid sequence of SEQ ID NO: 15;

c) an antibody whose VH comprises the amino acid sequence of SEQ ID NO: 15;

d) an antibody whose heavy chain (HC) comprises the amino acid sequences of SEQ ID NOs: 15 and 26;

e) an antibody whose L-CDR1-3 comprise the amino acid sequences of SEQ ID NOs: 44-46, respectively;

f) an antibody whose light chain variable domain (VL) is at least 90% identical in sequence to the amino acid sequence of SEQ ID NO: 16;

g) an antibody whose VL comprises the amino acid sequence of SEQ ID NO: 16;

h) an antibody whose light chain (LC) comprises the amino acid sequences of SEQ ID NOs: 16 and 28;

i) an antibody whose H-CDR1-3 and L-CDR1-3 comprise the amino acid sequences of SEQ ID NOs: 61, 62 and 43 and 44-46, respectively;

j) an antibody whose VH is at least 90% identical in sequence to the amino acid sequence of SEQ ID NO: 15 and whose VL is at least 90% identical in sequence to the amino acid sequence of SEQ ID NO: 16;

k) an antibody whose VH comprises the amino acid sequence of SEQ ID NO: 15 and whose VL comprises the amino acid sequence of SEQ ID NO: 16; and

l) an antibody whose HC comprises the amino acid sequences of SEQ ID NOs: 15 and 26 and whose LC comprises the amino acid sequences of SEQ ID NOs: 16 and 28.

[0073] In one embodiment, 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: 63-65, respectively;

b) an antibody whose heavy chain variable domain (VH) is at least 90% identical in sequence to the amino acid sequence of SEQ ID NO: 17;

c) an antibody whose VH comprises the amino acid sequence of SEQ ID NO: 17;

d) an antibody whose heavy chain (HC) comprises the amino acid sequences of SEQ ID NOs: 17 and 26;

e) an antibody whose L-CDR1-3 comprise the amino acid sequences of SEQ ID NOs: 32-34, respectively;

f) an antibody whose light chain variable domain (VL) is at least 90% identical in sequence to the amino acid sequence of SEQ ID NO: 18;

g) an antibody whose VL comprises the amino acid sequence of SEQ ID NO: 18;

h) an antibody whose light chain (LC) comprises the amino acid sequences of SEQ ID NOs: 18 and 28;

i) an antibody whose H-CDR1-3 and L-CDR1-3 comprise the amino acid sequences of SEQ ID NOs: 63-65 and 32-34, respectively;

j) an antibody whose VH is at least 90% identical in sequence to the amino acid sequence of SEQ ID NO: 17 and whose VL is at least 90% identical in sequence to the amino acid sequence of SEQ ID NO: 18;

k) an antibody whose VH comprises the amino acid sequence of SEQ ID NO: 17 and whose VL comprises the amino acid sequence of SEQ ID NO: 18; and

l) an antibody whose HC comprises the amino acid sequences of SEQ ID NOs: 17 and 26 and whose LC comprises the amino acid sequences of SEQ ID NOs: 18 and 28.

[0074] In one embodiment, 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: 66-68, respectively;

b) an antibody whose heavy chain variable domain (VH) is at least 90% identical in sequence to the amino acid sequence of SEQ ID NO: 19;

c) an antibody whose VH comprises the amino acid sequence of SEQ ID NO: 19;

d) an antibody whose heavy chain (HC) comprises the amino acid sequences of SEQ ID NOs: 19 and 26;

e) an antibody whose L-CDR1-3 comprise the amino acid sequences of SEQ ID NOs: 38, 45 and 55, respectively;

f) an antibody whose light chain variable domain (VL) is at least 90% identical in sequence to the amino acid sequence of SEQ ID NO: 20;

g) an antibody whose VL comprises the amino acid sequence of SEQ ID NO: 20;

h) an antibody whose light chain (LC) comprises the amino acid sequences of SEQ ID NOs: 20 and 28;

i) an antibody whose H-CDR1-3 and L-CDR1-3 comprise the amino acid sequences of SEQ ID NOs: 66-68 and 38, 45 and 55, respectively;

j) an antibody whose VH is at least 90% identical in sequence to the amino acid sequence of SEQ ID NO: 19 and whose VL is at least 90% identical in sequence to the amino acid sequence of SEQ ID NO: 20;

k) an antibody whose VH comprises the amino acid sequence of SEQ ID NO: 19 and whose VL comprises the amino acid sequence of SEQ ID NO: 20; and

l) an antibody whose HC comprises the amino acid sequences of SEQ ID NOs: 19 and 26 and whose LC comprises the amino acid sequences of SEQ ID NOs: 20 and 28.

[0075] In one embodiment, 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: 69-71, respectively;

b) an antibody whose heavy chain variable domain (VH) is at least 90% identical in sequence to the amino acid sequence of SEQ ID NO: 21;

c) an antibody whose VH comprises the amino acid sequence of SEQ ID NO: 21;

d) an antibody whose heavy chain (HC) comprises the amino acid sequences of SEQ ID NOs: 21 and 26;

e) an antibody whose L-CDR1-3 comprise the amino acid sequences of SEQ ID NOs: 72, 45 and 73, respectively;

f) an antibody whose light chain variable domain (VL) is at least 90% identical in sequence to the amino acid sequence of SEQ ID NO: 22;

g) an antibody whose VL comprises the amino acid sequence of SEQ ID NO: 22;

h) an antibody whose light chain (LC) comprises the amino acid sequences of SEQ ID NOs: 22 and 28;

i) an antibody whose H-CDR1-3 and L-CDR1-3 comprise the amino acid sequences of SEQ ID NOs: 69-71 and 72, 45 and 73, respectively;

j) an antibody whose VH is at least 90% identical in sequence to the amino acid sequence of SEQ ID NO: 21 and whose VL is at least 90% identical in sequence to the amino acid sequence of SEQ ID NO: 22;

k) an antibody whose VH comprises the amino acid sequence of SEQ ID NO: 21 and whose VL comprises the amino acid sequence of SEQ ID NO: 22; and

l) an antibody whose HC comprises the amino acid sequences of SEQ ID NOs: 21 and 26 and whose LC comprises the amino acid sequences of SEQ ID NOs: 22 and 28.

[0076] In one embodiment, 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: 74-76, respectively;

b) an antibody whose heavy chain variable domain (VH) is at least 90% identical in sequence to the amino acid sequence of SEQ ID NO: 23;

c) an antibody whose VH comprises the amino acid sequence of SEQ ID NO: 23;

d) an antibody whose heavy chain (HC) comprises the amino acid sequences of SEQ ID NOs: 23 and 26;

e) an antibody whose L-CDR1-3 comprise the amino acid sequences of SEQ ID NOs: 57, 33 and 34, respectively;

f) an antibody whose light chain variable domain (VL) is at least 90% identical in sequence to the amino acid sequence of SEQ ID NO: 24;

g) an antibody whose VL comprises the amino acid sequence of SEQ ID NO: 24;

h) an antibody whose light chain (LC) comprises the amino acid sequences of SEQ ID NOs: 24 and 28;

i) an antibody whose H-CDR1-3 and L-CDR1-3 comprise the amino acid sequences of SEQ ID NOs: 74-76 and 57, 33 and 34, respectively;

j) an antibody whose VH is at least 90% identical in sequence to the amino acid sequence of SEQ ID NO: 23 and whose VL is at least 90% identical in sequence to the amino acid sequence of SEQ ID NO: 24;

k) an antibody whose VH comprises the amino acid sequence of SEQ ID NO: 23 and whose VL comprises the amino acid sequence of SEQ ID NO: 24; and

l) an antibody whose HC comprises the amino acid sequences of SEQ ID NOs: 23 and 26 and whose LC comprises the amino acid sequences of SEQ ID NOs: 24 and 28.

[0077] In other embodiments, the anti-PD-1 antibody has a VH and VL that are at least 90% identical in amino acid sequence to the the VH and VL, respectively, of any one of antibodies 18040, 18049, 18098, 18113, 18201, 18247, 18250, 18325, 18366, 18400, 18413 and 18483, e.g., at least 92%, 95%, 96%, 97%, 98%, or 99%

identical in sequence to the VH and VL of any of said antibodies. In some embodiments, an antigen-binding portion of the anti-PD-1 antibody has said VH and VL.

[0078] In some embodiments, the anti-PD-1 antibody has a VH and VL that comprise the VH and VL amino acid sequences, respectively, of any one of antibodies 18040, 18049, 18098, 18113, 18201, 18247, 18250, 18325, 18366, 18400, 18413 and 18483. In some embodiments, an antigen-binding portion of the anti-PD-1 antibody has said VH and VL.

[0079] In some embodiments, the anti-PD-1 antibody or antigen-binding portion of the invention comprises the H-CDR1-3 and L-CDR1-3 amino acid sequences of:

a) SEQ ID NOs: 29, 30, 31, 32, 33, and 34, respectively;
b) SEQ ID NOs: 35, 36, 37, 38, 39, and 40, respectively;
c) SEQ ID NOs: 41, 42, 43, 44, 45, and 46, respectively;
d) SEQ ID NOs: 29, 47, 48, 49, 50, and 51, respectively;
e) SEQ ID NOs: 52, 53, 54, 38, 45, and 55, respectively;
f) SEQ ID NOs: 29, 56, 48, 57, 33, and 51, respectively;
g) SEQ ID NOs: 58, 59, 60, 57, 33, and 34, respectively;
h) SEQ ID NOs: 61, 62, 43, 44, 45, and 46, respectively;
i) SEQ ID NOs: 63, 64, 65, 32, 33, and 34, respectively;
j) SEQ ID NOs: 66, 67, 68, 38, 45, and 55, respectively;
k) SEQ ID NOs: 69, 70, 71, 72, 45, and 73, respectively; or
l) SEQ ID NOs: 74, 75, 76, 57, 33, and 34, respectively.

[0080] In some embodiments, the anti-PD-1 antibody or antigen-binding portion of the invention comprises a VH and a VL that are 80%, 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, or 99% identical to the amino acid sequences of:

a) SEQ ID NOs: 1 and 2, respectively;
b) SEQ ID NOs: 3 and 4, respectively;
c) SEQ ID NOs: 5 and 6, respectively;
d) SEQ ID NOs: 7 and 8, respectively;
e) SEQ ID NOs: 9 and 10, respectively;
f) SEQ ID NOs: 11 and 12, respectively;
g) SEQ ID NOs: 13 and 14, respectively;
h) SEQ ID NOs: 15 and 16, respectively;
i) SEQ ID NOs: 17 and 18, respectively;

j) SEQ ID NOs: 19 and 20, respectively;

k) SEQ ID NOs: 21 and 22, respectively; or

l) SEQ ID NOs: 23 and 24, respectively.

[0081] In some embodiments, the anti-PD-1 antibody or antigen-binding portion of the invention comprises a VH and a VL that have the amino acid sequences of:

a) SEQ ID NOs: 1 and 2, respectively;

b) SEQ ID NOs: 3 and 4, respectively;

c) SEQ ID NOs: 5 and 6, respectively;

d) SEQ ID NOs: 7 and 8, respectively;

e) SEQ ID NOs: 9 and 10, respectively;

f) SEQ ID NOs: 11 and 12, respectively;

g) SEQ ID NOs: 13 and 14, respectively;

h) SEQ ID NOs: 15 and 16, respectively;

i) SEQ ID NOs: 17 and 18, respectively;

j) SEQ ID NOs: 19 and 20, respectively;

k) SEQ ID NOs: 21 and 22, respectively; or

l) SEQ ID NOs: 23 and 24, respectively.

[0082] In some embodiments, the anti-PD-1 antibody comprises:

a) an HC with the amino acid sequences of SEQ ID NOs: 1 and 26 and an LC with the amino acid sequences of SEQ ID NOs: 2 and 28;

b) an HC with the amino acid sequences of SEQ ID NOs: 3 and 26 and an LC with the amino acid sequences of SEQ ID NOs: 4 and 28;

c) an HC with the amino acid sequences of SEQ ID NOs: 5 and 26 and an LC with the amino acid sequences of SEQ ID NOs: 6 and 28;

d) an HC with the amino acid sequences of SEQ ID NOs: 7 and 26 and an LC with the amino acid sequences of SEQ ID NOs: 8 and 28;

e) an HC with the amino acid sequences of SEQ ID NOs: 9 and 26 and an LC with the amino acid sequences of SEQ ID NOs: 10 and 28;

f) an HC with the amino acid sequences of SEQ ID NOs: 11 and 26 and an LC with the amino acid sequences of SEQ ID NOs: 12 and 28;

g) an HC with the amino acid sequences of SEQ ID NOs: 13 and 26 and an LC with the amino acid sequences of SEQ ID NOs: 14 and 28;

h) an HC with the amino acid sequences of SEQ ID NOs: 15 and 26 and an LC with the amino acid sequences of SEQ ID NOs: 16 and 28;

i) an HC with the amino acid sequences of SEQ ID NOs: 17 and 26 and an LC with the amino acid sequences of SEQ ID NOs: 18 and 28;

j) an HC with the amino acid sequences of SEQ ID NOs: 19 and 26 and an LC with the amino acid sequences of SEQ ID NOs: 20 and 28;

k) an HC with the amino acid sequences of SEQ ID NOs: 21 and 26 and an LC with the amino acid sequences of SEQ ID NOs: 22 and 28; or

l) an HC with the amino acid sequences of SEQ ID NOs: 23 and 26 and an LC with the amino acid sequences of SEQ ID NOs: 24 and 28.

[0083] In one embodiment, the anti-PD-1 antibody has a VH and VL that comprise the VH and VL amino acid sequences, respectively, of antibody 18040, i.e., where the VH comprises the amino acid sequence of SEQ ID NO: 1 and the VL comprises the amino acid sequence of SEQ ID NO: 2. In this embodiment, the VH may comprise the amino acid sequence of SEQ ID NO: 1, wherein X in position 35 is S, and/or X in position 84 is N. In certain embodiments, the VL may also comprise the amino acid sequence of SEQ ID NO: 2, wherein X in position 40 is A, and/or X in position 55 is Y.

[0084] In one embodiment, the anti-PD-1 antibody has a VH and VL that comprise the VH and VL amino acid sequences, respectively, of antibody 18049, i.e., where the VH comprises the amino acid sequence of SEQ ID NO: 3 and the VL comprises the amino acid sequence of SEQ ID NO: 4. In this embodiment, the VL may comprise the amino acid sequence of SEQ ID NO: 4, wherein X in position 1 is D, and/or X in position 3 is Q. In some embodiments, the VL comprises D in position 1 and Q in position 3. In certain embodiments, the VL may also comprise the amino acid sequence of SEQ ID NO: 4, wherein X in position 53 is S.

[0085] In one embodiment, the anti-PD-1 antibody has a VH and VL that comprise the VH and VL amino acid sequences, respectively, of antibody 18098, i.e., where the VH comprises the amino acid sequence of SEQ ID NO: 5 and the VL comprises the amino acid sequence of SEQ ID NO: 6. In this embodiment, the VH may comprise the amino acid sequence of SEQ ID NO: 5, wherein: X in position 1 is E, and/or X in position 5 is V. In some embodiments, the VH comprises E in position 1 and V in position 5. In certain embodiments, the VH may also comprise the amino acid sequence of SEQ ID NO: 5, wherein X in position 13 is Q, X in position 35 is S, X in position 46 is E, X in position 50 is A, X in position 77 is N, X in position 80 is Y,

and/or X in position 115 is M. The VL may comprise the amino acid sequence of SEQ ID NO: 6, wherein X in position 4 is M.

[0086] In one embodiment, the anti-PD-1 antibody has a VH and VL that comprise the VH and VL amino acid sequences, respectively, of antibody 18113, i.e., where the VH comprises the amino acid sequence of SEQ ID NO: 7 and the VL comprises the amino acid sequence of SEQ ID NO: 8. In this embodiment, the VH may comprise the amino acid sequence of SEQ ID NO: 7 wherein X in position 64 is V. The VL may comprise the amino acid sequence of SEQ ID NO: 8, wherein X in position 3 is V, and/or X in position 4 is M. In some embodiments, the VL comprises V in position 3 and M in position 4. In certain embodiments, the VL may also comprise the amino acid sequence of SEQ ID NO: 8 wherein X in position 69 is S.

[0087] In one embodiment, the anti-PD-1 antibody has a VH and VL that comprise the VH and VL amino acid sequences, respectively, of antibody 18201, i.e., where the VH comprises the amino acid sequence of SEQ ID NO: 9 and the VL comprises the amino acid sequence of SEQ ID NO: 10. In this embodiment, the VH may comprise the amino acid sequence of SEQ ID NO: 9 wherein X in position 5 is Q. In some embodiments, the VH may also comprise the amino acid sequence of SEQ ID NO: 9, wherein X in position 59 is N, X in position 76 is K, and/or X in position 83 is L. The VL may comprise the amino acid sequence of SEQ ID NO: 10 wherein X in position 1 is A.

[0088] In one embodiment, the anti-PD-1 antibody has a VH and VL that comprise the VH and VL amino acid sequences, respectively, of antibody 18247, i.e., where the VH comprises the amino acid sequence of SEQ ID NO: 11 and the VL comprises the amino acid sequence of SEQ ID NO: 12. In this embodiment, the VH may comprise the amino acid sequence of SEQ ID NO: 11, wherein X in position 10 is G, and/or X in position 16 is G. The VL may comprise the amino acid sequence of SEQ ID NO: 12, wherein: X in position 1 is D, and/or X in position 4 is M. In some embodiments, the VL comprises D in position 1 and M in position 4. In certain embodiments, the VL may also comprise the amino acid sequence of SEQ ID NO: 12, wherein X in position 55 is Y, and/or X in position 93 is Y.

[0089] In one embodiment, the anti-PD-1 antibody has a VH and VL that comprise the VH and VL amino acid sequences, respectively, of antibody 18250, i.e., where the VH comprises the amino acid sequence of SEQ ID NO: 13 and the VL comprises the amino acid sequence of SEQ ID NO: 14. In this embodiment, the VH may

comprise the amino acid sequence of SEQ ID NO: 13 wherein X in position 5 is V. In some embodiments, the VH may also comprise the amino acid sequence of SEQ ID NO: 13, wherein X in position 50 is Y, X in position 59 is Y, and/or X in position 61 is A. The VL may comprise the amino acid sequence of SEQ ID NO: 14, wherein X in position 3 is V, and/or X in position 4 is M. In some embodiments, the VL comprises V in position 3 and M in position 4. In certain embodiments, the VL may also comprise the amino acid sequence of SEQ ID NO: 14 wherein X in position 55 is Y.

[0090] In one embodiment, the anti-PD-1 antibody has a VH and VL that comprise the VH and VL amino acid sequences, respectively, of antibody 18325, i.e., where the VH comprises the amino acid sequence of SEQ ID NO: 15 and the VL comprises the amino acid sequence of SEQ ID NO: 16. In this embodiment, the VH may comprise the amino acid sequence of SEQ ID NO: 15, wherein X in position 1 is E, X in position 5 is V, and/or X in position 6 is E. In some embodiments, the VH comprises E in position 1, V in position 5 and E in position 6. In certain embodiments, the VH may also comprise the amino acid sequence of SEQ ID NO: 15, wherein X in position 35 is S, X in position 49 is S, X in position 50 is A, X in position 73 is D, and/or X in position 78 is T. The VL may comprise the amino acid sequence of SEQ ID NO: 16, wherein X in position 1 is D, X in position 3 is Q, and/or X in position 4 is M. In some embodiments, the VL comprises D in position 1, Q in position 3 and M in position 4.

[0091] In one embodiment, the anti-PD-1 antibody has a VH and VL that comprise the VH and VL amino acid sequences, respectively, of antibody 18366, i.e., where the VH comprises the amino acid sequence of SEQ ID NO: 17 and the VL comprises the amino acid sequence of SEQ ID NO: 18. In this embodiment, the VH may comprise the amino acid sequence of SEQ ID NO: 17, wherein X in position 1 is E, and/or X in position 6 is E. In some embodiments, the VH comprises E in position 1 and E in position 6. The VL may comprise the amino acid sequence of SEQ ID NO: 18 wherein X in position 1 is D. In certain embodiments, the VL may also comprise the amino acid sequence of SEQ ID NO: 18 wherein X in position 40 is A, and/or X in position 55 is Y.

[0092] In one embodiment, the anti-PD-1 antibody has a VH and VL that comprise the VH and VL amino acid sequences, respectively, of antibody 18400, i.e., where the VH comprises the amino acid sequence of SEQ ID NO: 19 and the VL comprises

the amino acid sequence of SEQ ID NO: 20. In this embodiment, the VH may comprise the amino acid sequence of SEQ ID NO: 19 wherein X in position 2 is V. In certain embodiments, the VH may also comprise the amino acid sequence of SEQ ID NO: 19, wherein X in position 43 is G, X in position 49 is I, X in position 59 is N, X in position 70 is I, and/or X in position 111 is Q. The VL may comprise the amino acid sequence of SEQ ID NO: 20, wherein X in position 1 is A, and/or X in position 3 is Q. In some embodiments, the VL comprises A in position 1 and Q in position 3. In certain embodiments, the VL may also comprise the amino acid sequence of SEQ ID NO: 20, wherein X in position 10 is S.

[0093] In one embodiment, the anti-PD-1 antibody has a VH and VL that comprise the VH and VL amino acid sequences, respectively, of antibody 18413, i.e., where the VH comprises the amino acid sequence of SEQ ID NO: 21 and the VL comprises the amino acid sequence of SEQ ID NO: 22. In this embodiment, the VH may comprise the amino acid sequence of SEQ ID NO: 21, wherein X in position 48 is V, and/or X in position 50 is A. The VL may comprise the amino acid sequence of SEQ ID NO: 22 wherein X in position 4 is M. In certain embodiments, the VL may also comprise the amino acid sequence of SEQ ID NO: 22, wherein X in position 12 is S.

[0094] In one embodiment, the anti-PD-1 antibody has a VH and VL that comprise the VH and VL amino acid sequences, respectively, of antibody 18483, i.e., where the VH comprises the amino acid sequence of SEQ ID NO: 23 and the VL comprises the amino acid sequence of SEQ ID NO: 24. In this embodiment, the VH may comprise the amino acid sequence of SEQ ID NO: 23, wherein X in position 1 is E, X in position 5 is V, and/or X in position 6 is E. In some embodiments, the VL comprises E in position 1, V in position 5 and E in position 6. In certain embodiments, the VH may also comprise the amino acid sequence of SEQ ID NO: 23, wherein X in position 35 is S. The VL may comprise the amino acid sequence of SEQ ID NO: 24 wherein X in position 3 is V. In certain embodiments, the VL may also comprise the amino acid sequence of SEQ ID NO: 24, wherein X in position 40 is A, and/or X in position 55 is Y.

[0095] In certain embodiments, the anti-PD-1 antibody or antigen-binding portion comprises the H-CDR1-3 and L-CDR1-3 amino acid sequences of an antibody selected from 18040, 18049, 18098, 18113, 18201, 18247, 18250, 18325, 18366, 18400, 18413, and 18483, and further utilizes the same heavy and/or light chain germline sequences as the selected antibody.

[0096] In certain embodiments, the anti-PD-1 antibody or antigen-binding portion comprises the H-CDR1-3 and L-CDR1-3 amino acid sequences of an antibody selected from 18040, 18049, 18098, 18113, 18201, 18247, 18250, 18325, 18366, 18400, 18413, and 18483, and further comprises framework regions (FRs) that are 80%, 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, or 99% identical to the FRs of the selected antibody.

[0097] In some embodiments, any of the anti-PD-1 antibodies or antigen-binding portions described herein may inhibit binding of PD-L1 or PD-L2, or both, to PD-1.

[0098] In some embodiments, any of the anti-PD-1 antibodies or antigen-binding portions described herein may have at least one of the following properties:

a) binds to cynomolgus PD-1 with a K_D of, for example, 4×10^{-8} M or less;

b) binds to mouse PD-1 with a K_D of, for example, 2×10^{-8} M or less;

c) binds to human PD-1 with a K_D of 3×10^{-9} M or less;

d) inhibits the interaction of PD-1 with PD-L1 at a concentration of 10 μg/ml;

e) stimulates IL-2 production in an SEB whole blood assay; and

f) stimulates IFN-γ production in a one-way mixed lymphocyte reaction assay.

[0099] In some embodiments, any of the anti-PD1 antibodies or antigen-binding portions described herein may bind to human PD-1 with a K_D of 5×10^{-9} M or less, 4×10^{-9} M or less, 3×10^{-9} M or less, 2×10^{-9} M or less, 1×10^{-9} M or less, 9×10^{-10} M or less, 8×10^{-10} M or less, 7×10^{-10} M or less, 6×10^{-10} M or less, 5×10^{-10} M or less, 4×10^{-10} M or less, 3×10^{-10} M or less, 2×10^{-10} M or less, or 1×10^{-10} M or less. In certain embodiments, the K_D is determined using surface plasmon resonance.

[0100] In some embodiments, any of the anti-PD1 antibodies or antigen-binding portions described herein may inhibit the interaction of PD-1 with PD-L1 at a concentration of 50, 40, 30, 20, 10, 9, 8, 7, 6, 5, 4, 3, 2, or 1 μg/ml.

[0101] 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 VL or VH is isolated using methods well-known in the art such that it does not include nucleic acid sequences encoding CL or CH. The nucleic acid molecules encoding VL or VH then are operatively linked to a nucleic acid sequence encoding a CL or CH, respectively, from a different class of immunoglobulin molecule. This may be achieved using a vector or nucleic acid molecule that comprises a CL or CH 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 IgG1 to IgG2. A κ light chain constant region can be changed, e.g., 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.

[0102] 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 IgG1, IgG2a or IgG2b, IgG3, or IgG4. In one embodiment, the antibody is an IgG1. In another embodiment, the antibody is an IgG4.

[0103] 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.

[0104] 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.

[0105] 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 IgG1 antibodies. Additionally or alternatively, the amino acid residue at position 228 may be mutated, for example to Pro. In some embodiments, 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.

[0106] In some embodiments, where the antibody is of the IgG4 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.

[0107] 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. In such embodiments, 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.

[0108] 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 VH domain of an anti-PD-1 antibody is linked to a first polypeptide, while the VL 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 VH and VL domains can interact with one another to form an antigen-binding site. In another preferred embodiment, the VH domain is separated from the VL domain by a linker such that the VH and VL domains can interact with one another (e.g., single-chain antibodies). The VH-linker-VL 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 bi-specific antibody.

[0109] To create a single chain antibody (scFv), the VH- and VL-encoding DNA fragments are operatively linked to another fragment encoding a flexible linker, e.g., encoding the amino acid sequence (Gly4 -Ser)3 (SEQ ID NO: 115), such that the VH and VL sequences can be expressed as a contiguous single-chain protein, with the VL and VH 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 VH and VL are used; bivalent, if two VH and VL are used; or polyvalent, if more than two VH and VL are used. Bi-specific or polyvalent antibodies may be generated that bind specifically to human PD-1 and to another molecule, for instance.

[0110] In other embodiments, other modified antibodies may be prepared using anti-PD-1 antibody-encoding nucleic acid molecules. For instance, "kappa bodies" (Ill 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.

[0111] 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 described herein. 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 bi-specific 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).

[0112] 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 bi-specific 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, Il.

[0113] 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.

[0114] 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, 125I, 131I), fluorescent labels (e.g., FITC, rhodamine, lanthanide phosphors), enzymatic labels (e.g., horseradish peroxidase, β-galactosidase, 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.

[0115] 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.

[0116] 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.

Bi-specific Binding Molecules

[0117] In a further aspect, the invention provides a bi-specific binding molecule having the binding specificity of an anti-PD-1 antibody described 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 bi-specific binding molecules are known in the art, and examples of different types of bi-specific binding molecules are given elsewhere herein.

Nucleic Acid Molecules and Vectors

[0118] The present invention also provides nucleic acid molecules and sequences encoding anti-PD-1 antibodies or antigen-binding portions thereof described herein. 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.

[0119] 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.

[0120] In any of the above embodiments, the nucleic acid molecules may be isolated.

[0121] In a further aspect, the present invention provides a vector suitable for expressing one of the chains of an antibody or antigen-binding portion thereof as described herein. 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”).

[0122] 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.

[0123] 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 source that produces such an antibody or portion. In various embodiments, the nucleic acid molecules are 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.

[0124] In some embodiments, a nucleic acid molecule of the invention can comprise a nucleotide sequence encoding a VH 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 VL 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.

[0125] In a further aspect of the invention, nucleic acid molecules encoding the variable domain of the heavy (VH) and/or light (VL) chains may be "converted" to full-length antibody genes. In one embodiment, nucleic acid molecules encoding the VH or VL 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) regions, respectively, such that the VH segment is operatively linked to the CH segment(s) within the vector, and/or the VL segment is operatively linked to the CL segment within the vector. In another embodiment, nucleic acid molecules encoding the VH and/or VL domains are converted into full-length antibody genes by linking, e.g., ligating, a nucleic acid molecule encoding a VH and/or VL domains to a nucleic acid molecule encoding a CH and/or CL region 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.

[0126] 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, bi-specific antibodies, single chain antibodies, immunoadhesins, diabodies, mutated antibodies and antibody derivatives, as described herein.

[0127] 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. In some embodiments, the oligonucleotides are from highly variable domains of the heavy and light chains of the antibody of interest. In some embodiments, the oligonucleotides 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.

[0128] 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.

[0129] 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.

[0130] 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, and the like. 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).

[0131] A convenient vector is one that encodes a functionally complete human CH or CL immunoglobulin sequence, with appropriate restriction sites engineered so that any VH or VL sequence can easily be inserted and expressed, as described above. The HC- and LC-encoding 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).

[0132] 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, etc. 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.

[0133] 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.

[0134] 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

[0135] 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 as defined herein, 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.

[0136] 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 may comprise, e.g., a vector according to the invention described above. The invention also provides host cells that comprise, e.g., 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.

[0137] 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.

[0138] 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, etc. Bacterial host cells include *E. coli* and *Streptomyces* species. Yeast host cells include *Schizosaccharomyces pombe*, *Saccharomyces cerevisiae* and *Pichia pastoris*.

[0139] 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.

[0140] 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 post-translational modification(s).

Pharmaceutical Compositions

[0141] 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 as described herein. In some embodiments, the compositions are intended for amelioration, prevention, and/or treatment of a PD-1-related disorder and/or cancer. As used herein, a PD-1-related or -mediated disorder refers to a disorder, disease or condition that improves, or slows down in its progression, by modulation of PD-1 activity. 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, colon, ovary, brain, prostate, kidney, soft tissues, the hematopoietic system, head and neck, liver, bladder, breast, stomach, uterus and pancreas.

[0142] Generally, the antibodies, antigen-binding portions thereof, and bi-specific binding molecules of the invention are suitable to be administered as a formulation in association with one or more pharmaceutically acceptable excipient(s), e.g., as described below.

[0143] Pharmaceutical compositions of the invention will comprise one or more anti-PD-1 antibodies or binding portions or bi-specific binding molecules of the invention, e.g., one or two anti-PD-1 antibodies, binding portions, or bi-specific binding molecules. In one embodiment, the composition comprises a single anti-PD-1 antibody of the invention or binding portion thereof.

[0144] 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.

[0145] 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, and the like that are physiologically compatible. Some examples of pharmaceutically acceptable excipients are water, saline, phosphate buffered saline, dextrose, glycerol, ethanol and the like, as well as combinations thereof. In many cases, it will be preferable to include isotonic agents, for example, sugars, polyalcohols such as mannitol, sorbitol, or sodium chloride in the composition. 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.

[0146] 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.

[0147] 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 one-third of such a dosage.

[0148] 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.

[0149] 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, but is not limited to, 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, and the like. In particular, parenteral administration is contemplated to include, but is not limited to, subcutaneous, intraperitoneal, intramuscular, intrasternal, intravenous, intraarterial, intrathecal, intraventricular, intraurethral, intracranial, intratumoral, and intrasynovial injection or infusions; and kidney dialytic infusion techniques. Regional perfusion is also contemplated. Particular embodiments include the intravenous and the subcutaneous routes.

[0150] 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, but are not limited to, suspensions, solutions, emulsions in oily or aqueous vehicles, pastes, and the like. Such formulations may further comprise one or more additional ingredients including, but not limited to, 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.

[0151] For example, in one aspect, sterile injectable solutions can be prepared by incorporating the anti-PD-1 antibody or antigen-binding portion thereof, bi-specific binding molecule, 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., slow-release coatings).

[0152] 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.

[0153] 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.

[0154] 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.

[0155] 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.

[0156] 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.

[0157] 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.

[0158] 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.

[0159] 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.

[0160] 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.

[0161] 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

[0162] 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 is immune-suppressed. For example, a physician can boost the anti-cancer activity of a patient's own immune system by administering an anti-PD-1 antibody, antibody-binding portion, composition, or bi-specific binding molecule of the present invention, alone or in combination with other therapeutic agents (sequentially or concurrently). The anti-PD-1 antibody or portion, composition, or bi-specific binding molecule modulates the activity of PD-1 in immune cells, resulting in enhancement of anti-cancer immunity. In certain embodiments, the antibody or antigen-binding portion thereof, composition, or bi-specific binding molecule is for use in the treatment of cancer, e.g., cancers that originate in tissues such as skin, lung, intestine, colon, ovary, brain, prostate, kidney, soft tissues, the hematopoietic system, head and neck, liver, bladder, breast, stomach, uterus and pancreas, and any cancers or other conditions which rely on PD-1 activity and/or in which the patient expresses or overexpresses PD1, PD-L1, and/or PD-L2. Cancers treated by the anti-PD-1 antibodies, antigen-binding portions thereof, anti-PD-1 compositions, and/or bi-specific 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, gastric cancer, microsatellite instability-high cancer, hepatocellular carcinoma, mesothelioma, Merkel cell carcinoma, glioma, multiple myeloma, diffuse large B cell lymphoma, Hodgkin's lymphoma, and renal cell carcinoma (RCC).

[0163] 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., 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, neuroendocrine tumors, 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, 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, urological cancer, bone cancer, thoracic cancer, respiratory tract cancer, adenoid cystic carcinoma, cervical cancer, astrocytoma, chordoma, hematologic neoplasms, neuroblastoma, oral cavity cancer, cutaneous squamous cell carcinoma, thyroid cancer, Kaposi sarcoma, anal cancer, gallbladder cancer, thymic cancer, uterine cancer, diffuse large B cell lymphoma, follicular lymphoma, mesothelioma, or solid tumors. The cancer may be, e.g., at an early, intermediate, late, or metastatic stage.

[0164] In some embodiments, the anti-PD-1 antibodies, antigen-binding portions, compositions, and/or bi-specific binding molecules of the invention may be used to treat viral and/or parasitic infections, e.g., where the pathogens inhibit the host immune response. For example, the pathogen may be, e.g., HIV, hepatitis (A, B, or C), human papilloma virus (HPV), lymphocytic choriomeningitis virus (LCMV), adenovirus, flavivirus, echovirus, rhinovirus, coxsackie virus, cornovirus, respiratory syncytial virus, mumps virus, rotavirus, measles virus, rubella virus, parvovirus, vaccinia virus, human T-cell lymphotrophic virus (HTLV), dengue virus, molluscum virus, poliovirus, rabies virus, John Cunningham (JC) virus, arboviral encephalitis virus, simian immunodeficiency virus (SIV), influenza, herpes, *Giardia*, malaria, *Leishmania*, *Staphylococcus aureus*, or *Pseudomonas aeruginosa*.

[0165] In some embodiments, the anti-PD-1 antibodies, antigen-binding portions, compositions, and/or bi-specific binding molecules of the invention may be used to treat a patient who is, or is at risk of being, immunocompromised (e.g., due to chemotherapeutic or radiation therapy).

[0166] "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.

[0167] "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, for example, result in tumor shrinkage, increased survival, elimination of cancer cells, decreased disease progression, reversal of metastasis, or other clinical endpoints desired by healthcare professionals.

[0168] The anti-PD-1 antibodies or antigen-binding portions thereof, compositions, or bi-specific binding molecules of the invention may be administered 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.

[0169] As used herein, the terms "co-administration", "co-administered" and "in combination with," referring to the antibodies and antigen-binding portions thereof, compositions, and bi-specific binding molecules 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 / antigen-binding portion / antibody composition / bi-specific binding molecule 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 / antigen-binding portion / antibody composition / bi-specific binding molecule 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 / antigen-binding portion / antibody composition / bi-specific binding molecule 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 / antigen-binding portion / antibody composition / bi-specific binding molecule 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.

[0170] The antibodies and antigen-binding portions thereof, antibody compositions, and bi-specific binding molecules of the invention may be administered without additional therapeutic treatments, i.e., as a stand-alone therapy (monotherapy). Alternatively, treatment with the antibodies and antigen-binding portions thereof, compositions, and bi-specific binding molecules of the invention may include at least one additional therapeutic treatment (combination therapy), e.g., another immunostimulatory agent, an anti-cancer agent, an anti-viral agent, or a vaccine (e.g., a tumor vaccine). In some embodiments, the antibody or antigen-binding portion thereof, composition, or bi-specific binding molecule 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.

[0171] By combining the antibodies, antigen-binding portions, compositions, or bi-specific binding molecules 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 proliferator-activated 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.

[0172] Pharmaceutical articles comprising an anti-PD-1 antibody or antigen-binding portion thereof, composition, or bi-specific binding molecule 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.

[0173] An anti-PD-1 antibody or antigen-binding portion thereof, antibody composition, or bi-specific binding molecule of the invention may also be used in combination with other anti-cancer therapies such as vaccines, cytokines, enzyme inhibitors, immunostimulatory compounds, 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.

[0174] It is also contemplated that an anti-PD-1 antibody or antigen-binding portion thereof, antibody composition, or bi-specific binding molecule 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 inhibit ligand-induced receptor phosphorylation by competing for the intracellular Mg-ATP binding site.

[0175] In some embodiments, the antibody or antigen-binding portion thereof, composition, or bi-specific binding molecule may be used in combination with

another medication/drug that mediates immune system activation, including, but not limited to, an agent that modulates the expression or activity of A2AR, BTLA, 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, LILRB2, CMTM6, 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 or antigen-binding portion thereof, composition, or bi-specific binding molecule 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 or antigen-binding portion thereof, composition, or bi-specific binding molecule of the invention may be administered in combination with ipilimumab.

[0176] In certain aspects, the antibodies and antigen-binding portions, compositions, and bi-specific binding molecules 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 anti-PD1 antibody or antigen-binding portion thereof, bi-specific antibody, or antibody composition of the invention may be administered in combination with pembrolizumab and/or nivolumab.

[0177] It is understood that the antibodies and antigen-binding portions thereof, antibody compositions, and bi-specific binding molecules 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. The invention also provides kits and articles of manufacture comprising the antibodies and antigen-binding portions thereof, antibody compositions, and/or bi-specific binding molecules described herein.

Dose and Route of Administration

[0178] The antibodies or antigen-binding portions thereof, compositions, and bi-specific binding molecules 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.

[0179] 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.

[0180] 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.

[0181] 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, the present invention encompasses intra-patient dose-escalation as determined by the skilled artisan. Determining appropriate dosages and regimens are well-known in the relevant art and would be understood to be encompassed by the skilled artisan once provided the teachings disclosed herein.

[0182] It is contemplated that a suitable dose of an antibody or antigen-binding portion thereof, composition, or bi-specific binding molecule 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, antigen-binding portion, composition, or bi-specific binding molecule 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.

[0183] 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, antigen-binding portion, composition, or bi-specific binding molecule 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

[0184] 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. The invention further encompasses kits (e.g., diagnostic kits) comprising the antibodies described herein.

[0185] 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, although methods and materials similar or equivalent to those described herein can also be used in the practice or testing of the present invention. In case of conflict, the present specification, including definitions, will control.

[0186] 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.

[0187] 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.

[0188] All publications and other references mentioned herein are incorporated by reference in their entirety. 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.

[0189] In order that this invention may be better understood, the following examples are set forth. These examples are for purposes of illustration only and are not to be construed as limiting the scope of the invention in any manner.

Examples

Example 1. Generation and screening of anti-PD-1 antibody repertoires

Materials and methods

[0190] OmniRat® rats (Osborn et al., *J Immunol.* 2013, 190(4):1481-90), an engineered rat strain from OMT (Open Monoclonal Technology, Inc.) capable of producing human antibodies, were immunized with human, cynomolgus, or mouse PD-1 antigens. Cloning of antibody genes from single-cell sorted antibody-secreting B cells (ASC) derived from the rats was performed by means of the Symplex™ antibody discovery technology (Meijer et al., *J Mol Biol* 2006, 358(3):764-72; US 7,749,697; WO 2008/104184).

[0191] A Symplex™ antibody library was prepared from single-cell sorted B cells from the immunized OMT rats, the library containing cognate VH and VL encoding pairs for each sorted B cell. The antibody repertoire expression constructs encoded fully human immunoglobulins in IgG1 format carrying two mutations (L234A/L235A) known to reduce effector function of the Fc region of IgG1 antibodies (Hezareh et al., *J Virol.* 75(24): 12161-8 (2001)).

[0192] CHO-S cells were transfected in 384-well format with expression constructs to display human, cynomolgus or mouse PD-1 using the FreeStyle™ MAX reagent (Invitrogen). Furthermore, another cell population was transfected with a control vector encoding the irrelevant protein human VEGFR2 and subsequently used as a negative control. In order to allow for a multiplexed screening setup, control cells labelled with intermediate intensity carboxyfluorescein succinimidyl ester ($CFSE^{inter}$), cyno PD-1 transfected cells labelled with high intensity CFSE ($CFSE^{high}$), and non-labelled human PD-1-transfected cells, were mixed at a ratio of 1:1:1, at a density of 1x10E6 cells per ml. 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 parallel, antibodies were screened for binding to human ($CFSE^{pos}$) and mouse PD-1 ($CFSE^{neg}$) in a similar setup. Samples were acquired using high throughput flow cytometry (iQue® Screener, Intellicyt) and data was analyzed using ForeCyt® software by plotting CFSE vs. IgG binding (AF647). PD-1-specific primary hits were identified as antibody clones binding to both human ($CSFE^{neg}$) and cynomolgus PD-1-transfected cells ($CFSE^{high}$), but not to control cells ($CFSE^{inter}$), and plate numbers and plate coordinates were collected for hit picking and subsequent sequence analysis. A number of primary hits exhibiting additional reactivity towards mouse PD-1 in the second screening ($CFSE^{neg}$) were also selected for further analysis.

Results

[0193] Figures 1(a) – 1(d) show representative flow cytometry dot plots for four antibody clones exhibiting different reactivity towards PD-1 orthologs:
(a) an antibody clone binding non-specifically to CHO-S cells,
(b) an antibody clone binding specifically to human PD-1-transfected cells only,
(c) an antibody clone binding specifically to human and cynomolgus PD-1, and
(d) an antibody clone binding to all three PD-1 species tested in the screening.

[0194] The upper dot plots in each of (a), (b), (c) and (d) represent a first screening round testing antibodies for binding to human PD-1 (huPD1) and cynomolgus PD-1 (cynoPD1) as compared to negative control cells (neg). The lower dot plots represent a second screening round testing antibodies for binding to mouse PD-1 (moPD1) and human PD-1 (huPD1). The x-axis (horizontal) shows CFSE, and the y-axis (vertical) shows human IgG binding.

Example 2. Antibody sequences

[0195] Screening hits were analyzed by DNA sequencing and antibody-encoding DNA sequences were extracted. 488 primary hits exhibiting cross reactivity to both human and cynomolgus PD-1 were sequenced. This revealed that the anti-PD-1 hit repertoire contained 254 unique antibodies representing 140 genetic clusters. Selected antibody clones were individually expressed and tested functionally as described below. Twelve antibodies exhibiting functional activity in *in vitro* assays are described hereafter. The numbering of the protein sequences of the twelve antibody VH and VL domains is shown in Table 1. Sequence numbering of the

immunoglobulin constant regions (Ig heavy chain (IgHC) with L234A/L235A mutations and Ig kappa light chain (IgKV)) used to clone the variable VH and VL genes is shown in Table 2. Sequence numbering of the CDRs of the twelve functional antibodies is shown in Table 3. 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 amino-terminal to the IMGT-CDR3 (Cys).

Table 1. Numbering of antibody variable domain amino acid sequences

Antibody number	VH protein SEQ ID NO.	VL protein SEQ ID NO.
18040	1	2
18049	3	4
18098	5	6
18113	7	8
18201	9	10
18247	11	12
18250	13	14
18325	15	16
18366	17	18
18400	19	20
18413	21	22
18483	23	24

Table 2. Numbering of antibody constant region DNA and amino acid sequences

Sequence name	DNA SEQ ID NO.	Protein SEQ ID NO.
IgHC	25	26
IgKC	27	28

Table 3. SEQ ID NOs for the amino acid sequences of the heavy chain CDR1, CDR2 and CDR3 and light chain CDR1 and CDR3 of anti-PD-1 antibodies

Antibody	H-CDR1	H-CDR2	H-CDR3	L-CDR1	L-CDR2	L-CDR3
18040	29	30	31	32	33	34
18049	35	36	37	38	39	40
18098	41	42	43	44	45	46
18113	29	47	48	49	50	51

18201	52	53	54	38	45	55
18247	29	56	48	57	33	51
18250	58	59	60	57	33	34
18325	61	62	43	44	45	46
18366	63	64	65	32	33	34
18400	66	67	68	38	45	55
18413	69	70	71	72	45	73
18483	74	75	76	57	33	34

Example 3. Framework mutations in antibody sequences

[0196] Alignment of the VH and VL amino acid sequences of Example 2 to human germline sequences was performed to reveal the germline genes from which the VH and VL sequences originate. Table 4 shows the assignment of the closest V- and J-germline gene for VH and VL of each clone, as well as information on framework mutations as described in the following.

[0197] Since the antibody genes were isolated by RT-PCR using the Symplex™ antibody discovery technology (Mejier et al., J Mol Biol 358:764-772 (2006); WO 2005/042774) with degenerate primers, the initial six amino acids are prone to mutations that do not arise during the maturation of the antibody sequence. Hence, these primer-derived mutations can be mutated back to germline sequence without risk of reduced binding affinity. The numbers and specific amino acid substitutions of VH and VL in these “Symplex-corrected” variant sequences are shown in Table 4. Furthermore, antibodies harbouring somatic hypermutations in framework regions of variable domains, i.e., mutations in the VH or VL outside the CDRs, can be changed back to the sequence of the closest V- or J-germline. The numbers and specific amino acid substitutions in antibody frameworks that may be changed to that of germline in these “germlined” variant sequences are also shown in Table 4. It will be apparent that the “germlined” variant mutations indicated in Table 4 (see the columns “Number of VH framework mutations in germlined variant” and “Mutations of VH framework mutations in germlined variant”) include any “Symplex-corrected” mutations as well as mutations outside of the initial six amino acid positions.

[0198] As noted above, alteration of degenerate primer-derived mutations in the first six amino acids in each VH or VL sequence is not expected to deteriorate the binding properties compared to the original antibody. Therefore, it is preferred that

the anti-PD-1 antibodies of the invention include the "Symplex-corrected" mutations indicated in the tables below.

[0199] As for the "germlined variant" mutations that are outside the initial six amino acid positions of each sequence, any one or more of these mutations may be selected. Determination of whether an individual mutation has a negative effect on the antigen-binding properties of an antibody may be performed by preparing germlined variant VH and VL sequences with the indicated mutation and comparing the antigen-binding properties of the antibody with those of the parent with the corresponding original or Symplex-corrected sequences. In the event that a reduction in binding affinity or other altered binding property is observed, variants may, for example, be tested using a 2x2 VH/VL matrix with a Symplex-corrected variant and a germlined variant of the heavy and light chains of each antibody, i.e., in this case four combinations for each antibody. This allows determination of whether one or the other, or both, of the germlined VH and VL sequences are contributing to any observed altered binding properties. Alternatively or additionally, a series of corresponding antibodies in which single germlined variant mutations are avoided may be tested to determine specific mutations that are influencing binding of the germlined variant having all mutations listed.

[0200] Table 5 shows the VH and VL sequence numbers for the original sequences as well as the corresponding Symplex-corrected and germlined variants. It will be apparent from the numbers in Table 5 that in some cases there is no difference between the original sequence and the Symplex-corrected sequence, or between the Symplex-corrected sequence and the germlined sequence. Mutations to germline framework residues are based on the IMGT definitions. In the appended sequence listing the resulting amino acid substitutions are underlined and marked by bold type.

Table 4 (Part 1: VH sequence framework mutations)

Antibody	Closest V-, J-germline	# of VH FR mutations in Symplex-corrected variant	VH FR mutations in Symplex-corrected variant	# of VH FR mutations in germlined variant	VH FR mutations in germlined variant
18040	IGHV3-11*01, IGHJ5*02	0		2	N35S, D84N
18049	IGHV3-33*04,	0		0	

Antibody	Closest V-, J-germline	# of VH FR mutations in Symplex-corrected variant	VH FR mutations in Symplex-corrected variant	# of VH FR mutations in germlined variant	VH FR mutations in germlined variant
	IGHJ4*02				
18098	IGHV3-23*04, IGHJ3*02	2	Q1E, Q5V	9	Q1E, Q5V, R13Q, N35S, V46E, T50A, S77N, F80Y, T115M
18113	IGHV3-11*01, IGHJ5*02	0		1	A64V
18201	IGHV4-4*02, IGHJ3*02	1	V5Q	4	V5Q, T59N, R76K, M83L
18247	IGHV3-11*01, IGHJ4*02	0		2	D10G, R16G
18250	IGHV3-11*01, IGHJ4*03	1	Q5V	4	Q5V, H50Y, D59Y, V61A
18325	IGHV3-23*04, IGHJ3*02	3	Q1E, Q5V, Q6E	8	Q1E, Q5V, Q6E, N35S, A49S, T50A, G73D, M78T
18366	IGHV3-7*02, IGHJ5*02	2	Q1E, Q6E	2	Q1E, Q6E
18400	IGHV4-4*02, IGHJ3*02	1	L2V	6	L2V, K43G, V49I, S59N, M70I, P111Q
18413	IGHV3-23*04, IGHJ4*03	0		2	L48V, T50A
18483	IGHV3-7*02, IGHJ5*02	3	Q1E, Q5V, Q6E	4	Q1E, Q5V, Q6E, N35S

Table 4 (Part 2: VL sequence framework mutations)

Antibody	Closest V- , J-germline	# of VL FR mutations in Symplex-corrected variant	VL FR mutations in Symplex-corrected variant	# of VL FR mutations in germlined variant	VL FR mutations in germlined variant
18040	IGKV4-1*01, IGKJ1*01	1	E1D	3	E1D, F40A, F55Y
18049	IGKV1-17*01, IGKJ1*01	2	E1D, V3Q	3	E1D, V3Q, N53S
18098	IGKV1D-12*01, IGKJ1*01	1	L4M	1	L4M
18113	IGKV4-1*01, IGKJ1*01	2	Q3V, L4M	3	Q3V, L4M, R69S
18201	IGKV1-6*01, IGKJ1*01	1	D1A	1	D1A
18247	IGKV4-1*01, IGKJ1*01	2	E1D, L4M	4	E1D, L4M, F55Y, F93Y
18250	IGKV4-1*01, IGKJ2*01	2	Q3V, L4M	3	Q3V, L4M, S55Y

18325	IGKV1D-12*02, IGKJ1*01	3	E1D, V3Q, L4M	3	E1D, V3Q, L4M
18366	IGKV4-1*01, IGKJ2*01	1	E1D	3	E1D, L40A, F55Y
18400	IGKV1-6*01, IGKJ1*01	2	E1A, V3Q	3	E1A, V3Q, P10S
18413	IGKV1D-12*01, IGKJ4*02	1	L4M	2	L4M, P12S
18483	IGKV4-1*01, IGKJ2*01	1	Q3V	3	Q3V, L40A, F55Y

Table 5. SEQ ID NOs for the amino acid sequences of the VH and VL of anti-PD-1 antibodies, including for Symplex-corrected and germlined variants

Antibody number	VH protein sequence number	Symplex-corrected VH variant	Germlined VH variant	VL protein sequence number	Symplex-corrected VL variant	Germlined VL variant
18040	1	1	96	2	84	106
18049	3	3	3	4	85	107
18098	5	77	97	6	86	86
18113	7	7	98	8	87	108
18201	9	78	99	10	88	88
18247	11	11	100	12	89	109
18250	13	79	101	14	90	110
18325	15	80	102	16	91	91
18366	17	81	81	18	92	111
18400	19	82	103	20	93	112
18413	21	21	104	22	94	113
18483	23	83	105	24	95	114

Example 4. Flow cytometric analysis of anti-PD-1 antibodies for PD-L1 blocking activity

[0201] This example describes testing of the anti-PD-1 antibodies for PD-L1 blocking activity by means of flow cytometry.

Methods

[0202] PD-L1 ligand blocking activity was investigated in a cellular assay, in which human PD-1 was 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). The CHO-S cells were transiently

transfected to express human PD-1. Cells were 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) with 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

[0203] The results of the competition experiment are presented in Figures 2(a) – 2(h), where the X-axis (horizontal) shows PD-L1 binding, and the Y-axis (vertical) shows human IgG binding. All anti-PD-1 antibodies tested were able to inhibit the interaction of PD-1 with PD-L1 at a final antibody concentration of 10 µg/ml. Binding of PD-L1 to PD-1-expressing cells in the presence of a non-specific antibody was used as a negative control for PD-L1 blocking. In addition, a representative plot for binding of PD-L1 in the presence of a non-blocking antibody is presented.

Example 5. Measurement of PD-1 antibody affinities against human and cynomolgus PD-1 ECD antigen

[0204] This example demonstrates how twelve functional anti-PD-1 antibodies exhibit strong binding affinity for human PD-1, with K_D in the range of low nM to intermediate pM. The same antibodies also cross-react with cynomolgus PD-1 with K_D in the range of intermediate to low nM. Antibodies 18201 and 18400 also cross react with mouse PD-1 with K_D in the range of intermediate or low nM.

Methods

[0205] The kinetic binding analysis was performed by Surface Plasmon Resonance (SPR) 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 G-a-hu-IgG Fc SensEye® SPR sensors (Ssens BV, The Netherlands). Anti-PD-1 antibodies expressed in IgG_1 LALA format (i.e., having the mutations L234A/L235A) were diluted to 2.5 nM in PBS-T (1x PBS with 0.05% Tween 20, pH 7.4). Antibodies

were spotted onto a G-a-hu-IgG Fc SensEye® for 15 minutes using a Continuous Flow Microspotter (CFM, Wasatch Microfluidics, Salt Lake City, US). After spotting, the SensEye® was positioned in the IBIS MX96 biosensor, and antibodies were chemically fixed to the sensor surface using Fix It Kit (Ssens BV, The Netherlands). Kinetic analysis was performed by applying a kinetic titration series (Karlsson et. al., Anal. Biochem. 349(1):136-47 (2006)), where monomeric PD-1 antigen was injected in increasing concentrations from 2 nM to 100 nM without application of surface regeneration steps after each antigen injection. Binding was tested to human PD-1 ECD (Acro Biosystems cat. no. PD1-H52219), mouse PD-1 ECD (Sino Biological. cat. no. 50124-M08H) and cynomolgus PD-1 ECD (Acro Biosystems cat. no. PD1-C5223) in three separate experiments. Antigen association was performed for 15 minutes and antigen dissociation was performed for 60 minutes. The kinetic analysis was performed at 25°C. After completion, 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 k_a), off-rate (k_{off} or k_d) and affinity (K_D) constants.

Results

[0206] The Surface Plasmon Resonance (SPR) results are shown below in Table 6. Generally, antibodies were of high affinity and all tested antibodies cross-reacted with cynomolgus PD-1. Antibodies 18201 and 18400 also cross-reacted with mouse PD-1, indicating that the epitopes recognized by these two antibodies were unique as compared to the other PD-1 antibodies, including the reference antibodies, pembrolizumab and nivolumab, which did not cross-react with mouse PD-1. These properties are advantageous because they allow the antibodies to be directly tested in non-human primate or murine models prior to testing in human subjects.

Table 6. Kinetics of anti-PD-1 antibodies' binding to human, cynomolgus or mouse PD-1 ECD as measured by Surface Plasmon Resonance (SPR). (N.B. = not binding)

Antibody	PD-1 ECD	k_{on} ($M^{-1}s^{-1}$)	k_{off} (s^{-1})	K_D (M)
18040	Human	2.6E+05	1.1E-04	4.1E-10
18040	Cynomolgus	3.4E+05	9.7E-04	2.9E-09
18040	Mouse	N.B.	N.B.	N.B.
18049	Human	3.4E+04	6.0E-05	1.8E-09
18049	Cynomolgus	3.8E+04	8.0E-05	2.1E-09

Antibody	PD-1 ECD	k_{on} ($M^{-1}s^{-1}$)	k_{off} (s^{-1})	K_D (M)
18049	Mouse	N.B.	N.B.	N.B.
18098	Human	1.0E+05	2.3E-04	2.3E-09
18098	Cynomolgus	3.9E+04	1.3E-03	3.4E-08
18098	Mouse	N.B.	N.B.	N.B.
18113	Human	1.7E+05	1.0E-04	6.0E-10
18113	Cynomolgus	1.4E+05	7.6E-04	5.3E-09
18113	Mouse	N.B.	N.B.	N.B.
18201	Human	2.9E+05	1.3E-04	4.3E-10
18201	Cynomolgus	1.9E+05	2.3E-04	1.2E-09
18201	Mouse	3.8E+04	7.3E-05	1.9E-09
18247	Human	1.1E+05	1.5E-04	1.4E-09
18247	Cynomolgus	9.8E+04	2.7E-03	2.8E-08
18247	Mouse	N.B.	N.B.	N.B.
18250	Human	3.5E+05	2.9E-04	8.3E-10
18250	Cynomolgus	2.8E+05	1.8E-03	6.4E-09
18250	Mouse	N.B.	N.B.	N.B.
18325	Human	1.3E+05	1.1E-04	8.2E-10
18325	Cynomolgus	1.4E+05	6.2E-04	4.3E-09
18325	Mouse	N.B.	N.B.	N.B.
18366	Human	3.0E+05	4.0E-04	1.3E-09
18366	Cynomolgus	2.2E+05	6.4E-03	2.9E-08
18366	Mouse	N.B.	N.B.	N.B.
18400	Human	2.3E+05	3.5E-04	1.6E-09
18400	Cynomolgus	2.3E+05	8.7E-04	3.7E-09
18400	Mouse	4.7E+03	5.0E-05	1.1E-08
18413	Human	7.7E+04	1.1E-04	1.5E-09
18413	Cynomolgus	9.7E+04	6.2E-04	6.4E-09
18413	Mouse	N.B.	N.B.	N.B.
18483	Human	5.8E+05	1.7E-04	2.9E-10
18483	Cynomolgus	2.6E+05	8.0E-04	3.1E-09
18483	Mouse	N.B.	N.B.	N.B.
nivolumab analogue	Human	2.4E+05	1.9E-04	8.0E-10
nivolumab analogue	Cynomolgus	4.1E+05	6.2E-04	1.5E-09
nivolumab analogue	Mouse	N.B.	N.B.	N.B.
pembrolizumab / Keytruda	Human	4.2E+05	1.4E-03	3.4E-09
pembrolizumab / Keytruda	Cynomolgus	3.5E+05	1.3E-03	3.6E-09
pembrolizumab / Keytruda	Mouse	N.B.	N.B.	N.B.

Example 6. *In vitro* functional evaluation of anti-PD-1 monoclonal antibodies

[0207] This example demonstrates how the twelve anti-PD-1 antibodies perform in two different functional assays: the Staphylococcal Enterotoxin B (SEB) whole blood assay and a one-way mixed lymphocyte reaction (MLR). The ability of the twelve anti-PD-1 mAbs to stimulate IL-2 production in the SEB treated whole blood assay or interferon-gamma (IFN- γ) production in the one-way MLR assay was evaluated as described below.

Methods

[0208] 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 cytokine release, including IL-2 and IFN-γ. SEB was added at 1 μg/ml to whole blood, and after two days of culture, supernatants were harvested and IL-2 levels were determined by regular ELISA.

[0209] In the one-way MLR assay, dendritic cells (DCs) and CD4-positive ($CD4^+$) T-cells isolated from two different healthy donors were co-cultured to induce an alloantigen specific reaction, resulting in cytokine production and T-cell activation/proliferation. Dendritic cells were differentiated from $CD14^+$ monocytes by six days of culture with 20 ng/ml granulocyte-macrophage colony-stimulating factor (GM-CSF) and 20 ng/ml interleukin-4 (IL-4), and mixed in a 1:10 ratio with $CD4^+$ T-cells isolated from peripheral blood mononuclear cells (PBMCs) from healthy donor material. After 5 days of culture, supernatants were harvested, and IFN-γ levels were determined using the Meso Scale electrochemiluminescence (MSD) cytokine assay.

[0210] Dose-response curves were generated by two-fold dilutions of the antibodies with a starting concentration of 50 μg/ml.

Results

[0211] Dose-response curves of the twelve antibodies in the SEB whole blood assay and one-way MLR assay are shown in Figures 3(a) – 3(f) and Figures 4(a) – 4(f), respectively. Each point on the graph represents the average of three replicates, with the error bars representing the SEM. All of the antibodies were

found to induce a dose-dependent increase in IL-2 production in the SEB whole blood assay and in IFN-γ production in the MLR assay.

Example 7. Epitope binning of anti-PD-1 monoclonal antibodies

[0212] This example illustrates how anti-PD-1 antibodies were grouped into epitope bins based on paired competition patterns. Antibodies belonging to different epitope bins recognize different epitopes on the PD-1 ECD.

Methods

[0213] 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 G-a-hu-IgG Fc SensEye® SPR sensor (Ssens BV, The Netherlands). A total of eighteen anti-PD-1 antibodies (human, IgG1) were diluted to 10 µg/mL in PBS buffer containing 0.05% Tween 20 (PBS-T), pH 7.0. Antibodies were captured onto the anti-Fc sensor surface by spotting for 15 minutes using a Continuous Flow Microspotter. After spotting, the SensEye® was positioned in the IBIS MX96 biosensor and residual anti-Fc sites blocked by injection of 30 µg/mL non-specific human IgG1. Captured antibodies were conjugated to the surface using a FixIt kit (Ssens BV, The Netherlands). 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 eighteen 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

[0214] The competition pattern of eighteen anti-PD-1 antibodies is presented in Figure 5. Antibodies 12866 and 12807 were not found to have functional activity in cell-based assays (data not shown), but were included because they recognize

distinct epitopes useful for characterizing the other epitope bins. The tested anti-PD-1 antibodies could be assigned to two main non-overlapping epitope bins. Functional antibodies belonging to epitope bin 1 all cross blocked each other, and could be further divided into sub bins based on blocking of antibodies 12866 and 12807. For instance, antibodies that blocked both mAbs 12866 and 12807 were assigned to epitope bin 1C. Epitope bin 1C includes antibodies 18366, 18483, 18113, 18247, 18040, and 18250. Antibodies in epitope bin 1D include nivolumab analogue ("Nivo") and 18049 and were characterized by blocking mAb 12866 but not 12807. Antibodies belonging to epitope bin 1E were characterized by only blocking mAb 12807. Epitope bin 1E includes pembrolizumab analogue ("Pembro") and antibodies 18098, 18201, 18400, 18413, and 18325.

[0215] Antibodies 12760 and 13112 do not block PD-L1 and PD-L2 ligand, and were assigned to 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. Antibodies 12760 and 13112 likely bind to a site on PD-1 that does not overlap with the PD-L1 and PD-L2 ligand binding site.

List of Sequences

SEQ ID NO: 1
QVQLVESGGGLVKPGGSLRLSCAASGFTFSDYYM**X**WIRQAPGKGLEWVSYISSTGSTIYYAD
SVKGRFTISRDNAKNSLYLQM**X**SLRAEDTAVYYCARATNWGSDYWGQGTLVTVSS

X in position 35 is N or S
X in position 84 is D or N

SEQ ID NO: 2
XIVMTQSPDSLAVSLGERATINCKSSQSVLYSSNNKNYL**X**WYQQKPGQPPKLLI**X**WASTRES
GVPDRFSGSGSGTDFTLTISSLQAEDVAVYYCQQYYSTPYTFGQGTKVEIK

X in position 1 is E or D
X in position 40 is F or A
X in position 55 is F or Y

SEQ ID NO: 3
QVQLVESGGGVVQPGRSLRLSCAASGFTFSNYGMHWVRQAPGKGLEWVAVIWYDGSDKYYAD
SVKGRFTISRDNSKNTLYLQMNSLRAEDTAVYYCAGGGNYYGDFWGQGTLVTVSS

SEQ ID NO: 4
XI**X**MTQSPSSLSASVGDRVTITCRASQGIRNDLGWYQQKPGKAPKRLIYVAS**X**LQSGVPSRF
SGSGSGTEFTLTISSLQPEDFATYYCLQYNSYPWTFGQGTKVEIK

X in position 1 is E or D
X in position 3 is V or Q
X in position 53 is N or S

SEQ ID NO: 5
XVQL**X**ESGGGLV**X**PGGSLRLSCAASGFTFSSFAM**X**WVRQAPGKGL**X**WVS**X**ITGGGTTSYYAD
SVKGRFTISRDNSK**X**TL**X**LQMNSLRAEDTAVYYCAKWGSWSAGAFDIWGQGT**X**VTVSS

X in position 1 is Q or E
X in position 5 is Q or V
X in position 13 is R or Q
X in position 35 is N or S
X in position 46 is V or E
X in position 50 is T or A
X in position 77 is S or N
X in position 80 is F or Y
X in position 115 is T or M

SEQ ID NO: 6
DIQ**X**TQSPSSVSASVGDRVTITCRASQGISSWLAWYQQKPGKAPKLLIYAASSLQSGVPSRF
SGSGSGTDFTLTISSLQPEDFATYYCQQANSFPWTFGQGTKVEIK

X in position 4 is L or M

SEQ ID NO: 7

QVQLVESGGGLVKPGGSLRLSCAASGFTFSDYYMSWIRQAPGKGLEWVSYISSSGSTIYYAD
S**X**KGRFTISRDNAKNSLYLQMNSLRAEDTAVYYCARDTNWAFDYWGQGTLVTVSS

X in position 64 is A or V

SEQ ID NO: 8
DI**XX**TQSPDSLAVSLGERATINCKSSQSVFYSANNKNYLAWYQQKPGQPPKLLIYWTSTRES
GVPDRF**X**GSGSGTDFTLTISSLQAEDVAVYYCQQFYSTPRTFGQGTKVEIK

X in position 3 is Q or V
X in position 4 is L or M
X in position 69 is R or S

SEQ ID NO: 9
QVQL**X**ESGPGLVKPSGTLSLTCAVSGGSISSNNWWSWVRQPPGKGLEWIGEIYHDGTT**X**YNP
SLKSRVTISVDKS**X**NQFSLK**X**SSVTAADTAVYYCARGDWGSGAFDIWGQGTMVTVSS

X in position 5 is V or Q
X in position 59 is T or N
X in position 76 is R or K
X in position 83 is M or L

SEQ ID NO: 10
XIQMTQSPSSLSASVGDRVTITCRASQGIRNDLGWYQQKPGKAPKLLIYAASSLQSGVPSRF
SGSGSGTDFTLTISSLQPEDFATYYCLQDYNYPRTFGQGTKVEIK

X in position 1 is D or A

SEQ ID NO: 11
QVQLVESGG**X**LVKPG**X**SLRLSCAASGFTFSDYYMSWIRQAPGKGLEWVSYISSSSSTIYYAD
SVKGRFTISRDNAKNSLYLQMNSLRAEDTAVYYCARDTNWAFDYWGQGTLVTVSS

X in position 10 is D or G
X in position 16 is R or G

SEQ ID NO: 12
XIV**X**TQSPDSLAVSLGERATINCKSSQSVFYSSNNKNYLAWYQQKPGQPPKLLI**X**WASTRES
GVPDRFSGSGSGTDFTLTISSLQAEDVAVY**X**CQQFYSTPRTFGQGTKVEIK

X in position 1 is E or D
X in position 4 is L or M
X in position 55 is F or Y
X in position 93 is F or Y

SEQ ID NO: 13
QVQL**X**ESGGGLVKPGGSLRLSCAASGFTFRDYYMSWIRQAPGKGLEWVS**X**ISSSGSII**X**Y**X**D
SVKGRFTISRDNAKNSLYLQMNSLRAEDTAVYYCARDTNWALDYWGQGTLVTVSS

X in position 5 is Q or V
X in position 50 is H or Y
X in position 59 is D or Y

X in position 61 is V or A

SEQ ID NO: 14
DI**XX**TQSPDSLAVSLGERATINCKSSQSVFYSSNNKNYLAWYQQKPGQPPKLLI**X**WASTRES
GVPDRFSGSGSGTDFTLTISSLQAEDVAVYYCQQYYSTPYTFGQGTKLEIK

X in position 3 is Q or V
X in position 4 is L or M
X in position 55 is S or Y

SEQ ID NO: 15
XVQL**XX**SGGGLVQPGGSLRLSCAASGFTFSSHVM**X**WVRQAPGKGLEWV**XX**ISGSGVDTYYAD
SVKGRFTISR**X**NSKN**X**LYLQMNSLRAEDTAVYYCAKWGSWSAGAFDIWGQGTMVTVSS

X in position 1 is Q or E
X in position 5 is Q or V
X in position 6 is Q or E
X in position 35 is N or S
X in position 49 is A or S
X in position 50 is T or A
X in position 73 is G or D
X in position 78 is M or T

SEQ ID NO: 16
XI**XX**TQSPSSVSASVGDRVTITCRASQGISSWLAWYQQKPGKAPKLLIYAASSLQSGVPSRF
SGSGSGTDFTLTISSLQPEDFATYYCQQANSFPWTFGQGTKVEIK

X in position 1 is E or D
X in position 3 is V or Q
X in position 4 is L or M

SEQ ID NO: 17
XVQLV**X**SGGGLVQPGGSLRLSCAASGFTFSSYWMSWVRQAPGKGLEWVANIKQDGSEKYYVD
SVKGRFTISRDNAKNSLYLQMNSLRAEDTAVYYCARDTNWGFDNWGQGTLVTVSS

X in position 1 is Q or E
X in position 6 is Q or E

SEQ ID NO: 18
XIVMTQSPDSLAVSLGERATINCKSSQSVLYSSNNKNYL**X**WYQQKPGQPPKLLI**X**WASTRES
GVPDRFSGSGSGTDFTLTISSLQAEDVAVYYCQQYYSTPYTFGQGTKLEIK

X in position 1 is E or D
X in position 40 is L or A
X in position 55 is F or Y

SEQ ID NO: 19
Q**X**QLQESGPGLVKPSGTLSLTCAVSGGSISSSNWWSWVRQPP**X**KGLEW**X**GEIFHDGTT**X**YNP
SLKSRVT**X**SVDKSKNQFSLKLSSVTAADTAVYYCARGNWGSGALDIWG**X**GTMVTVSS

X in position 2 is L or V

X in position 43 is K or G
X in position 49 is V or I
X in position 59 is S or N
X in position 70 is M or I
X in position 111 is P or Q

SEQ ID NO: 20
XI**X**MTQSPS**X**LSASVGDRVTITCRASQGIRNDLGWYQQKPGKAPKLLIYAASSLQSGVPSRF
SGSGSGTDFTLTISSLQPEDFATYYCLQDYNYPRTFGQGTKVEIK

X in position 1 is E or A
X in position 3 is V or Q
X in position 10 is P or S

SEQ ID NO: 21
EVQLVESGGGLVQPGGSLRLSCAASGFTFSSFVMSWVRQAPGKGLEW**X**S**X**ISGGGGSTYYAD
SVKGRFTISRDNSKNTLYLQMNSLRAEDTAVYYCAKDWDLYYFDYWGQGTLVTVSS

X in position 48 is L or V
X in position 50 is T or A

SEQ ID NO: 22
DIQ**X**TQSPSSV**X**ASVGDRVTITCRASQGISNWLAWYQQKPGKAPKLLIYAASSLQSGVPSRF
SGSGSGTDFTLTISSLQPEDFATYYCQQANSFPLTFGGGTKVEIK

X in position 4 is L or M
X in position 12 is P or S

SEQ ID NO: 23
XVQL**XX**SGGGLVQPGGSLRLSCAASGFTFSDYWM**X**WVRQAPGKGLEWVANIKEDGNEKYYVD
SVKGRFTISRDNAKNSLYLQMNSLRAEDTAVYYCARDTNWGSDYWGQGTLVTVSS

X in position 1 is Q or E
X in position 5 is Q or V
X in position 6 is Q or E
X in position 35 is N or S

SEQ ID NO: 24
DI**X**MTQSPDSLAVSLGERATINCKSSQSVFYSSNNKNYL**X**WYQQKPGQPPKLLI**X**WASTRES
GVPDRFSGSGSGTDFTLTISSLQAEDVAVYYCQQYYSTPYTFGQGTKLEIK

X in position 3 is Q or V
X in position 40 is L or A
X in position 55 is F or Y

Constant antibody sequences

SEQ ID NO: 25 (IgHC DNA sequence)
GCCTCCACCAAGGGCCCATCGGTCTTCCCCCTGGCACCCTCCTCCAAGAGCACCTCTGGGGG
CACAGCGGCCCTGGGCTGCCTGGTCAAGGACTACTTCCCCGAACCGGTGACGGTGTCGTGGA
ACTCAGGCGCCCTGACCAGCGGCGTGCACACCTTCCCGGCTGTCCTACAGTCCTCAGGACTC
TACTCCCTCAGCAGCGTGGTGACCGTGCCCTCCAGCAGCTTGGGCACCCAGACCTACATCTG

CAACGTGAATCACAAGCCCAGCAACACCAAGGTGGACAAGAGAGTTGAGCCCAAATCTTGTG
ACAAAACTCACACATGCCCACCGTGCCCAGCACCTGAAGCCGCCGGGGGACCGTCAGTCTTC
CTCTTCCCCCCAAAACCCAAGGACACCCTCATGATCTCCCGGACCCCTGAGGTCACATGCGT
GGTGGTGGACGTGAGCCACGAAGACCCTGAGGTCAAGTTCAACTGGTACGTGGACGGCGTGG
AGGTGCATAATGCCAAGACAAAGCCGCGGGAGGAGCAGTACAACAGCACGTACCGTGTGGTC
AGCGTCCTCACCGTCCTGCACCAGGACTGGCTGAATGGCAAGGAGTACAAGTGCAAGGTCTC
CAACAAAGCCCTCCCAGCCCCCATCGAGAAAACCATCTCCAAAGCCAAAGGGCAGCCCCGAG
AACCACAGGTGTACACCCTGCCCCCATCCCGGGAGGAGATGACCAAGAACCAGGTCAGCCTG
ACCTGCCTGGTCAAAGGCTTCTATCCCAGCGACATCGCCGTGGAGTGGGAGAGCAATGGGCA
GCCGGAGAACAACTACAAGACCACGCCTCCCGTGCTGGACTCCGACGGCTCCTTCTTCCTCT
ATAGCAAGCTCACCGTGGACAAGAGCAGGTGGCAGCAGGGGAACGTCTTCTCATGCTCCGTG
ATGCATGAGGCTCTGCACAACCACTACACGCAGAAGAGCCTCTCCCTGTCCCCGGGTAAA

SEQ ID NO: 26 (IgHC protein sequence)
ASTKGPSVFPLAPSSKSTSGGTAALGCLVKDYFPEPVTVSWNSGALTSGVHTFPAVLQSSGL
YSLSSVVTVPSSSLGTQTYICNVNHKPSNTKVDKRVEPKSCDKTHTCPPCPAPEAAGGPSVF
LFPPKPKDTLMISRTPEVTCVVVDVSHEDPEVKFNWYVDGVEVHNAKTKPREEQYNSTYRVV
SVLTVLHQDWLNGKEYKCKVSNKALPAPIEKTISKAKGQPREPQVYTLPPSREEMTKNQVSL
TCLVKGFYPSDIAVEWESNGQPENNYKTTPPVLDSDGSFFLYSKLTVDKSRWQQGNVFSCSV
MHEALHNHYTQKSLSLSPGK

SEQ ID NO: 27 (IgKC DNA sequence)
CGTACGGTGGCTGCACCATCTGTCTTCATCTTCCCGCCATCTGATGAGCAGTTGAAATCTGG
AACTGCCTCTGTTGTGTGCCTGCTGAATAACTTCTATCCCAGAGAGGCCAAAGTACAGTGGA
AGGTGGATAACGCCCTCCAATCGGGTAACTCCCAGGAGAGTGTCACAGAGCAGGACAGCAAG
GACAGCACCTACAGCCTCAGCAGCACCCTGACGCTGAGCAAAGCAGACTACGAGAAACACAA
AGTCTACGCCTGCGAAGTCACCCATCAGGGCCTGAGCTCGCCCGTCACAAAGAGCTTCAACA
GGGGAGAGTGT

SEQ ID NO: 28 (IgKC protein sequence)
RTVAAPSVFIFPPSDEQLKSGTASVVCLLNNFYPREAKVQWKVDNALQSGNSQESVTEQDSK
DSTYSLSSTLTLSKADYEKHKVYACEVTHQGLSSPVTKSFNRGEC

SEQ ID NO: 29 (18040/18113/18247 H-CDR1)
GFTFSDYY

SEQ ID NO: 30 (18040 H-CDR2)
ISSTGSTI

SEQ ID NO: 31 (18040 H-CDR3)
CARATNWGSDY

SEQ ID NO: 32 (18040/18366 L-CDR1)
QSVLYSSNNKNY

SEQ ID NO: 33 (18040/18247/18250/18366/18483 L-CDR2)
WAS

SEQ ID NO: 34 (18040/18250/18366/18483 L-CDR3)
CQQYYSTPYT

SEQ ID NO: 35 (18049 H-CDR1)
GFTFSNYG

SEQ ID NO: 36 (18049 H-CDR2)
IWYDGSDK

SEQ ID NO: 37 (18049 H-CDR3)
CAGGGNYYGDF

SEQ ID NO: 38 (18049/18201/18400 L-CDR1)
QGIRND

SEQ ID NO: 39 (18049 L-CDR2)
VAS

SEQ ID NO: 40 (18049 L-CDR3)
CLQYNSYPWT

SEQ ID NO: 41 (18098 H-CDR1)
GFTFSSFA

SEQ ID NO: 42 (18098 H-CDR2)
ITGGGTTS

SEQ ID NO: 43 (18098/18325 H-CDR3)
CAKWGSWSAGAFDI

SEQ ID NO: 44 (18098/18325 L-CDR1)
QGISSW

SEQ ID NO: 45 (18098/18201/18325/18400/18413 L-CDR2)
AAS

SEQ ID NO: 46 (18098/18325 L-CDR3)
CQQANSFPWT

SEQ ID NO: 47 (18113 H-CDR2)
ISSSGSTI

SEQ ID NO: 48 (18113/18247 H-CDR3)
CARDTNWAFDY

SEQ ID NO: 49 (18113 L-CDR1)
QSVFYSANNKNY

SEQ ID NO: 50 (18113 L-CDR2)
WTS

SEQ ID NO: 51 (18113/18247 L-CDR3)
CQQFYSTPRT

SEQ ID NO: 52 (18201 H-CDR1)
GGSISSNNW

SEQ ID NO: 53 (18201 H-CDR2)
IYHDGTT

SEQ ID NO: 54 (18201 H-CDR3)
CARGDWGSGAFDI

SEQ ID NO: 55 (18201/18400 L-CDR3)
CLQDYNYPRT

SEQ ID NO: 56 (18247 H-CDR2)
ISSSSSTI

SEQ ID NO: 57 (18247/18250/18483 L-CDR1)
QSVFYSSNNKNY

SEQ ID NO: 58 (18250 H-CDR1)
GFTFRDYY

SEQ ID NO: 59 (18250 H-CDR2)
ISSSGSII

SEQ ID NO: 60 (18250 H-CDR3)
CARDTNWALDY

SEQ ID NO: 61 (18325 H-CDR1)
GFTFSSHV

SEQ ID NO: 62 (18325 H-CDR2)
ISGSGVDT

SEQ ID NO: 63 (18366 H-CDR1)
GFTFSSYW

SEQ ID NO: 64 (18366 H-CDR2)
IKQDGSEK

SEQ ID NO: 65 (18366 H-CDR3)
CARDTNWGFDN

SEQ ID NO: 66 (18400 H-CDR1)
GGSISSSNW

SEQ ID NO: 67 (18400 H-CDR2)
IFHDGTT

SEQ ID NO: 68 (18400 H-CDR3)
CARGNWGSGALDI

SEQ ID NO: 69 (18413 H-CDR1)
GFTFSSFV

SEQ ID NO: 70 (18413 H-CDR2)
ISGGGGST

SEQ ID NO: 71 (18413 H-CDR3)
CAKDWDLYYFDY

SEQ ID NO: 72 (18413 L-CDR1)
QGISNW

SEQ ID NO: 73 (18413 L-CDR3)
CQQANSFPLT

SEQ ID NO: 74 (18483 H-CDR1)
GFTFSDYW

SEQ ID NO: 75 (18483 H-CDR2)
IKEDGNEK

SEQ ID NO: 76 (18483 H-CDR3)
CARDTNWGSDY

SEQ ID NO: 77
EVQL**V**ESGGGLV**Q**PGGSLRLSCAASGFTFSSFAM**S**WVRQAPGKGLVWVSTITGGGTTSYY
ADSVKGRFTISRDNSKSTLFLQMNSLRAEDTAVYYCAKWGSWSAGAFDIWGQGTTVTVSS

SEQ ID NO: 78
QVQL**Q**ESGPGLVKPSGTLSLTCAVSGGSISSNNWWSWVRQPPGKGLEWIGEIYHDGTTTY
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SEQ ID NO: 79
QVQL**V**ESGGGLVKPGGSLRLSCAASGFTFRDYYMSWIRQAPGKGLEWVSHISSSGSIIDY
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SEQ ID NO: 80
EVQL**VE**SGGGLVQPGGSLRLSCAASGFTFSSHVMNWVRQAPGKGLEWVATISGSGVDTYY
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SEQ ID NO: 81
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SEQ ID NO: 82
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SEQ ID NO: 83
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SEQ ID NO: 84
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SEQ ID NO: 85
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SEQ ID NO: 86
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SEQ ID NO: 87
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SEQ ID NO: 88
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SEQ ID NO: 89
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SEQ ID NO: 98
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SEQ ID NO: 99
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SEQ ID NO: 100
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SEQ ID NO: 106
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SEQ ID NO: 107
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SEQ ID NO: 109

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SEQ ID NO: 110
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SEQ ID NO: 111
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SEQ ID NO: 112
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SEQ ID NO: 113
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SEQ ID NO: 115
GGGGSGGGGSGGGGS

Claims

1. An anti-PD-1 antibody or an antigen-binding portion thereof, wherein said antibody comprises H-CDR1-3 and L-CDR1-3 amino acid sequences of SEQ ID NOs: 52, 53, 54, 38, 45, and 55, respectively; or
SEQ ID NOs: 66, 67, 68, 38, 45, and 55, respectively.

2. An anti-PD-1 antibody or an antigen-binding portion thereof, wherein said antibody comprises a heavy chain variable domain (VH) and a light chain variable domain (VL) that comprise:
   a) SEQ ID NOs: 9 and 10, respectively;
   b) SEQ ID NOs: 78 and 10, respectively;
   c) SEQ ID NOs: 99 and 10, respectively;
   d) SEQ ID NOs: 9 and 88, respectively;
   e) SEQ ID NOs: 78 and 88, respectively;
   f) SEQ ID NOs: 99 and 88, respectively;
   g) SEQ ID NOs: 19 and 20, respectively;
   h) SEQ ID NOs: 82 and 20, respectively;
   i) SEQ ID NOs: 103 and 20, respectively;
   j) SEQ ID NOs: 19 and 93, respectively;
   k) SEQ ID NOs: 82 and 93, respectively;
   l) SEQ ID NOs: 103 and 93, respectively;
   m) SEQ ID NOs: 19 and 112, respectively;
   n) SEQ ID NOs: 82 and 112, respectively; or
   o) SEQ ID NOs: 103 and 112, respectively.

3. The anti-PD-1 antibody of claim 1 or claim 2, wherein the antibody is of isotype IgG.

4. The anti-PD-1 antibody of claim 3, wherein the antibody is of isotype IgG subclass IgG1.

5. The anti-PD-1 antibody of claim 3, wherein

a) the antibody is of isotype IgG subclass IgG1, wherein one or both of the amino acid residues at positions 234 and 235 are mutated to Ala; or

b) the antibody is of isotype IgG subclass IgG4, wherein the amino acid residue at position 228 is mutated to Pro.

6. The anti-PD-1 antibody of any one of claims 1-5 or antigen-binding portion of claim 1 or claim 2, wherein the antibody or portion has at least one of the following properties:

a) binds to cynomolgus PD-1 with a K_D of 4 x 10^{-8} M or less;

b) binds to mouse PD-1 with a K_D of 2 x 10^{-8} M or less;

c) binds to human PD-1 with a K_D of 3 x 10^{-9} M or less;

d) inhibits the interaction of PD-1 with PD-L1 at a concentration of 10 µg/mL;

e) stimulates IL-2 production in an SEB whole blood assay; and

f) stimulates IFN-γ production in a one-way mixed lymphocyte reaction assay.

7. An anti-PD-1 antibody that comprises:

a) a heavy chain (HC) with the amino acid sequences of SEQ ID NOs: 9 and 26 and a light chain (LC) with the amino acid sequences of SEQ ID NOs: 10 and 28;

b) an HC with the amino acid sequences of SEQ ID NOs: 78 and 26 and an LC with the amino acid sequences of SEQ ID NOs: 10 and 28;

c) an HC with the amino acid sequences of SEQ ID NOs: 99 and 26 and an LC with the amino acid sequences of SEQ ID NOs: 10 and 28;

d) an HC with the amino acid sequences of SEQ ID NOs: 9 and 26 and an LC with the amino acid sequences of SEQ ID NOs: 88 and 28;

e) an HC with the amino acid sequences of SEQ ID NOs: 78 and 26 and an LC with the amino acid sequences of SEQ ID NOs: 88 and 28;

f) an HC with the amino acid sequences of SEQ ID NOs: 99 and 26 and an LC with the amino acid sequences of SEQ ID NOs: 88 and 28;

g) an HC with the amino acid sequences of SEQ ID NOs: 19 and 26 and an LC with the amino acid sequences of SEQ ID NOs: 20 and 28;

h) an HC with the amino acid sequences of SEQ ID NOs: 82 and 26 and an LC with the amino acid sequences of SEQ ID NOs: 20 and 28;

i) an HC with the amino acid sequences of SEQ ID NOs: 103 and 26 and an LC with the amino acid sequences of SEQ ID NOs: 20 and 28;

j) an HC with the amino acid sequences of SEQ ID NOs: 19 and 26 and an LC with the amino acid sequences of SEQ ID NOs: 93 and 28;

k) an HC with the amino acid sequences of SEQ ID NOs: 82 and 26 and an LC with the amino acid sequences of SEQ ID NOs: 93 and 28;

l) an HC with the amino acid sequences of SEQ ID NOs: 103 and 26 and an LC with the amino acid sequences of SEQ ID NOs: 93 and 28;

m) an HC with the amino acid sequences of SEQ ID NOs: 19 and 26 and an LC with the amino acid sequences of SEQ ID NOs: 112 and 28;

n) an HC with the amino acid sequences of SEQ ID NOs: 82 and 26 and an LC with the amino acid sequences of SEQ ID NOs: 112 and 28; or

o) an HC with the amino acid sequences of SEQ ID NOs: 103 and 26 and an LC with the amino acid sequences of SEQ ID NOs: 112 and 28.

8. A pharmaceutical composition comprising the anti-PD-1 antibody according to any one of claims 1 to 7 or antigen-binding portion according to any one of claims 1, 2 or 6 and a pharmaceutically acceptable excipient.

9. Isolated nucleic acid molecule(s) comprising a nucleotide sequence that encodes the heavy chain sequence, and a nucleotide sequence that encodes the light chain sequence, of the anti-PD-1 antibody of any one of claims 1-7 or antigen-binding portion of any one of claims 1, 2 or 6.

10. Vector(s) comprising the isolated nucleic acid molecule(s) of claim 9, wherein said vector(s) further comprise an expression control sequence.

11. A host cell comprising a nucleotide sequence that encodes the heavy chain sequence, and a nucleotide sequence that encodes the light chain sequence, of the anti-PD-1 antibody of any one of claims 1-7 or antigen-binding portion of any one of claims 1, 2 or 6.

12. A method for producing the antibody of any one of claims 1-7 or antigen-binding portion of any one of claims 1, 2 or 6, comprising providing the host cell according to claim 11, cultivating said host cell under conditions suitable for expression of the antibody or portion, and isolating the resulting antibody or portion.

13. A bi-specific binding molecule comprising the binding domain of the anti-PD-1 antibody of any one of claims 1-7 and the binding domain of another, distinct antibody.

14. A method for enhancing immunity in a patient in need thereof, comprising administering to said patient the anti-PD-1 antibody of any one of claims 1-7 or antigen-binding portion of any one of claims 1, 2 or 6, the pharmaceutical composition of claim 8, or the bi-specific binding molecule of claim 13.

15. Use of the anti-PD-1 antibody of any one of claims 1-7 or antigen-binding portion of any one of claims 1, 2 or 6, the pharmaceutical composition of claim 8, or the bi-specific binding molecule of claim 13, for the manufacture of a medicament for enhancing immunity in a patient.

16. A method for treating cancer in a patient, comprising administering to said patient the antibody of any one of claims 1-7 or antigen-binding portion of any one of claims 1, 2 or 6, the pharmaceutical composition of claim 8, or the bi-specific binding molecule of claim 13.

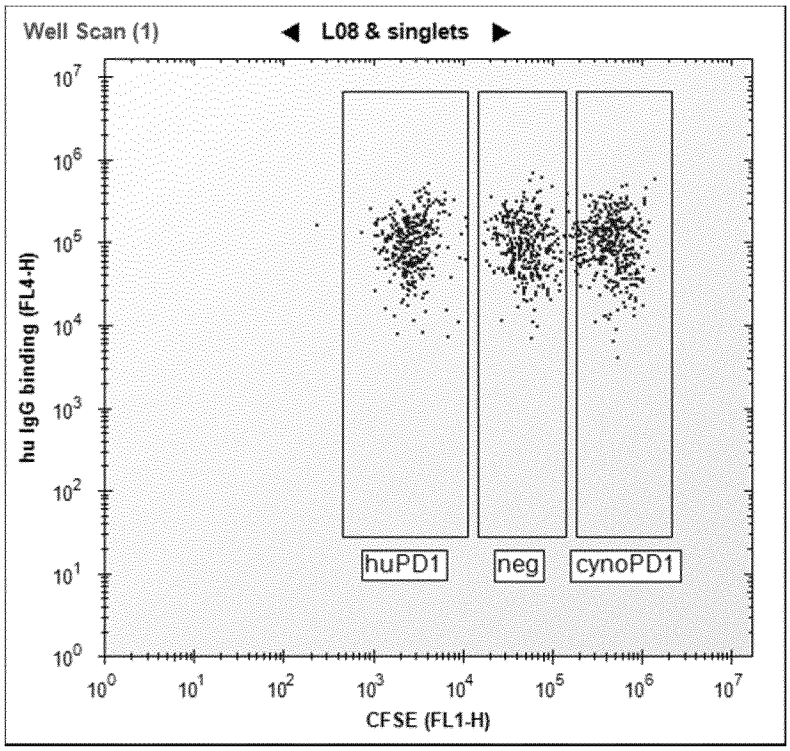
17. Use of the anti-PD-1 antibody of any one of claims 1-7 or antigen-binding portion of any one of claims 1, 2 or 6, the pharmaceutical composition of claim 8, or the bi-specific binding molecule of claim 13, for the manufacture of a medicament for treating cancer in a patient.

18. The method of claim 16 or the use of claim 17, wherein the cancer originates in a tissue selected from the group consisting of skin, lung, intestine, colon, ovary, brain, prostate, kidney, soft tissues, hematopoietic system, head and neck, liver, bladder, breast, stomach, uterus and pancreas.

19. The method of claim 16 or the use of claim 17, 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, bladder cancer, gastric cancer, renal cell carcinoma, hepatocellular carcinoma, colorectal cancer, or Hodgkin's lymphoma.

20. The method of any one of claims 16, 18, and 19 or the use of any one of claims 17-19, wherein the treatment further comprises an immunostimulatory agent, a vaccine, a chemotherapeutic agent, an anti-neoplastic agent, an anti-angiogenic agent, a tyrosine kinase inhibitor, a PD-1 pathway inhibitor, or radiation therapy.

Figure 1A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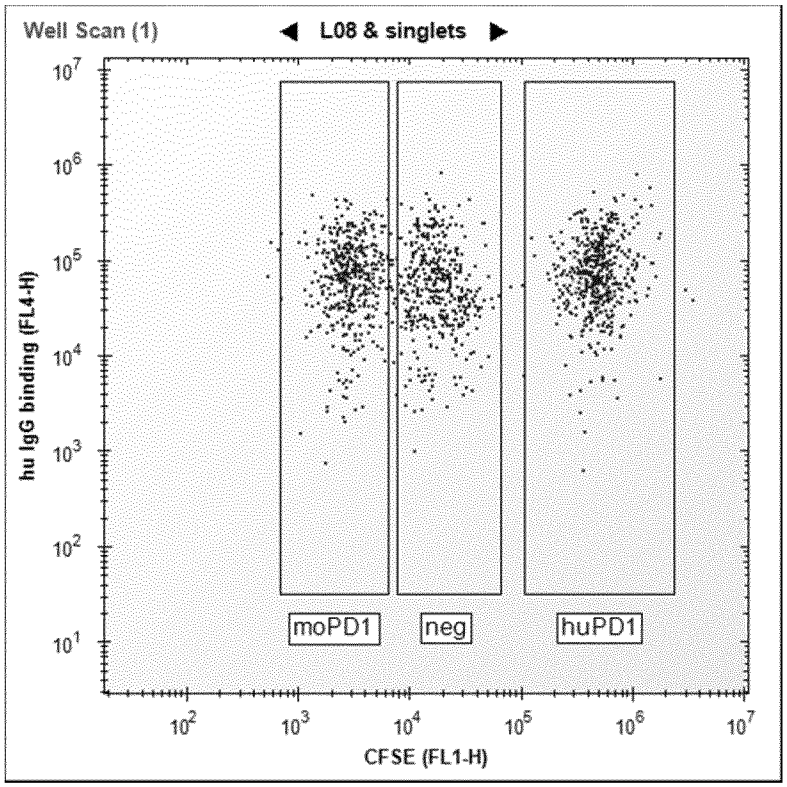
CFSE (FL1-H)
huPD1
neg
cynoPD1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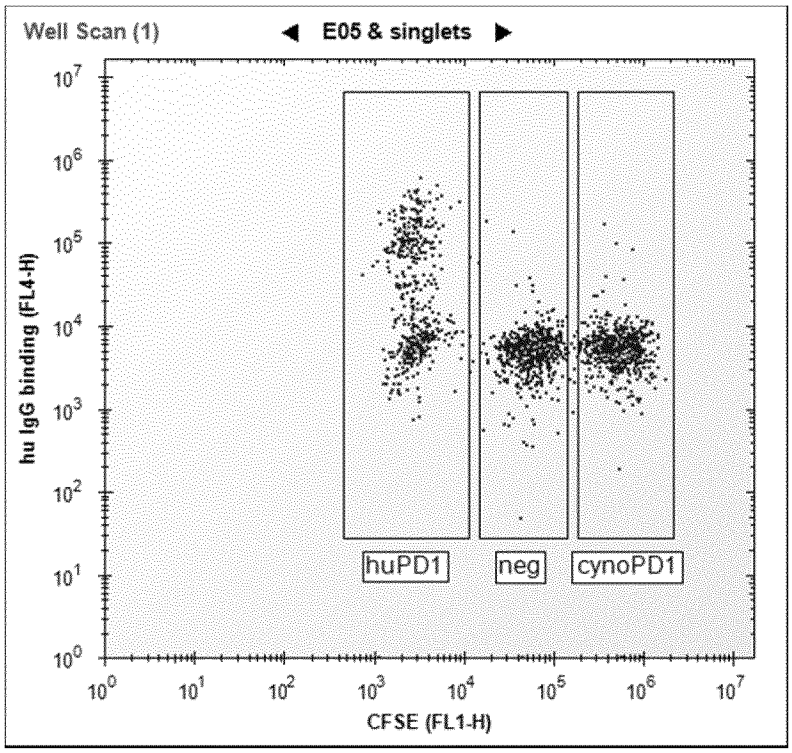
L08 & singlets
hu IgG binding (FL4-H)
CFSE (FL1-H)
moPD1
neg
huPD1

Figure 1B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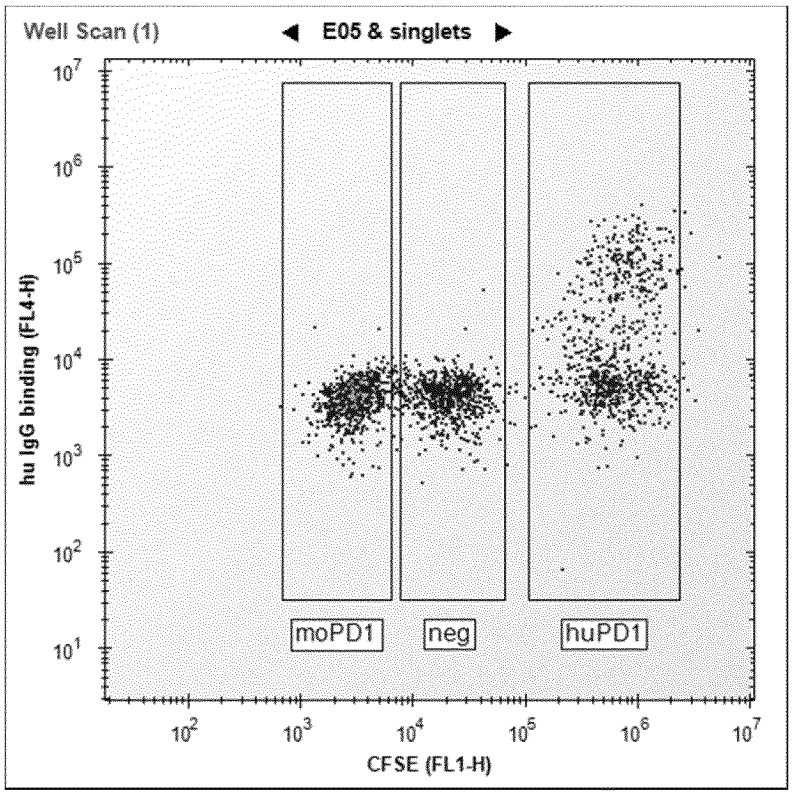
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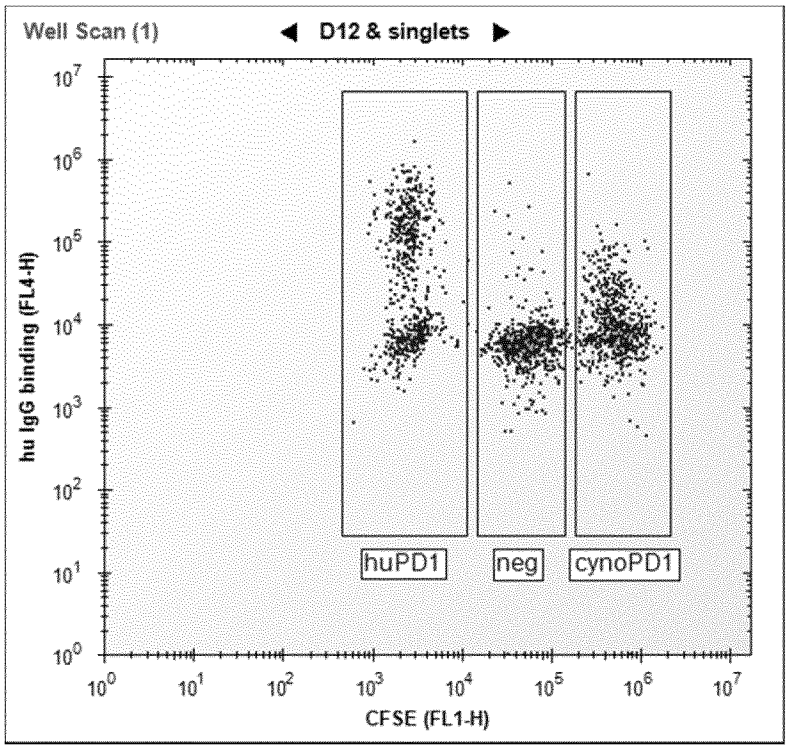
hu IgG binding (FL4-H)
CFSE (FL1-H)
moPD1
neg
huPD1

Figure 1C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CFSE (FL1-H)
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neg
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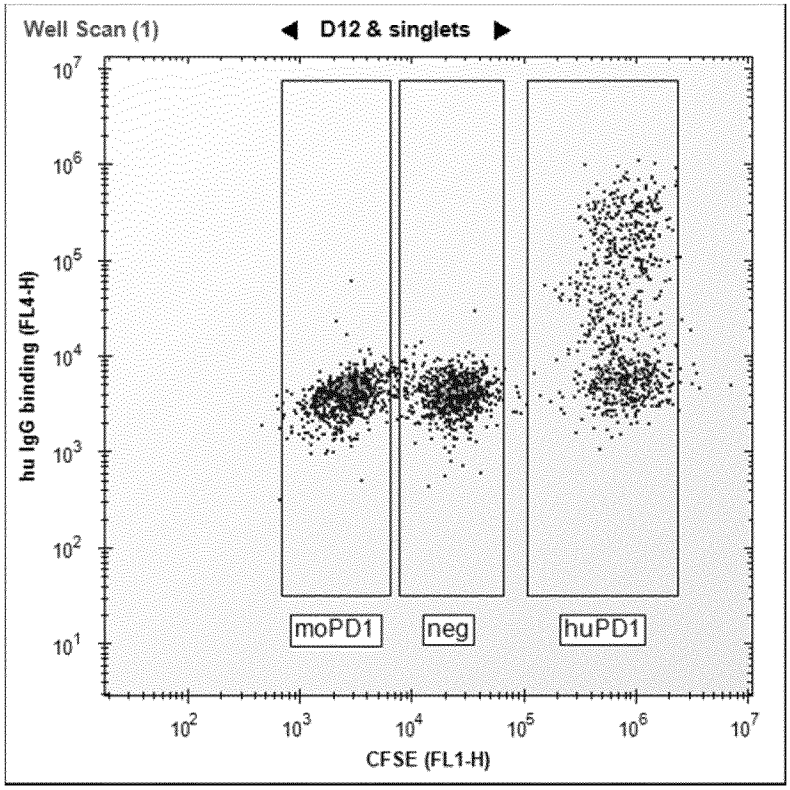
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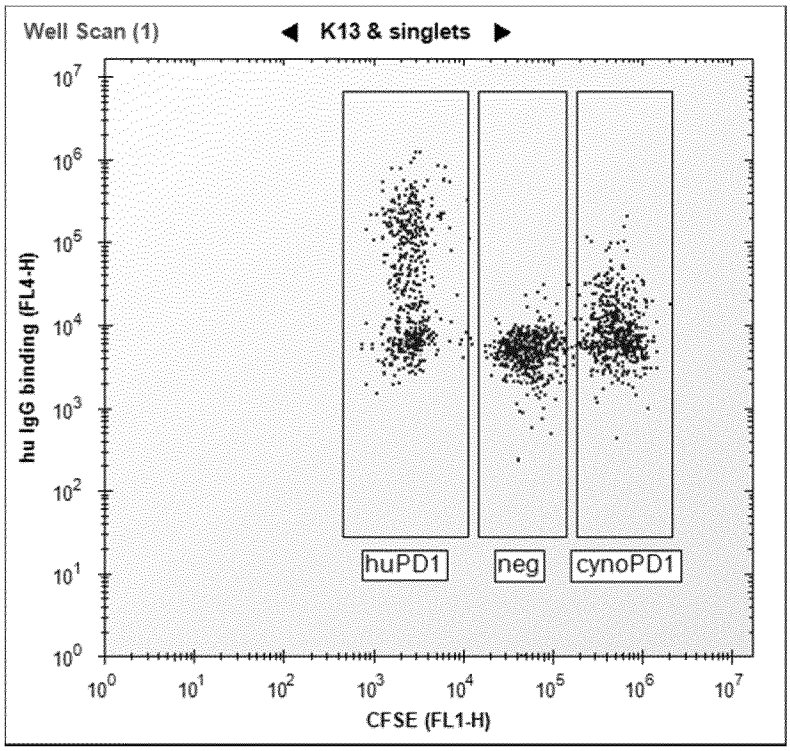
CFSE (FL1-H)
moPD1
neg
huPD1

Figure 1D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CFSE (FL1-H)
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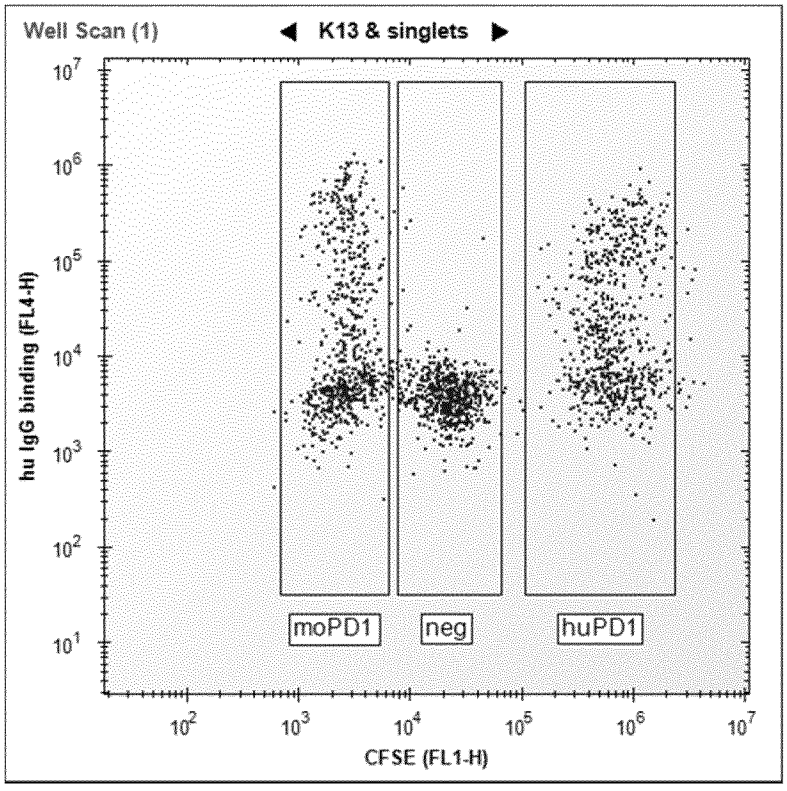
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CFSE (FL1-H)
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neg
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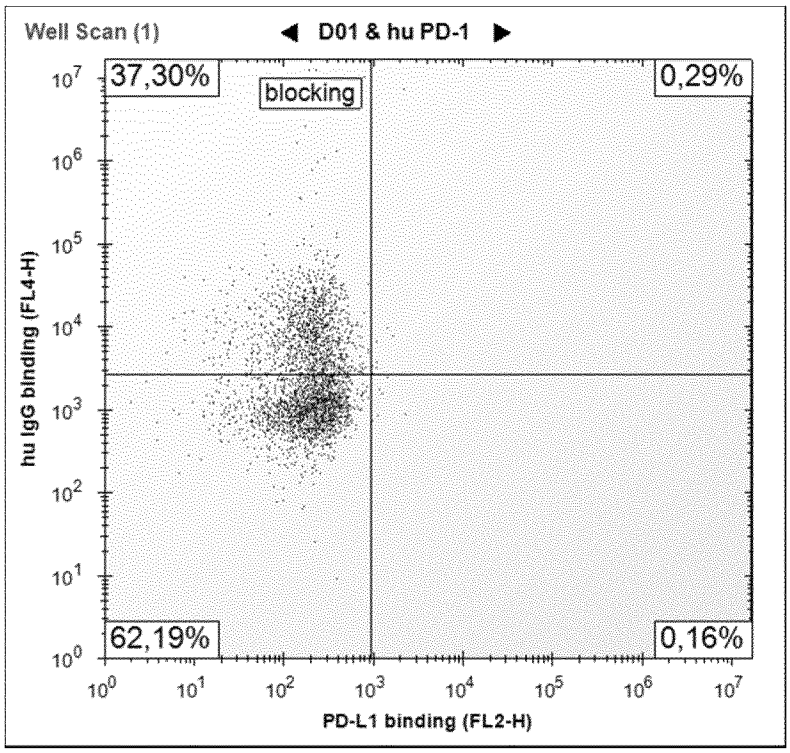
Figure 2A



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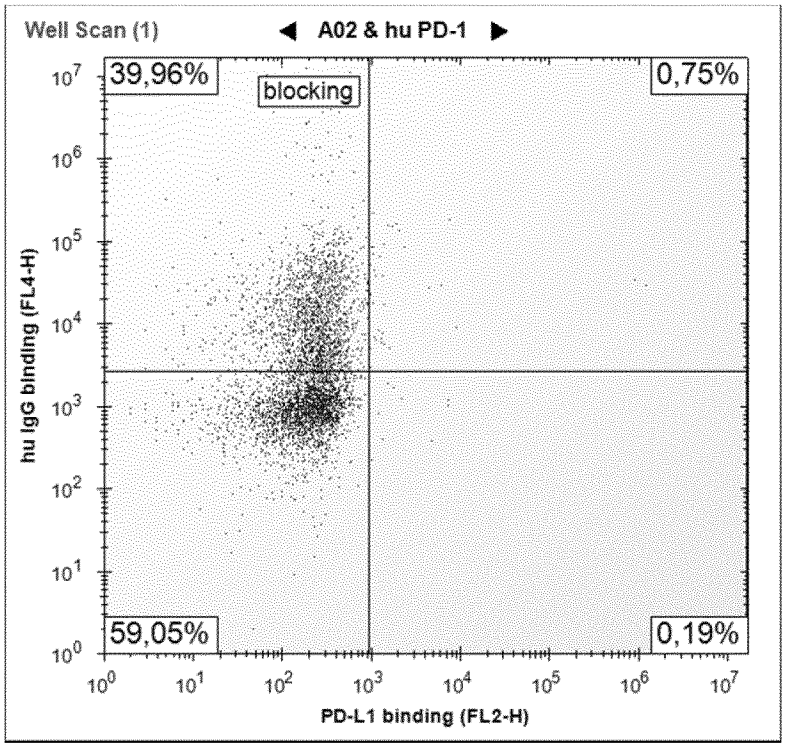

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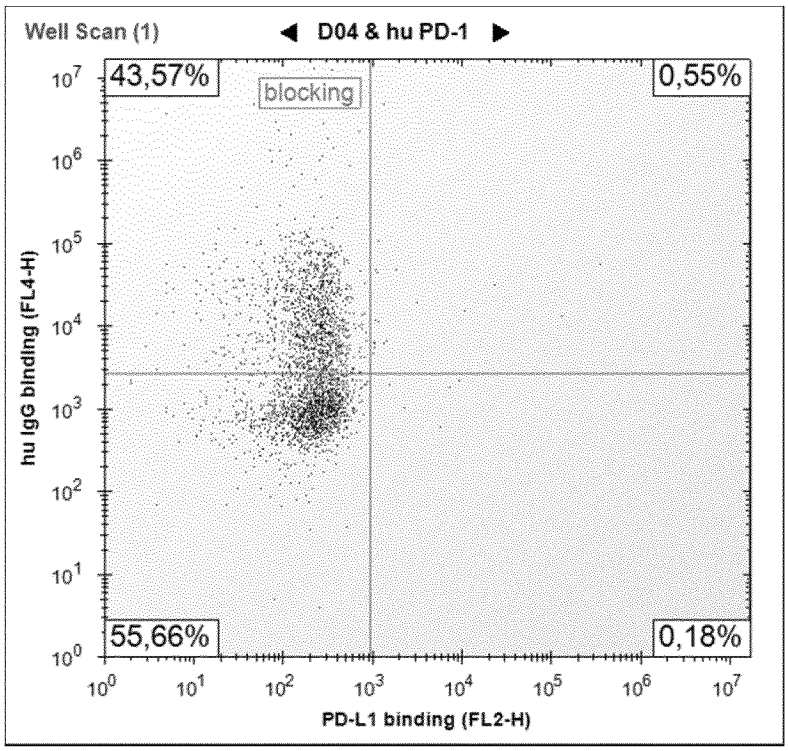
Figure 2B



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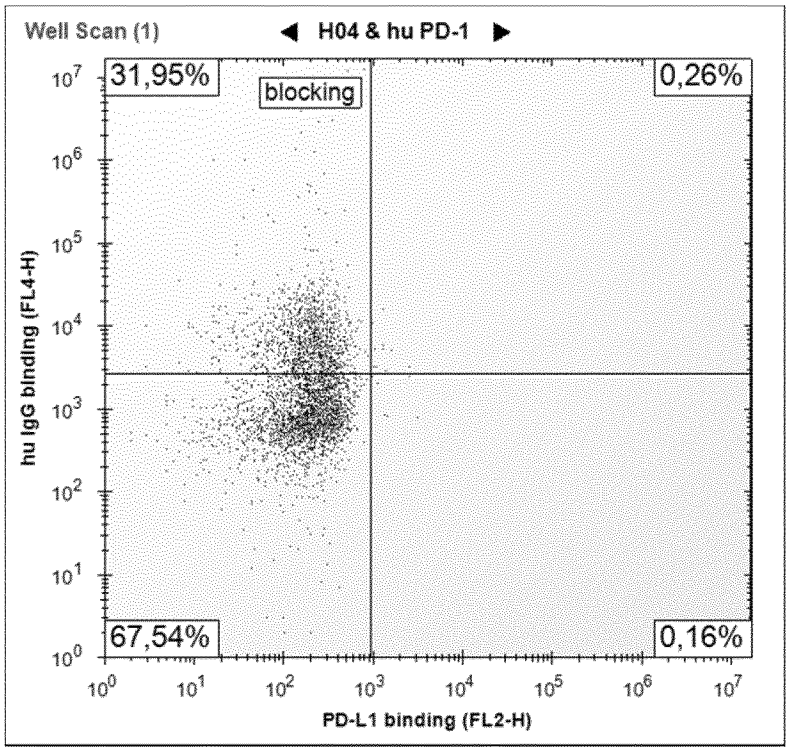

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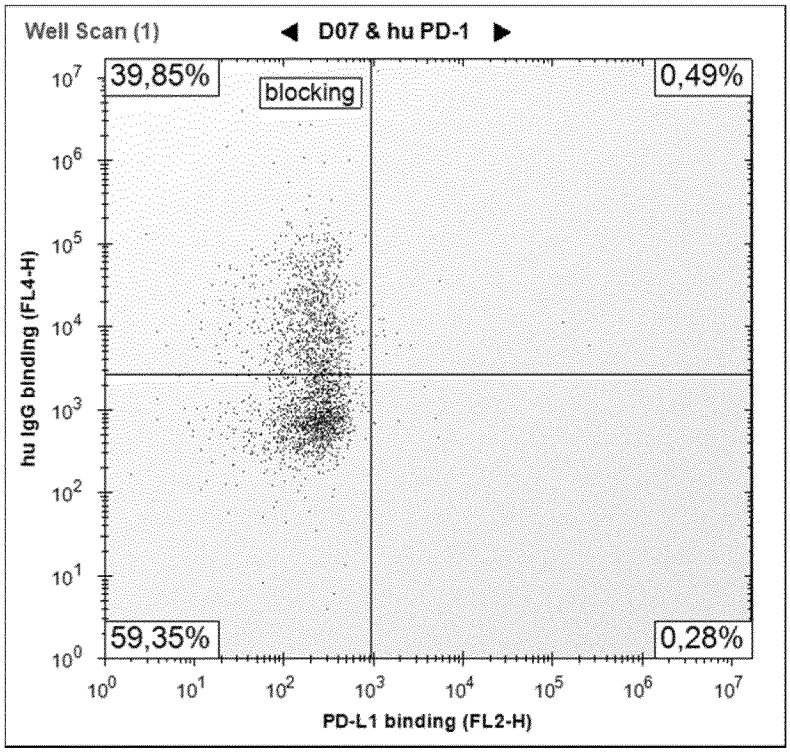
Figure 2C



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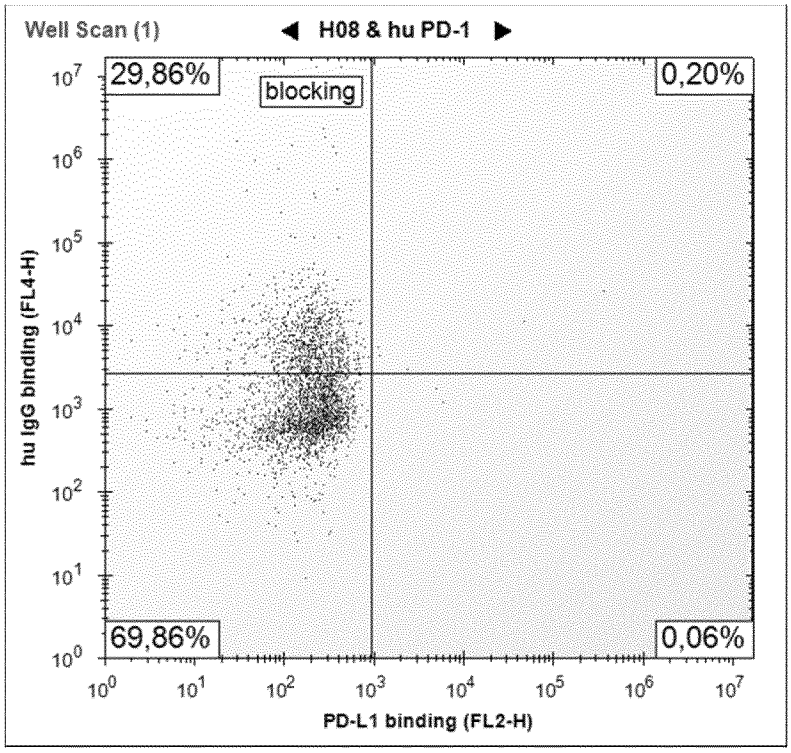

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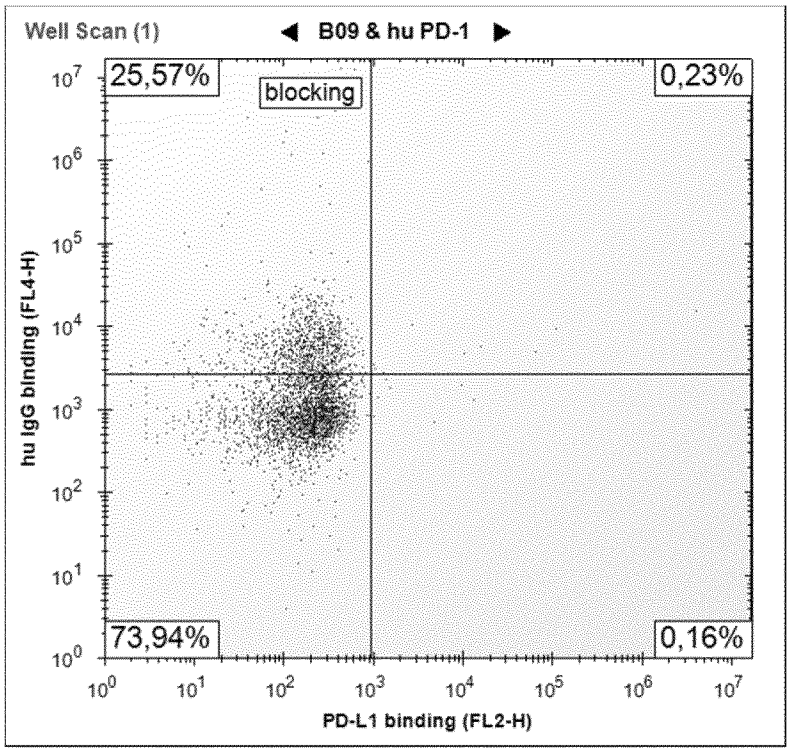
Figure 2D



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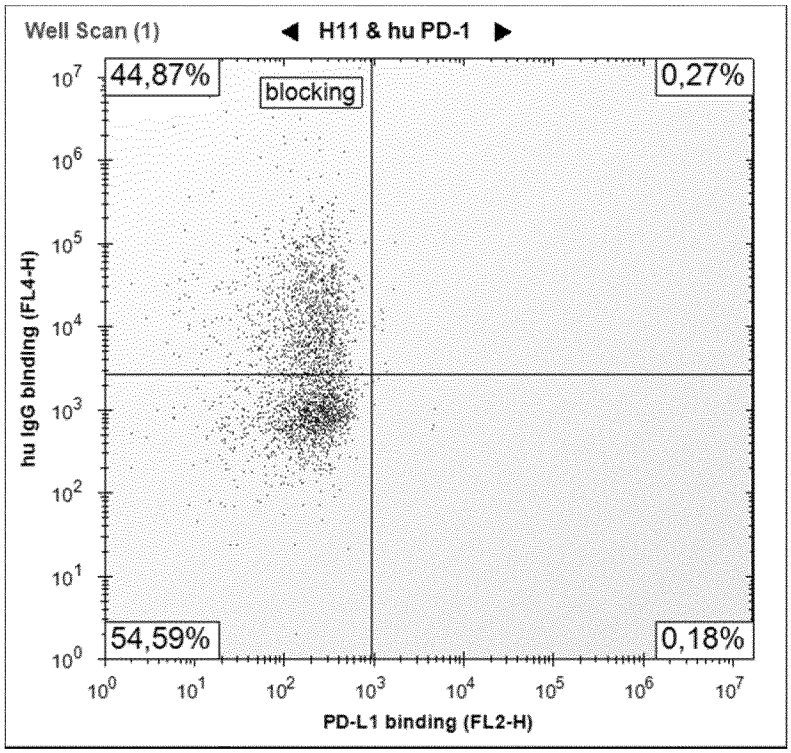

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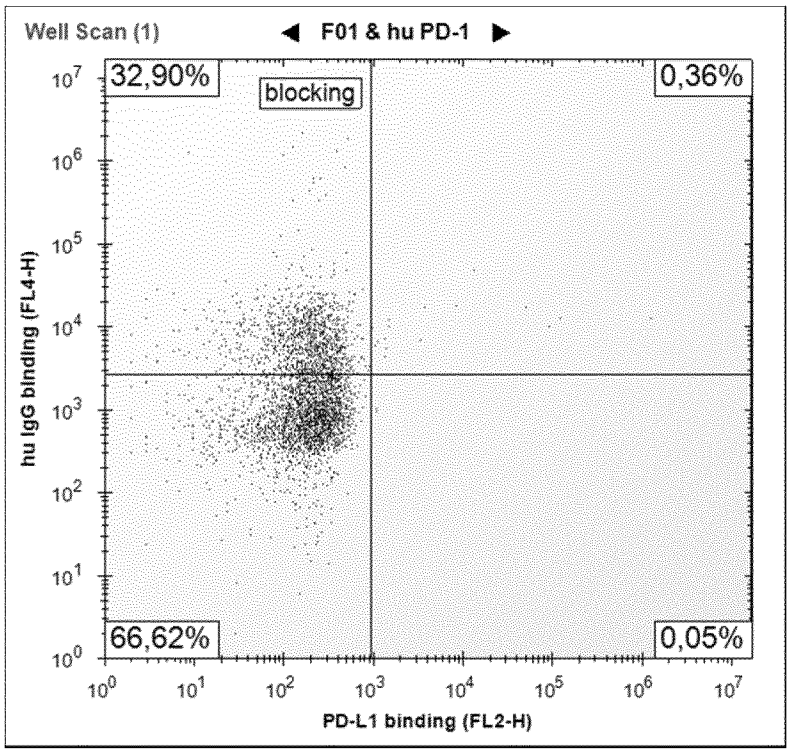

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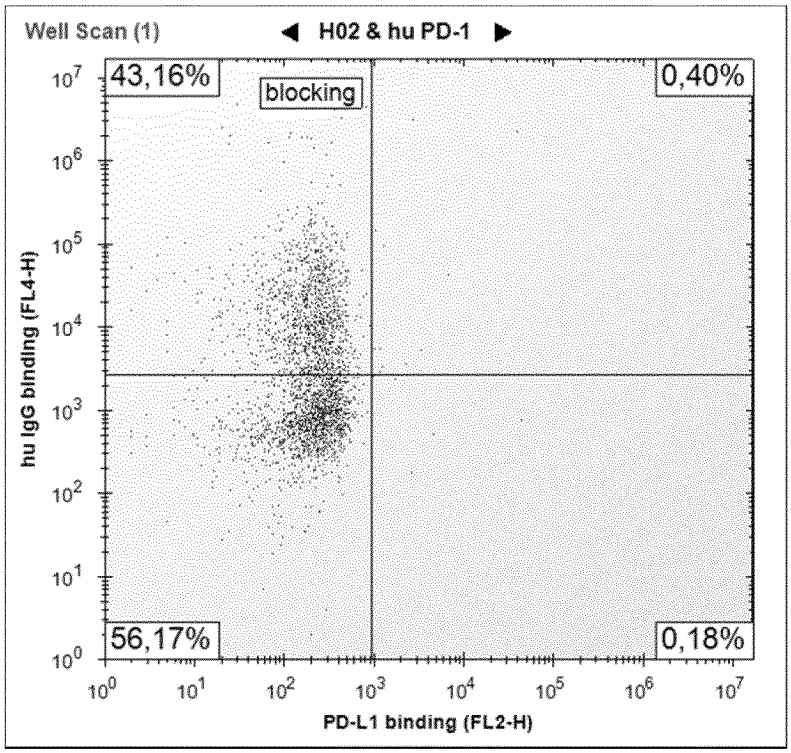

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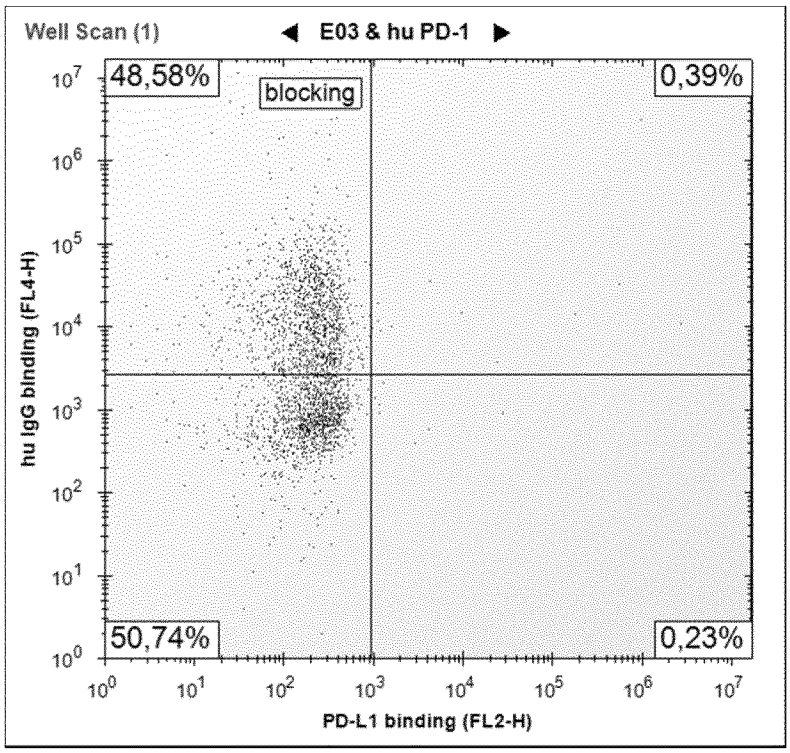
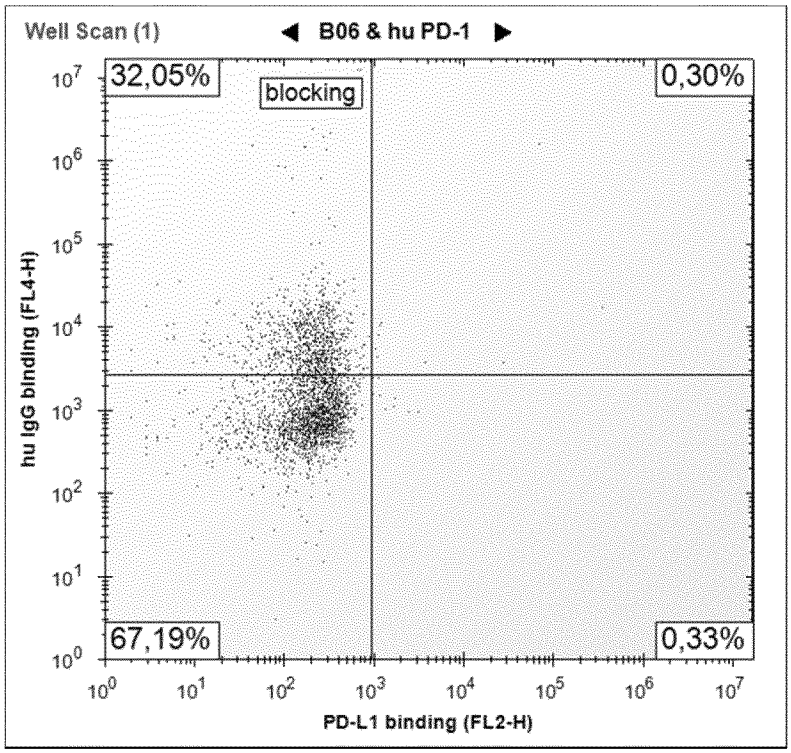
Figure 2F

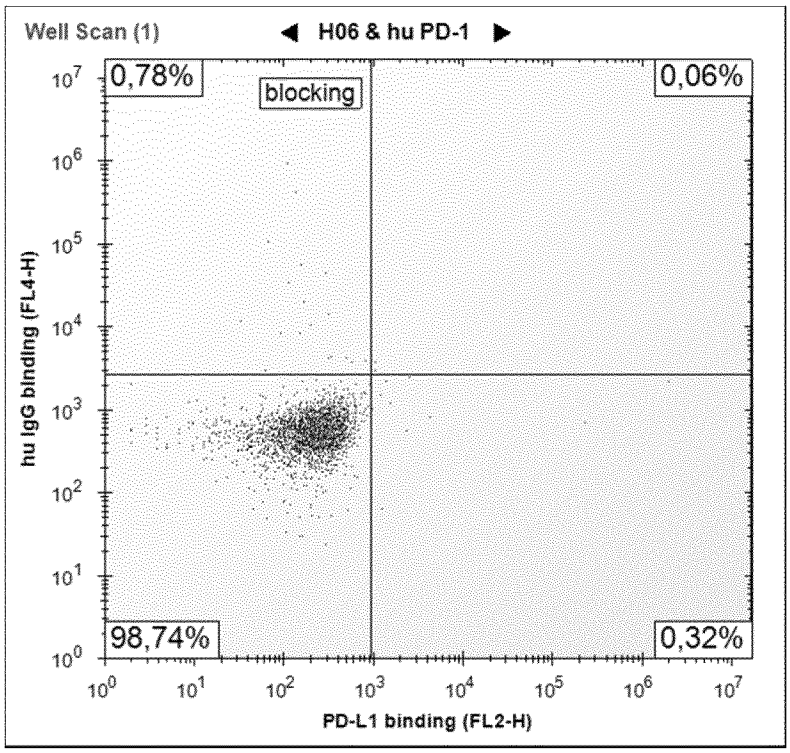


18413

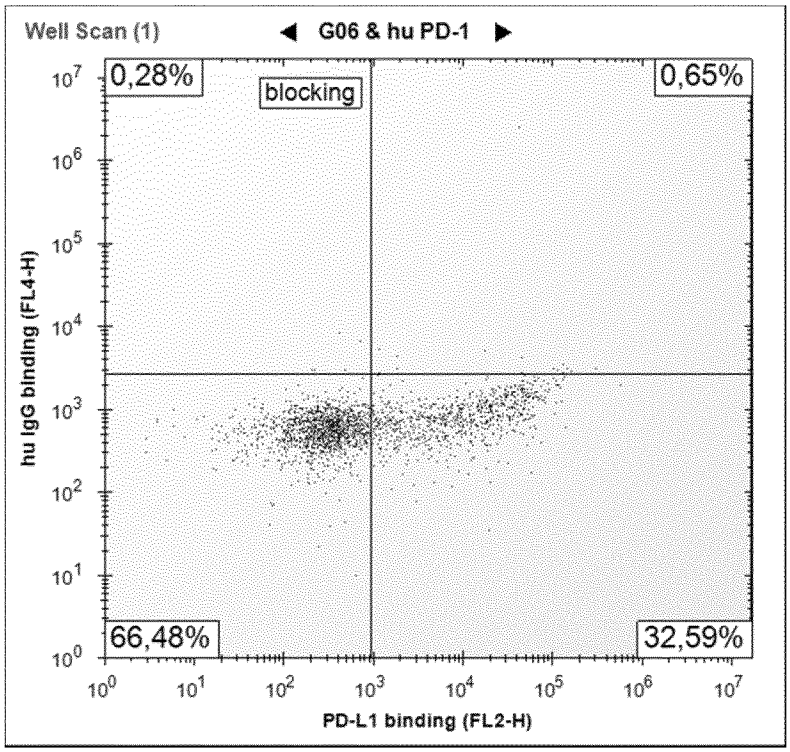


18483

Figure 2G

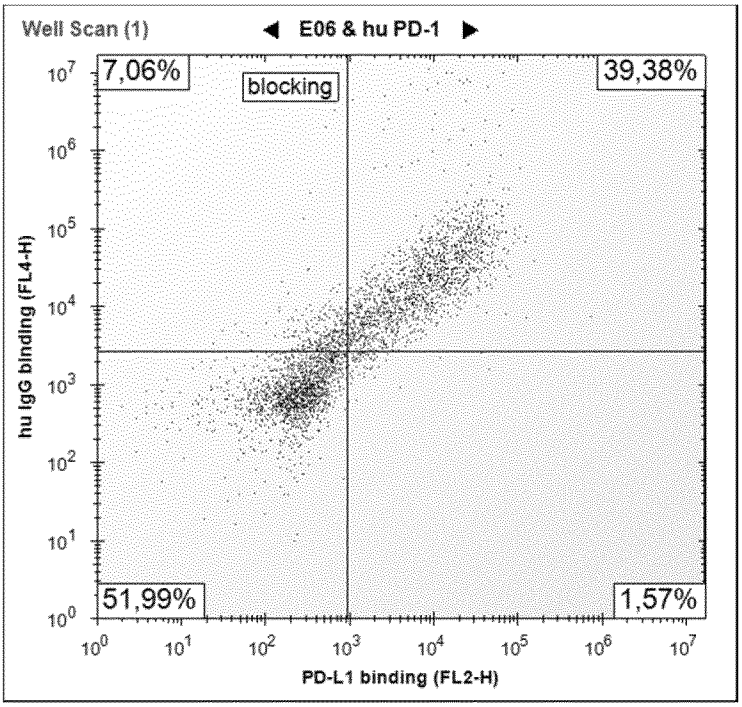


no PD-L1-PE
no mAb



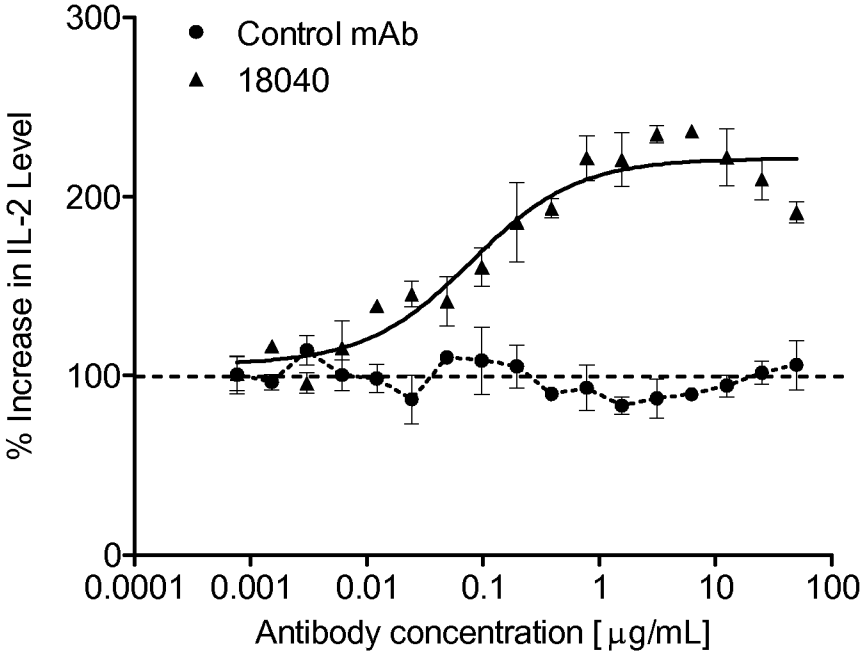
control mAb

Figure 2H



non-blocking mAb

Figure 3A


300
200
100
0
Control mAb
18040
% Increase in IL-2 Level
0.0001 0.001 0.01 0.1 1 10 100
Antibody concentration [µg/mL]

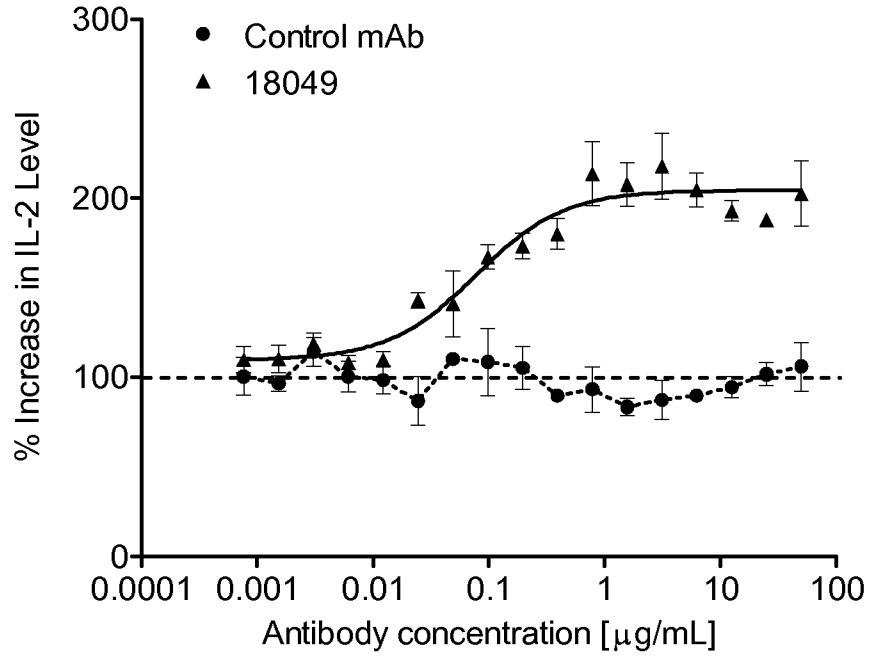
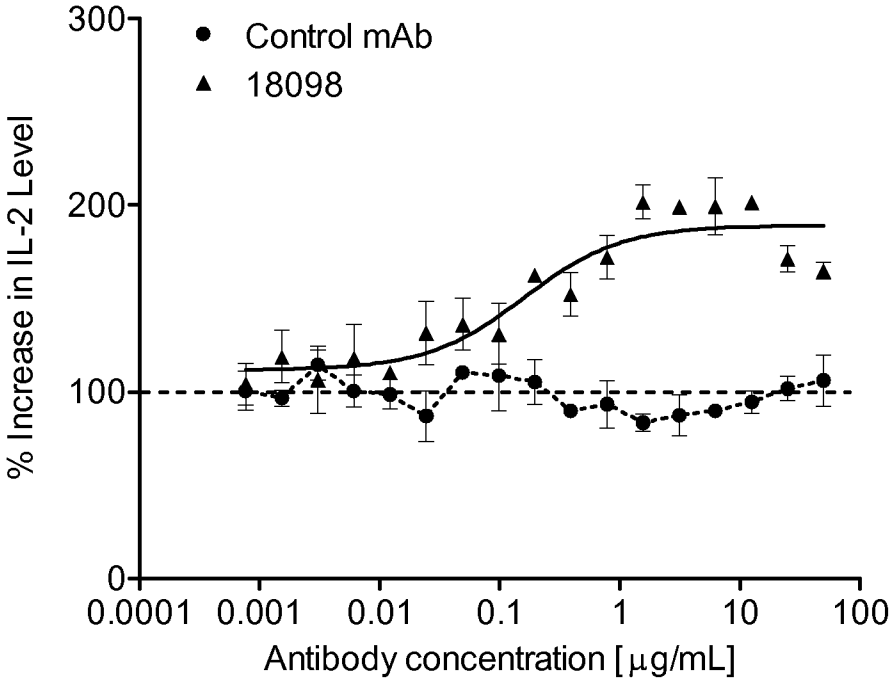

300
200
100
0
Control mAb
18049
% Increase in IL-2 Level
0.0001 0.001 0.01 0.1 1 10 100
Antibody concentration [µg/mL]

Figure 3B


300
200
100
0
Control mAb
18098
% Increase in IL-2 Level
0.0001
0.001
0.01
0.1
1
10
100
Antibody concentration [μg/mL]

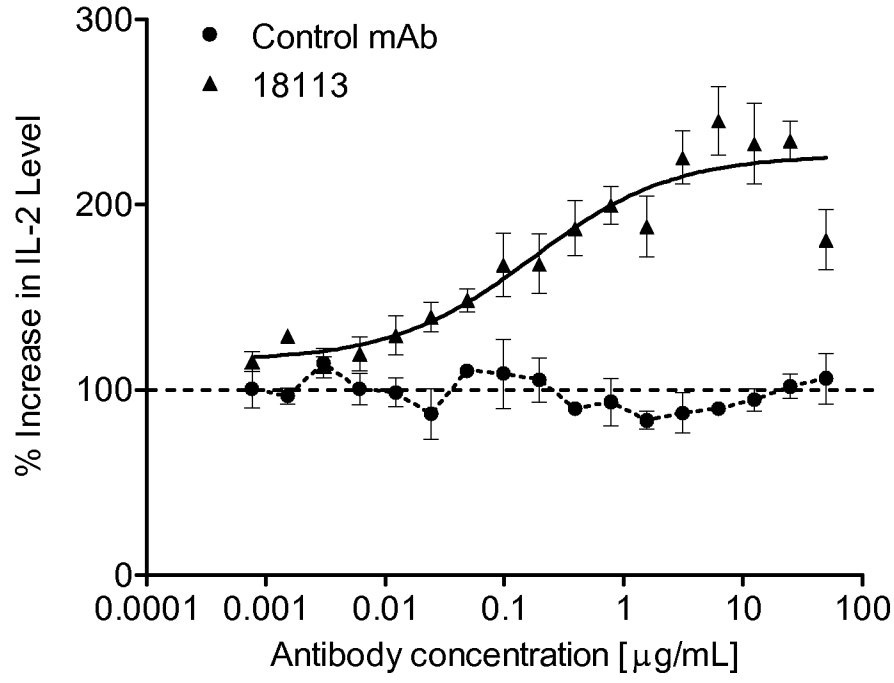
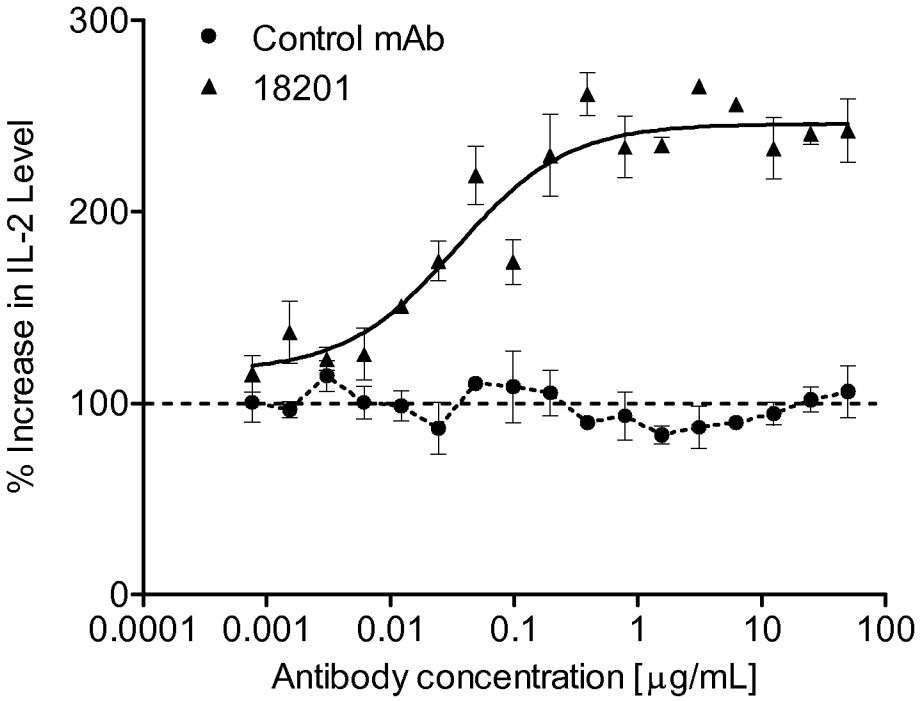

300
200
100
0
Control mAb
18113
% Increase in IL-2 Level
0.0001
0.001
0.01
0.1
1
10
100
Antibody concentration [μg/mL]

Figure 3C


300
200
100
0
% Increase in IL-2 Level
Control mAb
18201
0.0001
0.001
0.01
0.1
1
10
100
Antibody concentration [μg/mL]

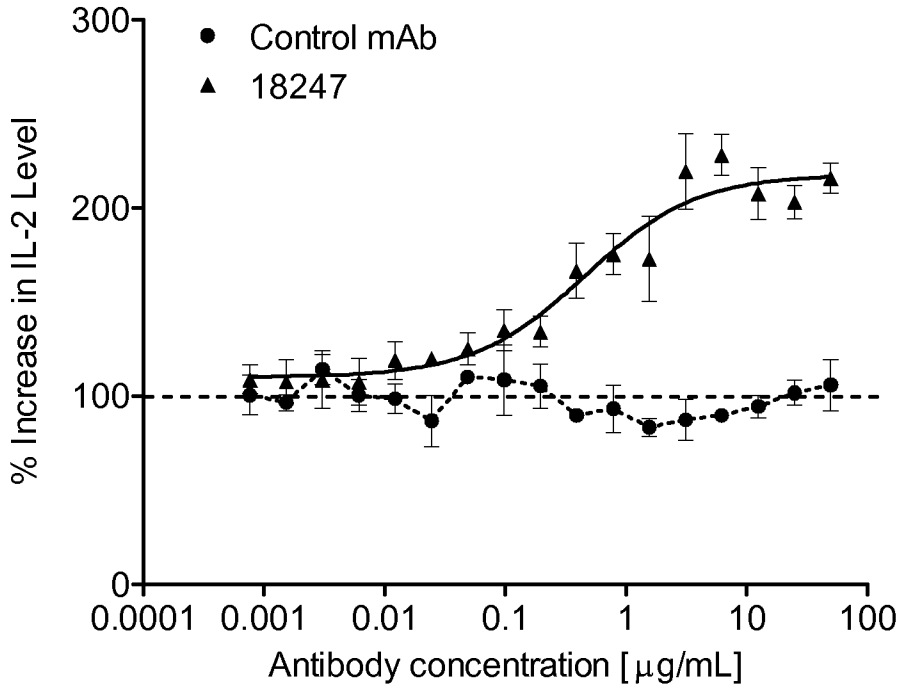
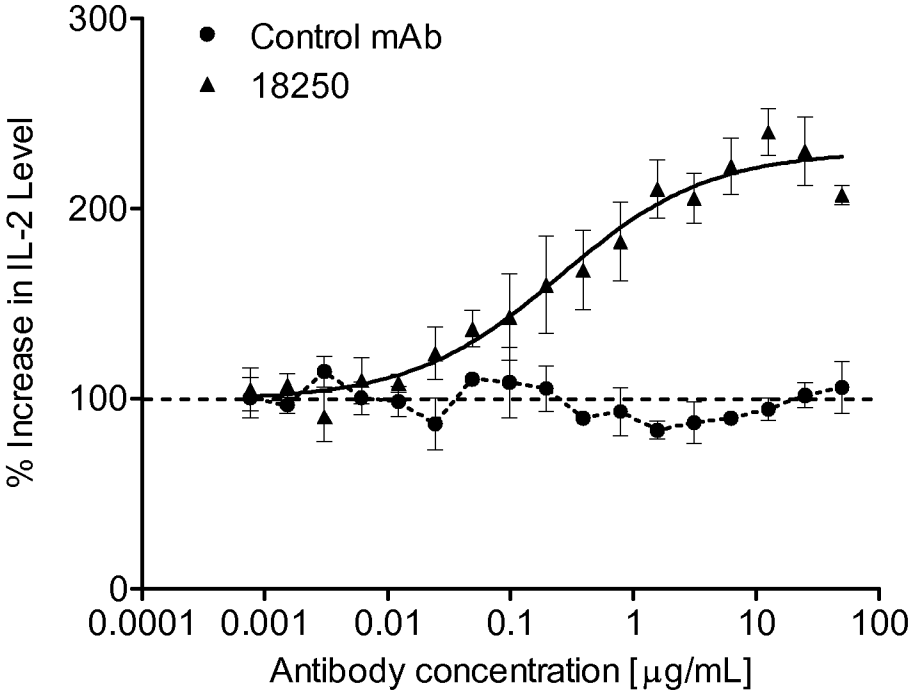

300
200
100
0
% Increase in IL-2 Level
Control mAb
18247
0.0001
0.001
0.01
0.1
1
10
100
Antibody concentration [μg/mL]

Figure 3D


300
200
100
0
% Increase in IL-2 Level
Control mAb
18250
0.0001
0.001
0.01
0.1
1
10
100
Antibody concentration [µg/mL]

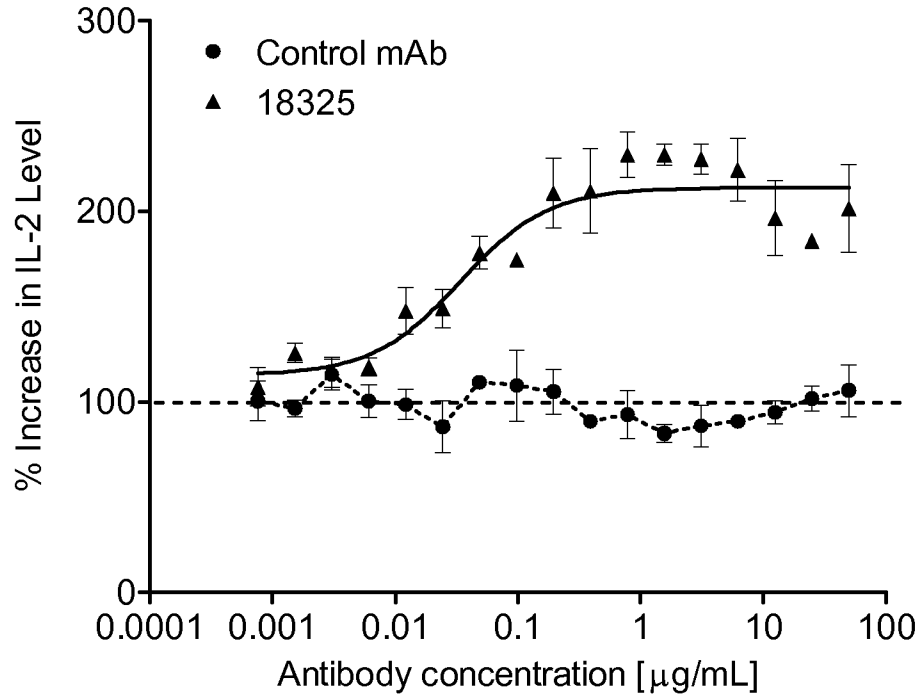

300
200
100
0
% Increase in IL-2 Level
Control mAb
18325
0.0001
0.001
0.01
0.1
1
10
100
Antibody concentration [µg/mL]

Figure 3E

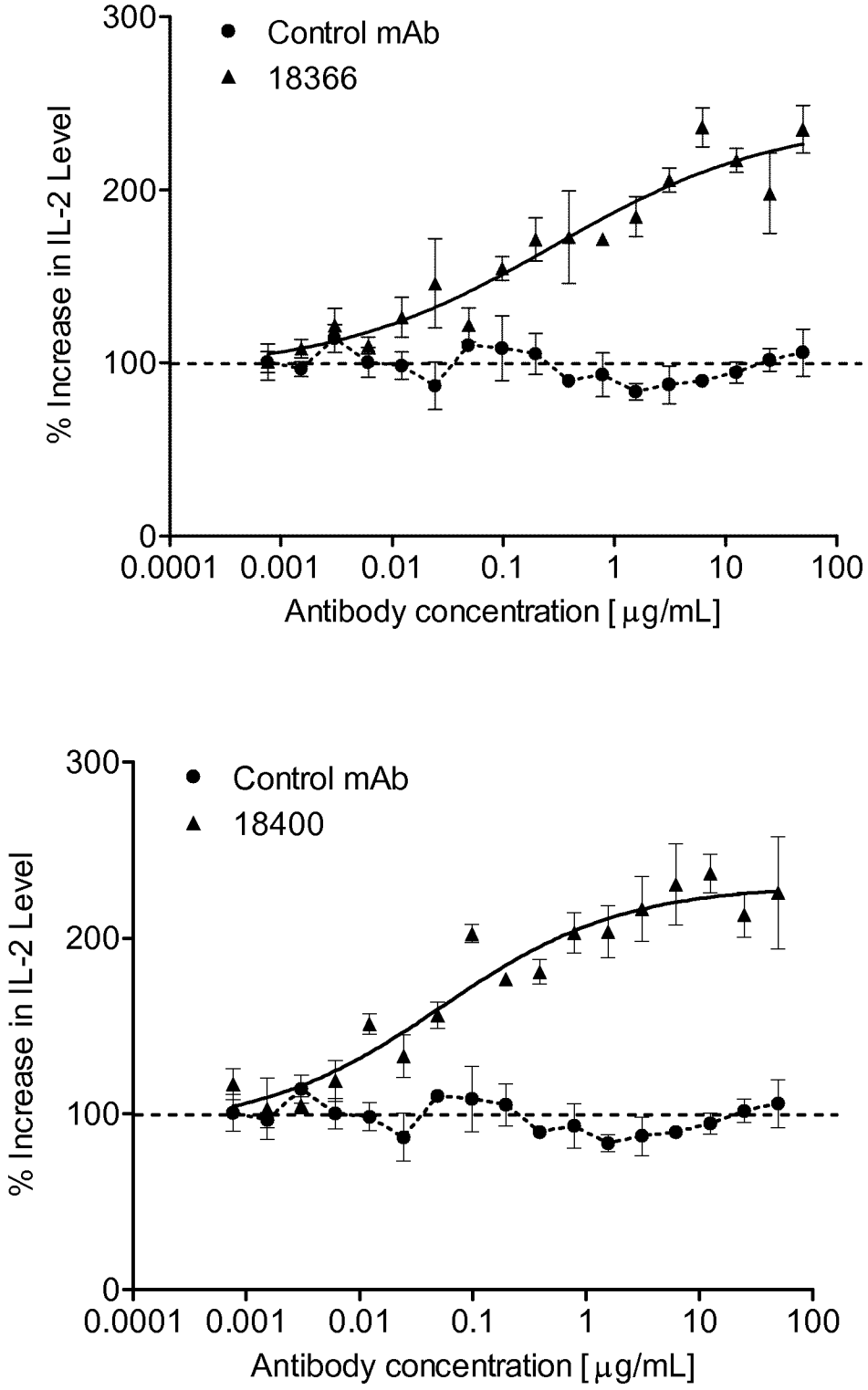
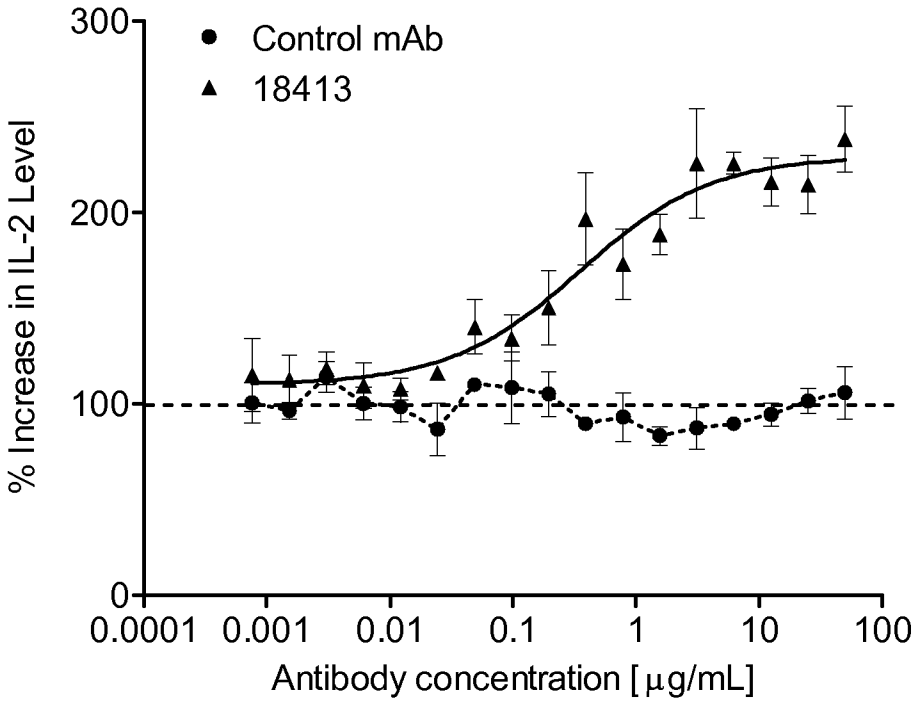

300
200
100
0
% Increase in IL-2 Level
Control mAb
18366
0.0001
0.001
0.01
0.1
1
10
100
Antibody concentration [μg/mL]
300
200
100
0
% Increase in IL-2 Level
Control mAb
18400
0.0001
0.001
0.01
0.1
1
10
100
Antibody concentration [μg/mL]

Figure 3F


300
200
100
0
% Increase in IL-2 Level
Control mAb
18413
0.0001
0.001
0.01
0.1
1
10
100
Antibody concentration [μg/mL]

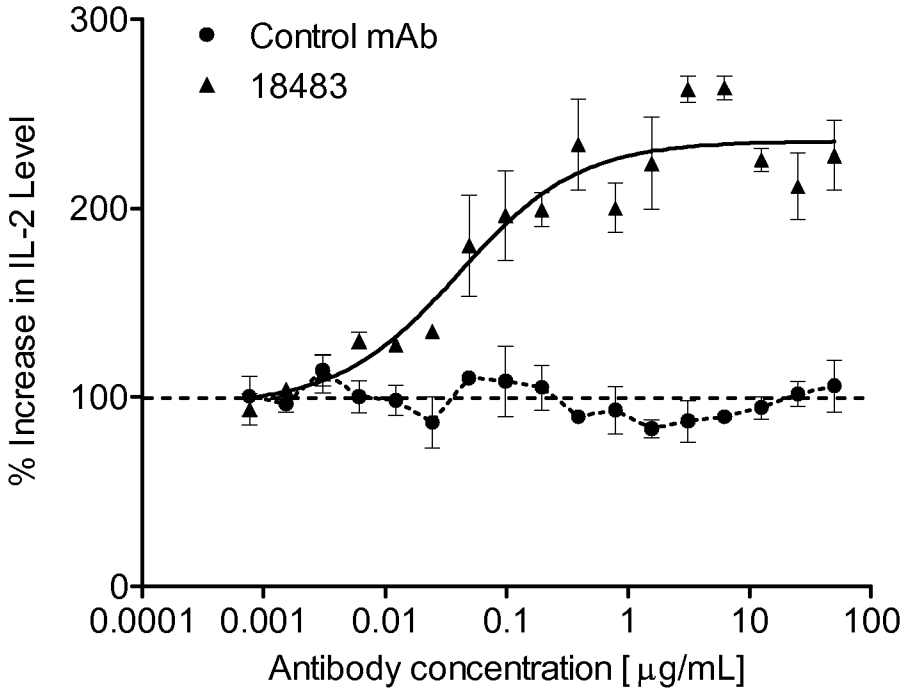
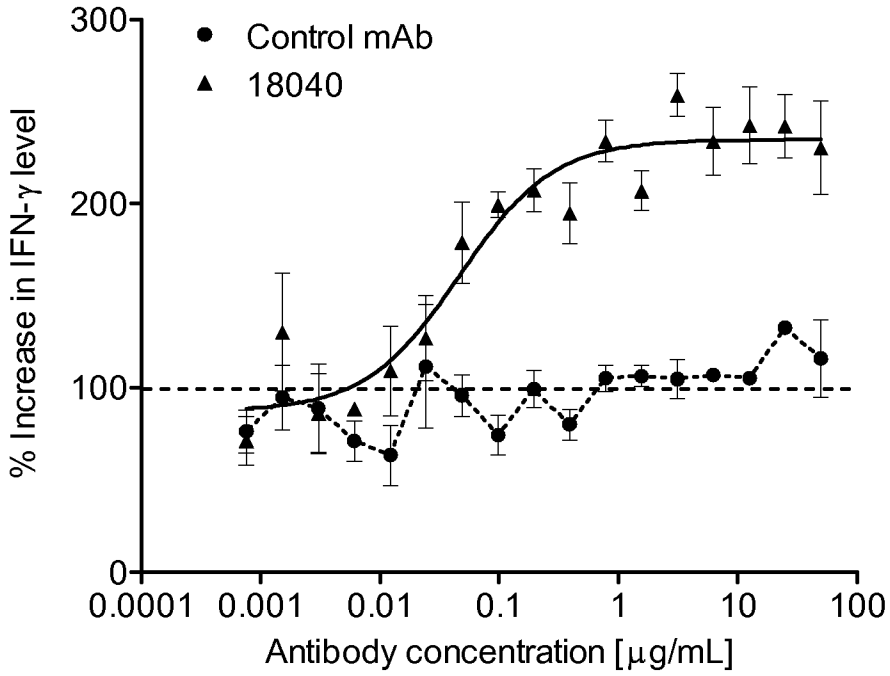

300
200
100
0
% Increase in IL-2 Level
Control mAb
18483
0.0001
0.001
0.01
0.1
1
10
100
Antibody concentration [μg/mL]

Figure 4A


300
200
100
0
% Increase in IFN-γ level
Control mAb
18040
0.0001
0.001
0.01
0.1
1
10
100
Antibody concentration [μg/mL]

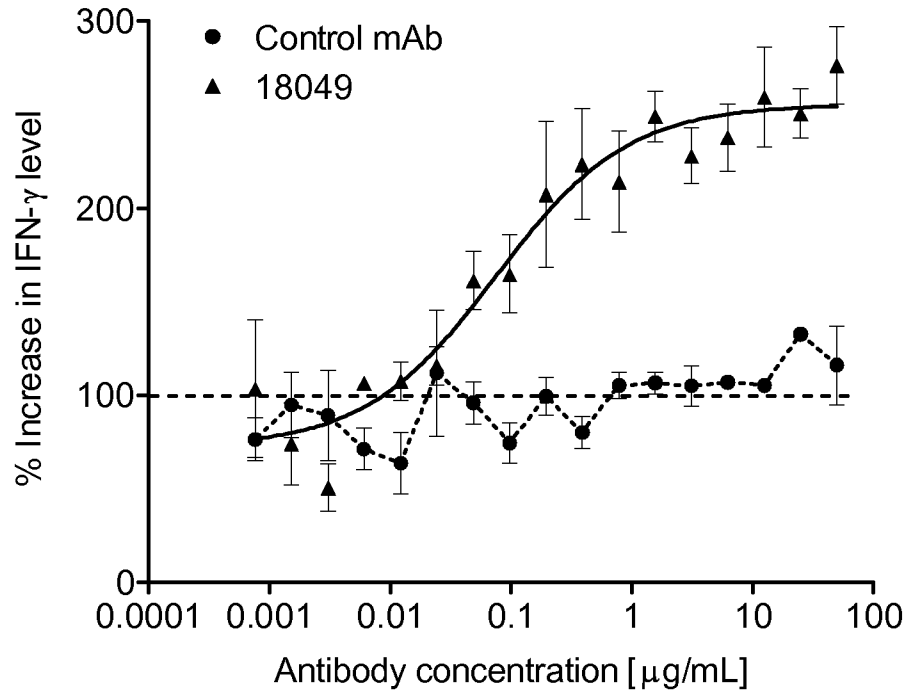
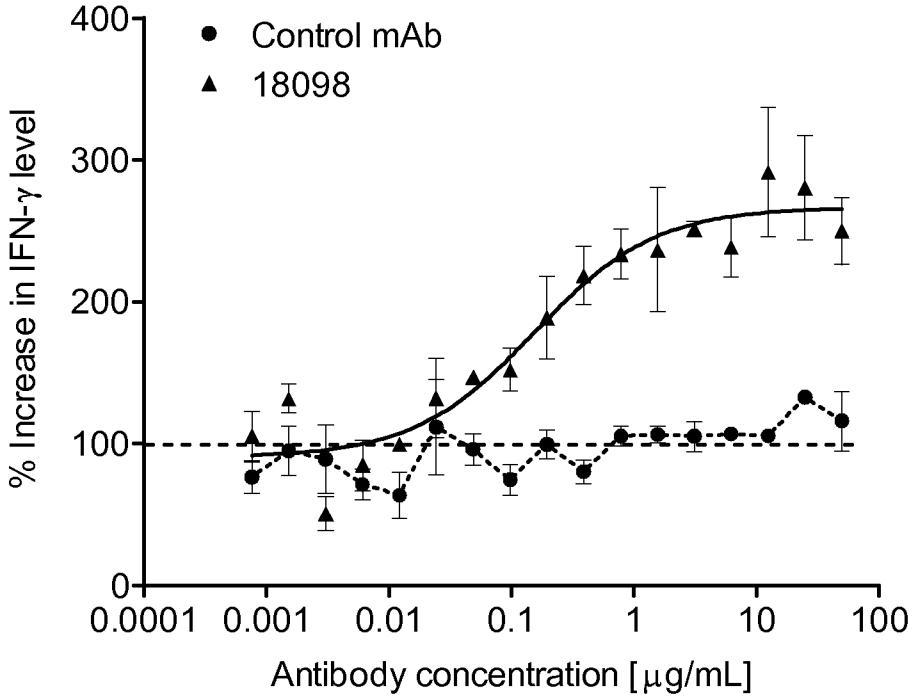

300
200
100
0
% Increase in IFN-γ level
Control mAb
18049
0.0001
0.001
0.01
0.1
1
10
100
Antibody concentration [μg/mL]

Figure 4B


Control mAb
18098
% Increase in IFN-γ level
400
300
200
100
0
0.0001
0.001
0.01
0.1
1
10
100
Antibody concentration [μg/mL]

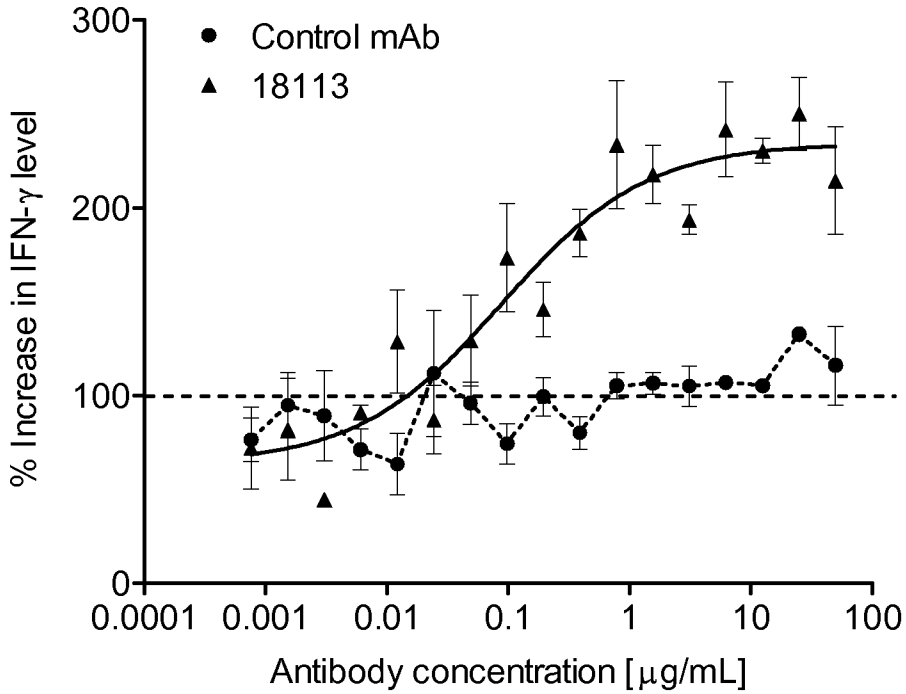
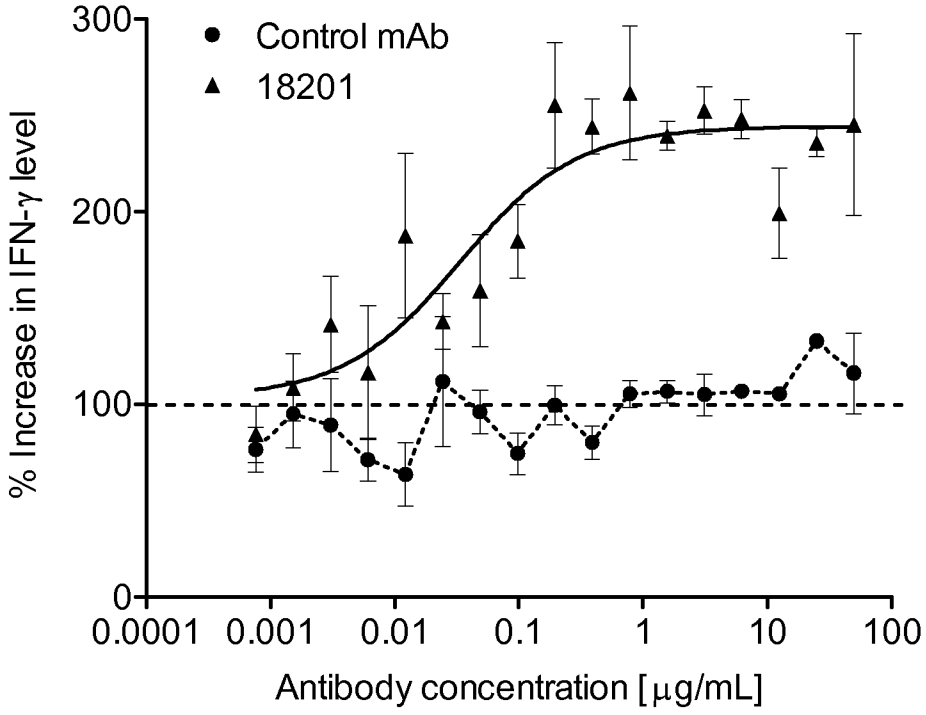

Control mAb
18113
% Increase in IFN-γ level
300
200
100
0
0.0001
0.001
0.01
0.1
1
10
100
Antibody concentration [μg/mL]

Figure 4C


300
200
100
0
% Increase in IFN-γ level
Control mAb
18201
0.0001
0.001
0.01
0.1
1
10
100
Antibody concentration [μg/mL]

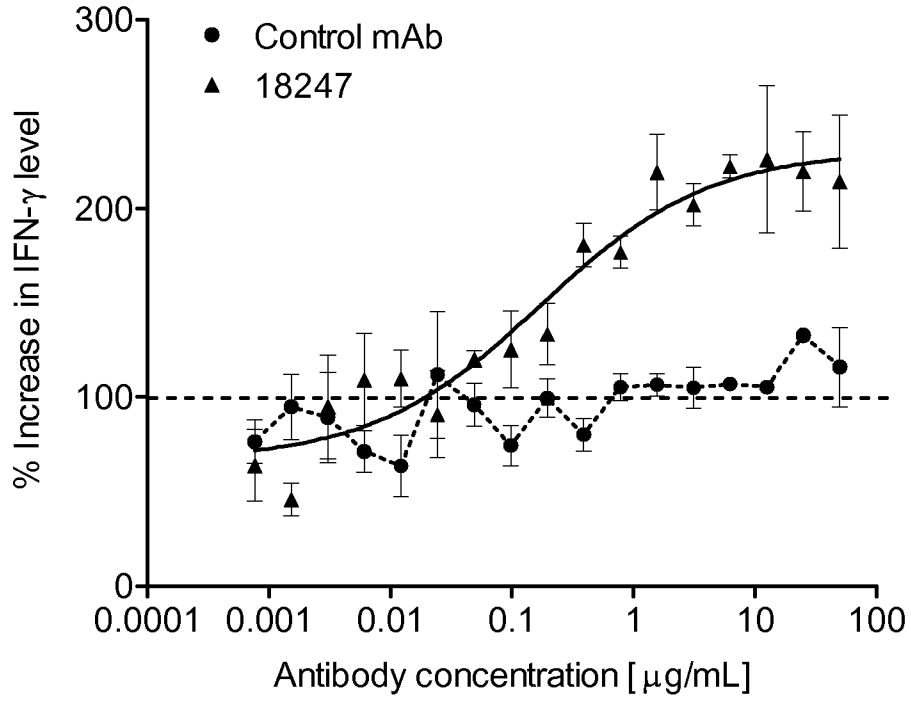
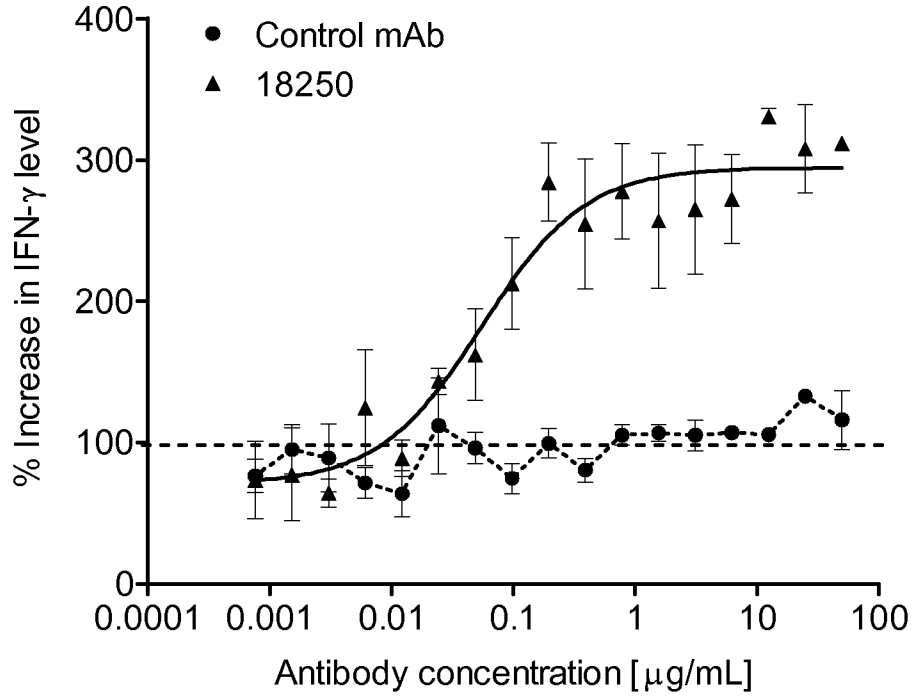

300
200
100
0
% Increase in IFN-γ level
Control mAb
18247
0.0001
0.001
0.01
0.1
1
10
100
Antibody concentration [μg/mL]

Figure 4D


Control mAb
18250
% Increase in IFN-γ level
400
300
200
100
0
0.0001
0.001
0.01
0.1
1
10
100
Antibody concentration [μg/mL]

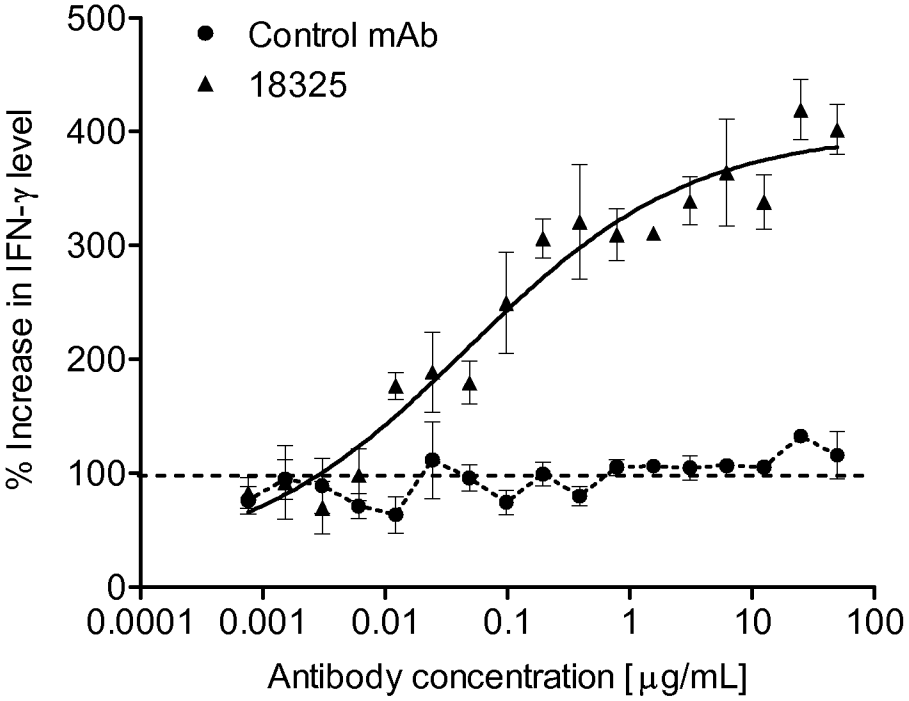
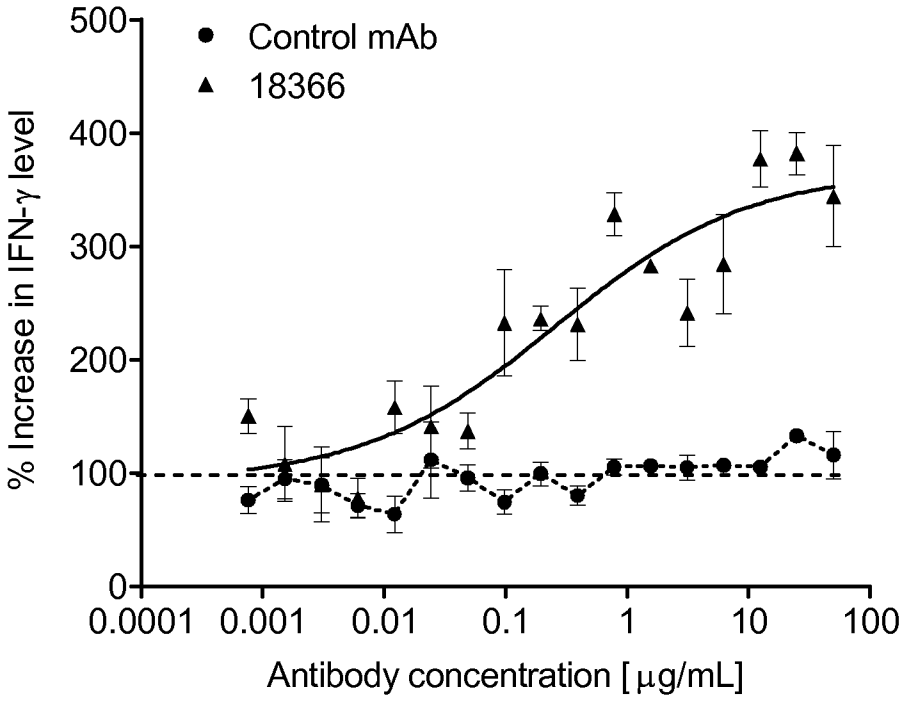

Control mAb
18325
% Increase in IFN-γ level
500
400
300
200
100
0
0.0001
0.001
0.01
0.1
1
10
100
Antibody concentration [μg/mL]

Figure 4E


Control mAb
18366
% Increase in IFN-γ level
500
400
300
200
100
0
0.0001
0.001
0.01
0.1
1
10
100
Antibody concentration [μg/mL]

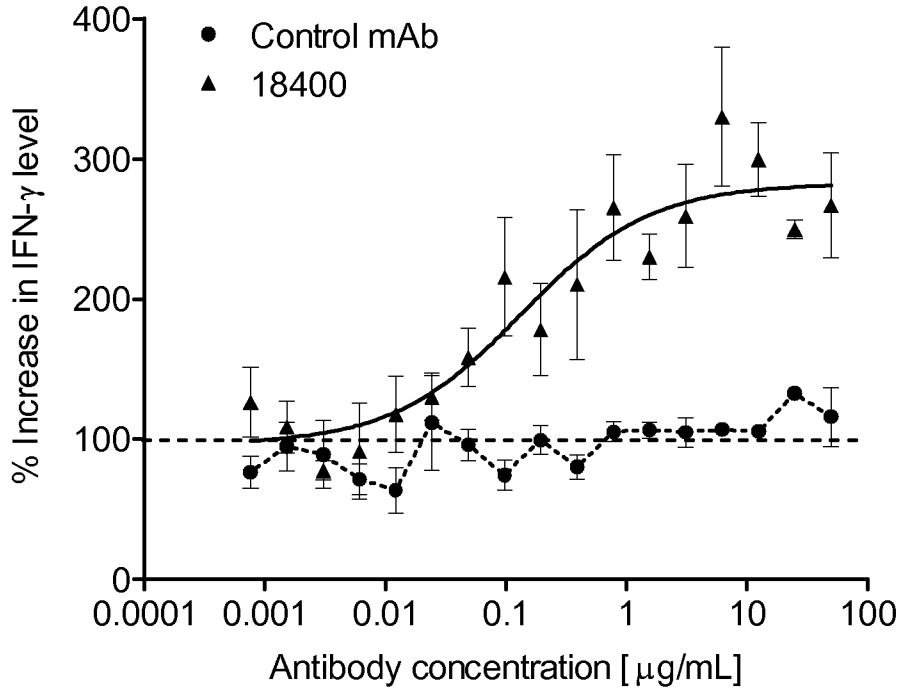
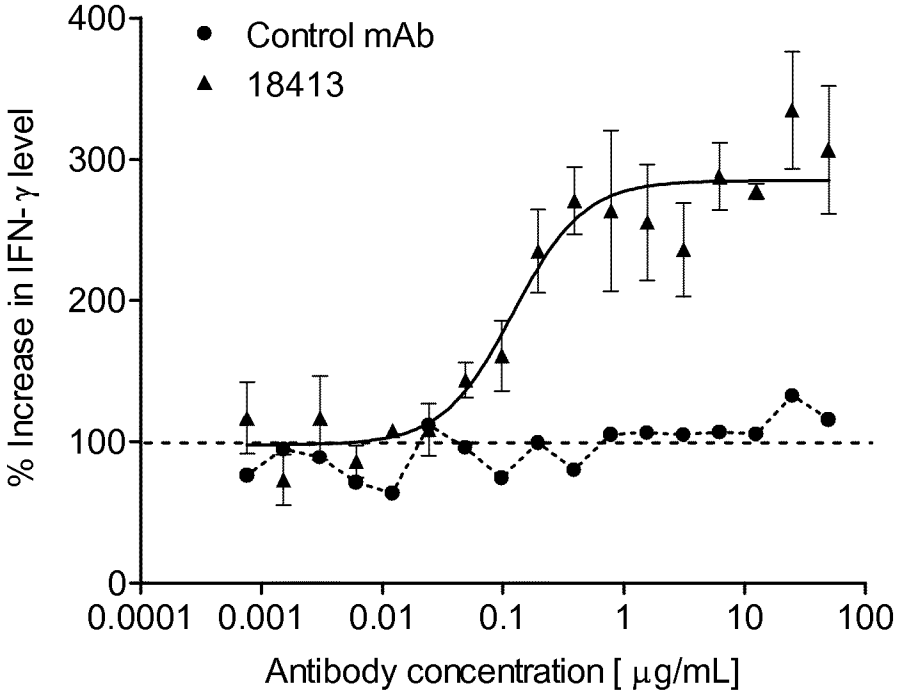

Control mAb
18400
% Increase in IFN-γ level
400
300
200
100
0
0.0001
0.001
0.01
0.1
1
10
100
Antibody concentration [μg/mL]

Figure 4F


Control mAb
18413
% Increase in IFN-γ level
400
300
200
100
0
0.0001
0.001
0.01
0.1
1
10
100
Antibody concentration [μg/mL]

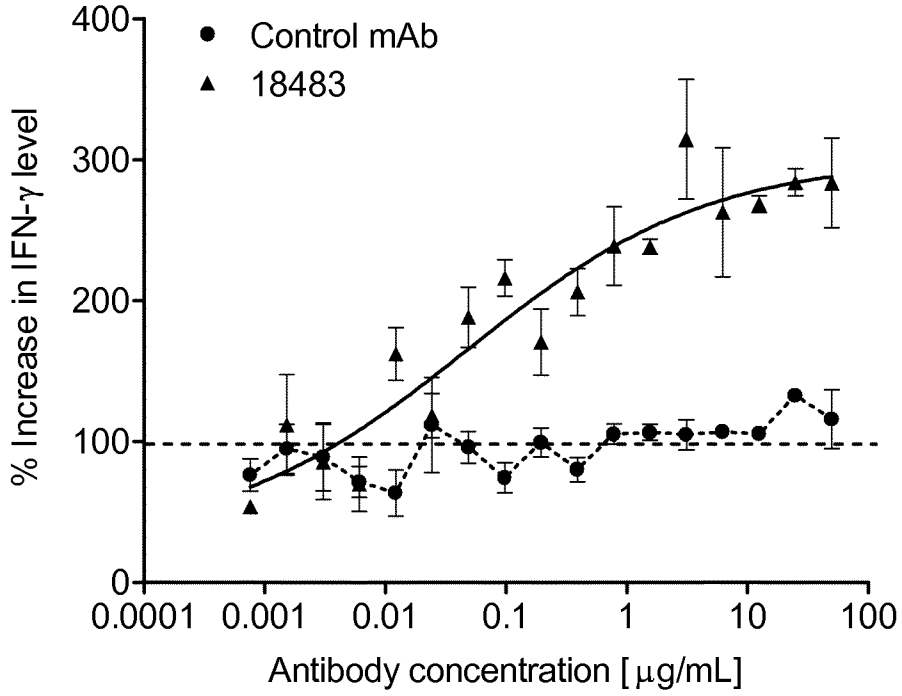
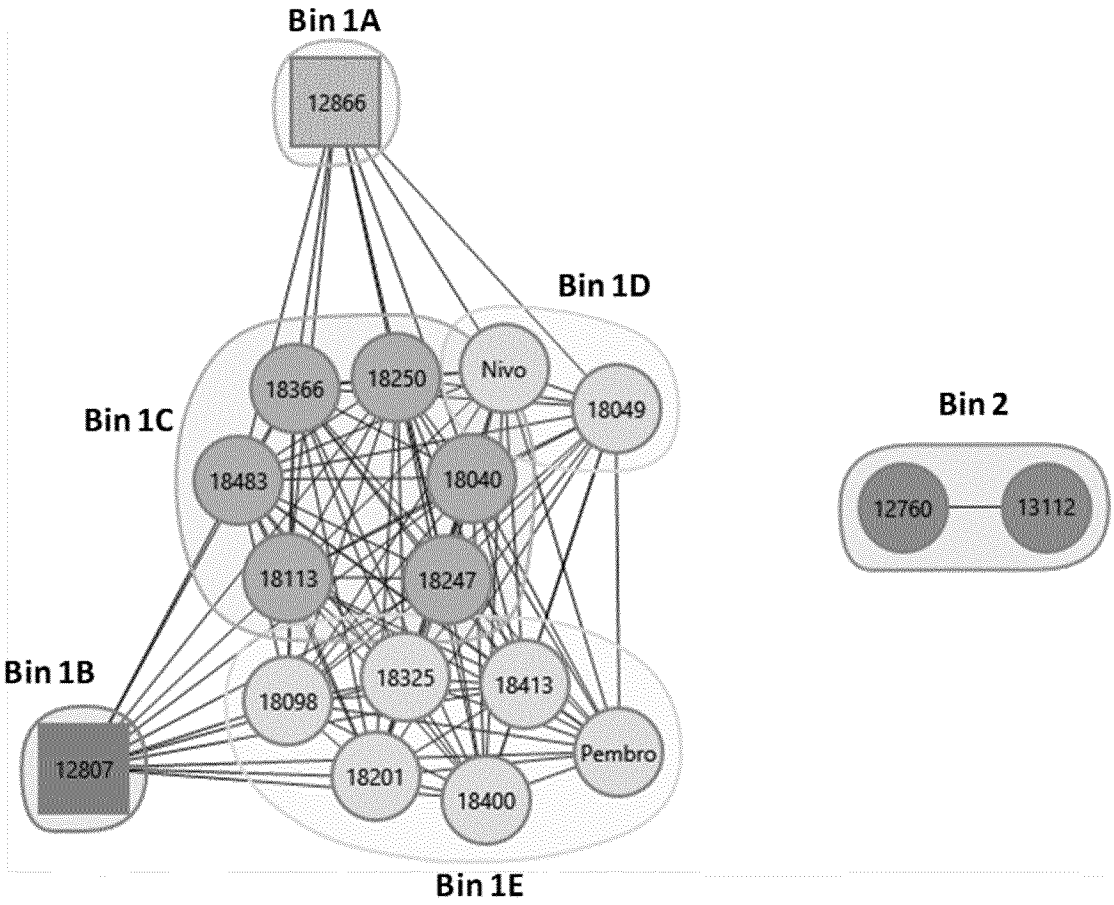

Control mAb
18483
% Increase in IFN-γ level
400
300
200
100
0
0.0001
0.001
0.01
0.1
1
10
100
Antibody concentration [μg/mL]

Figure 5


Bin 1A
12866
Bin 1D
Nivo
18366
18250
18049
Bin 1C
18483
18040
18113
18247
Bin 2
12760
13112
Bin 1B
18098
18325
18413
12807
Pembro
18201
18400
Bin 1E

SEQUENCE LISTING

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<120> ANTI-PD-1 ANTIBODIES AND COMPOSITIONS

<130> SPG18767PCT

<140> Not yet assigned
<141> Not yet assigned

<150> 62/424,163
<151> 2016-11-18

<160> 115

<170> PatentIn version 3.5

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<223> /note="Description of Artificial Sequence: Synthetic
polypeptide"

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<223> /replace="Ser"

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1 5 10 15

Ser Leu Arg Leu Ser Cys Ala Ala Ser Gly Phe Thr Phe Ser Asp Tyr

20 25 30

Tyr Met Asn Trp Ile Arg Gln Ala Pro Gly Lys Gly Leu Glu Trp Val
35 40 45

Ser Tyr Ile Ser Ser Thr Gly Ser Thr Ile Tyr Tyr Ala Asp Ser Val
50 55 60

Lys Gly Arg Phe Thr Ile Ser Arg Asp Asn Ala Lys Asn Ser Leu Tyr
65 70 75 80

Leu Gln Met Asp Ser Leu Arg Ala Glu Asp Thr Ala Val Tyr Tyr Cys
85 90 95

Ala Arg Ala Thr Asn Trp Gly Ser Asp Tyr Trp Gly Gln Gly Thr Leu
100 105 110

Val Thr Val Ser Ser
115

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<220>
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<220>
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<223> /note="Variant residues given in the sequence have no
preference with respect to those in the annotations
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Glu Ile Val Met Thr Gln Ser Pro Asp Ser Leu Ala Val Ser Leu Gly
1 5 10 15

Glu Arg Ala Thr Ile Asn Cys Lys Ser Ser Gln Ser Val Leu Tyr Ser
20 25 30

Ser Asn Asn Lys Asn Tyr Leu Phe Trp Tyr Gln Gln Lys Pro Gly Gln
35 40 45

Pro Pro Lys Leu Leu Ile Phe Trp Ala Ser Thr Arg Glu Ser Gly Val
50 55 60

Pro Asp Arg Phe Ser Gly Ser Gly Ser Gly Thr Asp Phe Thr Leu Thr
65 70 75 80

Ile Ser Ser Leu Gln Ala Glu Asp Val Ala Val Tyr Tyr Cys Gln Gln
85 90 95

Tyr Tyr Ser Thr Pro Tyr Thr Phe Gly Gln Gly Thr Lys Val Glu Ile
100 105 110

Lys

<210> 3
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<212> PRT
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<220>
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<223> /note="Description of Artificial Sequence: Synthetic
polypeptide"

<400> 3

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Gln Val Gln Leu Val Glu Ser Gly Gly Gly Val Val Gln Pro Gly Arg
1               5                   10                  15


Ser Leu Arg Leu Ser Cys Ala Ala Ser Gly Phe Thr Phe Ser Asn Tyr
            20                  25                  30


Gly Met His Trp Val Arg Gln Ala Pro Gly Lys Gly Leu Glu Trp Val
        35                  40                  45


Ala Val Ile Trp Tyr Asp Gly Ser Asp Lys Tyr Tyr Ala Asp Ser Val
    50                  55                  60


Lys Gly Arg Phe Thr Ile Ser Arg Asp Asn Ser Lys Asn Thr Leu Tyr
65                  70                  75                  80


Leu Gln Met Asn Ser Leu Arg Ala Glu Asp Thr Ala Val Tyr Tyr Cys
                85                  90                  95


Ala Gly Gly Gly Asn Tyr Tyr Gly Asp Phe Trp Gly Gln Gly Thr Leu
            100                 105                 110


Val Thr Val Ser Ser
        115


<210> 4
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<223> /note="Description of Artificial Sequence: Synthetic
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<220>
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<220>
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preference with respect to those in the annotations
for variant positions"

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Glu Ile Val Met Thr Gln Ser Pro Ser Ser Leu Ser Ala Ser Val Gly
1 5 10 15

Asp Arg Val Thr Ile Thr Cys Arg Ala Ser Gln Gly Ile Arg Asn Asp
20 25 30

Leu Gly Trp Tyr Gln Gln Lys Pro Gly Lys Ala Pro Lys Arg Leu Ile
35 40 45

Tyr Val Ala Ser Asn Leu Gln Ser Gly Val Pro Ser Arg Phe Ser Gly
50 55 60

Ser Gly Ser Gly Thr Glu Phe Thr Leu Thr Ile Ser Ser Leu Gln Pro
65 70 75 80

Glu Asp Phe Ala Thr Tyr Tyr Cys Leu Gln Tyr Asn Ser Tyr Pro Trp
85 90 95

Thr Phe Gly Gln Gly Thr Lys Val Glu Ile Lys
100 105

<210> 5
<211> 120
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<213> Artificial Sequence

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<223> /replace="Glu"

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<220>
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preference with respect to those in the annotations
for variant positions"

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Gln Val Gln Leu Gln Glu Ser Gly Gly Gly Leu Val Arg Pro Gly Gly
1   5   10   15

Ser Leu Arg Leu Ser Cys Ala Ala Ser Gly Phe Thr Phe Ser Ser Phe
            20   25   30

Ala Met Asn Trp Val Arg Gln Ala Pro Gly Lys Gly Leu Val Trp Val
        35   40   45

Ser Thr Ile Thr Gly Gly Gly Thr Thr Ser Tyr Tyr Ala Asp Ser Val
    50   55   60

Lys Gly Arg Phe Thr Ile Ser Arg Asp Asn Ser Lys Ser Thr Leu Phe
65   70   75   80

Leu Gln Met Asn Ser Leu Arg Ala Glu Asp Thr Ala Val Tyr Tyr Cys
                85   90   95

Ala Lys Trp Gly Ser Trp Ser Ala Gly Ala Phe Asp Ile Trp Gly Gln
            100   105   110

Gly Thr Thr Val Thr Val Ser Ser
        115   120

<210> 6
<211> 107
<212> PRT
<213> Artificial Sequence

<220>
<221> source
<223> /note="Description of Artificial Sequence: Synthetic
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<220>
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<220>
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<222> (1)..(107)
<223> /note="Variant residues given in the sequence have no
preference with respect to those in the annotations
for variant positions"

<400> 6
Asp Ile Gln Leu Thr Gln Ser Pro Ser Ser Val Ser Ala Ser Val Gly
1 5 10 15

Asp Arg Val Thr Ile Thr Cys Arg Ala Ser Gln Gly Ile Ser Ser Trp
20 25 30

Leu Ala Trp Tyr Gln Gln Lys Pro Gly Lys Ala Pro Lys Leu Leu Ile
35 40 45

Tyr Ala Ala Ser Ser Leu Gln Ser Gly Val Pro Ser Arg Phe Ser Gly
50 55 60

Ser Gly Ser Gly Thr Asp Phe Thr Leu Thr Ile Ser Ser Leu Gln Pro
65 70 75 80

Glu Asp Phe Ala Thr Tyr Tyr Cys Gln Gln Ala Asn Ser Phe Pro Trp
85 90 95

Thr Phe Gly Gln Gly Thr Lys Val Glu Ile Lys
100 105

<210> 7
<211> 117
<212> PRT
<213> Artificial Sequence

<220>
<221> source
<223> /note="Description of Artificial Sequence: Synthetic
polypeptide"

<220>
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<222> (64)..(64)
<223> /replace="Val"

<220>
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<222> (1)..(117)
<223> /note="Variant residues given in the sequence have no
preference with respect to those in the annotations
for variant positions"

<400> 7
Gln Val Gln Leu Val Glu Ser Gly Gly Gly Leu Val Lys Pro Gly Gly
1 5 10 15

Ser Leu Arg Leu Ser Cys Ala Ala Ser Gly Phe Thr Phe Ser Asp Tyr
20 25 30

Tyr Met Ser Trp Ile Arg Gln Ala Pro Gly Lys Gly Leu Glu Trp Val
35 40 45

Ser Tyr Ile Ser Ser Ser Gly Ser Thr Ile Tyr Tyr Ala Asp Ser Ala
50 55 60

Lys Gly Arg Phe Thr Ile Ser Arg Asp Asn Ala Lys Asn Ser Leu Tyr
65 70 75 80

Leu Gln Met Asn Ser Leu Arg Ala Glu Asp Thr Ala Val Tyr Tyr Cys
85 90 95

Ala Arg Asp Thr Asn Trp Ala Phe Asp Tyr Trp Gly Gln Gly Thr Leu
100 105 110

Val Thr Val Ser Ser
115

<210> 8
<211> 113
<212> PRT
<213> Artificial Sequence

<220>
<221> source
<223> /note="Description of Artificial Sequence: Synthetic
polypeptide"

<220>
<221> VARIANT
<222> (3)..(3)

<223> /replace="Val"

<220>
<221> VARIANT
<222> (4)..(4)
<223> /replace="Met"

<220>
<221> VARIANT
<222> (69)..(69)
<223> /replace="Ser"

<220>
<221> MISC_FEATURE
<222> (1)..(113)
<223> /note="Variant residues given in the sequence have no
preference with respect to those in the annotations
for variant positions"

<400> 8
Asp Ile Gln Leu Thr Gln Ser Pro Asp Ser Leu Ala Val Ser Leu Gly
1 5 10 15

Glu Arg Ala Thr Ile Asn Cys Lys Ser Ser Gln Ser Val Phe Tyr Ser
20 25 30

Ala Asn Asn Lys Asn Tyr Leu Ala Trp Tyr Gln Gln Lys Pro Gly Gln
35 40 45

Pro Pro Lys Leu Leu Ile Tyr Trp Thr Ser Thr Arg Glu Ser Gly Val
50 55 60

Pro Asp Arg Phe Arg Gly Ser Gly Ser Gly Thr Asp Phe Thr Leu Thr
65 70 75 80

Ile Ser Ser Leu Gln Ala Glu Asp Val Ala Val Tyr Tyr Cys Gln Gln
85 90 95

Phe Tyr Ser Thr Pro Arg Thr Phe Gly Gln Gly Thr Lys Val Glu Ile
100 105 110

Lys

<210> 9
<211> 119
<212> PRT
<213> Artificial Sequence

<220>
<221> source
<223> /note="Description of Artificial Sequence: Synthetic
polypeptide"

<220>
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<222> (5)..(5)
<223> /replace="Gln"

<220>
<221> VARIANT
<222> (59)..(59)
<223> /replace="Asn"

<220>
<221> VARIANT
<222> (76)..(76)
<223> /replace="Lys"

<220>
<221> VARIANT
<222> (83)..(83)
<223> /replace="Leu"

<220>
<221> MISC_FEATURE
<222> (1)..(119)
<223> /note="Variant residues given in the sequence have no
preference with respect to those in the annotations
for variant positions"

<400> 9
Gln Val Gln Leu Val Glu Ser Gly Pro Gly Leu Val Lys Pro Ser Gly
1 5 10 15

Thr Leu Ser Leu Thr Cys Ala Val Ser Gly Gly Ser Ile Ser Ser Asn
20 25 30

Asn Trp Trp Ser Trp Val Arg Gln Pro Pro Gly Lys Gly Leu Glu Trp
35 40 45

Ile Gly Glu Ile Tyr His Asp Gly Thr Thr Thr Tyr Asn Pro Ser Leu

50 55 60

Lys Ser Arg Val Thr Ile Ser Val Asp Lys Ser Arg Asn Gln Phe Ser
65 70 75 80

Leu Lys Met Ser Ser Val Thr Ala Ala Asp Thr Ala Val Tyr Tyr Cys
85 90 95

Ala Arg Gly Asp Trp Gly Ser Gly Ala Phe Asp Ile Trp Gly Gln Gly
100 105 110

Thr Met Val Thr Val Ser Ser
115

<210> 10
<211> 107
<212> PRT
<213> Artificial Sequence

<220>
<221> source
<223> /note="Description of Artificial Sequence: Synthetic polypeptide"

<220>
<221> VARIANT
<222> (1)..(1)
<223> /replace="Ala"

<220>
<221> MISC_FEATURE
<222> (1)..(107)
<223> /note="Variant residues given in the sequence have no preference with respect to those in the annotations for variant positions"

<400> 10
Asp Ile Gln Met Thr Gln Ser Pro Ser Ser Leu Ser Ala Ser Val Gly
1 5 10 15

Asp Arg Val Thr Ile Thr Cys Arg Ala Ser Gln Gly Ile Arg Asn Asp
20 25 30

Leu Gly Trp Tyr Gln Gln Lys Pro Gly Lys Ala Pro Lys Leu Leu Ile

```
        35                  40                  45

Tyr Ala Ala Ser Ser Leu Gln Ser Gly Val Pro Ser Arg Phe Ser Gly
    50                  55                  60

Ser Gly Ser Gly Thr Asp Phe Thr Leu Thr Ile Ser Ser Leu Gln Pro
65                  70                  75                  80

Glu Asp Phe Ala Thr Tyr Tyr Cys Leu Gln Asp Tyr Asn Tyr Pro Arg
                85                  90                  95

Thr Phe Gly Gln Gly Thr Lys Val Glu Ile Lys
            100                 105
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<210> 11
<211> 117
<212> PRT
<213> Artificial Sequence

<220>
<221> source
<223> /note="Description of Artificial Sequence: Synthetic
polypeptide"

<220>
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<222> (10)..(10)
<223> /replace="Gly"

<220>
<221> VARIANT
<222> (16)..(16)
<223> /replace="Gly"

<220>
<221> MISC_FEATURE
<222> (1)..(117)
<223> /note="Variant residues given in the sequence have no
preference with respect to those in the annotations
for variant positions"

<400> 11

```
Gln Val Gln Leu Val Glu Ser Gly Gly Asp Leu Val Lys Pro Gly Arg
1               5                   10                  15
```

```
Ser Leu Arg Leu Ser Cys Ala Ala Ser Gly Phe Thr Phe Ser Asp Tyr
            20                  25                  30


Tyr Met Ser Trp Ile Arg Gln Ala Pro Gly Lys Gly Leu Glu Trp Val
        35                  40                  45


Ser Tyr Ile Ser Ser Ser Ser Ser Thr Ile Tyr Tyr Ala Asp Ser Val
    50                  55                  60


Lys Gly Arg Phe Thr Ile Ser Arg Asp Asn Ala Lys Asn Ser Leu Tyr
65                  70                  75                  80


Leu Gln Met Asn Ser Leu Arg Ala Glu Asp Thr Ala Val Tyr Tyr Cys
                85                  90                  95


Ala Arg Asp Thr Asn Trp Ala Phe Asp Tyr Trp Gly Gln Gly Thr Leu
            100                 105                 110


Val Thr Val Ser Ser
        115


<210> 12
<211> 113
<212> PRT
<213> Artificial Sequence

<220>
<221> source
<223> /note="Description of Artificial Sequence: Synthetic
      polypeptide"


<220>
<221> VARIANT
<222> (1)..(1)
<223> /replace="Asp"

<220>
<221> VARIANT
<222> (4)..(4)
<223> /replace="Met"

<220>
<221> VARIANT
<222> (55)..(55)
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<223> /replace="Tyr"

<220>
<221> VARIANT
<222> (93)..(93)
<223> /replace="Tyr"

<220>
<221> MISC_FEATURE
<222> (1)..(113)
<223> /note="Variant residues given in the sequence have no
preference with respect to those in the annotations
for variant positions"

<400> 12
Glu Ile Val Leu Thr Gln Ser Pro Asp Ser Leu Ala Val Ser Leu Gly
1 5 10 15

Glu Arg Ala Thr Ile Asn Cys Lys Ser Ser Gln Ser Val Phe Tyr Ser
20 25 30

Ser Asn Asn Lys Asn Tyr Leu Ala Trp Tyr Gln Gln Lys Pro Gly Gln
35 40 45

Pro Pro Lys Leu Leu Ile Phe Trp Ala Ser Thr Arg Glu Ser Gly Val
50 55 60

Pro Asp Arg Phe Ser Gly Ser Gly Ser Gly Thr Asp Phe Thr Leu Thr
65 70 75 80

Ile Ser Ser Leu Gln Ala Glu Asp Val Ala Val Tyr Phe Cys Gln Gln
85 90 95

Phe Tyr Ser Thr Pro Arg Thr Phe Gly Gln Gly Thr Lys Val Glu Ile
100 105 110

Lys

<210> 13
<211> 117
<212> PRT
<213> Artificial Sequence

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<220>
<221> source
<223> /note="Description of Artificial Sequence: Synthetic
      polypeptide"


<220>
<221> VARIANT
<222> (5)..(5)
<223> /replace="Val"

<220>
<221> VARIANT
<222> (50)..(50)
<223> /replace="Tyr"

<220>
<221> VARIANT
<222> (59)..(59)
<223> /replace="Tyr"

<220>
<221> VARIANT
<222> (61)..(61)
<223> /replace="Ala"

<220>
<221> MISC_FEATURE
<222> (1)..(117)
<223> /note="Variant residues given in the sequence have no
      preference with respect to those in the annotations
      for variant positions"

<400> 13
Gln Val Gln Leu Gln Glu Ser Gly Gly Gly Leu Val Lys Pro Gly Gly
1               5                   10                  15


Ser Leu Arg Leu Ser Cys Ala Ala Ser Gly Phe Thr Phe Arg Asp Tyr
            20                  25                  30


Tyr Met Ser Trp Ile Arg Gln Ala Pro Gly Lys Gly Leu Glu Trp Val
        35                  40                  45


Ser His Ile Ser Ser Ser Gly Ser Ile Ile Asp Tyr Val Asp Ser Val
    50                  55                  60


Lys Gly Arg Phe Thr Ile Ser Arg Asp Asn Ala Lys Asn Ser Leu Tyr
65                  70                  75                  80
```

```
Leu Gln Met Asn Ser Leu Arg Ala Glu Asp Thr Ala Val Tyr Tyr Cys
                85                  90                  95


Ala Arg Asp Thr Asn Trp Ala Leu Asp Tyr Trp Gly Gln Gly Thr Leu
            100                 105                 110


Val Thr Val Ser Ser
        115


<210> 14
<211> 113
<212> PRT
<213> Artificial Sequence

<220>
<221> source
<223> /note="Description of Artificial Sequence: Synthetic
      polypeptide"


<220>
<221> VARIANT
<222> (3)..(3)
<223> /replace="Val"

<220>
<221> VARIANT
<222> (4)..(4)
<223> /replace="Met"

<220>
<221> VARIANT
<222> (55)..(55)
<223> /replace="Tyr"

<220>
<221> MISC_FEATURE
<222> (1)..(113)
<223> /note="Variant residues given in the sequence have no
      preference with respect to those in the annotations
      for variant positions"

<400> 14
Asp Ile Gln Leu Thr Gln Ser Pro Asp Ser Leu Ala Val Ser Leu Gly
1               5                   10                  15
```

```
Glu Arg Ala Thr Ile Asn Cys Lys Ser Ser Gln Ser Val Phe Tyr Ser
            20                  25                  30


Ser Asn Asn Lys Asn Tyr Leu Ala Trp Tyr Gln Gln Lys Pro Gly Gln
        35                  40                  45


Pro Pro Lys Leu Leu Ile Ser Trp Ala Ser Thr Arg Glu Ser Gly Val
    50                  55                  60


Pro Asp Arg Phe Ser Gly Ser Gly Ser Gly Thr Asp Phe Thr Leu Thr
65                  70                  75                  80


Ile Ser Ser Leu Gln Ala Glu Asp Val Ala Val Tyr Tyr Cys Gln Gln
                85                  90                  95


Tyr Tyr Ser Thr Pro Tyr Thr Phe Gly Gln Gly Thr Lys Leu Glu Ile
            100                 105                 110


Lys



<210> 15
<211> 120
<212> PRT
<213> Artificial Sequence

<220>
<221> source
<223> /note="Description of Artificial Sequence: Synthetic
      polypeptide"


<220>
<221> VARIANT
<222> (1)..(1)
<223> /replace="Glu"

<220>
<221> VARIANT
<222> (5)..(5)
<223> /replace="Val"

<220>
<221> VARIANT
<222> (6)..(6)
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<223> /replace="Glu"

<220>
<221> VARIANT
<222> (35)..(35)
<223> /replace="Ser"

<220>
<221> VARIANT
<222> (49)..(49)
<223> /replace="Ser"

<220>
<221> VARIANT
<222> (50)..(50)
<223> /replace="Ala"

<220>
<221> VARIANT
<222> (73)..(73)
<223> /replace="Asp"

<220>
<221> VARIANT
<222> (78)..(78)
<223> /replace="Thr"

<220>
<221> MISC_FEATURE
<222> (1)..(120)
<223> /note="Variant residues given in the sequence have no
preference with respect to those in the annotations
for variant positions"

<400> 15
Gln Val Gln Leu Gln Gln Ser Gly Gly Gly Leu Val Gln Pro Gly Gly
1 5 10 15

Ser Leu Arg Leu Ser Cys Ala Ala Ser Gly Phe Thr Phe Ser Ser His
20 25 30

Val Met Asn Trp Val Arg Gln Ala Pro Gly Lys Gly Leu Glu Trp Val
35 40 45

Ala Thr Ile Ser Gly Ser Gly Val Asp Thr Tyr Tyr Ala Asp Ser Val
50 55 60

Lys Gly Arg Phe Thr Ile Ser Arg Gly Asn Ser Lys Asn Met Leu Tyr

65 70 75 80

Leu Gln Met Asn Ser Leu Arg Ala Glu Asp Thr Ala Val Tyr Tyr Cys
85 90 95

Ala Lys Trp Gly Ser Trp Ser Ala Gly Ala Phe Asp Ile Trp Gly Gln
100 105 110

Gly Thr Met Val Thr Val Ser Ser
115 120

<210> 16
<211> 107
<212> PRT
<213> Artificial Sequence

<220>
<221> source
<223> /note="Description of Artificial Sequence: Synthetic polypeptide"

<220>
<221> VARIANT
<222> (1)..(1)
<223> /replace="Asp"

<220>
<221> VARIANT
<222> (3)..(3)
<223> /replace="Gln"

<220>
<221> VARIANT
<222> (4)..(4)
<223> /replace="Met"

<220>
<221> MISC_FEATURE
<222> (1)..(107)
<223> /note="Variant residues given in the sequence have no preference with respect to those in the annotations for variant positions"

<400> 16
Glu Ile Val Leu Thr Gln Ser Pro Ser Ser Val Ser Ala Ser Val Gly
1 5 10 15

```
Asp Arg Val Thr Ile Thr Cys Arg Ala Ser Gln Gly Ile Ser Ser Trp
            20                  25                  30


Leu Ala Trp Tyr Gln Gln Lys Pro Gly Lys Ala Pro Lys Leu Leu Ile
        35                  40                  45


Tyr Ala Ala Ser Ser Leu Gln Ser Gly Val Pro Ser Arg Phe Ser Gly
    50                  55                  60


Ser Gly Ser Gly Thr Asp Phe Thr Leu Thr Ile Ser Ser Leu Gln Pro
65                  70                  75                  80


Glu Asp Phe Ala Thr Tyr Tyr Cys Gln Gln Ala Asn Ser Phe Pro Trp
                85                  90                  95


Thr Phe Gly Gln Gly Thr Lys Val Glu Ile Lys
            100                 105


<210> 17
<211> 117
<212> PRT
<213> Artificial Sequence

<220>
<221> source
<223> /note="Description of Artificial Sequence: Synthetic
      polypeptide"


<220>
<221> VARIANT
<222> (1)..(1)
<223> /replace="Glu"

<220>
<221> VARIANT
<222> (6)..(6)
<223> /replace="Glu"

<220>
<221> MISC_FEATURE
<222> (1)..(117)
<223> /note="Variant residues given in the sequence have no
      preference with respect to those in the annotations
      for variant positions"
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<400> 17
Gln Val Gln Leu Val Gln Ser Gly Gly Gly Leu Val Gln Pro Gly Gly
1   5   10   15

Ser Leu Arg Leu Ser Cys Ala Ala Ser Gly Phe Thr Phe Ser Ser Tyr
            20   25   30

Trp Met Ser Trp Val Arg Gln Ala Pro Gly Lys Gly Leu Glu Trp Val
        35   40   45

Ala Asn Ile Lys Gln Asp Gly Ser Glu Lys Tyr Tyr Val Asp Ser Val
    50   55   60

Lys Gly Arg Phe Thr Ile Ser Arg Asp Asn Ala Lys Asn Ser Leu Tyr
65   70   75   80

Leu Gln Met Asn Ser Leu Arg Ala Glu Asp Thr Ala Val Tyr Tyr Cys
                85   90   95

Ala Arg Asp Thr Asn Trp Gly Phe Asp Asn Trp Gly Gln Gly Thr Leu
            100   105   110

Val Thr Val Ser Ser
        115

<210> 18
<211> 113
<212> PRT
<213> Artificial Sequence

<220>
<221> source
<223> /note="Description of Artificial Sequence: Synthetic
      polypeptide"

<220>
<221> VARIANT
<222> (1)..(1)
<223> /replace="Asp"

<220>
<221> VARIANT

<222> (40)..(40)
<223> /replace="Ala"

<220>
<221> VARIANT
<222> (55)..(55)
<223> /replace="Tyr"

<220>
<221> MISC_FEATURE
<222> (1)..(113)
<223> /note="Variant residues given in the sequence have no
preference with respect to those in the annotations
for variant positions"

<400> 18
Glu Ile Val Met Thr Gln Ser Pro Asp Ser Leu Ala Val Ser Leu Gly
1 5 10 15

Glu Arg Ala Thr Ile Asn Cys Lys Ser Ser Gln Ser Val Leu Tyr Ser
20 25 30

Ser Asn Asn Lys Asn Tyr Leu Leu Trp Tyr Gln Gln Lys Pro Gly Gln
35 40 45

Pro Pro Lys Leu Leu Ile Phe Trp Ala Ser Thr Arg Glu Ser Gly Val
50 55 60

Pro Asp Arg Phe Ser Gly Ser Gly Ser Gly Thr Asp Phe Thr Leu Thr
65 70 75 80

Ile Ser Ser Leu Gln Ala Glu Asp Val Ala Val Tyr Tyr Cys Gln Gln
85 90 95

Tyr Tyr Ser Thr Pro Tyr Thr Phe Gly Gln Gly Thr Lys Leu Glu Ile
100 105 110

Lys

<210> 19
<211> 119
<212> PRT
<213> Artificial Sequence

<220>
<221> source
<223> /note="Description of Artificial Sequence: Synthetic
polypeptide"

<220>
<221> VARIANT
<222> (2)..(2)
<223> /replace="Val"

<220>
<221> VARIANT
<222> (43)..(43)
<223> /replace="Gly"

<220>
<221> VARIANT
<222> (49)..(49)
<223> /replace="Ile"

<220>
<221> VARIANT
<222> (59)..(59)
<223> /replace="Asn"

<220>
<221> VARIANT
<222> (70)..(70)
<223> /replace="Ile"

<220>
<221> VARIANT
<222> (111)..(111)
<223> /replace="Gln"

<220>
<221> MISC_FEATURE
<222> (1)..(119)
<223> /note="Variant residues given in the sequence have no
preference with respect to those in the annotations
for variant positions"

<400> 19
Gln Leu Gln Leu Gln Glu Ser Gly Pro Gly Leu Val Lys Pro Ser Gly
1 5 10 15

Thr Leu Ser Leu Thr Cys Ala Val Ser Gly Gly Ser Ile Ser Ser Ser
20 25 30

```
Asn Trp Trp Ser Trp Val Arg Gln Pro Pro Lys Lys Gly Leu Glu Trp
        35                  40                  45


Val Gly Glu Ile Phe His Asp Gly Thr Thr Ser Tyr Asn Pro Ser Leu
    50                  55                  60


Lys Ser Arg Val Thr Met Ser Val Asp Lys Ser Lys Asn Gln Phe Ser
65                  70                  75                  80


Leu Lys Leu Ser Ser Val Thr Ala Ala Asp Thr Ala Val Tyr Tyr Cys
                85                  90                  95


Ala Arg Gly Asn Trp Gly Ser Gly Ala Leu Asp Ile Trp Gly Pro Gly
            100                 105                 110


Thr Met Val Thr Val Ser Ser
        115


<210> 20
<211> 107
<212> PRT
<213> Artificial Sequence

<220>
<221> source
<223> /note="Description of Artificial Sequence: Synthetic
      polypeptide"


<220>
<221> VARIANT
<222> (1)..(1)
<223> /replace="Ala"

<220>
<221> VARIANT
<222> (3)..(3)
<223> /replace="Gln"

<220>
<221> VARIANT
<222> (10)..(10)
<223> /replace="Ser"

<220>
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<221> MISC_FEATURE
<222> (1)..(107)
<223> /note="Variant residues given in the sequence have no
preference with respect to those in the annotations
for variant positions"

<400> 20
Glu Ile Val Met Thr Gln Ser Pro Ser Pro Leu Ser Ala Ser Val Gly
1 5 10 15

Asp Arg Val Thr Ile Thr Cys Arg Ala Ser Gln Gly Ile Arg Asn Asp
20 25 30

Leu Gly Trp Tyr Gln Gln Lys Pro Gly Lys Ala Pro Lys Leu Leu Ile
35 40 45

Tyr Ala Ala Ser Ser Leu Gln Ser Gly Val Pro Ser Arg Phe Ser Gly
50 55 60

Ser Gly Ser Gly Thr Asp Phe Thr Leu Thr Ile Ser Ser Leu Gln Pro
65 70 75 80

Glu Asp Phe Ala Thr Tyr Tyr Cys Leu Gln Asp Tyr Asn Tyr Pro Arg
85 90 95

Thr Phe Gly Gln Gly Thr Lys Val Glu Ile Lys
100 105

<210> 21
<211> 118
<212> PRT
<213> Artificial Sequence

<220>
<221> source
<223> /note="Description of Artificial Sequence: Synthetic
polypeptide"

<220>
<221> VARIANT
<222> (48)..(48)
<223> /replace="Val"

<220>

<221> VARIANT
<222> (50)..(50)
<223> /replace="Ala"

<220>
<221> MISC_FEATURE
<222> (1)..(118)
<223> /note="Variant residues given in the sequence have no
preference with respect to those in the annotations
for variant positions"

<400> 21
Glu Val Gln Leu Val Glu Ser Gly Gly Gly Leu Val Gln Pro Gly Gly
1 5 10 15

Ser Leu Arg Leu Ser Cys Ala Ala Ser Gly Phe Thr Phe Ser Ser Phe
20 25 30

Val Met Ser Trp Val Arg Gln Ala Pro Gly Lys Gly Leu Glu Trp Leu
35 40 45

Ser Thr Ile Ser Gly Gly Gly Gly Ser Thr Tyr Tyr Ala Asp Ser Val
50 55 60

Lys Gly Arg Phe Thr Ile Ser Arg Asp Asn Ser Lys Asn Thr Leu Tyr
65 70 75 80

Leu Gln Met Asn Ser Leu Arg Ala Glu Asp Thr Ala Val Tyr Tyr Cys
85 90 95

Ala Lys Asp Trp Asp Leu Tyr Tyr Phe Asp Tyr Trp Gly Gln Gly Thr
100 105 110

Leu Val Thr Val Ser Ser
115

<210> 22
<211> 107
<212> PRT
<213> Artificial Sequence

<220>
<221> source
<223> /note="Description of Artificial Sequence: Synthetic

polypeptide"

<220>
<221> VARIANT
<222> (4)..(4)
<223> /replace="Met"

<220>
<221> VARIANT
<222> (12)..(12)
<223> /replace="Ser"

<220>
<221> MISC_FEATURE
<222> (1)..(107)
<223> /note="Variant residues given in the sequence have no preference with respect to those in the annotations for variant positions"

<400> 22
Asp Ile Gln Leu Thr Gln Ser Pro Ser Ser Val Pro Ala Ser Val Gly
1 5 10 15

Asp Arg Val Thr Ile Thr Cys Arg Ala Ser Gln Gly Ile Ser Asn Trp
20 25 30

Leu Ala Trp Tyr Gln Gln Lys Pro Gly Lys Ala Pro Lys Leu Leu Ile
35 40 45

Tyr Ala Ala Ser Ser Leu Gln Ser Gly Val Pro Ser Arg Phe Ser Gly
50 55 60

Ser Gly Ser Gly Thr Asp Phe Thr Leu Thr Ile Ser Ser Leu Gln Pro
65 70 75 80

Glu Asp Phe Ala Thr Tyr Tyr Cys Gln Gln Ala Asn Ser Phe Pro Leu
85 90 95

Thr Phe Gly Gly Gly Thr Lys Val Glu Ile Lys
100 105

<210> 23
<211> 117
<212> PRT

<213> Artificial Sequence

<220>
<221> source
<223> /note="Description of Artificial Sequence: Synthetic
polypeptide"

<220>
<221> VARIANT
<222> (1)..(1)
<223> /replace="Glu"

<220>
<221> VARIANT
<222> (5)..(5)
<223> /replace="Val"

<220>
<221> VARIANT
<222> (6)..(6)
<223> /replace="Glu"

<220>
<221> VARIANT
<222> (35)..(35)
<223> /replace="Ser"

<220>
<221> MISC_FEATURE
<222> (1)..(117)
<223> /note="Variant residues given in the sequence have no
preference with respect to those in the annotations
for variant positions"

<400> 23
Gln Val Gln Leu Gln Gln Ser Gly Gly Gly Leu Val Gln Pro Gly Gly
1 5 10 15

Ser Leu Arg Leu Ser Cys Ala Ala Ser Gly Phe Thr Phe Ser Asp Tyr
20 25 30

Trp Met Asn Trp Val Arg Gln Ala Pro Gly Lys Gly Leu Glu Trp Val
35 40 45

Ala Asn Ile Lys Glu Asp Gly Asn Glu Lys Tyr Tyr Val Asp Ser Val
50 55 60

```
Lys Gly Arg Phe Thr Ile Ser Arg Asp Asn Ala Lys Asn Ser Leu Tyr
65                  70                  75                  80


Leu Gln Met Asn Ser Leu Arg Ala Glu Asp Thr Ala Val Tyr Tyr Cys
                85                  90                  95


Ala Arg Asp Thr Asn Trp Gly Ser Asp Tyr Trp Gly Gln Gly Thr Leu
            100                 105                 110


Val Thr Val Ser Ser
        115


<210> 24
<211> 113
<212> PRT
<213> Artificial Sequence

<220>
<221> source
<223> /note="Description of Artificial Sequence: Synthetic
      polypeptide"


<220>
<221> VARIANT
<222> (3)..(3)
<223> /replace="Val"

<220>
<221> VARIANT
<222> (40)..(40)
<223> /replace="Ala"

<220>
<221> VARIANT
<222> (55)..(55)
<223> /replace="Tyr"

<220>
<221> MISC_FEATURE
<222> (1)..(113)
<223> /note="Variant residues given in the sequence have no
      preference with respect to those in the annotations
      for variant positions"

<400> 24
Asp Ile Gln Met Thr Gln Ser Pro Asp Ser Leu Ala Val Ser Leu Gly
1               5                   10                  15
```

Glu Arg Ala Thr Ile Asn Cys Lys Ser Ser Gln Ser Val Phe Tyr Ser
            20   25   30

Ser Asn Asn Lys Asn Tyr Leu Leu Trp Tyr Gln Gln Lys Pro Gly Gln
        35   40   45

Pro Pro Lys Leu Leu Ile Phe Trp Ala Ser Thr Arg Glu Ser Gly Val
    50   55   60

Pro Asp Arg Phe Ser Gly Ser Gly Ser Gly Thr Asp Phe Thr Leu Thr
65   70   75   80

Ile Ser Ser Leu Gln Ala Glu Asp Val Ala Val Tyr Tyr Cys Gln Gln
                85   90   95

Tyr Tyr Ser Thr Pro Tyr Thr Phe Gly Gln Gly Thr Lys Leu Glu Ile
            100   105   110

Lys

<210> 25
<211> 990
<212> DNA
<213> Artificial Sequence

<220>
<221> source
<223> /note="Description of Artificial Sequence: Synthetic
      polynucleotide"

<400> 25
gcctccacca agggcccatc ggtcttcccc ctggcaccct cctccaagag cacctctggg 60

ggcacagcgg ccctgggctg cctggtcaag gactacttcc ccgaaccggt gacggtgtcg 120

tggaactcag gcgccctgac cagcggcgtg cacaccttcc cggctgtcct acagtcctca 180

ggactctact ccctcagcag cgtggtgacc gtgccctcca gcagcttggg cacccagacc 240

tacatctgca acgtgaatca caagcccagc aacaccaagg tggacaagag agttgagccc 300

aaatcttgtg acaaaactca cacatgccca ccgtgcccag cacctgaagc cgccggggga 360

ccgtcagtct tcctcttccc cccaaaaccc aaggacaccc tcatgatctc ccggacccct 420

gaggtcacat gcgtggtggt ggacgtgagc cacgaagacc ctgaggtcaa gttcaactgg 480

tacgtggacg gcgtggaggt gcataatgcc aagacaaagc cgcgggagga gcagtacaac 540

agcacgtacc gtgtggtcag cgtcctcacc gtcctgcacc aggactggct gaatggcaag 600

gagtacaagt gcaaggtctc caacaaagcc ctcccagccc ccatcgagaa aaccatctcc 660

aaagccaaag ggcagccccg agaaccacag gtgtacaccc tgcccccatc ccgggaggag 720

atgaccaaga accaggtcag cctgacctgc ctggtcaaag gcttctatcc cagcgacatc 780

gccgtggagt gggagagcaa tgggcagccg gagaacaact acaagaccac gcctcccgtg 840

ctggactccg acggctcctt cttcctctat agcaagctca ccgtggacaa gagcaggtgg 900

cagcagggga acgtcttctc atgctccgtg atgcatgagg ctctgcacaa ccactacacg 960

cagaagagcc tctccctgtc cccgggtaaa 990

<210> 26
<211> 330
<212> PRT
<213> Artificial Sequence

<220>
<221> source
<223> /note="Description of Artificial Sequence: Synthetic
polypeptide"

<400> 26
Ala Ser Thr Lys Gly Pro Ser Val Phe Pro Leu Ala Pro Ser Ser Lys
1 5 10 15

Ser Thr Ser Gly Gly Thr Ala Ala Leu Gly Cys Leu Val Lys Asp Tyr
20 25 30

Phe Pro Glu Pro Val Thr Val Ser Trp Asn Ser Gly Ala Leu Thr Ser
35 40 45

Gly Val His Thr Phe Pro Ala Val Leu Gln Ser Ser Gly Leu Tyr Ser
50 55 60

Leu Ser Ser Val Val Thr Val Pro Ser Ser Ser Leu Gly Thr Gln Thr

```
65                  70                  75                  80

Tyr Ile Cys Asn Val Asn His Lys Pro Ser Asn Thr Lys Val Asp Lys
                85                  90                  95

Arg Val Glu Pro Lys Ser Cys Asp Lys Thr His Thr Cys Pro Pro Cys
            100                 105                 110

Pro Ala Pro Glu Ala Ala Gly Gly Pro Ser Val Phe Leu Phe Pro Pro
        115                 120                 125

Lys Pro Lys Asp Thr Leu Met Ile Ser Arg Thr Pro Glu Val Thr Cys
    130                 135                 140

Val Val Val Asp Val Ser His Glu Asp Pro Glu Val Lys Phe Asn Trp
145                 150                 155                 160

Tyr Val Asp Gly Val Glu Val His Asn Ala Lys Thr Lys Pro Arg Glu
                165                 170                 175

Glu Gln Tyr Asn Ser Thr Tyr Arg Val Val Ser Val Leu Thr Val Leu
            180                 185                 190

His Gln Asp Trp Leu Asn Gly Lys Glu Tyr Lys Cys Lys Val Ser Asn
        195                 200                 205

Lys Ala Leu Pro Ala Pro Ile Glu Lys Thr Ile Ser Lys Ala Lys Gly
    210                 215                 220

Gln Pro Arg Glu Pro Gln Val Tyr Thr Leu Pro Pro Ser Arg Glu Glu
225                 230                 235                 240

Met Thr Lys Asn Gln Val Ser Leu Thr Cys Leu Val Lys Gly Phe Tyr
                245                 250                 255

Pro Ser Asp Ile Ala Val Glu Trp Glu Ser Asn Gly Gln Pro Glu Asn
            260                 265                 270

Asn Tyr Lys Thr Thr Pro Pro Val Leu Asp Ser Asp Gly Ser Phe Phe
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275 280 285

Leu Tyr Ser Lys Leu Thr Val Asp Lys Ser Arg Trp Gln Gln Gly Asn
290 295 300

Val Phe Ser Cys Ser Val Met His Glu Ala Leu His Asn His Tyr Thr
305 310 315 320

Gln Lys Ser Leu Ser Leu Ser Pro Gly Lys
325 330

<210> 27
<211> 321
<212> DNA
<213> Artificial Sequence

<220>
<221> source
<223> /note="Description of Artificial Sequence: Synthetic polynucleotide"

<400> 27
cgtacggtgg ctgcaccatc tgtcttcatc ttcccgccat ctgatgagca gttgaaatct 60

ggaactgcct ctgttgtgtg cctgctgaat aacttctatc ccagagaggc caaagtacag 120

tggaaggtgg ataacgccct ccaatcgggt aactcccagg agagtgtcac agagcaggac 180

agcaaggaca gcacctacag cctcagcagc accctgacgc tgagcaaagc agactacgag 240

aaacacaaag tctacgcctg cgaagtcacc catcagggcc tgagctcgcc cgtcacaaag 300

agcttcaaca ggggagagtg t 321

<210> 28
<211> 107
<212> PRT
<213> Artificial Sequence

<220>
<221> source
<223> /note="Description of Artificial Sequence: Synthetic polypeptide"

<400> 28
Arg Thr Val Ala Ala Pro Ser Val Phe Ile Phe Pro Pro Ser Asp Glu
1 5 10 15

```
Gln Leu Lys Ser Gly Thr Ala Ser Val Val Cys Leu Leu Asn Asn Phe
            20                  25                  30


Tyr Pro Arg Glu Ala Lys Val Gln Trp Lys Val Asp Asn Ala Leu Gln
        35                  40                  45


Ser Gly Asn Ser Gln Glu Ser Val Thr Glu Gln Asp Ser Lys Asp Ser
    50                  55                  60


Thr Tyr Ser Leu Ser Ser Thr Leu Thr Leu Ser Lys Ala Asp Tyr Glu
65                  70                  75                  80


Lys His Lys Val Tyr Ala Cys Glu Val Thr His Gln Gly Leu Ser Ser
                85                  90                  95


Pro Val Thr Lys Ser Phe Asn Arg Gly Glu Cys
            100                 105


<210> 29
<211> 8
<212> PRT
<213> Artificial Sequence

<220>
<221> source
<223> /note="Description of Artificial Sequence: Synthetic
      peptide"

<400> 29
Gly Phe Thr Phe Ser Asp Tyr Tyr
1               5


<210> 30
<211> 8
<212> PRT
<213> Artificial Sequence

<220>
<221> source
<223> /note="Description of Artificial Sequence: Synthetic
      peptide"

<400> 30
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Ile Ser Ser Thr Gly Ser Thr Ile
1 5

<210> 31
<211> 11
<212> PRT
<213> Artificial Sequence

<220>
<221> source
<223> /note="Description of Artificial Sequence: Synthetic peptide"

<400> 31
Cys Ala Arg Ala Thr Asn Trp Gly Ser Asp Tyr
1 5 10

<210> 32
<211> 12
<212> PRT
<213> Artificial Sequence

<220>
<221> source
<223> /note="Description of Artificial Sequence: Synthetic peptide"

<400> 32
Gln Ser Val Leu Tyr Ser Ser Asn Asn Lys Asn Tyr
1 5 10

<210> 33
<211> 3
<212> PRT
<213> Artificial Sequence

<220>
<221> source
<223> /note="Description of Artificial Sequence: Synthetic peptide"

<400> 33
Trp Ala Ser
1

<210> 34
<211> 10
<212> PRT

<213> Artificial Sequence

<220>
<221> source
<223> /note="Description of Artificial Sequence: Synthetic
peptide"

<400> 34
Cys Gln Gln Tyr Tyr Ser Thr Pro Tyr Thr
1 5 10

<210> 35
<211> 8
<212> PRT
<213> Artificial Sequence

<220>
<221> source
<223> /note="Description of Artificial Sequence: Synthetic
peptide"

<400> 35
Gly Phe Thr Phe Ser Asn Tyr Gly
1 5

<210> 36
<211> 8
<212> PRT
<213> Artificial Sequence

<220>
<221> source
<223> /note="Description of Artificial Sequence: Synthetic
peptide"

<400> 36
Ile Trp Tyr Asp Gly Ser Asp Lys
1 5

<210> 37
<211> 11
<212> PRT
<213> Artificial Sequence

<220>
<221> source
<223> /note="Description of Artificial Sequence: Synthetic
peptide"

<400> 37
Cys Ala Gly Gly Gly Asn Tyr Tyr Gly Asp Phe
1   5   10

<210> 38
<211> 6
<212> PRT
<213> Artificial Sequence

<220>
<221> source
<223> /note="Description of Artificial Sequence: Synthetic
      peptide"

<400> 38
Gln Gly Ile Arg Asn Asp
1   5

<210> 39
<211> 3
<212> PRT
<213> Artificial Sequence

<220>
<221> source
<223> /note="Description of Artificial Sequence: Synthetic
      peptide"

<400> 39
Val Ala Ser
1

<210> 40
<211> 10
<212> PRT
<213> Artificial Sequence

<220>
<221> source
<223> /note="Description of Artificial Sequence: Synthetic
      peptide"

<400> 40
Cys Leu Gln Tyr Asn Ser Tyr Pro Trp Thr
1   5   10

<210> 41
<211> 8

<212> PRT
<213> Artificial Sequence

<220>
<221> source
<223> /note="Description of Artificial Sequence: Synthetic peptide"

<400> 41
Gly Phe Thr Phe Ser Ser Phe Ala
1 5

<210> 42
<211> 8
<212> PRT
<213> Artificial Sequence

<220>
<221> source
<223> /note="Description of Artificial Sequence: Synthetic peptide"

<400> 42
Ile Thr Gly Gly Gly Thr Thr Ser
1 5

<210> 43
<211> 14
<212> PRT
<213> Artificial Sequence

<220>
<221> source
<223> /note="Description of Artificial Sequence: Synthetic peptide"

<400> 43
Cys Ala Lys Trp Gly Ser Trp Ser Ala Gly Ala Phe Asp Ile
1 5 10

<210> 44
<211> 6
<212> PRT
<213> Artificial Sequence

<220>
<221> source
<223> /note="Description of Artificial Sequence: Synthetic peptide"

<400> 44
Gln Gly Ile Ser Ser Trp
1   5

<210> 45
<211> 3
<212> PRT
<213> Artificial Sequence

<220>
<221> source
<223> /note="Description of Artificial Sequence: Synthetic
      peptide"

<400> 45
Ala Ala Ser
1

<210> 46
<211> 10
<212> PRT
<213> Artificial Sequence

<220>
<221> source
<223> /note="Description of Artificial Sequence: Synthetic
      peptide"

<400> 46
Cys Gln Gln Ala Asn Ser Phe Pro Trp Thr
1   5   10

<210> 47
<211> 8
<212> PRT
<213> Artificial Sequence

<220>
<221> source
<223> /note="Description of Artificial Sequence: Synthetic
      peptide"

<400> 47
Ile Ser Ser Ser Gly Ser Thr Ile
1   5

<210> 48

<211> 11
<212> PRT
<213> Artificial Sequence

<220>
<221> source
<223> /note="Description of Artificial Sequence: Synthetic peptide"

<400> 48
Cys Ala Arg Asp Thr Asn Trp Ala Phe Asp Tyr
1 5 10

<210> 49
<211> 12
<212> PRT
<213> Artificial Sequence

<220>
<221> source
<223> /note="Description of Artificial Sequence: Synthetic peptide"

<400> 49
Gln Ser Val Phe Tyr Ser Ala Asn Asn Lys Asn Tyr
1 5 10

<210> 50
<211> 3
<212> PRT
<213> Artificial Sequence

<220>
<221> source
<223> /note="Description of Artificial Sequence: Synthetic peptide"

<400> 50
Trp Thr Ser
1

<210> 51
<211> 10
<212> PRT
<213> Artificial Sequence

<220>
<221> source
<223> /note="Description of Artificial Sequence: Synthetic

peptide"

<400> 51
Cys Gln Gln Phe Tyr Ser Thr Pro Arg Thr
1 5 10

<210> 52
<211> 9
<212> PRT
<213> Artificial Sequence

<220>
<221> source
<223> /note="Description of Artificial Sequence: Synthetic peptide"

<400> 52
Gly Gly Ser Ile Ser Ser Asn Asn Trp
1 5

<210> 53
<211> 7
<212> PRT
<213> Artificial Sequence

<220>
<221> source
<223> /note="Description of Artificial Sequence: Synthetic peptide"

<400> 53
Ile Tyr His Asp Gly Thr Thr
1 5

<210> 54
<211> 13
<212> PRT
<213> Artificial Sequence

<220>
<221> source
<223> /note="Description of Artificial Sequence: Synthetic peptide"

<400> 54
Cys Ala Arg Gly Asp Trp Gly Ser Gly Ala Phe Asp Ile
1 5 10

<210> 55
<211> 10
<212> PRT
<213> Artificial Sequence

<220>
<221> source
<223> /note="Description of Artificial Sequence: Synthetic peptide"

<400> 55
Cys Leu Gln Asp Tyr Asn Tyr Pro Arg Thr
1 5 10

<210> 56
<211> 8
<212> PRT
<213> Artificial Sequence

<220>
<221> source
<223> /note="Description of Artificial Sequence: Synthetic peptide"

<400> 56
Ile Ser Ser Ser Ser Ser Thr Ile
1 5

<210> 57
<211> 12
<212> PRT
<213> Artificial Sequence

<220>
<221> source
<223> /note="Description of Artificial Sequence: Synthetic peptide"

<400> 57
Gln Ser Val Phe Tyr Ser Ser Asn Asn Lys Asn Tyr
1 5 10

<210> 58
<211> 8
<212> PRT
<213> Artificial Sequence

<220>
<221> source

<223> /note="Description of Artificial Sequence: Synthetic
peptide"

<400> 58
Gly Phe Thr Phe Arg Asp Tyr Tyr
1 5

<210> 59
<211> 8
<212> PRT
<213> Artificial Sequence

<220>
<221> source
<223> /note="Description of Artificial Sequence: Synthetic
peptide"

<400> 59
Ile Ser Ser Ser Gly Ser Ile Ile
1 5

<210> 60
<211> 11
<212> PRT
<213> Artificial Sequence

<220>
<221> source
<223> /note="Description of Artificial Sequence: Synthetic
peptide"

<400> 60
Cys Ala Arg Asp Thr Asn Trp Ala Leu Asp Tyr
1 5 10

<210> 61
<211> 8
<212> PRT
<213> Artificial Sequence

<220>
<221> source
<223> /note="Description of Artificial Sequence: Synthetic
peptide"

<400> 61
Gly Phe Thr Phe Ser Ser His Val
1 5

<210> 62
<211> 8
<212> PRT
<213> Artificial Sequence

<220>
<221> source
<223> /note="Description of Artificial Sequence: Synthetic peptide"

<400> 62
Ile Ser Gly Ser Gly Val Asp Thr
1 5

<210> 63
<211> 8
<212> PRT
<213> Artificial Sequence

<220>
<221> source
<223> /note="Description of Artificial Sequence: Synthetic peptide"

<400> 63
Gly Phe Thr Phe Ser Ser Tyr Trp
1 5

<210> 64
<211> 8
<212> PRT
<213> Artificial Sequence

<220>
<221> source
<223> /note="Description of Artificial Sequence: Synthetic peptide"

<400> 64
Ile Lys Gln Asp Gly Ser Glu Lys
1 5

<210> 65
<211> 11
<212> PRT
<213> Artificial Sequence

<220>

<221> source
<223> /note="Description of Artificial Sequence: Synthetic peptide"

<400> 65
Cys Ala Arg Asp Thr Asn Trp Gly Phe Asp Asn
1 5 10

<210> 66
<211> 9
<212> PRT
<213> Artificial Sequence

<220>
<221> source
<223> /note="Description of Artificial Sequence: Synthetic peptide"

<400> 66
Gly Gly Ser Ile Ser Ser Ser Asn Trp
1 5

<210> 67
<211> 7
<212> PRT
<213> Artificial Sequence

<220>
<221> source
<223> /note="Description of Artificial Sequence: Synthetic peptide"

<400> 67
Ile Phe His Asp Gly Thr Thr
1 5

<210> 68
<211> 13
<212> PRT
<213> Artificial Sequence

<220>
<221> source
<223> /note="Description of Artificial Sequence: Synthetic peptide"

<400> 68
Cys Ala Arg Gly Asn Trp Gly Ser Gly Ala Leu Asp Ile
1 5 10

<210> 69
<211> 8
<212> PRT
<213> Artificial Sequence

<220>
<221> source
<223> /note="Description of Artificial Sequence: Synthetic
peptide"

<400> 69
Gly Phe Thr Phe Ser Ser Phe Val
1 5

<210> 70
<211> 8
<212> PRT
<213> Artificial Sequence

<220>
<221> source
<223> /note="Description of Artificial Sequence: Synthetic
peptide"

<400> 70
Ile Ser Gly Gly Gly Gly Ser Thr
1 5

<210> 71
<211> 12
<212> PRT
<213> Artificial Sequence

<220>
<221> source
<223> /note="Description of Artificial Sequence: Synthetic
peptide"

<400> 71
Cys Ala Lys Asp Trp Asp Leu Tyr Tyr Phe Asp Tyr
1 5 10

<210> 72
<211> 6
<212> PRT
<213> Artificial Sequence

<220>
<221> source
<223> /note="Description of Artificial Sequence: Synthetic peptide"

<400> 72
Gln Gly Ile Ser Asn Trp
1 5

<210> 73
<211> 10
<212> PRT
<213> Artificial Sequence

<220>
<221> source
<223> /note="Description of Artificial Sequence: Synthetic peptide"

<400> 73
Cys Gln Gln Ala Asn Ser Phe Pro Leu Thr
1 5 10

<210> 74
<211> 8
<212> PRT
<213> Artificial Sequence

<220>
<221> source
<223> /note="Description of Artificial Sequence: Synthetic peptide"

<400> 74
Gly Phe Thr Phe Ser Asp Tyr Trp
1 5

<210> 75
<211> 8
<212> PRT
<213> Artificial Sequence

<220>
<221> source
<223> /note="Description of Artificial Sequence: Synthetic peptide"

<400> 75
Ile Lys Glu Asp Gly Asn Glu Lys

1 5

<210> 76
<211> 11
<212> PRT
<213> Artificial Sequence

<220>
<221> source
<223> /note="Description of Artificial Sequence: Synthetic peptide"

<400> 76
Cys Ala Arg Asp Thr Asn Trp Gly Ser Asp Tyr
1 5 10

<210> 77
<211> 120
<212> PRT
<213> Artificial Sequence

<220>
<221> source
<223> /note="Description of Artificial Sequence: Synthetic polypeptide"

<400> 77
Glu Val Gln Leu Val Glu Ser Gly Gly Gly Leu Val Gln Pro Gly Gly
1 5 10 15

Ser Leu Arg Leu Ser Cys Ala Ala Ser Gly Phe Thr Phe Ser Ser Phe
20 25 30

Ala Met Ser Trp Val Arg Gln Ala Pro Gly Lys Gly Leu Val Trp Val
35 40 45

Ser Thr Ile Thr Gly Gly Gly Thr Thr Ser Tyr Tyr Ala Asp Ser Val
50 55 60

Lys Gly Arg Phe Thr Ile Ser Arg Asp Asn Ser Lys Ser Thr Leu Phe
65 70 75 80

Leu Gln Met Asn Ser Leu Arg Ala Glu Asp Thr Ala Val Tyr Tyr Cys
85 90 95

```
Ala Lys Trp Gly Ser Trp Ser Ala Gly Ala Phe Asp Ile Trp Gly Gln
            100                 105                 110


Gly Thr Thr Val Thr Val Ser Ser
        115                 120


<210> 78
<211> 119
<212> PRT
<213> Artificial Sequence

<220>
<221> source
<223> /note="Description of Artificial Sequence: Synthetic
      polypeptide"

<400> 78
Gln Val Gln Leu Gln Glu Ser Gly Pro Gly Leu Val Lys Pro Ser Gly
1               5                   10                  15


Thr Leu Ser Leu Thr Cys Ala Val Ser Gly Gly Ser Ile Ser Ser Asn
            20                  25                  30


Asn Trp Trp Ser Trp Val Arg Gln Pro Pro Gly Lys Gly Leu Glu Trp
        35                  40                  45


Ile Gly Glu Ile Tyr His Asp Gly Thr Thr Thr Tyr Asn Pro Ser Leu
    50                  55                  60


Lys Ser Arg Val Thr Ile Ser Val Asp Lys Ser Arg Asn Gln Phe Ser
65                  70                  75                  80


Leu Lys Met Ser Ser Val Thr Ala Ala Asp Thr Ala Val Tyr Tyr Cys
                85                  90                  95


Ala Arg Gly Asp Trp Gly Ser Gly Ala Phe Asp Ile Trp Gly Gln Gly
            100                 105                 110


Thr Met Val Thr Val Ser Ser
        115
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<210> 79
<211> 117
<212> PRT
<213> Artificial Sequence

<220>
<221> source
<223> /note="Description of Artificial Sequence: Synthetic polypeptide"

<400> 79
Gln Val Gln Leu Val Glu Ser Gly Gly Gly Leu Val Lys Pro Gly Gly
1   5   10   15

Ser Leu Arg Leu Ser Cys Ala Ala Ser Gly Phe Thr Phe Arg Asp Tyr
            20   25   30

Tyr Met Ser Trp Ile Arg Gln Ala Pro Gly Lys Gly Leu Glu Trp Val
        35   40   45

Ser His Ile Ser Ser Ser Gly Ser Ile Ile Asp Tyr Val Asp Ser Val
    50   55   60

Lys Gly Arg Phe Thr Ile Ser Arg Asp Asn Ala Lys Asn Ser Leu Tyr
65   70   75   80

Leu Gln Met Asn Ser Leu Arg Ala Glu Asp Thr Ala Val Tyr Tyr Cys
                85   90   95

Ala Arg Asp Thr Asn Trp Ala Leu Asp Tyr Trp Gly Gln Gly Thr Leu
            100   105   110

Val Thr Val Ser Ser
        115

<210> 80
<211> 120
<212> PRT
<213> Artificial Sequence

<220>
<221> source
<223> /note="Description of Artificial Sequence: Synthetic polypeptide"

<400> 80
Glu Val Gln Leu Val Glu Ser Gly Gly Gly Leu Val Gln Pro Gly Gly
1   5   10   15

Ser Leu Arg Leu Ser Cys Ala Ala Ser Gly Phe Thr Phe Ser Ser His
            20   25   30

Val Met Asn Trp Val Arg Gln Ala Pro Gly Lys Gly Leu Glu Trp Val
        35   40   45

Ala Thr Ile Ser Gly Ser Gly Val Asp Thr Tyr Tyr Ala Asp Ser Val
    50   55   60

Lys Gly Arg Phe Thr Ile Ser Arg Gly Asn Ser Lys Asn Met Leu Tyr
65   70   75   80

Leu Gln Met Asn Ser Leu Arg Ala Glu Asp Thr Ala Val Tyr Tyr Cys
                85   90   95

Ala Lys Trp Gly Ser Trp Ser Ala Gly Ala Phe Asp Ile Trp Gly Gln
            100   105   110

Gly Thr Met Val Thr Val Ser Ser
        115   120

<210> 81
<211> 117
<212> PRT
<213> Artificial Sequence

<220>
<221> source
<223> /note="Description of Artificial Sequence: Synthetic
      polypeptide"

<400> 81
Glu Val Gln Leu Val Glu Ser Gly Gly Gly Leu Val Gln Pro Gly Gly
1   5   10   15

Ser Leu Arg Leu Ser Cys Ala Ala Ser Gly Phe Thr Phe Ser Ser Tyr
            20   25   30

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Trp Met Ser Trp Val Arg Gln Ala Pro Gly Lys Gly Leu Glu Trp Val
        35                  40                  45


Ala Asn Ile Lys Gln Asp Gly Ser Glu Lys Tyr Tyr Val Asp Ser Val
    50                  55                  60


Lys Gly Arg Phe Thr Ile Ser Arg Asp Asn Ala Lys Asn Ser Leu Tyr
65                  70                  75                  80


Leu Gln Met Asn Ser Leu Arg Ala Glu Asp Thr Ala Val Tyr Tyr Cys
                85                  90                  95


Ala Arg Asp Thr Asn Trp Gly Phe Asp Asn Trp Gly Gln Gly Thr Leu
            100                 105                 110


Val Thr Val Ser Ser
        115


<210> 82
<211> 119
<212> PRT
<213> Artificial Sequence

<220>
<221> source
<223> /note="Description of Artificial Sequence: Synthetic
      polypeptide"

<400> 82
Gln Val Gln Leu Gln Glu Ser Gly Pro Gly Leu Val Lys Pro Ser Gly
1               5                   10                  15


Thr Leu Ser Leu Thr Cys Ala Val Ser Gly Gly Ser Ile Ser Ser Ser
            20                  25                  30


Asn Trp Trp Ser Trp Val Arg Gln Pro Pro Lys Lys Gly Leu Glu Trp
        35                  40                  45


Val Gly Glu Ile Phe His Asp Gly Thr Thr Ser Tyr Asn Pro Ser Leu
    50                  55                  60
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```
Lys Ser Arg Val Thr Met Ser Val Asp Lys Ser Lys Asn Gln Phe Ser
65                  70                  75                  80


Leu Lys Leu Ser Ser Val Thr Ala Ala Asp Thr Ala Val Tyr Tyr Cys
                85                  90                  95


Ala Arg Gly Asn Trp Gly Ser Gly Ala Leu Asp Ile Trp Gly Pro Gly
            100                 105                 110


Thr Met Val Thr Val Ser Ser
        115


<210> 83
<211> 117
<212> PRT
<213> Artificial Sequence

<220>
<221> source
<223> /note="Description of Artificial Sequence: Synthetic
      polypeptide"

<400> 83
Glu Val Gln Leu Val Glu Ser Gly Gly Gly Leu Val Gln Pro Gly Gly
1               5                   10                  15


Ser Leu Arg Leu Ser Cys Ala Ala Ser Gly Phe Thr Phe Ser Asp Tyr
            20                  25                  30


Trp Met Asn Trp Val Arg Gln Ala Pro Gly Lys Gly Leu Glu Trp Val
        35                  40                  45


Ala Asn Ile Lys Glu Asp Gly Asn Glu Lys Tyr Tyr Val Asp Ser Val
    50                  55                  60


Lys Gly Arg Phe Thr Ile Ser Arg Asp Asn Ala Lys Asn Ser Leu Tyr
65                  70                  75                  80


Leu Gln Met Asn Ser Leu Arg Ala Glu Asp Thr Ala Val Tyr Tyr Cys
                85                  90                  95


Ala Arg Asp Thr Asn Trp Gly Ser Asp Tyr Trp Gly Gln Gly Thr Leu
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100 105 110

Val Thr Val Ser Ser
115

<210> 84
<211> 113
<212> PRT
<213> Artificial Sequence

<220>
<221> source
<223> /note="Description of Artificial Sequence: Synthetic polypeptide"

<400> 84
Asp Ile Val Met Thr Gln Ser Pro Asp Ser Leu Ala Val Ser Leu Gly
1 5 10 15

Glu Arg Ala Thr Ile Asn Cys Lys Ser Ser Gln Ser Val Leu Tyr Ser
20 25 30

Ser Asn Asn Lys Asn Tyr Leu Phe Trp Tyr Gln Gln Lys Pro Gly Gln
35 40 45

Pro Pro Lys Leu Leu Ile Phe Trp Ala Ser Thr Arg Glu Ser Gly Val
50 55 60

Pro Asp Arg Phe Ser Gly Ser Gly Ser Gly Thr Asp Phe Thr Leu Thr
65 70 75 80

Ile Ser Ser Leu Gln Ala Glu Asp Val Ala Val Tyr Tyr Cys Gln Gln
85 90 95

Tyr Tyr Ser Thr Pro Tyr Thr Phe Gly Gln Gly Thr Lys Val Glu Ile
100 105 110

Lys

<210> 85
<211> 107

<212> PRT
<213> Artificial Sequence

<220>
<221> source
<223> /note="Description of Artificial Sequence: Synthetic
polypeptide"

<400> 85
Asp Ile Gln Met Thr Gln Ser Pro Ser Ser Leu Ser Ala Ser Val Gly
1 5 10 15

Asp Arg Val Thr Ile Thr Cys Arg Ala Ser Gln Gly Ile Arg Asn Asp
20 25 30

Leu Gly Trp Tyr Gln Gln Lys Pro Gly Lys Ala Pro Lys Arg Leu Ile
35 40 45

Tyr Val Ala Ser Asn Leu Gln Ser Gly Val Pro Ser Arg Phe Ser Gly
50 55 60

Ser Gly Ser Gly Thr Glu Phe Thr Leu Thr Ile Ser Ser Leu Gln Pro
65 70 75 80

Glu Asp Phe Ala Thr Tyr Tyr Cys Leu Gln Tyr Asn Ser Tyr Pro Trp
85 90 95

Thr Phe Gly Gln Gly Thr Lys Val Glu Ile Lys
100 105

<210> 86
<211> 107
<212> PRT
<213> Artificial Sequence

<220>
<221> source
<223> /note="Description of Artificial Sequence: Synthetic
polypeptide"

<400> 86
Asp Ile Gln Leu Thr Gln Ser Pro Ser Ser Val Ser Ala Ser Val Gly
1 5 10 15

```
Asp Arg Val Thr Ile Thr Cys Arg Ala Ser Gln Gly Ile Ser Ser Trp
            20                  25                  30

Leu Ala Trp Tyr Gln Gln Lys Pro Gly Lys Ala Pro Lys Leu Leu Ile
        35                  40                  45

Tyr Ala Ala Ser Ser Leu Gln Ser Gly Val Pro Ser Arg Phe Ser Gly
    50                  55                  60

Ser Gly Ser Gly Thr Asp Phe Thr Leu Thr Ile Ser Ser Leu Gln Pro
65                  70                  75                  80

Glu Asp Phe Ala Thr Tyr Tyr Cys Gln Gln Ala Asn Ser Phe Pro Trp
                85                  90                  95

Thr Phe Gly Gln Gly Thr Lys Val Glu Ile Lys
            100                 105


<210> 87
<211> 113
<212> PRT
<213> Artificial Sequence

<220>
<221> source
<223> /note="Description of Artificial Sequence: Synthetic
      polypeptide"

<400> 87
Asp Ile Val Met Thr Gln Ser Pro Asp Ser Leu Ala Val Ser Leu Gly
1               5                   10                  15

Glu Arg Ala Thr Ile Asn Cys Lys Ser Ser Gln Ser Val Phe Tyr Ser
            20                  25                  30

Ala Asn Asn Lys Asn Tyr Leu Ala Trp Tyr Gln Gln Lys Pro Gly Gln
        35                  40                  45

Pro Pro Lys Leu Leu Ile Tyr Trp Thr Ser Thr Arg Glu Ser Gly Val
    50                  55                  60

Pro Asp Arg Phe Arg Gly Ser Gly Ser Gly Thr Asp Phe Thr Leu Thr
```

65 70 75 80

Ile Ser Ser Leu Gln Ala Glu Asp Val Ala Val Tyr Tyr Cys Gln Gln
85 90 95

Phe Tyr Ser Thr Pro Arg Thr Phe Gly Gln Gly Thr Lys Val Glu Ile
100 105 110

Lys

<210> 88
<211> 107
<212> PRT
<213> Artificial Sequence

<220>
<221> source
<223> /note="Description of Artificial Sequence: Synthetic polypeptide"

<400> 88
Ala Ile Gln Met Thr Gln Ser Pro Ser Ser Leu Ser Ala Ser Val Gly
1 5 10 15

Asp Arg Val Thr Ile Thr Cys Arg Ala Ser Gln Gly Ile Arg Asn Asp
20 25 30

Leu Gly Trp Tyr Gln Gln Lys Pro Gly Lys Ala Pro Lys Leu Leu Ile
35 40 45

Tyr Ala Ala Ser Ser Leu Gln Ser Gly Val Pro Ser Arg Phe Ser Gly
50 55 60

Ser Gly Ser Gly Thr Asp Phe Thr Leu Thr Ile Ser Ser Leu Gln Pro
65 70 75 80

Glu Asp Phe Ala Thr Tyr Tyr Cys Leu Gln Asp Tyr Asn Tyr Pro Arg
85 90 95

Thr Phe Gly Gln Gly Thr Lys Val Glu Ile Lys
100 105

<210> 89
<211> 113
<212> PRT
<213> Artificial Sequence

<220>
<221> source
<223> /note="Description of Artificial Sequence: Synthetic
polypeptide"

<400> 89
Asp Ile Val Met Thr Gln Ser Pro Asp Ser Leu Ala Val Ser Leu Gly
1 5 10 15

Glu Arg Ala Thr Ile Asn Cys Lys Ser Ser Gln Ser Val Phe Tyr Ser
20 25 30

Ser Asn Asn Lys Asn Tyr Leu Ala Trp Tyr Gln Gln Lys Pro Gly Gln
35 40 45

Pro Pro Lys Leu Leu Ile Phe Trp Ala Ser Thr Arg Glu Ser Gly Val
50 55 60

Pro Asp Arg Phe Ser Gly Ser Gly Ser Gly Thr Asp Phe Thr Leu Thr
65 70 75 80

Ile Ser Ser Leu Gln Ala Glu Asp Val Ala Val Tyr Phe Cys Gln Gln
85 90 95

Phe Tyr Ser Thr Pro Arg Thr Phe Gly Gln Gly Thr Lys Val Glu Ile
100 105 110

Lys

<210> 90
<211> 113
<212> PRT
<213> Artificial Sequence

<220>
<221> source

<223> /note="Description of Artificial Sequence: Synthetic
polypeptide"

<400> 90
Asp Ile Val Met Thr Gln Ser Pro Asp Ser Leu Ala Val Ser Leu Gly
1 5 10 15

Glu Arg Ala Thr Ile Asn Cys Lys Ser Ser Gln Ser Val Phe Tyr Ser
20 25 30

Ser Asn Asn Lys Asn Tyr Leu Ala Trp Tyr Gln Gln Lys Pro Gly Gln
35 40 45

Pro Pro Lys Leu Leu Ile Ser Trp Ala Ser Thr Arg Glu Ser Gly Val
50 55 60

Pro Asp Arg Phe Ser Gly Ser Gly Ser Gly Thr Asp Phe Thr Leu Thr
65 70 75 80

Ile Ser Ser Leu Gln Ala Glu Asp Val Ala Val Tyr Tyr Cys Gln Gln
85 90 95

Tyr Tyr Ser Thr Pro Tyr Thr Phe Gly Gln Gly Thr Lys Leu Glu Ile
100 105 110

Lys

<210> 91
<211> 107
<212> PRT
<213> Artificial Sequence

<220>
<221> source
<223> /note="Description of Artificial Sequence: Synthetic
polypeptide"

<400> 91
Asp Ile Gln Met Thr Gln Ser Pro Ser Ser Val Ser Ala Ser Val Gly
1 5 10 15

Asp Arg Val Thr Ile Thr Cys Arg Ala Ser Gln Gly Ile Ser Ser Trp

```
            20                  25                  30

Leu Ala Trp Tyr Gln Gln Lys Pro Gly Lys Ala Pro Lys Leu Leu Ile
        35                  40                  45

Tyr Ala Ala Ser Ser Leu Gln Ser Gly Val Pro Ser Arg Phe Ser Gly
    50                  55                  60

Ser Gly Ser Gly Thr Asp Phe Thr Leu Thr Ile Ser Ser Leu Gln Pro
65                  70                  75                  80

Glu Asp Phe Ala Thr Tyr Tyr Cys Gln Gln Ala Asn Ser Phe Pro Trp
                85                  90                  95

Thr Phe Gly Gln Gly Thr Lys Val Glu Ile Lys
            100                 105


<210> 92
<211> 113
<212> PRT
<213> Artificial Sequence

<220>
<221> source
<223> /note="Description of Artificial Sequence: Synthetic
      polypeptide"

<400> 92
Asp Ile Val Met Thr Gln Ser Pro Asp Ser Leu Ala Val Ser Leu Gly
1               5                   10                  15

Glu Arg Ala Thr Ile Asn Cys Lys Ser Ser Gln Ser Val Leu Tyr Ser
            20                  25                  30

Ser Asn Asn Lys Asn Tyr Leu Leu Trp Tyr Gln Gln Lys Pro Gly Gln
        35                  40                  45

Pro Pro Lys Leu Leu Ile Phe Trp Ala Ser Thr Arg Glu Ser Gly Val
    50                  55                  60

Pro Asp Arg Phe Ser Gly Ser Gly Ser Gly Thr Asp Phe Thr Leu Thr
65                  70                  75                  80
```

```
Ile Ser Ser Leu Gln Ala Glu Asp Val Ala Val Tyr Tyr Cys Gln Gln
                85                  90                  95


Tyr Tyr Ser Thr Pro Tyr Thr Phe Gly Gln Gly Thr Lys Leu Glu Ile
            100                 105                 110


Lys



<210> 93
<211> 107
<212> PRT
<213> Artificial Sequence

<220>
<221> source
<223> /note="Description of Artificial Sequence: Synthetic
      polypeptide"

<400> 93
Ala Ile Gln Met Thr Gln Ser Pro Ser Pro Leu Ser Ala Ser Val Gly
1               5                   10                  15


Asp Arg Val Thr Ile Thr Cys Arg Ala Ser Gln Gly Ile Arg Asn Asp
            20                  25                  30


Leu Gly Trp Tyr Gln Gln Lys Pro Gly Lys Ala Pro Lys Leu Leu Ile
        35                  40                  45


Tyr Ala Ala Ser Ser Leu Gln Ser Gly Val Pro Ser Arg Phe Ser Gly
    50                  55                  60


Ser Gly Ser Gly Thr Asp Phe Thr Leu Thr Ile Ser Ser Leu Gln Pro
65                  70                  75                  80


Glu Asp Phe Ala Thr Tyr Tyr Cys Leu Gln Asp Tyr Asn Tyr Pro Arg
                85                  90                  95


Thr Phe Gly Gln Gly Thr Lys Val Glu Ile Lys
            100                 105
```

<210> 94
<211> 107
<212> PRT
<213> Artificial Sequence

<220>
<221> source
<223> /note="Description of Artificial Sequence: Synthetic
polypeptide"

<400> 94
Asp Ile Gln Leu Thr Gln Ser Pro Ser Ser Val Pro Ala Ser Val Gly
1 5 10 15

Asp Arg Val Thr Ile Thr Cys Arg Ala Ser Gln Gly Ile Ser Asn Trp
20 25 30

Leu Ala Trp Tyr Gln Gln Lys Pro Gly Lys Ala Pro Lys Leu Leu Ile
35 40 45

Tyr Ala Ala Ser Ser Leu Gln Ser Gly Val Pro Ser Arg Phe Ser Gly
50 55 60

Ser Gly Ser Gly Thr Asp Phe Thr Leu Thr Ile Ser Ser Leu Gln Pro
65 70 75 80

Glu Asp Phe Ala Thr Tyr Tyr Cys Gln Gln Ala Asn Ser Phe Pro Leu
85 90 95

Thr Phe Gly Gly Gly Thr Lys Val Glu Ile Lys
100 105

<210> 95
<211> 113
<212> PRT
<213> Artificial Sequence

<220>
<221> source
<223> /note="Description of Artificial Sequence: Synthetic
polypeptide"

<400> 95
Asp Ile Val Met Thr Gln Ser Pro Asp Ser Leu Ala Val Ser Leu Gly

1 5 10 15

Glu Arg Ala Thr Ile Asn Cys Lys Ser Ser Gln Ser Val Phe Tyr Ser
20 25 30

Ser Asn Asn Lys Asn Tyr Leu Leu Trp Tyr Gln Gln Lys Pro Gly Gln
35 40 45

Pro Pro Lys Leu Leu Ile Phe Trp Ala Ser Thr Arg Glu Ser Gly Val
50 55 60

Pro Asp Arg Phe Ser Gly Ser Gly Ser Gly Thr Asp Phe Thr Leu Thr
65 70 75 80

Ile Ser Ser Leu Gln Ala Glu Asp Val Ala Val Tyr Tyr Cys Gln Gln
85 90 95

Tyr Tyr Ser Thr Pro Tyr Thr Phe Gly Gln Gly Thr Lys Leu Glu Ile
100 105 110

Lys

<210> 96
<211> 117
<212> PRT
<213> Artificial Sequence

<220>
<221> source
<223> /note="Description of Artificial Sequence: Synthetic
polypeptide"

<400> 96
Gln Val Gln Leu Val Glu Ser Gly Gly Gly Leu Val Lys Pro Gly Gly
1 5 10 15

Ser Leu Arg Leu Ser Cys Ala Ala Ser Gly Phe Thr Phe Ser Asp Tyr
20 25 30

Tyr Met Ser Trp Ile Arg Gln Ala Pro Gly Lys Gly Leu Glu Trp Val
35 40 45

```
Ser Tyr Ile Ser Ser Thr Gly Ser Thr Ile Tyr Tyr Ala Asp Ser Val
    50                  55                  60

Lys Gly Arg Phe Thr Ile Ser Arg Asp Asn Ala Lys Asn Ser Leu Tyr
65                  70                  75                  80

Leu Gln Met Asn Ser Leu Arg Ala Glu Asp Thr Ala Val Tyr Tyr Cys
                85                  90                  95

Ala Arg Ala Thr Asn Trp Gly Ser Asp Tyr Trp Gly Gln Gly Thr Leu
            100                 105                 110

Val Thr Val Ser Ser
        115


<210> 97
<211> 120
<212> PRT
<213> Artificial Sequence

<220>
<221> source
<223> /note="Description of Artificial Sequence: Synthetic
      polypeptide"

<400> 97
Glu Val Gln Leu Val Glu Ser Gly Gly Gly Leu Val Gln Pro Gly Gly
1               5                   10                  15

Ser Leu Arg Leu Ser Cys Ala Ala Ser Gly Phe Thr Phe Ser Ser Phe
            20                  25                  30

Ala Met Ser Trp Val Arg Gln Ala Pro Gly Lys Gly Leu Glu Trp Val
        35                  40                  45

Ser Ala Ile Thr Gly Gly Gly Thr Thr Ser Tyr Tyr Ala Asp Ser Val
    50                  55                  60

Lys Gly Arg Phe Thr Ile Ser Arg Asp Asn Ser Lys Asn Thr Leu Tyr
65                  70                  75                  80
```

```
Leu Gln Met Asn Ser Leu Arg Ala Glu Asp Thr Ala Val Tyr Tyr Cys
                85                  90                  95


Ala Lys Trp Gly Ser Trp Ser Ala Gly Ala Phe Asp Ile Trp Gly Gln
            100                 105                 110


Gly Thr Met Val Thr Val Ser Ser
        115                 120


<210> 98
<211> 117
<212> PRT
<213> Artificial Sequence

<220>
<221> source
<223> /note="Description of Artificial Sequence: Synthetic
      polypeptide"

<400> 98
Gln Val Gln Leu Val Glu Ser Gly Gly Gly Leu Val Lys Pro Gly Gly
1               5                   10                  15


Ser Leu Arg Leu Ser Cys Ala Ala Ser Gly Phe Thr Phe Ser Asp Tyr
            20                  25                  30


Tyr Met Ser Trp Ile Arg Gln Ala Pro Gly Lys Gly Leu Glu Trp Val
        35                  40                  45


Ser Tyr Ile Ser Ser Ser Gly Ser Thr Ile Tyr Tyr Ala Asp Ser Val
    50                  55                  60


Lys Gly Arg Phe Thr Ile Ser Arg Asp Asn Ala Lys Asn Ser Leu Tyr
65                  70                  75                  80


Leu Gln Met Asn Ser Leu Arg Ala Glu Asp Thr Ala Val Tyr Tyr Cys
                85                  90                  95


Ala Arg Asp Thr Asn Trp Ala Phe Asp Tyr Trp Gly Gln Gly Thr Leu
            100                 105                 110
```

Val Thr Val Ser Ser
        115

<210> 99
<211> 119
<212> PRT
<213> Artificial Sequence

<220>
<221> source
<223> /note="Description of Artificial Sequence: Synthetic
      polypeptide"

<400> 99
Gln Val Gln Leu Gln Glu Ser Gly Pro Gly Leu Val Lys Pro Ser Gly
1   5   10   15

Thr Leu Ser Leu Thr Cys Ala Val Ser Gly Gly Ser Ile Ser Ser Asn
            20   25   30

Asn Trp Trp Ser Trp Val Arg Gln Pro Pro Gly Lys Gly Leu Glu Trp
        35   40   45

Ile Gly Glu Ile Tyr His Asp Gly Thr Thr Asn Tyr Asn Pro Ser Leu
    50   55   60

Lys Ser Arg Val Thr Ile Ser Val Asp Lys Ser Lys Asn Gln Phe Ser
65   70   75   80

Leu Lys Leu Ser Ser Val Thr Ala Ala Asp Thr Ala Val Tyr Tyr Cys
                85   90   95

Ala Arg Gly Asp Trp Gly Ser Gly Ala Phe Asp Ile Trp Gly Gln Gly
            100   105   110

Thr Met Val Thr Val Ser Ser
        115

<210> 100
<211> 117
<212> PRT
<213> Artificial Sequence

<220>
<221> source
<223> /note="Description of Artificial Sequence: Synthetic
polypeptide"

<400> 100
Gln Val Gln Leu Val Glu Ser Gly Gly Gly Leu Val Lys Pro Gly Gly
1 5 10 15

Ser Leu Arg Leu Ser Cys Ala Ala Ser Gly Phe Thr Phe Ser Asp Tyr
20 25 30

Tyr Met Ser Trp Ile Arg Gln Ala Pro Gly Lys Gly Leu Glu Trp Val
35 40 45

Ser Tyr Ile Ser Ser Ser Ser Ser Thr Ile Tyr Tyr Ala Asp Ser Val
50 55 60

Lys Gly Arg Phe Thr Ile Ser Arg Asp Asn Ala Lys Asn Ser Leu Tyr
65 70 75 80

Leu Gln Met Asn Ser Leu Arg Ala Glu Asp Thr Ala Val Tyr Tyr Cys
85 90 95

Ala Arg Asp Thr Asn Trp Ala Phe Asp Tyr Trp Gly Gln Gly Thr Leu
100 105 110

Val Thr Val Ser Ser
115

<210> 101
<211> 117
<212> PRT
<213> Artificial Sequence

<220>
<221> source
<223> /note="Description of Artificial Sequence: Synthetic
polypeptide"

<400> 101
Gln Val Gln Leu Val Glu Ser Gly Gly Gly Leu Val Lys Pro Gly Gly
1 5 10 15

```
Ser Leu Arg Leu Ser Cys Ala Ala Ser Gly Phe Thr Phe Arg Asp Tyr
            20                  25                  30


Tyr Met Ser Trp Ile Arg Gln Ala Pro Gly Lys Gly Leu Glu Trp Val
        35                  40                  45


Ser Tyr Ile Ser Ser Ser Gly Ser Ile Ile Tyr Tyr Ala Asp Ser Val
    50                  55                  60


Lys Gly Arg Phe Thr Ile Ser Arg Asp Asn Ala Lys Asn Ser Leu Tyr
65                  70                  75                  80


Leu Gln Met Asn Ser Leu Arg Ala Glu Asp Thr Ala Val Tyr Tyr Cys
                85                  90                  95


Ala Arg Asp Thr Asn Trp Ala Leu Asp Tyr Trp Gly Gln Gly Thr Leu
            100                 105                 110


Val Thr Val Ser Ser
        115


<210> 102
<211> 120
<212> PRT
<213> Artificial Sequence

<220>
<221> source
<223> /note="Description of Artificial Sequence: Synthetic
      polypeptide"

<400> 102
Glu Val Gln Leu Val Glu Ser Gly Gly Gly Leu Val Gln Pro Gly Gly
1               5                   10                  15


Ser Leu Arg Leu Ser Cys Ala Ala Ser Gly Phe Thr Phe Ser Ser His
            20                  25                  30


Val Met Asn Trp Val Arg Gln Ala Pro Gly Lys Gly Leu Glu Trp Val
        35                  40                  45
```

```
Ser Ala Ile Ser Gly Ser Gly Val Asp Thr Tyr Tyr Ala Asp Ser Val
    50                  55                  60


Lys Gly Arg Phe Thr Ile Ser Arg Asp Asn Ser Lys Asn Thr Leu Tyr
65                  70                  75                  80


Leu Gln Met Asn Ser Leu Arg Ala Glu Asp Thr Ala Val Tyr Tyr Cys
                85                  90                  95


Ala Lys Trp Gly Ser Trp Ser Ala Gly Ala Phe Asp Ile Trp Gly Gln
            100                 105                 110


Gly Thr Met Val Thr Val Ser Ser
        115                 120


<210> 103
<211> 119
<212> PRT
<213> Artificial Sequence

<220>
<221> source
<223> /note="Description of Artificial Sequence: Synthetic
      polypeptide"

<400> 103
Gln Val Gln Leu Gln Glu Ser Gly Pro Gly Leu Val Lys Pro Ser Gly
1               5                   10                  15


Thr Leu Ser Leu Thr Cys Ala Val Ser Gly Gly Ser Ile Ser Ser Ser
            20                  25                  30


Asn Trp Trp Ser Trp Val Arg Gln Pro Pro Gly Lys Gly Leu Glu Trp
        35                  40                  45


Ile Gly Glu Ile Phe His Asp Gly Thr Thr Asn Tyr Asn Pro Ser Leu
    50                  55                  60


Lys Ser Arg Val Thr Ile Ser Val Asp Lys Ser Lys Asn Gln Phe Ser
65                  70                  75                  80


Leu Lys Leu Ser Ser Val Thr Ala Ala Asp Thr Ala Val Tyr Tyr Cys
```

85 90 95

Ala Arg Gly Asn Trp Gly Ser Gly Ala Leu Asp Ile Trp Gly Gln Gly
100 105 110

Thr Met Val Thr Val Ser Ser
115

<210> 104
<211> 118
<212> PRT
<213> Artificial Sequence

<220>
<221> source
<223> /note="Description of Artificial Sequence: Synthetic polypeptide"

<400> 104
Glu Val Gln Leu Val Glu Ser Gly Gly Gly Leu Val Gln Pro Gly Gly
1 5 10 15

Ser Leu Arg Leu Ser Cys Ala Ala Ser Gly Phe Thr Phe Ser Ser Phe
20 25 30

Val Met Ser Trp Val Arg Gln Ala Pro Gly Lys Gly Leu Glu Trp Val
35 40 45

Ser Ala Ile Ser Gly Gly Gly Gly Ser Thr Tyr Tyr Ala Asp Ser Val
50 55 60

Lys Gly Arg Phe Thr Ile Ser Arg Asp Asn Ser Lys Asn Thr Leu Tyr
65 70 75 80

Leu Gln Met Asn Ser Leu Arg Ala Glu Asp Thr Ala Val Tyr Tyr Cys
85 90 95

Ala Lys Asp Trp Asp Leu Tyr Tyr Phe Asp Tyr Trp Gly Gln Gly Thr
100 105 110

Leu Val Thr Val Ser Ser
115

<210> 105
<211> 117
<212> PRT
<213> Artificial Sequence

<220>
<221> source
<223> /note="Description of Artificial Sequence: Synthetic
polypeptide"

<400> 105
Glu Val Gln Leu Val Glu Ser Gly Gly Gly Leu Val Gln Pro Gly Gly
1 5 10 15

Ser Leu Arg Leu Ser Cys Ala Ala Ser Gly Phe Thr Phe Ser Asp Tyr
20 25 30

Trp Met Ser Trp Val Arg Gln Ala Pro Gly Lys Gly Leu Glu Trp Val
35 40 45

Ala Asn Ile Lys Glu Asp Gly Asn Glu Lys Tyr Tyr Val Asp Ser Val
50 55 60

Lys Gly Arg Phe Thr Ile Ser Arg Asp Asn Ala Lys Asn Ser Leu Tyr
65 70 75 80

Leu Gln Met Asn Ser Leu Arg Ala Glu Asp Thr Ala Val Tyr Tyr Cys
85 90 95

Ala Arg Asp Thr Asn Trp Gly Ser Asp Tyr Trp Gly Gln Gly Thr Leu
100 105 110

Val Thr Val Ser Ser
115

<210> 106
<211> 113
<212> PRT
<213> Artificial Sequence

<220>
<221> source

<223> /note="Description of Artificial Sequence: Synthetic
polypeptide"

<400> 106
Asp Ile Val Met Thr Gln Ser Pro Asp Ser Leu Ala Val Ser Leu Gly
1 5 10 15

Glu Arg Ala Thr Ile Asn Cys Lys Ser Ser Gln Ser Val Leu Tyr Ser
20 25 30

Ser Asn Asn Lys Asn Tyr Leu Ala Trp Tyr Gln Gln Lys Pro Gly Gln
35 40 45

Pro Pro Lys Leu Leu Ile Tyr Trp Ala Ser Thr Arg Glu Ser Gly Val
50 55 60

Pro Asp Arg Phe Ser Gly Ser Gly Ser Gly Thr Asp Phe Thr Leu Thr
65 70 75 80

Ile Ser Ser Leu Gln Ala Glu Asp Val Ala Val Tyr Tyr Cys Gln Gln
85 90 95

Tyr Tyr Ser Thr Pro Tyr Thr Phe Gly Gln Gly Thr Lys Val Glu Ile
100 105 110

Lys

<210> 107
<211> 107
<212> PRT
<213> Artificial Sequence

<220>
<221> source
<223> /note="Description of Artificial Sequence: Synthetic
polypeptide"

<400> 107
Asp Ile Gln Met Thr Gln Ser Pro Ser Ser Leu Ser Ala Ser Val Gly
1 5 10 15

Asp Arg Val Thr Ile Thr Cys Arg Ala Ser Gln Gly Ile Arg Asn Asp

```
            20                  25                  30

Leu Gly Trp Tyr Gln Gln Lys Pro Gly Lys Ala Pro Lys Arg Leu Ile
        35                  40                  45

Tyr Val Ala Ser Ser Leu Gln Ser Gly Val Pro Ser Arg Phe Ser Gly
    50                  55                  60

Ser Gly Ser Gly Thr Glu Phe Thr Leu Thr Ile Ser Ser Leu Gln Pro
65                  70                  75                  80

Glu Asp Phe Ala Thr Tyr Tyr Cys Leu Gln Tyr Asn Ser Tyr Pro Trp
                85                  90                  95

Thr Phe Gly Gln Gly Thr Lys Val Glu Ile Lys
            100                 105


<210> 108
<211> 113
<212> PRT
<213> Artificial Sequence

<220>
<221> source
<223> /note="Description of Artificial Sequence: Synthetic
      polypeptide"

<400> 108
Asp Ile Val Met Thr Gln Ser Pro Asp Ser Leu Ala Val Ser Leu Gly
1               5                   10                  15

Glu Arg Ala Thr Ile Asn Cys Lys Ser Ser Gln Ser Val Phe Tyr Ser
            20                  25                  30

Ala Asn Asn Lys Asn Tyr Leu Ala Trp Tyr Gln Gln Lys Pro Gly Gln
        35                  40                  45

Pro Pro Lys Leu Leu Ile Tyr Trp Thr Ser Thr Arg Glu Ser Gly Val
    50                  55                  60

Pro Asp Arg Phe Ser Gly Ser Gly Ser Gly Thr Asp Phe Thr Leu Thr
65                  70                  75                  80
```

```
Ile Ser Ser Leu Gln Ala Glu Asp Val Ala Val Tyr Tyr Cys Gln Gln
                85                  90                  95


Phe Tyr Ser Thr Pro Arg Thr Phe Gly Gln Gly Thr Lys Val Glu Ile
            100                 105                 110


Lys



<210> 109
<211> 113
<212> PRT
<213> Artificial Sequence

<220>
<221> source
<223> /note="Description of Artificial Sequence: Synthetic
      polypeptide"

<400> 109
Asp Ile Val Met Thr Gln Ser Pro Asp Ser Leu Ala Val Ser Leu Gly
1               5                   10                  15


Glu Arg Ala Thr Ile Asn Cys Lys Ser Ser Gln Ser Val Phe Tyr Ser
            20                  25                  30


Ser Asn Asn Lys Asn Tyr Leu Ala Trp Tyr Gln Gln Lys Pro Gly Gln
        35                  40                  45


Pro Pro Lys Leu Leu Ile Tyr Trp Ala Ser Thr Arg Glu Ser Gly Val
    50                  55                  60


Pro Asp Arg Phe Ser Gly Ser Gly Ser Gly Thr Asp Phe Thr Leu Thr
65                  70                  75                  80


Ile Ser Ser Leu Gln Ala Glu Asp Val Ala Val Tyr Tyr Cys Gln Gln
                85                  90                  95


Phe Tyr Ser Thr Pro Arg Thr Phe Gly Gln Gly Thr Lys Val Glu Ile
            100                 105                 110
```

Lys

<210> 110
<211> 113
<212> PRT
<213> Artificial Sequence

<220>
<221> source
<223> /note="Description of Artificial Sequence: Synthetic polypeptide"

<400> 110
Asp Ile Val Met Thr Gln Ser Pro Asp Ser Leu Ala Val Ser Leu Gly
1 5 10 15

Glu Arg Ala Thr Ile Asn Cys Lys Ser Ser Gln Ser Val Phe Tyr Ser
20 25 30

Ser Asn Asn Lys Asn Tyr Leu Ala Trp Tyr Gln Gln Lys Pro Gly Gln
35 40 45

Pro Pro Lys Leu Leu Ile Tyr Trp Ala Ser Thr Arg Glu Ser Gly Val
50 55 60

Pro Asp Arg Phe Ser Gly Ser Gly Ser Gly Thr Asp Phe Thr Leu Thr
65 70 75 80

Ile Ser Ser Leu Gln Ala Glu Asp Val Ala Val Tyr Tyr Cys Gln Gln
85 90 95

Tyr Tyr Ser Thr Pro Tyr Thr Phe Gly Gln Gly Thr Lys Leu Glu Ile
100 105 110

Lys

<210> 111
<211> 113
<212> PRT
<213> Artificial Sequence

<220>
<221> source
<223> /note="Description of Artificial Sequence: Synthetic
polypeptide"

<400> 111
Asp Ile Val Met Thr Gln Ser Pro Asp Ser Leu Ala Val Ser Leu Gly
1 5 10 15

Glu Arg Ala Thr Ile Asn Cys Lys Ser Ser Gln Ser Val Leu Tyr Ser
20 25 30

Ser Asn Asn Lys Asn Tyr Leu Ala Trp Tyr Gln Gln Lys Pro Gly Gln
35 40 45

Pro Pro Lys Leu Leu Ile Tyr Trp Ala Ser Thr Arg Glu Ser Gly Val
50 55 60

Pro Asp Arg Phe Ser Gly Ser Gly Ser Gly Thr Asp Phe Thr Leu Thr
65 70 75 80

Ile Ser Ser Leu Gln Ala Glu Asp Val Ala Val Tyr Tyr Cys Gln Gln
85 90 95

Tyr Tyr Ser Thr Pro Tyr Thr Phe Gly Gln Gly Thr Lys Leu Glu Ile
100 105 110

Lys

<210> 112
<211> 107
<212> PRT
<213> Artificial Sequence

<220>
<221> source
<223> /note="Description of Artificial Sequence: Synthetic
polypeptide"

<400> 112
Ala Ile Gln Met Thr Gln Ser Pro Ser Ser Leu Ser Ala Ser Val Gly
1 5 10 15

```
Asp Arg Val Thr Ile Thr Cys Arg Ala Ser Gln Gly Ile Arg Asn Asp
            20                  25                  30


Leu Gly Trp Tyr Gln Gln Lys Pro Gly Lys Ala Pro Lys Leu Leu Ile
        35                  40                  45


Tyr Ala Ala Ser Ser Leu Gln Ser Gly Val Pro Ser Arg Phe Ser Gly
    50                  55                  60


Ser Gly Ser Gly Thr Asp Phe Thr Leu Thr Ile Ser Ser Leu Gln Pro
65                  70                  75                  80


Glu Asp Phe Ala Thr Tyr Tyr Cys Leu Gln Asp Tyr Asn Tyr Pro Arg
                85                  90                  95


Thr Phe Gly Gln Gly Thr Lys Val Glu Ile Lys
            100                 105


<210> 113
<211> 107
<212> PRT
<213> Artificial Sequence

<220>
<221> source
<223> /note="Description of Artificial Sequence: Synthetic
      polypeptide"

<400> 113
Asp Ile Gln Leu Thr Gln Ser Pro Ser Ser Val Pro Ala Ser Val Gly
1               5                   10                  15


Asp Arg Val Thr Ile Thr Cys Arg Ala Ser Gln Gly Ile Ser Asn Trp
            20                  25                  30


Leu Ala Trp Tyr Gln Gln Lys Pro Gly Lys Ala Pro Lys Leu Leu Ile
        35                  40                  45


Tyr Ala Ala Ser Ser Leu Gln Ser Gly Val Pro Ser Arg Phe Ser Gly
    50                  55                  60
```

```
Ser Gly Ser Gly Thr Asp Phe Thr Leu Thr Ile Ser Ser Leu Gln Pro
65                  70                  75                  80


Glu Asp Phe Ala Thr Tyr Tyr Cys Gln Gln Ala Asn Ser Phe Pro Leu
                85                  90                  95


Thr Phe Gly Gly Gly Thr Lys Val Glu Ile Lys
            100                 105


<210> 114
<211> 113
<212> PRT
<213> Artificial Sequence

<220>
<221> source
<223> /note="Description of Artificial Sequence: Synthetic
      polypeptide"

<400> 114
Asp Ile Val Met Thr Gln Ser Pro Asp Ser Leu Ala Val Ser Leu Gly
1               5                   10                  15


Glu Arg Ala Thr Ile Asn Cys Lys Ser Ser Gln Ser Val Phe Tyr Ser
            20                  25                  30


Ser Asn Asn Lys Asn Tyr Leu Ala Trp Tyr Gln Gln Lys Pro Gly Gln
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Pro Pro Lys Leu Leu Ile Tyr Trp Ala Ser Thr Arg Glu Ser Gly Val
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Pro Asp Arg Phe Ser Gly Ser Gly Ser Gly Thr Asp Phe Thr Leu Thr
65                  70                  75                  80


Ile Ser Ser Leu Gln Ala Glu Asp Val Ala Val Tyr Tyr Cys Gln Gln
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Tyr Tyr Ser Thr Pro Tyr Thr Phe Gly Gln Gly Thr Lys Leu Glu Ile
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Lys

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