

ANTI-PD-1 MONOCLONAL ANTIBODY AND METHODS OF PREPARATION THEREOF**Sequence Listing**

[0001] This application is being filed electronically via EFS-Web and includes an electronically submitted sequence listing in .xml format in ST.26 format. The .xml file contains a sequence listing entitled "025-PCT-SEQ-Listing.xml" created on November 8, 2024 and having a size of 23,068 bytes and 24,576 bytes on disk. The sequence listing contained in this .xml file is part of the specification and is herein incorporated by reference in its entirety.

Technical Field of the Invention

[0002] The disclosure relates to the field of biomedicine, in particular to an anti-PD-1 monoclonal antibody, optionally combining another therapeutic agent such as pegylated human interferon- α 2b, and preparation methods and application thereof.

Background Art

[0003] The protein Programmed Death 1 (PD-1) is known in the art. Briefly, it is an inhibitory member of the CD28 family of receptors, that also includes CD28, CTLA-4, ICOS and BTLA. PD-1 is expressed on activated B cells, T cells, macrophages, and myeloid cells. The initial members of the family, CD28 and ICOS, were discovered by functional effects on augmenting T cell proliferation following the addition of monoclonal antibodies.

[0004] The PD-1 gene is a 50-55 kDa type I transmembrane protein that is part of the Ig gene superfamily. PD-1 contains a membrane proximal immunoreceptor tyrosine inhibitory motif (ITIM) and a membrane distal tyrosine-based switch motif (ITSM). Although structurally similar to CTLA-4, PD-1 lacks the MYPPPY motif that is critical for B7-1 and B7-2 binding. Two ligands for PD-1 have been identified. PD-L1 and PD-L2, that have been shown to downregulate T cell activation upon binding to PD-1. Both PD-L1 and PD-L2 are B7 homologs that bind to PD-1, but do not bind to other CD28 family members. One ligand for PD-1, PD-L1 is abundant in a variety of human cancers. The interaction between PD-1 and PD-L1 results in a decrease in tumor infiltrating lymphocytes, a decrease in T-cell receptor mediated proliferation, and immune evasion by the cancerous cells. Immune suppression can be reversed by inhibiting the local interaction of PD-1 with PD-L1, and the effect is additive when the interaction of PD-1 with PD-L2 is blocked as well. PD-1 has been found to play a role in autoimmune encephalomyelitis, systemic lupus erythematosus, graft-versus-host disease (GVHD), type 1 diabetes, and rheumatoid arthritis.

Disclosure of the Invention

[0005] In a first aspect, the present disclosure relates to an isolated antibody or an antigen-binding portion thereof that preferentially binds PD-1, including a heavy chain complementarity determining region one (VHCDR1) including the amino acid sequence as recited in SEQ ID NO: 9, SEQ ID NO: 18, SEQ ID NO: 19 or SEQ ID NO: 21, a heavy chain complementarity determining region two (VHCDR2) including the amino acid sequence as recited in SEQ ID NO: 10, and/or a heavy chain complementarity determining region three

(VHCDR3) including the amino acid sequence as recited in SEQ ID NO: 11 or SEQ ID NO: 20; and a light chain complementarity determining region one (VLCDR1) including the amino acid sequence as recited in SEQ ID NO: 12, a light chain complementarity determining region two (VLCDR2) including the amino acid sequence as recited in SEQ ID NO: 13, and/or a light chain complementarity determining region three (VLCDR3) including the amino acid sequence as recited in SEQ ID NO: 14.

[0006] In a second aspect, this disclosure is directed to an isolated antibody or an antigen-binding portion thereof that preferentially binds PD-1 including a heavy chain variable region (VH) comprising SEQ ID NO: 5 or SEQ ID NO: 22; and a light chain variable region (VL) comprising SEQ ID NO: 6.

[0007] In another aspect, the disclosure is drawn to an isolated antibody or an antigen-binding portion thereof preferentially binds PD-1, comprising: a heavy chain complementarity determining region one (VHCDR1), a heavy chain complementarity determining region two (VHCDR2), and/or a heavy chain complementarity determining region three (VHCDR3) of or obtained from (e.g., determined, derived or copied from) a heavy chain variable region amino acid sequence as recited in SEQ ID NO: 5; and a light chain complementarity determining region one (VLCDR1), a light chain complementarity determining region two (VLCDR2), and/or a light chain complementarity determining region three (VLCDR3) obtained from (e.g., determined, derived or copied from) a light chain variable region amino acid sequence as recited in SEQ ID NO: 6.

[0008] Further aspect includes the fact that said isolated antibody or an antigen-binding portion thereof that preferentially binds PD-1 further comprises a heavy chain constant region (HCCR) including the amino acid sequence recited in SEQ ID NO: 7; and wherein the HCCR optionally include one, two, or three conservatively modified amino acid substitutions.

[0009] Additional aspect includes that aspect 2's said isolated antibody or an antigen-binding portion thereof further comprises a light chain constant region (LCCR) including the amino acid sequence of SEQ ID No: 8; and wherein the LCCR optionally includes one, two, or three conservatively modified amino acid substitutions.

[0010] Yet more aspect of aspect 2 includes aspect 2's isolated antibody or an antigen-binding portion thereof further comprising a heavy chain constant region (HCCR) including the amino acid sequence recited in SEQ ID NO: 7, and wherein said HCCR optionally includes one, two, or three conservatively modified amino acid substitutions; and wherein said isolated antibody or an antigen-binding portion thereof further comprises a light chain constant region (LCCR) including the amino acid sequence of SEQ ID No: 8, wherein the LCCR optionally includes one, two, or three conservatively modified amino acid substitutions.

[0011] In some aspect, the antibody or the antigen-binding portion's VH further comprises a heavy chain signal peptide (HCSP) including the amino acids recited in SEQ ID NO: 15 on the N terminus of the VH.

[0012] In another aspect, the antibody or the antigen-binding portion thereof of the present disclosure includes a VL, wherein said VL further comprises a light chain signal peptide (LCSP) including the amino acids recited in SEQ ID NO: 16 on the N terminus of the VL.

[0013] In an aspect, the present disclosure provides antibody or the antigen-binding portion that is a human version or humanized from a different species, and can be IgG1, IgG2, IgG3 or IgG4.

[0014] In another aspect, the present disclosure includes a pharmaceutical composition for treating, suppressing and/or inhibiting a solid tumor comprising (i) the antibody or the antigen-binding portion thereof disclosed herein; and (ii) a pharmaceutically accepted carrier.

[0015] Yet in another aspect, a polynucleotide molecule encoding the antibody or the antigen-binding portion of the present disclosure is contemplated and made, which, in an embodiment, can be an expression vector. In an embodiment, such expression vector encodes a light chain signal peptide (LCSP) and/or a heavy chain signal peptide (HCSP) but does not comprise an intron. The expression vector can be utilized in a host cell, such as *E. Coli*.

[0016] In an aspect, the present disclosure relates to a method of preventing, treating and/or suppressing a tumor in a subject, comprising administering an effective amount of the antibody or the antigen-binding portion to the subject. Such tumors can include Hepatocellular carcinoma, or solid tumor comprising melanoma and/or renal cell carcinoma (RCC).

[0017] In a further aspect, the present disclosure is directed to a method of regulating the activity of one or more T cells in vitro, ex vivo, and/or in vivo in a subject, the method comprises: contacting the T cell with an antibody or an antigen-binding portion thereof which preferentially binds to human programmed death receptor 1 (hPD-1), wherein the antibody blocks and/or regulates the binding of human PD-L1 and/or human PD-L2 to hPD-1, and wherein said antibody or an antigen-binding portion thereof includes a heavy chain variable region (VH) comprising SEQ ID NO: 5 or SEQ ID NO:22; and a light chain variable region (VL) comprising SEQ ID NO: 6. Yet in another aspect, the antibody or antigen-binding portion thereof can further comprise a heavy chain constant region (HCCR) including the amino acid sequence recited in SEQ ID NO: 7, and the HCCR optionally includes one, two, or three conservatively modified amino acid substitutions and/or where the antibody or antigen-binding portion thereof further comprises a light chain constant region (LCCR) including the amino acid sequence of SEQ ID No: 8, and wherein the LCCR optionally includes one, two, or three conservatively modified amino acid substitutions.

[0018] In another aspect, the method of disclosed herein can regulate T cells such as one or more natural killer cells, one or more cytotoxic T cells, one or more helper T cells, one or more monocyte cells, one or more memory T cells, and/or one or more regulatory T cells.

[0019] In one aspect, the antibody or antigen-binding portion disclosed herein blocks binding of human PD-L1 and human PD-L2 to human PD-1 with an IC50 of about 1 nM or lower.

[0020] Another further aspect of the present disclosure includes a process for producing an antibody or an antigen-binding portion vector construct comprising culturing the host cell containing an expression vector of interest under conditions allowing the expression of said antibody or antigen-binding portion construct; and purifying and/or recovering the produced antibody or antigen-binding portion vector construct from the culture.

[0021] In an aspect, the antibody or an antigen-binding portion thereof for use in the treatment of solid tumor can optionally comprise another therapeutic agent. Such optional agent can be P1101 and/or another anti-human PD1 monoclonal antibody for treatment of solid tumor, including but not limited to RCC and/or

melanoma.

[0022] The present disclosure includes a combination for use in the treating, suppressing, preventing and/or inhibiting of a solid tumor in a subject, wherein the combination comprises an antibody or an antigen-binding portion thereof; and another therapeutic agent, wherein the antibody or an antigen-binding portion thereof includes a heavy chain variable region (VH) comprising SEQ ID NO: 5 or SEQ ID NO:22; and a light chain variable region (VL) comprising SEQ ID NO: 6.

[0023] Yet, the above combination, said antibody or antigen-binding portion thereof further comprises a heavy chain constant region (HCCR) including the amino acid sequence recited in SEQ ID NO: 7, wherein the HCCR optionally includes one, two, or three conservatively modified amino acid substitutions; and wherein said antibody or antigen-binding portion thereof further comprises a light chain constant region (LCCR) including the amino acid sequence of SEQ ID No: 8, and wherein the LCCR optionally includes one, two, or three conservatively modified amino acid substitutions.

[0024] Yet in another aspect, the present disclosure directs to a method of preventing, treating and/or suppressing a solid tumor in a subject such as human, comprising administering an effective amount of P1101 and P1801 in a serial or combination manner to the subject. The administration regime can be in any serial and/or serial manner. In some respects, P1101 is administered before P1801, or vice versa, and can be performed with various length of administration timing and combinations. The solid tumor treated includes Hepatocellular carcinoma, melanoma and/or renal cell carcinoma.

[0025] In one embodiment the present disclosure provides an isolated antibody or an antigen-binding portion thereof that preferentially binds PD-1, comprising: a heavy chain complementarity determining region one (VHCDR1) including the amino acid sequence as recited in SEQ ID NO: 9, SEQ ID NO: 18, SEQ ID NO: 19 or SEQ ID NO: 21, a heavy chain complementarity determining region two (VHCDR2) including the amino acid sequence as recited in SEQ ID NO: 10, and/or a heavy chain complementarity determining region three (VHCDR3) including the amino acid sequence as recited in SEQ ID NO: 11, SEQ ID NO:20 or SEQ ID NO:23; and a light chain complementarity determining region one (VLCDR1) including the amino acid sequence as recited in SEQ ID NO: 12, a light chain complementarity determining region two (VLCDR2) including the amino acid sequences as recited in SEQ ID NO: 13, and/or a light chain complementarity determining region three (VLCDR3) including the amino acid sequences as recited in and SEQ ID NO: 14.

[0026] Another embodiment the present disclosure provides an isolated antibody or an antigen-binding portion thereof that preferentially binds PD-1 comprising one or more paratopes comprising: at least one of the following residues: Phe2, Ser5, Ser6, and Tyr7 of a heavy chain variable sequence (VHCDR1) of SEQ ID NO: 19;

at least one of the following residues: Phe2, Ser5, and Tyr6 of a heavy chain variable sequence (VHCDR1) of SEQ ID NO: 21; at least one of the following residues: Thr13, Gly14, Gly15, Gly16, Ser17, and Tyr18 of a heavy chain variable sequence (VHCDR2) of SEQ ID NO: 10; at least one of the following residues: Tyr33, Leu34, Asn35, Asp38, and Tyr39 of a heavy chain variable sequence (VHCDR3) of SEQ ID NO: 11; or at least

one of the following residues: Ser31, Tyr33, Leu34, Asn35, Asp38, and Tyr39 of a heavy chain variable sequence (VHCDR3) of SEQ ID NO: 20.

[0027] In one embodiment, the present disclosure provides an isolated antibody or an antigen-binding portion thereof that preferentially binds PD-1 comprising one or more paratopes comprising: at least one of the following residues: Asn9 of a light chain variable sequence (VLCDR1) of SEQ ID NO: 12; at least one of the following residues: Tyr12 and Ser18 of a heavy chain variable sequence (VHCDR2) of SEQ ID NO: 13; or at least one of the following residues: Ser22, Asn23, and Trp25 of a heavy chain variable sequence (VHCDR3) of SEQ ID NO: 14.

[0028] Another embodiment of the present disclosure provides an isolated antibody or an antigen-binding portion thereof that preferentially binds PD-1 comprising one or more paratopes comprising: at least one of the following residues: Phe2, Ser5, Ser6, and Tyr7 of a heavy chain variable sequence (VHCDR1) of SEQ ID NO: 19; at least one of the following residues: Phe2, Ser5, and Tyr6 of a heavy chain variable sequence (VHCDR1) of SEQ ID NO: 21; at least one of the following residues: Thr13, Gly14, Gly15, Gly16, Ser17, and Tyr18 of a heavy chain variable sequence (VHCDR2) of SEQ ID NO: 10; at least one of the following residues: Tyr33, Leu34, Asn35, Asp38, and Tyr39 of a heavy chain variable sequence (VHCDR3) of SEQ ID NO: 11; at least one of the following residues: Ser31, Tyr33, Leu34, Asn35, Asp38, and Tyr39 of a heavy chain variable sequence (VHCDR3) of SEQ ID NO: 20; at least one of the following residues: Asn9 of a light chain variable sequence (VLCDR1) of SEQ ID NO: 12; at least one of the following residues: Tyr12 and Ser18 of a heavy chain variable sequence (VHCDR2) of SEQ ID NO: 13; or at least one of the following residues: Ser22, Asn23, and Trp25 of a heavy chain variable sequence (VHCDR3) of SEQ ID NO: 14.

[0029] In one embodiment the present disclosure provides an isolated antibody or an antigen-binding portion thereof preferentially binds PD-1 comprising: a heavy chain variable region (VH) comprising SEQ ID NO: 9 and a light chain variable region (VL) comprising SEQ ID NO: 6; or a heavy chain variable region (VH) comprising SEQ ID NO: 18 and a light chain variable region (VL) comprising SEQ ID NO: 6; or a heavy chain variable region (VH) comprising SEQ ID NO: 19 and a light chain variable region (VL) comprising SEQ ID NO: 6; or a heavy chain variable region (VH) comprising SEQ ID NO: 21 and a light chain variable region (VL) comprising SEQ ID NO: 6.

[0030] In one embodiment the present disclosure provides an isolated antibody or an antigen-binding portion thereof that preferentially binds PD-1 comprising a heavy chain variable region (VH) comprising SEQ ID NO: 5 or a heavy chain variable region (VH) comprising SEQ ID NO: 22; and a light chain variable region (VL) comprising SEQ ID NO: 6.

[0031] In any of the embodiments including the antibody or the antigen-binding portion thereof, said antibody or the antigen-binding portion thereof can further comprise a heavy chain constant region (HCCR) including the amino acid sequence recited in SEQ ID NO: 7; and wherein the HCCR optionally includes one, two, or three conservatively modified amino acid substitutions.

[0032] In any of the embodiments including the antibody or the antigen-binding portion thereof, said antibody

or the antigen-binding portion thereof can further comprise a light chain constant region (LCCR) including the amino acid sequence of SEQ ID No: 8; and wherein the LCCR optionally includes one, two, or three conservatively modified amino acid substitutions.

[0033] In any of the embodiments including the antibody or the antigen-binding portion thereof, said antibody or the antigen-binding portion thereof can further comprise a heavy chain constant region (HCCR) including the amino acid sequence recited in SEQ ID NO: 7, and wherein said HCCR optionally includes one, two, or three conservatively modified amino acid substitutions; and a light chain constant region (LCCR) including the amino acid sequence of SEQ ID No:8, wherein the LCCR optionally includes one, two, or three conservatively modified amino acid substitutions.

[0034] In any of the embodiments including the antibody or the antigen-binding portion thereof, said VH can further comprises a heavy chain signal peptide (HCSP) including the amino acids recited in SEQ ID NO: 15 on the N terminus of the VH. In any of the embodiments including the antibody or the antigen-binding portion thereof, said VL can further comprise a light chain signal peptide (LCSP) including the amino acids recited in SEQ ID NO: 16 on the N terminus of the VL. In any of the embodiments including the antibody or the antigen-binding portion thereof, the antibody can be human or humanized. In any of the embodiments including the antibody or the antigen-binding portion thereof, the antibody can be IgG4 isotype.

[0035] In one aspect the present disclosure provides a pharmaceutical composition for treating, suppressing and/or inhibiting a solid tumor comprising (i) the antibody or the antigen-binding portion thereof as disclosed in any of the embodiments listed herein; and (ii) a pharmaceutically accepted carrier.

[0036] In one embodiment the present disclosure provides a polynucleotide molecule encoding the antibody or the antigen-binding portion in any one of the embodiments listed herein.

[0037] In one embodiment the present disclosure provides an expression vector comprising the polynucleotide molecule encoding the antibody or the antigen-binding portion as disclosed in any of the embodiments listed herein, said expression vector which encodes an LCSP and/or HCSP does not comprise an intron. In one embodiment the present disclosure provides a host cell comprising the expression vector.

[0038] In one embodiment the present disclosure provides a method of preventing, treating and/or suppressing a tumor in a subject, comprising the steps of: administering an effective amount of the antibody or the antigen-binding portion as disclosed in any of the embodiments listed herein to the subject. The tumor includes Hepatocellular carcinoma, or solid tumor comprising melanoma and/or renal cell carcinoma (RCC).

[0039] In one embodiment the present disclosure provides a method of regulating the activity of one or more T cells *in vitro*, *ex vivo*, and/or *in vivo* in a subject, the method comprising the steps of: contacting the T cell with an antibody or an antigen-binding portion thereof which preferentially binds to human programmed death receptor 1 (hPD-1), wherein the antibody blocks and/or regulates the binding of human PD-L1 and/or human PD-L2 to hPD-1, and wherein said antibody or an antigen-binding portion thereof includes: a heavy chain complementarity determining region one (VHCDR1) including the amino acid sequence as recited in SEQ ID NO: 9, SEQ ID NO: 18, SEQ ID NO: 19 or SEQ ID NO: 21, a heavy chain complementarity determining

region two (VHCDR2) including the amino acid sequence as recited in SEQ ID NO: 10, and/or a heavy chain complementarity determining region three (VHCDR3) including the amino acid sequence as recited in SEQ ID NO: 11, SEQ ID NO:20 or SEQ ID NO:23; and a light chain complementarity determining region one (VLCDR1) including the amino acid sequence as recited in SEQ ID NO: 12, a light chain complementarity determining region two (VLCDR2) including the amino acid sequence as recited in SEQ ID NO: 13, and/or a light chain complementarity determining region three (VLCDR3) including the amino acid sequence as recited in and SEQ ID NO: 14.

[0040] In one embodiment of the method of regulating the activity of one or more T cells *in vitro*, *ex vivo*, and/or *in vivo* in a subject, said antibody or an antigen-binding portion thereof further comprises a heavy chain constant region (HCCR) including the amino acid sequence recited in SEQ ID NO: 7, and the HCCR optionally includes one, two, or three conservatively modified amino acid substitutions. In the method, said antibody or an antigen-binding portion thereof can further comprise a light chain constant region (LCCR) including the amino acid sequence of SEQ ID No: 8, wherein the LCCR optionally includes one, two, or three conservatively modified amino acid substitutions. In the method, said one or more T cells can comprise one or more natural killer cells, one or more cytotoxic T cells, one or more helper T cells, one or more monocyte cells, one or more memory T cells, and/or one or more regulatory T cells. In the method, the antibody or antigen-binding portion can block binding of human PD-L1 and human PD-L2 to human PD-1 with an IC₅₀ of about 1 nM or lower.

[0041] In one aspect, the present disclosure provides a process for producing an antibody or an antigen-binding portion vector construct comprising culturing the host cell as disclosed in any of the embodiments listed herein under conditions allowing the expression of said antibody or antigen-binding portion construct; and purifying and/or recovering the produced antibody or antigen-binding portion vector construct from the culture.

[0042] In another aspect, the present disclosure provides an antibody or an antigen-binding portion thereof for use in the treatment of solid tumors which comprises: a heavy chain variable region (VH) comprising SEQ ID NO: 5 or a heavy chain variable region (VH) comprising SEQ ID NO: 22; a light chain variable region (VL) comprising SEQ ID NO: 6; and optionally comprises another therapeutic agent. The antibody or an antigen-binding portion thereof can further comprise: a heavy chain constant region (HCCR) including the amino acid sequence recited in SEQ ID NO: 7, wherein the HCCR optionally includes one, two, or three conservatively modified amino acid substitutions; and a light chain constant region (LCCR) including the amino acid sequence of SEQ ID No: 8, and wherein the LCCR optionally include one, two, or three conservatively modified amino acid substitutions; and optionally comprises another therapeutic agent. Said another therapeutic agent can comprise P1101, P1801, and/or another anti-human PD1 monoclonal antibody. In the antibody or an antigen-binding portion thereof for use, said solid tumor can comprise Hepatocellular carcinoma, renal cell carcinoma and/or melanoma.

[0043] In one embodiment the present disclosure provides a combination for use in the treating, suppressing, preventing and/or inhibiting a solid tumor in a subject, wherein the combination comprises an antibody or an

antigen-binding portion thereof; and another therapeutic agent, wherein the antibody or an antigen-binding portion thereof includes: a heavy chain variable region (VH) comprising SEQ ID NO: 5 or a heavy chain variable region (VH) comprising SEQ ID NO: 22; and a light chain variable region (VL) comprising SEQ ID NO: 6. Said antibody or an antigen-binding portion thereof can further comprise a heavy chain constant region (HCCR) including the amino acid sequence recited in SEQ ID NO: 7, wherein the HCCR optionally includes one, two, or three conservatively modified amino acid substitutions; and a light chain constant region (LCCR) including the amino acid sequence of SEQ ID No: 8, and wherein the LCCR optionally includes one, two, or three conservatively modified amino acid substitutions. In the combination, said therapeutic agent can comprise P1101, P1801, and/or another anti-human PD1 monoclonal antibody. In the combination, said P1101, P1801, and/or another anti-human PD1 monoclonal antibody can be administered serially. In the combination, said P1101 and/or P1801 is administered before another anti-human PD1 monoclonal antibody. In the combination, said solid tumor can include Hepatocellular carcinoma, melanoma and/or renal cell carcinoma.

[0044] In one embodiment the present disclosure provides a recombinant protein which comprises (i) the antibody or an antigen-binding portion disclosed herein; and (ii) an optional tag sequence for expression detection and/or purification.

[0045] In another embodiment, any of the embodiments including the antibody or the antigen-binding portion thereof can be for use in a method of treating cancer, wherein the method comprises administering the isolated antibody or an antigen-binding portion thereof to a subject. In an embodiment, the cancer comprises Hepatocellular carcinoma, melanoma and/or renal cell carcinoma.

[0046] In an embodiment, any of the embodiments including the antibody or the antigen-binding portion thereof can be for use for the manufacture of a medicament for the therapeutic and/or prophylactic treatment of cancer. In an embodiment, the cancer comprises Hepatocellular carcinoma, melanoma and/or renal cell carcinoma.

Description of the Drawings

[0047] For a more complete understanding of the features and advantages of the present disclosure, reference is now made to the detailed description of the disclosure along with the accompanying figures and in which:

Figure 1(a) to 1(r) denote amino acid sequences of P1801. Figure 1(a) denotes the full heavy chain of P1801 including three CDRs and variable domain plus constant regions. It also includes heavy chain signal peptides, that will be eventually cleaved by the host cell. The full heavy chain sequence is denoted as SEQ ID NO: 1. Figure 1(b) denotes the full light chain of P1801 including three CDRs and variable domain plus constant regions. It also includes light chain signal peptides, that will be eventually cleaved by the host cell. The full light chain sequence is denoted as SEQ ID NO: 2. Figure 1(c) denotes the heavy chain of P1801 with no heavy chain signal peptide (SEQ ID No:3). Figure 1(d) denotes the light chain of P1801 with no light chain signal peptide (SEQ ID No:4). Figure 1(f) denotes the full heavy chain variable region of P1801 (SEQ ID No:5). Figure 1(g) denotes the full light chain variable region of P1801 (SEQ ID No:6). Figure 1(h) denotes the heavy chain constant region of P1801 (SEQ ID No:7). Figure 1(i) denotes the light chain constant region

of P1801 (SEQ ID No:8). Figure 1(j) denotes heavy chain CDR1 of P1801 (SEQ ID No:9). Figure 1(k) denotes heavy chain CDR2 of P1801 (SEQ ID No:10). Figure 1(l) denotes heavy chain CDR3 of P1801 (SEQ ID No:11). Figure 1(m) denotes light chain CDR1 of P1801 (SEQ ID No:12). Figure 1(n) denotes light chain CDR2 of P1801 (SEQ ID No:13). Figure 1(o) denotes light chain CDR3 of P1801 (SEQ ID No:14). Figure 1(p) denotes heavy chain signal peptide of P1801 (SEQ ID No:15). Figure 1(q) denotes light chain signal peptide of P1801 (SEQ ID No:16). Figure 1(r) denotes the hinge sequence is be located in heavy chains (SEQ ID No: 17). Figure 1(s) denotes an alternative heavy chain CDR1 of P1801 (SEQ ID No:18). Figure 1(t) denotes an alternative heavy chain CDR1 of P1801 (SEQ ID No:19). Figure 1(u) denotes an alternative heavy chain CDR1 of P1801 (SEQ ID No:20). Figure 1(v) denotes an alternative heavy chain CDR1 of P1801 (SEQ ID No:21). Figure 1(w) denotes an alternative full heavy chain variable region of P1801 (SEQ ID No:22). Figure 1(x) denotes an alternative heavy chain CDR3 of P1801 (SEQ ID No:23).

Figure 2 denotes the chemical structure of P1101. IFN stands for human interferon- α 2b. mPEG stands for polyethylene glycol polymer, and each mPEG has a molecular weight from about 10KD to 30 KD, and/or each mPEG has a molecular weight from about 20 kD.

Figure 3 denotes competition ELISA for testing interfered capacity of selected murine anti-hPD-1 mAb (mP1801) as compared to commercially known anti-PD1 mAb.

Figure 4(a) to Figure 4(c) denote different embodiments of administration regimens of a combination of molecules of present disclosure; for example, P1101 and an anti-PD1 monoclonal antibody, such as P1801. Solid arrow and dotted arrow can be molecule 1 such as P1101, and molecule 2 can be an anti-PD1 monoclonal antibody, such as P1801 respectively, or vice versa.

Figure 5 denotes another different embodiment of administration regimens of a combination of the molecules of present disclosure. For example, molecule 1 can be P1101 or an anti-PD1 monoclonal antibody, such as P1801. Molecule 2 can be P1101 or an anti-PD1 monoclonal antibody, such as P1801, or vice versa.

Figure 6 denotes yet another different embodiment of administration regimens of a combination of molecules disclosed in the present disclosure. For example, molecule 1 can be P1101 or an anti-PD1 monoclonal antibody, such as P1801. Molecule 2 can be P1101 or an anti-PD1 monoclonal antibody, such as P1801.

Figure 7 denotes another embodiment of administration regimens of a combination of molecules disclosed in the present disclosure. For example, Figure 7(a) shows molecule 1 can be hIgG4 (as isotope control), P1101 or an anti-PD1 monoclonal antibody such as P1801, Cemiplimab, Pembrolizumab or Novolumab administered individually. Figure 7(b) shows a version of alternating between molecule 1 and 2, where molecule 1 can be mP1101, molecule 2 can be P1801, and vice versa.

Figures 8(a) and 8(b) denote binding assay of P1801 as compared to various commercially available anti-PD1 mAb against PD1 antigen. The Y-Axis is wavelength, and the x-axis is time in seconds. As shown in

Figure 8(a) the second line from the top is P1801 and is marked with an arrow. As shown in Figure 8(b), the third line from the top is P1801 and is marked with an arrow.

Figure 9 denotes binding specificity of P1801. P1801 specifically binds to PD-1, but not with PD-L1, PD-L2, CTLA4 or CD28 as those are flat and undetectable.

Figure 10 denotes the mean RCC tumor volume from 7 different groups as described in protocols in Example 9.

Figure 11 denotes actual RCC tumor measure from group 1 of the Example 9.

Figure 12 denotes actual RCC tumor measure from group 6 of the Example 9.

Figure 13 denotes five different cohort that is used for a subject to determine effect dosage of P1101 + P1801.

Figure 14 denotes further refinement from Figure 13 dosage cohort for effective dosage pf P1101 + P1801. The top circle is for RCC and the bottom is for melanoma.

Figure 15 is another example of another cohort.

Figure 16 is an image generated with ChimeraX of the final 3D reconstruction of the PD1-AcroBio + PS 00066 + PS00067 at a nominal resolution of 2.9A.

Figure 17 is an image generated with ChimeraX of the final 2.9A map colored by the local resolution values calculated by cryoSPARC 4.4.

Figure 18 is an image of the structure of the P1801/PD1 complex solved by Cryo-Electron Microscopy.

Figure 19 is an image of the structure of the P1801/PL1/PD1 complex solved by Cryo-Electron Microscopy showing an overlay of P1801/PD1 and PD-L1/PD1.

Figure 20 is an image of the epitope mapping of the P1801 epitope.

Figure 21 is an image of the epitope mapping of the PD-L1 epitope.

Figure 22 is a model of the P1801 CDRs at the PD-1 binding interface.

Figure 23 is a graph of epitope binding anti-PD-1 Antibodies.

[0048] While the making and using of various embodiments of the present disclosure are discussed in detail below, it should be appreciated that the present disclosure provides many applicable inventive concepts that can be embodied in a wide variety of specific contexts. The specific embodiments discussed herein are merely illustrative of specific ways to make and use the disclosure and do not delimit the scope of the disclosure.

[0049] To facilitate the understanding of this disclosure, a number of terms are defined below. Terms defined herein have meanings as commonly understood by a person of ordinary skill in the areas relevant to the present disclosure. Terms such as “a”, “an” and “the” are not intended to refer to only a singular entity, but include the general class of which a specific example may be used for illustration. The terminology herein is used to describe specific embodiments of the disclosure, but their usage does not delimit the disclosure, except as outlined in the claims.

i. Definitions

[0050] As used herein, the term “isolated molecule” as referring to a molecule (where the molecule is, for example, a polypeptide, a polynucleotide, or an 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 substantially free of other molecules from the same source, e.g., species, cell from which it is expressed, library, etc., (3) is expressed by a cell from a different species, or (4) does not occur in nature. Thus, a molecule that is chemically synthesized, or expressed in a cellular system different from the system from which it naturally originates, will be “isolated” from its naturally associated components. A molecule also can be rendered substantially free of naturally associated components by isolation, using purification techniques well known in the art. Molecule purity or homogeneity may be assayed by a number of means known in the art. For example, the purity of a polypeptide sample may be assayed using polyacrylamide gel electrophoresis and staining of the gel to visualize the polypeptide using techniques well known in the art. For certain purposes, higher resolution may be provided by using HPLC or other means well known in the art of purification.

[0051] An “antibody” is an immunoglobulin molecule capable of specific binding to a target, such as a carbohydrate, polynucleotide, lipid, polypeptide, etc., through at least one antigen recognition site, located in the variable region of the immunoglobulin molecule. As used herein, the term encompasses not only intact polyclonal or monoclonal antibodies, but also, unless otherwise specified, any antigen binding portion thereof that competes with the intact antibody for specific binding, and any other modified configuration of the immunoglobulin molecule that comprises an antigen recognition site. Antigen binding portions include, for example, Fab, Fab', F(ab')₂, Fd, Fv, domain antibodies (dAbs, e.g., shark and camelid antibodies), fragments including complementarity determining regions (CDRs), single chain variable fragment antibodies (scFv), maxibodies, minibodies, intrabodies, diabodies, triabodies, tetrabodies, v-NAR and bis-scFv, and polypeptides that contain at least a portion of an immunoglobulin that is sufficient to confer specific antigen binding to the target polypeptide. An antibody includes an antibody of any class, such as IgG, IgA, or IgM (or sub-class thereof), and the antibody need not be of any particular class. Depending on the antibody amino acid sequence of the constant region of its heavy chains, immunoglobulins can be assigned to different classes. There are five major classes of immunoglobulins: IgA, IgD, IgE, IgG, and IgM, and several of these may be further divided into subclasses (isotypes), e.g., IgG1, IgG2, IgG3, IgG4, IgA1 and IgA2. The heavy-chain constant regions that correspond to the different classes of immunoglobulins are called alpha, delta, epsilon, gamma, and mu, respectively. The subunit structures and three-dimensional configurations of different classes of immunoglobulins are known in the art.

[0052] A “variable region” of an antibody refers to the variable region of the antibody light chain or the variable region of the antibody heavy chain, either alone or in combination. As known in the art, the variable regions of the heavy and light chains each consist of four framework regions (FRs) connected by three complementarity determining regions (CDRs) also known as hypervariable regions, and contribute to the formation of the antigen binding site of antibodies. If variants of a subject variable region are desired, particularly with substitution in amino acid residues outside of a CDR region (i.e., in the framework region),

appropriate amino acid substitution, preferably, conservative amino acid substitution, can be identified by comparing the subject variable region to the variable regions of other antibodies which contain CDR1 and CDR2 sequences in the same canonical class as the subject variable region.

[0053] In certain embodiments, definitive delineation of a CDR and identification of residues comprising the binding site of an antibody is accomplished by solving the structure of the antibody and/or solving the structure of the antibody-ligand complex. In certain embodiments, that can be accomplished by any of a variety of techniques known to those skilled in the art, such as X-ray crystallography. In certain embodiments, various methods of analysis can be employed to identify or approximate the CDR regions. In certain embodiments, various methods of analysis can be employed to identify or approximate the CDR regions. Examples of such methods include, but are not limited to, the Kabat definition, the Chothia definition, the AbM definition, the contact definition, and the conformational definition. In an embodiment, the present disclosure's CDRs are determined by the Kabat definition. In another embodiment, the CDRs are determined by the Chothia definition.

[0054] As used herein, the "EU index" or "EU numbering" system is based on the sequential numbering of the first human IgG sequenced (the EU antibody). Because the most common reference for this convention is the Kabat sequence manual, the EU index is sometimes erroneously used synonymously with the Kabat index. The EU index does not provide insertions and deletions, and thus in some cases comparisons of IgG positions across IgG subclass and species can be unclear, particularly in the hinge regions. Nonetheless, the convention has sufficed at enabling straightforward comparison between Fc regions in numerous Fc structure function studies.

[0055] As used herein, the "conformational definition" of CDRs, refers to the positions of the CDRs may be identified as the residues that make enthalpic contributions to antigen binding.

[0056] As used herein, a CDR may refer to CDRs defined by any approach known in the art, including combinations of approaches. The methods used herein may utilize CDRs defined according to any of these approaches. For any given embodiment containing more than one CDR, the CDRs may be defined in accordance with any of Kabat (Kabat et al., Sequences of Proteins of Immunological Interest, 5th Ed. Public Health Service, National Institutes of Health, Bethesda, Md. (1991), IMGT, Chothia (Chothia and Lesk, J. Mol. Biol. 196:901-917 (1987)), Martin, extended, AbM (AbM antibody model software using Oxford Molecular), contact, conformational definitions and/or any CDR determination method well known in the art.

[0057] CDRs are commonly considered as structured loops that are involved in antigen binding and exhibiting a hyper-variable amino acid composition. Defining a CDR based on antibody amino acid sequences can use different numbering schemes or utilize different definitions of CDR lengths. For example, the Kabat (and IMGT) CDR definitions are based on sequence alignments while the Chothia CDR definition better reflects the loop structure in antibodies' 3D architecture. The Kabat numbering scheme is often considered as the standard that is widely adopted for numbering antibody residues. The Chothia numbering scheme can be based on the alignment of antibody structures and ensures a better correspondence to the structural loops. Chothia numbering scheme possesses that topologically aligned residues from different antibodies are localized at the

same position number and that the Chothia CDR definition corresponds in most antibody sequences to the structural antigen-binding loops. The CDRs defined on the hypervariable amino acids according to Kabat and based on loop topology in Chothia's nomenclature have for some CDR's shifted location and/or comprise deviating loop lengths.

[0058] The Martin numbering scheme focuses on the structural alignment of different framework regions of unconventional lengths. The Martin numbering scheme highlighted residues that are absent in most sequences and structures and therefore define these as deletion positions and proposes a correction of the insertion point within the framework region 3 of the heavy chain domain. The Martin numbering scheme uses the numbering software, ABnum, and provides a numbering scheme that consists of the Chothia numbering system corrected by the ABnum software, which integrates sequences from Kabat, IMGT, and the PDB databases. IMGT numbering scheme is a reference in immunogenetics and immuno-informatics and provides a standardized numbering system for all the protein sequences of the immunoglobulin superfamily, including variable domains from antibody light and heavy chains as well as T cell receptor chains from different species. IMGT numbering scheme is based on amino acid sequence alignment of the germ-line V genes. IMGT numbering scheme covers the entire variable domains and developed various tools to analyze the full-length sequences. Alternatively, or additionally, any other new method or methods not mentioned in the present disclosure can be used to determine CDRs.

[0059] As known in the art, a “constant region” of an antibody refers to the constant region of the antibody light chain or the constant region of the antibody heavy chain, either alone or in combination.

[0060] As used herein, “monoclonal antibody” refers to an antibody obtained from a population of substantially homogeneous antibodies, i.e., the individual antibodies comprising the population are identical except for possible naturally-occurring mutations that may be present in minor amounts. Monoclonal antibodies are highly specific, being directed against a single antigenic site. Furthermore, in contrast to polyclonal antibody preparations, which typically include different antibodies directed against different determinants (epitopes), each monoclonal antibody is directed against a single determinant on the antigen. The modifier “monoclonal” indicates the character of the antibody as being obtained from a substantially homogeneous population of antibodies, and is not to be construed as requiring production of the antibody by any particular method. For example, the monoclonal antibodies to be used in accordance with the present disclosure may be made by the hybridoma method first described by Kohler and Milstein, 1975, Nature 256:495, or may be made by recombinant DNA methods such as described in U.S. Pat. No. 4,816,567. The monoclonal antibodies may also be isolated from phage libraries generated using the techniques known in the art.

[0061] As used herein, “humanized” antibody refers to forms of non-human (e.g., murine) antibodies that are chimeric immunoglobulins, immunoglobulin chains, or fragments thereof (such as Fv, Fab, Fab', F(ab')₂ or other antigen-binding subsequences of antibodies) that contain minimal sequence derived from non-human immunoglobulin. Humanized antibodies are human immunoglobulins (recipient antibody) in which residues

from a CDR of the recipient are replaced by residues from a CDR of a non-human species (donor antibody) such as mouse, rat, or rabbit having the desired specificity, affinity, and capacity. The humanized antibody may comprise residues that are found neither in the recipient antibody nor in the imported CDR or framework sequences, but are included to further refine and optimize antibody performance.

[0062] Terms such as “treating” or “treatment” or “to treat” or “alleviating” or “to alleviate” all refer to both (1) therapeutic measures that cure, slow down, lessen symptoms of, and/or halt progression of a diagnosed pathologic condition or disorder and/or (2) prophylactic or preventative measures that prevent and/or slow the development of a targeted pathologic condition or disorder. Thus, those in need of treatment include those already with the disorder; those prone to have the disorder; and those in whom the disorder is to be prevented. In certain aspects, a subject is successfully “treated” for cancer according to the methods of the present disclosure if the patient shows, e.g., total, partial, or transient remission of a certain type of cancer.

[0063] A “human antibody” is one which possesses an amino acid sequence which corresponds to that of an antibody produced by a human and/or has been made using any of the techniques for making human antibodies as disclosed herein. This definition of a human antibody specifically excludes a humanized antibody comprising non-human antigen binding residues.

[0064] The term “chimeric antibody” is intended to refer to antibodies in which the variable region sequences are derived from one species and the constant region sequences are derived from another species, such as an antibody in which the variable region sequences are derived from a mouse antibody and the constant region sequences are derived from a human antibody.

[0065] The term “suppress” and/or “regulate” PDL1 and/or PDL2 activity, as used herein, refer to the ability of anti-PD1 binding molecule, e.g., an antibody or antigen-binding fragment thereof to bind to PD1, which in turn, in some aspects, can suppress PDL1 and/or PDL2 activity by decreasing at least about 10%, or at least 15%, or at least 20%, or at least 25%, or at least 30%, or at least 35%, or at least 40%, or at least 45%, or at least 50%, or at least 55%, or at least 60%, or at least 65%, or at least 70%, or at least 75%, or at least 80%, or at least 85%, or at least 90%, or at least 95%, or at least 98%, or at least 99%, or 100% of PDL1 and/or PDL2 activity when cells or a sample are contacted with an anti-PD1 binding molecule, e.g., an antibody or antigen-binding fragment thereof of the present disclosure.

[0066] The term “epitope” refers to that portion of a molecule capable of being recognized by and bound by an antibody at one or more of the antibody's antigen-binding regions. Epitopes often consist of a surface grouping of molecules such as amino acids or sugar side chains and have specific three-dimensional structural characteristics as well as specific charge characteristics. In some embodiments, the epitope can be a protein epitope. Protein epitopes can be linear or conformational. In a linear epitope, all of the points of interaction between the protein and the interacting molecule (such as an antibody) occur linearly along the primary amino acid sequence of the protein. A “nonlinear epitope” or “conformational epitope” comprises noncontiguous polypeptides (or amino acids) within the antigenic protein to which an antibody specific to the epitope binds.

[0067] The term “antigenic epitope” as used herein, is defined as a portion of an antigen to which an antibody

can specifically bind as determined by any method well known in the art, for example, by conventional immunoassays. Once a desired epitope on an antigen is determined, it is possible to generate antibodies to that epitope. Alternatively, during the discovery process, the generation and characterization of antibodies may elucidate information about desirable epitopes. It can be performed to competitively screen antibodies for binding to the same epitope. An approach to achieve this is to conduct competition and cross-competition studies to find antibodies that compete or cross-compete with one another for binding to PD-1, e.g., the antibodies compete for binding to the antigen.

[0068] The present antibodies or antigen-binding fragments thereof contain “paratope” and “non-paratope” amino acid residues. A “paratope” amino acid of a present antibody or antigen-binding fragment thereof can have, for example, an atomic nucleus within about 4 Å of an antibody or an antigen-binding portion thereof preferentially binds PD-1 isoform. Because each isoform forms a structurally different complex with the present antibodies or antigen-binding fragments thereof, the paratope residues may be different for each isoform. An “antibody or an antigen-binding portion thereof preferentially binds PD-1 paratope residue,” for example, has an atomic nucleus within about 4 Å of an atomic nucleus of human antibody or an antigen-binding portion thereof preferentially binds PD-1. An “antibody or an antigen-binding portion thereof preferentially binds PD-1 paratope” residue can have, for example, an atomic nucleus within 4 about Å of an atomic nucleus of all isoforms.

[0069] A residue is designated a paratope residue irrespective of location within a CDR or FW (Framework) region, as defined by the Kabat nomenclature. Many paratope residues are located within CDR regions, as shown in TABLE 15, for example. However, some paratope residues are located within the FW regions. A “non-paratope” amino acid is any amino acid of the antibody or antigen-binding fragment thereof that is not a “paratope” amino acid, irrespective of whether the residue is located in a CDR or FW region.

[0070] The terms “Programmed Death 1,” “Programmed Cell Death 1,” “Protein PD-1,” “PD-1,” PD1,” “PDCD1,” “hPD-1” and “hPD-I” are all used interchangeably, and include variants, isoforms, species homologs of human PD-1, and analogs having at least one common epitope with PD-1. The complete PD-1 sequence can be found under GenBank Accession No. U64863 and is incorporate herein by reference.

[0071] The term “agonist” refers to a substance which promotes (i.e., induces, causes, enhances, or increases) the biological activity or effect of another molecule. The term agonist encompasses substances which bind receptor, such as an antibody, and substances which promote receptor function without binding thereto (e.g., by activating an associated protein).

[0072] The term “antagonist” or “inhibitor” refers to a substance that prevents, blocks, inhibits, neutralizes, or reduces a biological activity or effect of another molecule, such as a receptor.

[0073] As used herein, an “anti-PD-1 antibody” refers to an antibody that is able to inhibit PD-1 biological activity and/or downstream events(s) mediated by PD-1. Anti-PD-1 antibodies encompass antibodies that block, antagonize, suppress or reduce (to any degree including significantly) PD-1 biological activity, including downstream events mediated by PD-1, such as PD-L1 binding and downstream signaling, PD-L2

binding and downstream signaling, inhibition of T cell proliferation, inhibition of T cell activation, inhibition of IFN secretion, inhibition of IL-2 secretion, inhibition of TNF secretion, induction of IL-10, and inhibition of anti-tumor immune responses. For purposes of the present disclosure, it will be understood that the term “anti-PD-1 antibody” (interchangeably termed “PD-1 antibody”) encompasses all the previously identified terms, titles, and functional states and characteristics whereby PD-1 itself, a PD-1 biological activity, or the consequences of the biological activity, are substantially nullified, decreased, or neutralized in any degree. In some embodiments, an anti-PD-1 antibody binds PD-1 and upregulates an anti-tumor immune response. Examples of anti-PD-1 antibodies are provided herein, such as P1801. In an embodiment, P1801 is first generated as a murine version, and can later be humanized for human use. In some embodiments, RMP 1-14, which is a known version of the murine anti-PD1 monoclonal antibody can be used to demonstrate efficacy in mice models.

[0074] The terms “polypeptide”, “oligopeptide”, “peptide” and “protein” are used interchangeably herein to refer to chains of amino acids of any length. The chain may be linear or branched, it may comprise modified amino acids, and/or may be interrupted by non-amino acids. The terms also encompass an amino acid chain that has been modified naturally or by intervention; for example, disulfide bond formation, glycosylation, lipidation, acetylation, phosphorylation, or any other manipulation or modification, such as conjugation with a labeling component. Also included within the definition are, for example, polypeptides containing one or more analogs of an amino acid (including, for example, unnatural amino acids, etc.), as well as other modifications known in the art. It is understood that the polypeptides can occur as single chains or associated chains.

[0075] As known in the art, “polynucleotide,” or “nucleic acid,” as used interchangeably herein, refer to chains of nucleotides of any length, and include DNA and RNA. The nucleotides can be deoxyribonucleotides, ribonucleotides, modified nucleotides or bases, and/or their analogs, or any substrate that can be incorporated into a chain by DNA or RNA polymerase. A polynucleotide may comprise modified nucleotides, such as methylated nucleotides and their analogs. If present, modification to the nucleotide structure may be imparted before or after assembly of the chain. The sequence of nucleotides may be interrupted by non-nucleotide components. A polynucleotide may be further modified after polymerization, such as by conjugation with a labeling component. Other types of modifications include, for example, “caps”, substitution of one or more of the naturally occurring nucleotides with an analog, internucleotide modifications such as, for example, those with uncharged linkages (e.g., methyl phosphonates, phosphotriesters, phosphoamidates, carbamates, etc.) and with charged linkages (e.g., phosphorothioates, phosphorodithioates, etc.), those containing pendant moieties, such as, for example, proteins (e.g., nucleases, toxins, antibodies, signal peptides, poly-L-lysine, etc.), those with intercalators (e.g., acridine, psoralen, etc.), those containing chelators (e.g., metals, radioactive metals, boron, oxidative metals, etc.), those containing alkylators, those with modified linkages (e.g., alpha anomeric nucleic acids, etc.), as well as unmodified forms of the polynucleotide(s). Further, any of the hydroxyl groups ordinarily present in the sugars may be replaced, for example, by phosphonate groups, phosphate groups,

protected by standard protecting groups, or activated to prepare additional linkages to additional nucleotides, or may be conjugated to solid supports. The 5' and 3' terminal OH can be phosphorylated or substituted with amines or organic capping group moieties of from 1 to 20 carbon atoms. Other hydroxyls may also be derivatized to standard protecting groups. Polynucleotides can also contain analogous forms of ribose or deoxyribose sugars that are generally known in the art, including, for example, 2'-O-methyl-, 2'-O-allyl-, 2'-fluoro- or 2'-azido-ribose, carbocyclic sugar analogs, alpha- or beta-anomeric sugars, epimeric sugars such as arabinose, xyloses or lyxoses, pyranose sugars, furanose sugars, sedoheptuloses, acyclic analogs and abasic nucleoside analogs such as methyl riboside. One or more phosphodiester linkages may be replaced by alternative linking groups. These alternative linking groups include, but are not limited to, embodiments wherein phosphate is replaced by P(O)S ("thioate"), P(S)S ("dithioate"), (O)NR₂ ("amidate"), P(O)R, P(O)OR', CO or CH₂ ("formacetal"), in which each R or R' is independently H or substituted or unsubstituted alkyl (1-20 C) optionally containing an ether (—O—) linkage, aryl, alkenyl, cycloalkyl, cycloalkenyl or araldyl. Not all linkages in a polynucleotide need be identical. The preceding description applies to all polynucleotides referred to herein, including RNA and DNA.

[0076] An antibody that "preferentially binds" or "specifically binds" (used interchangeably herein) to an epitope are terms understood in the art, and methods to determine such specific or preferential binding are also known in the art. A molecule is said to exhibit "specific binding" or "preferential binding" if it reacts or associates more frequently, more rapidly, with greater duration and/or with greater affinity with a particular cell or substance than it does with alternative cells or substances. An antibody "specifically binds" or "preferentially binds" to a target if it binds with greater affinity, avidity, more readily, and/or with greater duration than it binds to other substances. For example, an antibody that specifically or preferentially binds to a PD-1 epitope is an antibody that binds this epitope with greater affinity, avidity, more readily, and/or with greater duration than it binds to other PD-1 epitopes or non-PD-1 epitopes. It is also understood by reading this definition that, for example, an antibody (or moiety or epitope) that specifically or preferentially binds to a first target may or may not specifically or preferentially bind to a second target. As such, "specific binding" or "preferential binding" does not necessarily require (although it can include) exclusive binding. Generally, but not necessarily, reference to binding means preferential binding.

[0077] As used herein, "substantially pure" refers to material which is at least 50% pure (i.e., free from contaminants), at least 90% pure, at least 95% pure, at least 98% pure, or at least 99% pure.

[0078] A "host cell" includes an individual cell or cell culture that can be or has been a recipient for vector(s) for incorporation of polynucleotide inserts. Host cells include progeny of a single host cell, and the progeny may not necessarily be completely identical (in morphology or in genomic DNA complement) to the original parent cell due to natural, accidental, or deliberate mutation. A host cell includes cells transfected *in vivo* with a polynucleotide(s) of this disclosure. One example is E. Coli.

[0079] As used herein, any concentration range, percentage range, ratio range or integer range is to be understood to include the value of any integer within the recited range and, when appropriate, fractions thereof

(such as one tenth and one hundredth of an integer), unless otherwise indicated.

[0080] As used in the art, “Fc receptor” and “FcR” describe a receptor that binds to the Fc region of an antibody. The preferred FcR is a native sequence human FcR. Moreover, a preferred FcR is one which binds an IgG antibody (a gamma receptor) and includes receptors of the Fc γ RI, Fc γ RII, and Fc γ RIII subclasses, including allelic variants and alternatively spliced forms of these receptors. Fc γ RII receptors include Fc γ RIIA (an “activating receptor”) and Fc γ RIIB (an “inhibiting receptor”), which have similar amino acid sequences that differ primarily in the cytoplasmic domains thereof. FcRs are reviewed and known in the art. “FcR” also includes the neonatal receptor, FcRn, which is responsible for the transfer of maternal IgGs to the fetus.

[0081] The term “compete”, as used herein with regard to an antibody, means that a first antibody, or an antigen-binding portion thereof, binds to an epitope in a manner sufficiently similar to the binding of a second antibody, or an antigen-binding portion thereof, such that the result of binding of the first antibody with its cognate epitope is detectably decreased in the presence of the second antibody compared to the binding of the first antibody in the absence of the second antibody. The alternative, where the binding of the second antibody to its epitope is also detectably decreased in the presence of the first antibody, can, but need not be the case. That is, a first antibody can inhibit the binding of a second antibody to its epitope without that second antibody inhibiting the binding of the first antibody to its respective epitope. However, where each antibody detectably inhibits the binding of the other antibody with its cognate epitope or ligand, whether to the same, greater, or lesser extent, the antibodies are said to “cross-compete” with each other for binding of their respective epitope(s). Both competing and cross-competing antibodies are encompassed by the present disclosure. Regardless of the mechanism by which such competition or cross-competition occurs (e.g., steric hindrance, conformational change, or binding to a common epitope, or portion thereof), the skilled artisan would appreciate, based upon the teachings provided herein, that such competing and/or cross-competing antibodies are encompassed and can be useful for the methods disclosed herein.

[0082] A “functional Fc region” possesses at least one effector function of a native sequence Fc region. Exemplary “effector functions” include C1q binding; complement dependent cytotoxicity; Fc receptor binding; antibody-dependent cell-mediated cytotoxicity; phagocytosis; down-regulation of cell surface receptors (e.g., B cell receptor), etc. Such effector functions generally require the Fc region to be combined with a binding domain (e.g., an antibody variable domain) and can be assessed using various assays known in the art for evaluating such antibody effector functions.

[0083] A “native sequence Fc region” comprises an amino acid sequence identical to the amino acid sequence of an Fc region found in nature. A “variant Fc region” comprises an amino acid sequence which differs from that of a native sequence Fc region by virtue of at least one amino acid modification, yet retains at least one effector function of the native sequence Fc region. Preferably, the variant Fc region has at least one amino acid substitution compared to a native sequence Fc region or to the Fc region of a parent polypeptide, e.g., from about one to about ten amino acid substitutions, from about one to about five amino acid substitutions in a native sequence Fc region or in the Fc region of the parent polypeptide. The variant Fc region herein can

possess at least about 80% sequence identity with a native sequence Fc region and/or with an Fc region of a parent polypeptide, at least about 90% sequence identity therewith, at least about 95%, at least about 96%, at least about 97%, at least about 98%, at least about 99%, or at least 99.5% sequence identity therewith.

[0084] As used herein, the term "dose" refers to a quantity of active ingredient(s) intended to be administered to a subject each time. The term "dosage" sometimes refers to plural form of the term dose. Each dose and dosage can contain constant and/or variable quantity of active ingredient(s), which may, depending on the context, be different in each dose or dosage. The unit dose or unit dosage may be in the form of a tablet, capsule, sachet, or liquid, etc. For example, a dose (e.g., in the form of one or two separate tablets) may be 0.5 mg of compound A or include 0.5 mg of compound A and 0.1 mg of compound B. Depending on the context, dose and dosage can be used interchangeably.

[0085] As used herein, an "effective dosage", "effective dose" or "effective amount" of drug, compound, or pharmaceutical composition is an amount sufficient to affect any one or more beneficial or desired results. In more specific aspects, an effective amount prevents, alleviates, ameliorates symptoms of disease, and/or prolongs the survival of the subject being treated. For prophylactic use, beneficial or desired results include, but not limited to: eliminating or reducing the risk, lessening the severity, or delaying the outset of the disease, including biochemical, histological and/or behavioral symptoms of the disease, its complications and intermediate pathological phenotypes presenting during development of the disease. For therapeutic use, beneficial or desired results include, but not limited to clinical results such as reducing one or more symptoms of a disease such as, for example, cancer including, for example without limitation, solid tumors, decreasing the dose of other medications required to treat the disease, enhancing the effect of another medication, and/or delaying the progression of the cancer in patients. An effective dosage can be administered in one or more administrations. For purposes of this disclosure, an effective dosage of drug, compound, or pharmaceutical composition is an amount sufficient to accomplish prophylactic or therapeutic treatment either directly or indirectly. As is understood in the clinical context, an effective dosage of a drug, compound, or pharmaceutical composition may or may not be achieved in conjunction with another drug, compound, or pharmaceutical composition. Thus, an "effective dosage" or "effective amount" may be considered in the context of administering one or more therapeutic agents, and a single agent may be considered to be given in an effective amount if, in conjunction with one or more other agents, a desirable result may be or is achieved.

[0086] An "individual" or a "subject" is a mammal, non-limiting example is a human. Mammals also include, but are not limited to, farm animals (e.g., cows, pigs, horses, chickens, etc.), sport animals, pets, primates, horses, dogs, cats, mice and rats.

[0087] As used herein, "vector" or "expression vector" means a construct, which is capable of delivering, and expressing one or more gene(s) or sequence(s) of interest in a host cell. Examples of vectors include, but are not limited to, viral vectors, naked DNA or RNA expression vectors, plasmid, cosmid or phage vectors, DNA or RNA expression vectors associated with cationic condensing agents, DNA or RNA expression vectors encapsulated in liposomes, and certain eukaryotic cells, such as producer cells.

[0088] As used herein, “expression control sequence” means a nucleic acid sequence that directs transcription of a nucleic acid. An expression control sequence can be a promoter, such as a constitutive or an inducible promoter, or an enhancer. The expression control sequence is operably linked to the nucleic acid sequence to be transcribed.

[0089] As used herein, “pharmaceutically acceptable carrier” or “pharmaceutical acceptable excipient” includes any material which, when combined with an active ingredient, allows the ingredient to retain biological activity or stability and is non-reactive with the subject's immune system. Examples include, but are not limited to, any of the standard pharmaceutical carriers such as a phosphate buffered saline solution, water, emulsions such as oil/water emulsion, and various types of wetting agents. Preferred diluents for aerosol or parenteral administration are phosphate buffered saline (PBS) or normal (0.9%) saline. Compositions comprising such carriers are formulated by known conventional methods.

[0090] The term “*k_{on}*”, as used herein, refers to the rate constant for association of an antibody to an antigen. Specifically, the rate constants (*k_{on}* and *k_{off}*) and equilibrium dissociation constants are measured using full-length antibodies and/or Fab antibody fragments (i.e., univalent) and PD-1.

[0091] The term “*k_{off}*”, as used herein, refers to the rate constant for dissociation of an antibody from the antibody/antigen complex.

[0092] The term “immune modulator” refers to a substance capable of altering (e.g., inhibiting, decreasing, increasing, enhancing, or stimulating) the immune response (as defined herein) or the working of any component of the innate, humoral or cellular immune system of a host mammal. Thus, the term “immune modulator” encompasses the “immune-effector-cell enhancer” as defined herein and the “immune-suppressive-cell inhibitor” as defined herein, as well as substance that affects other components of the immune system of a mammal.

[0093] The term “immune response” refers to any detectable response to a particular substance (such as an antigen or immunogen) by the immune system of a host mammal, such as innate immune responses (e.g., activation of Toll receptor signaling cascade), cell-mediated immune responses (e.g., responses mediated by T cells, such as antigen-specific T cells, and non-specific cells of the immune system), and humoral immune responses (e.g., responses mediated by B cells, such as generation and secretion of antibodies into the plasma, lymph, and/or tissue fluids).

[0094] The term “immunogenic” refers to the ability of a substance to cause, elicit, stimulate, or induce an immune response, or to improve, enhance, increase or prolong a pre-existing immune response, against a particular antigen, whether alone or when linked to a carrier, in the presence or absence of an adjuvant.

[0095] The term “immune-suppressive-cell inhibitor” or “ISC inhibitor” refers to a substance capable of reducing or suppressing the number or function of immune suppressive cells of a mammal. Examples of immune suppressive cells include regulatory T cells (“T regs”), myeloid-derived suppressor cells, and tumor-associated macrophages.

[0096] The term “intradermal administration”, “i.d.”, or “administered intradermally,” in the context of

administering a substance to a mammal including a human, refers to the delivery of the substance into the dermis layer of the skin of the mammal. The skin of a mammal is composed of an epidermis layer, a dermis layer, and a subcutaneous layer. The epidermis is the outer layer of the skin. The dermis, which is the middle layer of the skin, contains nerve endings, sweat glands and oil (sebaceous) glands, hair follicles, and blood vessels. The subcutaneous layer is made up of fat and connective tissue that houses larger blood vessels and nerves. In contrast in intradermal administration, “subcutaneous administration” refers to the administration of a substance into the subcutaneous layer and “topical administration” refers to the administration of a substance onto the surface of the skin.

[0097] The term “preventing” or “prevent” refers to (a) keeping a disorder from occurring or (b) delaying the onset of a disorder or onset of symptoms of a disorder.

[0098] The term “tumor-associated antigen” or “TAA” refers to an antigen which is specifically expressed by tumor cells or expressed at a higher frequency or density by tumor cells than by non-tumor cells of the same tissue type. Tumor-associated antigens may be antigens not normally expressed by the host; they may be mutated, truncated, misfolded, or otherwise abnormal manifestations of molecules normally expressed by the host; they may be identical to molecules normally expressed but expressed at abnormally high levels; or they may be expressed in a context or milieu that is abnormal. Tumor-associated antigens may be, for example, proteins or protein fragments, complex carbohydrates, gangliosides, haptens, nucleic acids, or any combination of these or other biological molecules.

[0099] The term “vaccine” refers to an immunogenic composition for administration to a mammal for eliciting an immune response against a particular antigen in the mammal. A vaccine typically contains an agent (known as “antigen” or “immunogen”) that resembles, or is derived from, the target of the immune response, such as a disease-causing micro-organism or tumor cells. A vaccine intended for the treatment of a tumor, such as a cancer, typically contains an antigen that is derived from a TAA found on the target tumor and is able to elicit immunogenicity against the TAA on the target tumor.

[0100] The term “vaccine-based immunotherapy regimen” refers to a therapeutic regimen in which a vaccine is administered in combination with one or more immune modulators. The vaccine and the immune modulators may be administered together in a single formulation or administered separately.

[0101] The term “antigen-binding portion” of an antibody (or simply “antibody portion”), as used herein, refers to one or more fragments of an antibody that retain the ability to specifically bind to an antigen (e.g., PD-1). It has been shown that the antigen-binding function of an antibody can be performed by fragments of a full-length antibody. Examples of binding fragments encompassed within the term “antigen-binding portion” of an antibody 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) a 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). Furthermore, although the two

domains of the Fv fragment, VL and VH, are coded for 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 regions pair to form monovalent molecules (known as single chain Fv (scFv)). Such single chain antibodies are also intended to be encompassed within the term “antigen-binding portion” of an antibody. These antibody fragments are obtained using conventional techniques known to those with skill in the art, and the fragments are screened for utility in the same manner as are intact antibodies.

[0102] The term “human antibody”, as used herein, is intended to include antibodies having variable regions in which both the framework and CDR regions are derived from human germline immunoglobulin sequences. Furthermore, if the antibody contains a constant region, the constant region also is derived from human germline immunoglobulin sequences. The human antibodies of the disclosure may include amino acid residues not encoded by human germline immunoglobulin sequences (e.g., mutations introduced by random or site-specific mutagenesis *in vitro* or by somatic mutation *in vivo*). However, the term “human antibody”, as used herein, is not intended to include antibodies in which CDR sequences derived from the germline of another mammalian species, such as a mouse, have been grafted onto human framework sequences.

[0103] The term “human monoclonal antibody” refers to antibodies displaying a single binding specificity which have variable regions in which both the framework and CDR regions are derived from human germline immunoglobulin sequences. In one embodiment, the human monoclonal antibodies are produced by a hybridoma which includes a B cell obtained from a transgenic nonhuman animal, e.g., a transgenic mouse, having a genome comprising a human heavy chain transgene and a light chain transgene fused to an immortalized cell.

[0104] The term “humanized antibody” or “recombinant human antibody”, as used herein, includes all human antibodies that are prepared, expressed, created or isolated by recombinant means, such as (a) antibodies isolated from an animal (e.g., a mouse) that is transgenic or transchromosomal for human immunoglobulin genes or a hybridoma prepared therefrom (described further below), (b) antibodies isolated from a host cell transformed to express the human antibody, e.g., from a transfectoma, (c) antibodies isolated from a recombinant, combinatorial human antibody library, and (d) antibodies prepared, expressed, created or isolated by any other means that involve splicing of human immunoglobulin gene sequences to other DNA sequences. Such recombinant human antibodies have variable regions in which the framework and CDR regions are derived from human germline immunoglobulin sequences. In certain embodiments, however, such recombinant human antibodies can be subjected to *in vitro* mutagenesis (or, when an animal transgenic for human Ig sequences is used, *in vivo* somatic mutagenesis) and thus the amino acid sequences of the VH and VL regions of the recombinant antibodies are sequences that, while derived from and related to human germline VH and VL sequences, may not naturally exist within the human antibody germline repertoire *in vivo*.

[0105] As used herein, “isotype” refers to the antibody class (e.g., IgM or IgG1) that is encoded by the heavy chain constant region genes.

[0106] The phrases “an antibody recognizing an antigen” and “an antibody specific for an antigen” are used

interchangeably herein with the term “an antibody which binds specifically to an antigen.”

[0107] The term “human antibody derivatives” refers to any modified form of the human antibody, e.g., a conjugate of the antibody and another agent or antibody.

[0108] The term “chimeric antibody” is intended to refer to antibodies in which the variable region sequences are derived from one species and the constant region sequences are derived from another species, such as an antibody in which the variable region sequences are derived from a mouse antibody and the constant region sequences are derived from a human antibody.

[0109] The term “Kassoc” or “Ka”, as used herein, is intended to refer to the association rate of a particular antibody-antigen interaction, whereas the term “Kdis” or “Kd,” as used herein, is intended to refer to the dissociation rate of a particular antibody-antigen interaction. The term “KD”, as used herein, is intended to refer to the dissociation constant, which is obtained from the ratio of Kd to KA (i.e., K_d/K_a) and is expressed as a molar concentration (M). KD values for antibodies can be determined using methods established in the art. A method for determining the KD of an antibody is by using surface plasmon resonance, such as using a biosensor system such as a Biacore (trademarked) system.

[0110] As used herein, the term “high affinity” for an IgG antibody refers to an antibody having a KD of about 10^{-8} M or less, about 10^{-9} M or less, or about 10^{-10} M or less for a target antigen. However, “high affinity” binding can vary for other antibody isotypes. For example, “high affinity” binding for an IgM isotype refers to an antibody having a KD of about 10^{-7} M or less, about 10^{-8} M or less, or about 10^{-9} M or less.

[0111] As used herein, “about” mean within an acceptable error range for the particular value as determined by one of ordinary skill in the art, which will depend in part on how the value is measured or determined, i.e., the limitations of the measurement system. For example, “about” can mean within 1 or more than 1 standard deviation per the practice in the art. Alternatively, “about” can mean a range of up to $\pm 5\%$, $\pm 10\%$, $\pm 15\%$, or $\pm 20\%$. Furthermore, particularly with respect to biological systems or processes, the terms can mean up to an order of magnitude or up to ± 3 -fold of a value. When particular values are provided in the application and claims, unless otherwise stated, the meaning of “about” should be assumed to be within an acceptable error range for that particular value. Reference to “about” a value or parameter herein includes (and describes) embodiments that are directed to that value or parameter *per se*. For example, description referring to “about X” includes description of “X”. Numeric ranges are inclusive of the numbers defining the range. In addition, or alternatively, throughout this disclosure, values expressed in a range format should be interpreted in a flexible manner to include not only the numerical values explicitly recited as the limits of the range, but also to include all the individual numerical values or sub-ranges encompassed within that range as if each numerical value and sub-range is explicitly recited. For example, a range of “about 0.1% to about 5%” or “about 0.1% to 5%” should be interpreted to include not just about 0.1% to about 5%, but also the individual values (e.g., 1%, 2%, 3%, and 4%) and the sub-ranges (e.g., 0.1% to 0.5%, 1.1% to 2.2%, 3.3% to 4.4%) within the indicated range and can be up to two decimals for such number. The statement “about X to Y” has the same meaning as “about X to about Y,” unless indicated otherwise. Likewise, the statement “about X, Y, or about Z” has the

same meaning as “about X, about Y, or about Z,” unless indicated otherwise.

[0112] As used herein, the term “conservative sequence modifications” is intended to refer to amino acid modifications that do not significantly affect or alter the binding characteristics of the antibody containing the amino acid sequence. Such conservative modifications include amino acid substitutions, additions and deletions. Modifications can be introduced into an antibody of the disclosure by standard techniques known in the art, such as site-directed mutagenesis and PCR-mediated mutagenesis. Conservative amino acid substitutions are ones in which the amino acid residue is replaced with an amino acid residue having a similar side chain. Families of amino acid residues having similar side chains have been defined in the art. These families include amino acids with basic side chains (e.g., lysine, arginine, histidine), acidic side chains (e.g., aspartic acid, glutamic acid), uncharged polar side chains (e.g., glycine, asparagine, glutamine, serine, threonine, tyrosine, cysteine, tryptophan), nonpolar side chains (e.g., alanine, valine, leucine, isoleucine, proline, phenylalanine, methionine), beta-branched side chains (e.g., threonine, valine, isoleucine) and aromatic side chains (e.g., tyrosine, phenylalanine, tryptophan, histidine). Thus, one or more amino acid residues within the CDR regions of an antibody of the disclosure can be replaced with other amino acid residues from the same side chain family and the altered antibody can be tested for retained function (i.e., the functions set forth in (c) through (I) above) using the functional assays described herein.

[0113] The term “treatment” or “therapy” refers to administering an active agent with the purpose to cure, heal, alleviate, relieve, alter, remedy, ameliorate, improve, or affect a condition (e.g., a disease), the symptoms of the condition, or to prevent or delay the onset of the symptoms, complications, biochemical indicia of a disease, or otherwise arrest or inhibit further development of the disease, condition, or disorder in a statistically significant manner.

[0114] An “adverse event” (AE) as used herein is any unfavorable and generally unintended, even undesirable, sign (including an abnormal laboratory finding), symptom, or disease associated with the use of a medical treatment. For example, an adverse event may be associated with activation of the immune system or expansion of immune system cells (e.g., T cells) in response to a treatment. A medical treatment may have one or more associated AEs and each AE may have the same or different level of severity. Reference to methods capable of “altering adverse events” means a treatment regime that decreases the incidence and/or severity of one or more AEs associated with the use of a different treatment regime.

[0115] As used herein, the term “drug” refers to any compound possessing a desired biological activity and a reactive functional group available for preparing the conjugate of the disclosure. The biological activity includes activity for use in the diagnosis, cure, mitigation, treatment, or prevention of a disease in human or other animals. Thus, so long as it has the needed reactive functional group, the compound involved by the term “drug” include drugs identified in the official national pharmacopeia as well as e.g., official Homeopathic Pharmacopeia of the United States, or official National Formulary, or any supplements thereof. Exemplary drugs are set forth in the Physician's Desk Reference (PDR) and in the Orange Book maintained by the U.S. Food and Drug Administration (FDA).

[0116] As used herein, the term “biologics drug” refers to any compound or protein possessing a desired biological activity and a reactive functional group available for preparing the conjugate of the disclosure. The biological activity includes activity for use in the diagnosis, cure, mitigation, treatment, or prevention of a disease in human or other animals. Thus, so long as it has the needed reactive functional group, the compound or protein involved by the term “biologics drug” include drugs identified in the official national pharmacopeia as well as e.g., in the Purple Book maintained by the U.S. Food and Drug Administration (FDA).

[0117] As used herein, the term HCC refers to Hepatocellular carcinoma which is a cancer that starts in liver. It's different from "secondary" liver cancers, which have spread to the liver from other organs.

[0118] The term “cytotoxic agents” refers to substances that inhibit or block cell expression activity, cell function and/or result in cell destruction. The term includes radioisotopes, chemotherapeutics, and toxins, such as small-molecular toxins or enzymatically active toxins (including fragments and/or variants thereof) derived from bacteria, fungi, plants or animals. Examples of cytotoxic agents include, but are not limited to: Auristatins (for example, Auristatin E, Auristatin F, MMAE and MMAF), chlortetracycline, metotanol, ricin, ricin A-chain, cobustatin, dokamicin, Dorastatin, adriamycin, daunorubicin, paclitaxel, cisplatin, cc1065, ethidium bromide, mitomycin, etoposide, tenoposide, vincristine, vinblastine, colchicine, dihydroxyanthracnose diketone, actinomycin, diphtheria toxin, Pseudomonas exotoxin (PE) A, PE40, abrin, abrin A chain, modeccin A chain, α -Sarcina, gelonin, mitogellin, retstrictocin, phenomycin, enomycin, curicin, crocotin, calicheamicins, Sapaonaria officinalis inhibitor, as well as glucocorticoid and other chemotherapy agents, as well as radioisotopes such as At211, I131, I125, Y90, Re186, Re188, Sm153, Bi212 or Bi213, P32 and Lu (including Lu177). Antibodies can also be conjugated to anticancer prodrug activating enzymes that can convert a prodrug into the active form.

[0119] Figure 1(a) denotes the full heavy chain of P1801 including three CDRs and variable domain plus constant regions. It also includes heavy chain signal peptides, that will be eventually cleaved by the host cell. The full heavy chain sequence is denoted as SEQ ID NO: 1. Figure 1(b) denotes the full light chain of P1801 including three CDRs and variable domain plus constant regions. It also includes light chain signal peptides, that will be eventually cleaved by the host cell. The full light chain sequence is denoted as SEQ ID NO: 2. Figure 1(c) denotes the heavy chain of P1801 with no heavy chain signal peptide (SEQ ID No:3). Figure 1(d) denotes the light chain of P1801 with no light chain signal peptide (SEQ ID No:4). Figure 1(f) denotes the full heavy chain variable region of P1801 (SEQ ID No:5). Figure 1(g) denotes the full light chain variable region of P1801 (SEQ ID No:6). Figure 1(h) denotes the heavy chain constant region of P1801 (SEQ ID No:7). Figure 1(i) denotes the light chain constant region of P1801 (SEQ ID No:8). Figure 1(j) denotes heavy chain CDR1 of P1801 (SEQ ID No:9). Figure 1(k) denotes heavy chain CDR2 of P1801 (SEQ ID No:10). Figure 1(l) denotes heavy chain CDR3 of P1801 (SEQ ID No:11). Figure 1(m) denotes light chain CDR1 of P1801 (SEQ ID No:12). Figure 1(n) denotes light chain CDR2 of P1801 (SEQ ID No:13). Figure 1(o) denotes light chain CDR3 of P1801 (SEQ ID No:14). Figure 1(p) denotes heavy chain signal peptide of P1801 (SEQ ID No:15). Figure 1(q) denotes light chain signal peptide of P1801 (SEQ ID No:16). Figure 1(r) denotes the hinge

sequence is located in heavy chains (SEQ ID No: 17). Figure 1(s) denotes an alternative heavy chain CDR1 of P1801 (SEQ ID No:18). Figure 1(t) denotes an alternative heavy chain CDR1 of P1801 (SEQ ID No:19). Figure 1(u) denotes an alternative heavy chain CDR1 of P1801 (SEQ ID No:20). Figure 1(v) denotes an alternative heavy chain CDR1 of P1801 (SEQ ID No:21). Figure 1(w) denotes an alternative heavy chain CDR2 of P1801 (SEQ ID No:10). Figure 1(x) denotes an alternative heavy chain CDR3 of P1801 (SEQ ID No:23).

[0120] As used herein, the term “P1801” refers to the anti-PD-1 monoclonal antibody of the present disclosure as denoted in Figure 1. Detailed amino sequences such as heavy/light chains and all CDRs or alternative CDRs are denoted in Figure 1(a) to 1(o) and Figure 1(a) to 1(o) plus Figure 1(s), without signal peptides. Figure 1 denotes the mapping of human P1801 with alternative CDR in Figure 1(s).

[0121] As used herein, the term “P1101”, “Ropeg”, and/or “pegylated human interferon- α 2b” are used interchangeably. P1101 is known in the art, for example, see U.S. Patent Numbers: US 8,143,214 B2, US 8,273,343, US 8,617,532, and/or US 8,106,160 B2, the content of all of which are incorporate herein in their entirety. Also see U.S. Patent Application Numbers: US20170326206A1, US20220152156A1, US20220362343A1, and/or US20230057788A1, the content of all of which are incorporate herein in their entirety. Briefly, for example, the chemical formula, method of manufacturing, and its uses are disclosed therein. For convenience, its structure is briefly denoted in Figure 2. More specifically, the interferon is the version that is functional in human subjects for P1101.

[0122] As used herein, the term “mP1101” refers to the same structure of pegylation and linker as P1101, except that the interferon is the mouse version which is functional in murine subjects.

ii. Composition and Molecular Profile

Peptide Sequence

[0123] Figure 1(a) to 1(x) show the various amino acid sequence embodiments of P1801. Table 1 below shows molecular characteristics of P1801.

Table 1

Property	Description
Molecular formula	C ₆₄₁₀ H ₉₈₅₄ N ₁₆₉₀ O ₂₀₁₄ S ₄₂
Molecular Weight	146,799 Da
Physical Form/ Appearance	Colorless clear liquid, essentially free from visible particles
Extinction coefficient at 280 nm	1.621 mLmg ⁻¹ cm ⁻¹
pI	6.6

[0124] In certain embodiments, the antibody of the present disclosure comprises a heavy chain variable region

from a particular germline heavy chain immunoglobulin gene and/or a light chain variable region from a particular germline light chain immunoglobulin gene.

[0125] In an embodiment, the nucleic acid encoding P1801 and the vector expressing such amino acids contains nucleic acid molecules encoding signal peptides (SEQ ID No:15 for heavy chain and SEQ ID No:16 for light chain), where the signal peptide is located at the N terminus of heavy chain and/or light chain variable domain. In addition, in an embodiment, the nucleic acid encoding the light chain signal peptide does not include any intron. These signal peptides can be first expressed in a host cell, and later cleaved away by the host cell before secretion or release to solution outside of the host cell resulting in P1801.

[0126] Nucleic Acid Molecules Encoding Antibody P1801

[0127] Another aspect of the present disclosure pertains to nucleic acid molecules that encode the antibodies of the disclosure. The nucleic acids may be present in whole cells, in a cell lysate, or in a partially purified or substantially pure form. A nucleic acid is “isolated” or “rendered substantially pure” when purified away from other cellular components or other contaminants, e.g., other cellular nucleic acids or proteins, by standard techniques, including alkaline/SDS treatment, CsCl banding, column chromatography, agarose gel electrophoresis and others well known in the art. For example, DNA or RNA and may or may not contain intronic sequences. In a preferred embodiment, the nucleic acid is an cDNA molecule.

[0128] Nucleic acids of the present disclosure can be obtained using standard molecular biology techniques. For antibodies expressed by hybridomas (e.g., hybridomas prepared from transgenic mice carrying human immunoglobulin genes as described further below), cDNAs encoding the light and heavy chains of the antibody made by the hybridoma can be obtained by standard PCR amplification or cDNA cloning techniques. For antibodies obtained from an immunoglobulin gene library (e.g., using phage display techniques), nucleic acid encoding the antibody can be recovered from the library.

[0129] Once DNA fragments encoding VH and VL segments are obtained, these DNA fragments can be further manipulated by standard recombinant DNA techniques, for example to convert the variable region genes to full-length antibody chain genes, to Fab fragment genes or to a scFv gene. In these manipulations, a VL- or VH-encoding DNA fragment is operatively linked to another DNA fragment encoding another protein, such as an antibody constant region or a flexible linker. The term “operatively linked”, as used in this context, is intended to mean that the two DNA fragments are joined such that the amino acid sequences encoded by the two DNA fragments remain in-frame.

[0130] In an embodiment, the isolated DNA encoding the VH region can be converted to a full-length heavy chain gene by operatively linking the VH-encoding DNA to another DNA molecule encoding heavy chain constant regions (CH1, CH2 and CH3). The sequences of human heavy chain constant region genes are known in the art, and DNA fragments encompassing these regions can be obtained by standard PCR amplification. The heavy chain constant region can be an IgG1, IgG2, IgG3, IgG4, IgA, IgE, IgM or IgD constant region, particularly be IgG1 or IgG4's constant region. For a Fab fragment heavy chain gene, the VH-encoding DNA can be operatively linked to another DNA molecule encoding only the heavy chain CH1 constant region.

[0131] In an embodiment, the isolated DNA encoding the VL region can be converted to a full-length light chain gene (as well as a Fab light chain gene) by operatively linking the VL-encoding DNA to another DNA molecule encoding the light chain constant region, CL. The sequences of human light chain constant region genes are known in the art and DNA fragments encompassing these regions can be obtained by standard PCR amplification. The light chain constant region can be a kappa or lambda constant region, or kappa constant region.

[0132] To create a scFv gene, 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, such that the VH and VL sequences can be expressed as a contiguous single-chain protein, with the VL and VH regions joined by the flexible linker.

iii. Method of Making

[0133] The disclosure also provides methods of generating, selecting, and making anti-PD-1 antibodies. The antibodies of this disclosure can be made by procedures known in the art. In some embodiments, antibodies may be made recombinantly and expressed using any method known in the art.

Cloning

[0134] Cloning vectors may be constructed according to known techniques, or may be selected from a large number of cloning vectors available in the art. While the cloning vector selected may vary according to the host cell intended to be used, end product cloning vectors will generally have the ability to self-replicate, carry possess a single target for a particular restriction endonuclease, and/or can carry genes for a marker that can be used in selecting clones containing the vector. Suitable examples include plasmids and bacterial viruses, e.g., pUC18, pUC19, Bluescript (e.g., pBS SK+) and its derivatives, mp18, mp19, pBR322, pMB9, ColE1, pCR1, RP4, phage DNAs, and shuttle vectors such as pSA3 and pAT28. These and many other commercial cloning vectors are available from various vendors.

[0135] Expression vectors are further provided in this disclosure. Expression vectors generally are replicable polynucleotide constructs that contain a polynucleotide according to the disclosure. It is implied that an expression vector must be replicable in the host cells either as episomes or as an integral part of the chromosomal DNA. Suitable expression vectors include but are not limited to plasmids, viral vectors, including adenoviruses, adeno-associated viruses, retroviruses, cosmids, and expression vector(s) disclosed in PCT Publication No. WO 87/04462. Vector components may generally include, but are not limited to, one or more of the following: a signal sequence; an origin of replication; one or more marker genes; suitable transcriptional controlling elements (such as promoters, enhancers and terminators). For expression (i.e., translation), one or more translational controlling elements are also usually required, such as ribosome binding sites, translation initiation sites, and stop codons.

[0136] The vectors containing the polynucleotides of interest can be introduced into the host cell by any of a number of appropriate means, including electroporation, transfection employing calcium chloride, rubidium chloride, calcium phosphate, DEAE-dextran, or other substances; microprojectile bombardment; lipofection;

and infection (e.g., where the vector is an infectious agent such as vaccinia virus). The choice of introducing vectors or polynucleotides depends on features of the host cell.

[0137] The disclosure herein also provides host cells comprising any of the polynucleotides described herein. Any host cells capable of over-expressing heterologous DNAs can be used for the purpose of isolating the genes encoding the antibody, polypeptide or protein of interest. Non-limiting examples of mammalian host cells include but not limited to COS, HeLa, and CHO cells. See also PCT Publication No. WO 87/04462. Suitable non-mammalian host cells include prokaryotes (such as *E. coli* or *B. subtilis*) and yeast (such as *S. cerevisiae*, *S. pombe*; or *K. lactis*). In an aspect, the host cells express the cDNAs at a level of about 5-fold higher, 10-fold higher, or even 20-fold higher than that of the corresponding endogenous antibody or protein of interest, if present, in the host cells. In an embodiment, P1801 can be made by using Chinese hamster ovary (CHO) DG44 cells is with dhfr gene deficiency cell line(dhfr-) and comes from one of the CHO cell lineages.

[0138] Screening the host cells for a specific binding to PD-1 or a PD-1 domain is affected by an immunoassay or fluorescence activated cell sorting (FACS). A cell overexpressing the antibody or protein of interest can be identified.

[0139] An expression vector can be used to direct expression of an anti-PD-1 antibody. One skilled in the art is familiar with administration of expression vectors to obtain expression of an exogenous protein *in vivo*. See, e.g., U.S. Pat. Nos. 6,436,908; 6,413,942; and 6,376,471, all of the contents are incorporated herein in reference in their entirety. Administration of expression vectors includes local or systemic administration, including injection, oral administration, particle gun or catheterized administration, and topical administration. In another embodiment, the expression vector is administered directly to the sympathetic trunk or ganglion, or into a coronary artery, atrium, ventricle, or pericardium.

[0140] Vectors can be transfected to host cell and stored in a Master Cell Bank. Typically, one or two Master Cell Bank (MCB) vials are taken from a liquid nitrogen container. After thawing at about 37°C, the MCB vial is transferred into a 125 ml Tri-forest shake flask with pre-warmed Dynamis basic medium under a biosafety cabinet. The cells can be then cultured at about 37°C in an INFORS incubator with shaking.

[0141] Seed culture passage: After about 72-96 hours of cell growth, the initial seed culture is transferred into a 500 ml Tri-forest shake flask with Dynamis basic medium and about 30ml cells under a biosafety cabinet. This culture passage can be repeated one or more times as needed.

[0142] After cell growth, cell medium can be inoculated into a SUB50 with a basic medium LC100B. Cell density is determined by sampling the culture and measured using a Beckman Vi-Cell counter. The pH of the fed-batch cell culture is controlled. The dissolved oxygen (DO) level can be maintained at about 45%, while stirring. The feeding medium and components can be individually added based on a fixed schedule. Antifoam emulsion is added as needed. The inoculation cell density can be about 1.5×10^6 cells/ml. Once the cells reach the peak cell density, the cell culture is then harvested. After harvesting, the fed-batch culture can be filtrated one or more times. After filtration, the culture is collected in a 50L storage bag and stored at about 4°C. Methods known in the art for purifying antibodies and other proteins also include, for example, those described

in U.S. Patent Publication Nos. 2008/0312425, 2008/0177048, and 2009/0187005, each of which is hereby incorporated by reference herein in its entirety.

General Properties

[0143] P1801 blocks the interaction of the immune-checkpoint PD-1 and its ligand PD-L1 to disrupt the mechanism of immune suppression. P1801 may belong to the IgG4 subclass of human antibodies, which have been shown to exhibit unique properties. The human IgG4 antibodies can be functionally monovalent *in vivo*, because of the possibility of exchanging half-molecules (one heavy and one light chain) among themselves, a process known as Fab-arm exchange. The Fab domains of P1801 are connected to the Fc by the hinge region with amino acid sequence ESKYGPPCPPCP (SEQ ID No: 17) located within the heavy chain constant region, which has a S288P mutation to reduce the Fab-arm exchange. The IgG4 shows a low affinity for complement component C1q (C1q) and Fc receptors resulting in weakly induced complement toxicity and antibody-dependent cell activation. These properties of IgG4 are preferred in cancer immunotherapy when recruitment of host effector function is undesirable.

[0144] In another embodiment, P1801 can keep all the variable regions and change the IgG types to IgG1, IgG2, IgG3, or other types.

Hybridoma Screening

[0145] Anti-PD1 antibodies, e.g., P1801, can also be generated from hybridoma screening. For example, the murine antibodies were selected as the one of the top clones for its high binding affinity shown in ELISA. It has specific binding to human PD1 and has the ability to block the binding of PD1 and PDL1/PDL2. The murine version of P1801 was humanized and was formatted as human immunoglobulin subtype IgG4, because IgG4 has fewer immune responses, like ADCC and CDC, than the other subtypes IgG1 and IgG3; besides. The DNA sequences of P1801 were codon optimized for expression. The expression vectors of P1801 can be synthesized by DNA2.0.

Phage Display

[0146] In some embodiments, antibodies may be prepared and selected by phage display technology. See, for example, U.S. Pat. Nos. 5,565,332; 5,580,717; 5,733,743; and 6,265,150, all of which are incorporated in reference in their entirety. Alternatively, the phage display technology can be used to produce human antibodies and antibody fragments *in vitro*, from immunoglobulin variable (V) domain gene repertoires from unimmunized donors. According to this technique, antibody V domain genes are cloned in-frame into either a major or minor coat protein gene of a filamentous bacteriophage, such as M13 or fd, and displayed as functional antibody fragments on the surface of the phage particle. Because the filamentous particle contains a single-stranded DNA copy of the phage genome, selections based on the functional properties of the antibody also result in selection of the gene encoding the antibody exhibiting those properties. Thus, the phage mimics some of the properties of the B cell. Phage display can be performed in a variety of formats. Several sources of V-gene segments can be used for phage display. Known art reported isolation of a diverse array of anti-oxazolone antibodies from a small random combinatorial library of V genes derived from the spleens of immunized mice.

A repertoire of V genes from human donors can be constructed and antibodies to a diverse array of antigens (including self-antigens) can be isolated essentially following the techniques known in the art. In a natural immune response, antibody genes accumulate mutations at a high rate (somatic hypermutation). Some of the changes introduced will confer higher affinity, and B cells displaying high-affinity surface immunoglobulin are preferentially replicated and differentiated during subsequent antigen challenge. This natural process can be mimicked by employing the technique known as “chain shuffling” known in the art. In this method, the affinity of “primary” human antibodies obtained by phage display can be improved by sequentially replacing the heavy and light chain V region genes with repertoires of naturally occurring variants (repertoires) of V domain genes obtained from unimmunized donors. This technique allows the production of antibodies and antibody fragments with affinities in the pM-nM range. A strategy for making very large phage antibody repertoires (also known as “the mother-of-all libraries”) has been described and known in the art. Gene shuffling can also be used to derive human antibodies from rodent antibodies, where the human antibody has similar affinities and specificities to the starting rodent antibody. According to this method, which is also referred to as “epitope imprinting”, the heavy or light chain V domain gene of rodent antibodies obtained by phage display technique is replaced with a repertoire of human V domain genes, creating rodent-human chimeras. Selection on antigen results in isolation of human variable regions capable of restoring a functional antigen-binding site, i.e., the epitope governs (imprints) the choice of partner. When the process is repeated in order to replace the remaining rodent V domain, a human antibody is obtained (see PCT Publication No. WO 93/06213). Unlike traditional humanization of rodent antibodies by CDR grafting, this technique provides completely human antibodies, which have no framework or CDR residues of rodent origin.

[0147] In some embodiments, antibodies may be made using hybridoma technology. It is contemplated that any mammalian subject including humans or antibody producing cells therefrom can be manipulated to serve as the basis for production of mammalian, including human, hybridoma cell lines. The route and schedule of immunization of the host animal are generally in keeping with known techniques for antibody stimulation and production, as further described herein. Typically, the host animal is inoculated intraperitoneally, intramuscularly, orally, subcutaneously, intraplantarly, and/or intradermally with an amount of immunogen, including as described herein.

[0148] Hybridomas can be prepared from the lymphocytes and immortalized myeloma cells using the general somatic cell hybridization technique. Available myeloma lines can be used in the hybridization. Generally, the technique involves fusing myeloma cells and lymphoid cells using a fusogen such as polyethylene glycol, or by electrical means well known to those skilled in the art. After the fusion, the cells are separated from the fusion medium and grown in a selective growth medium, such as hypoxanthine-aminopterin-thymidine (HAT) medium, to eliminate unhybridized parent cells. Any of the media described herein, supplemented with or without serum, can be used for culturing hybridomas that secrete monoclonal antibodies. As another alternative to the cell fusion technique, EBV immortalized B cells may be used to produce the PD-1 monoclonal antibodies of the subject disclosure. The hybridomas or other immortalized B-cells are expanded and subcloned, if desired,

and supernatants are assayed for anti-immunogen activity by conventional immunoassay procedures (e.g., radioimmunoassay, enzyme immunoassay, or fluorescence immunoassay).

[0149] Hybridomas that may be used as source of antibodies encompass all derivatives, progeny cells of the parent hybridomas that produce monoclonal antibodies specifically or preferentially binds for PD-1, or a portion thereof.

[0150] Hybridomas that produce such antibodies may be grown *in vitro* or *in vivo* using known procedures. Monoclonal antibodies can be isolated from the culture media or body fluids, by conventional immunoglobulin purification procedures such as ammonium sulfate precipitation, gel electrophoresis, dialysis, chromatography, and ultrafiltration, if desired. Undesired activity, if present, can be removed, for example, by running the preparation over adsorbents made of the immunogen attached to a solid phase and eluting or releasing the desired antibodies off the immunogen. Immunization of a host animal with a PD-1 polypeptide, or a fragment containing the target amino acid sequence conjugated to a protein that is immunogenic in the species to be immunized, e.g., keyhole limpet hemocyanin, serum albumin, bovine thyroglobulin, or soybean trypsin inhibitor using a bifunctional or derivatizing agent, for example, maleimidobenzoyl sulfosuccinimide ester (conjugation through cysteine residues), N-hydroxysuccinimide (through lysine residues), glutaraldehyde, succinic anhydride, SOCl_2 , or $\text{R}'\text{N}=\text{C}=\text{NR}$, where R and R' are different alkyl groups, can yield a population of antibodies (e.g., monoclonal antibodies).

[0151] In an embodiment, P1801 (monoclonal or polyclonal) of interest may be sequenced and the polynucleotide sequence may then be cloned into a vector for expression or propagation. The sequence encoding the antibody of interest may be maintained in vector in a host cell and the host cell can then be expanded and frozen for future use. Production of recombinant monoclonal antibodies in cell culture can be carried out through cloning of antibody genes from B cells by means known in the art. See, for example: U.S. Pat. No. 7,314,622.

Humanization

[0152] In some embodiments, the polynucleotide sequence can be used for genetic manipulation to “humanize” the antibody or to improve the affinity, or other characteristics of the antibody. Antibodies can also be customized for use, for example, in dogs, cats, primates, equines and bovines.

[0153] In some embodiments, fully human antibodies can be obtained by using commercially available mice that have been engineered to express specific human immunoglobulin proteins. Transgenic animals that are designed to produce a more desirable (e.g., fully human antibodies) or more robust immune response may also be used for generation of humanized or human antibodies. Examples of such technology are Xenomouse (trademark) from Abgenix, Inc., HuMAb-Mouse (trademarked) and TC Mouse (trademark) from Medarex, Inc.

[0154] Antibodies can also be made recombinantly by first isolating the antibodies and antibody producing cells from host animals, obtaining the gene sequence, and using the gene sequence to express the antibody recombinantly in host cells (e.g., CHO cells). Another method which may be employed is to express the antibody sequence in plants (e.g., tobacco) or transgenic milk. Methods for expressing antibodies

recombinantly in plants or milk have been disclosed. Methods for making derivatives of antibodies, e.g., domain, single chain, etc. are also known in the art.

[0155] Immunoassays and flow cytometry sorting techniques such as fluorescence activated cell sorting (FACS) can also be employed to isolate antibodies that are specific for PD-1.

[0156] DNA encoding the monoclonal antibodies is readily isolated and sequenced using conventional procedures (e.g., by using oligonucleotide probes that are capable of binding specifically to genes encoding the heavy and light chains of the monoclonal antibodies). The hybridoma cells serve as a source of such DNA. Once isolated, the DNA can be placed into expression vectors (such as expression vectors disclosed in PCT Publication No. WO 87/04462), which are then transfected into host cells such as E. coli cells, simian COS cells, Chinese hamster ovary (CHO) cells, or myeloma cells that do not otherwise produce immunoglobulin protein, to obtain the synthesis of monoclonal antibodies in the recombinant host cells. See, e.g., PCT Publication No. WO 87/04462. The DNA also may be modified, for example, by substituting the coding sequence for human heavy and light chain constant domains in place of the homologous murine sequences, or by covalently joining to the immunoglobulin coding sequence all or part of the coding sequence for a non-immunoglobulin polypeptide. In that manner, “chimeric” or “hybrid” antibodies are prepared that have the binding specificity of a PD-1 monoclonal antibody herein.

[0157] Antibody fragments can be produced by proteolytic or other degradation of the antibodies, by recombinant methods as described above or by chemical synthesis. Polypeptides of the antibodies, especially shorter polypeptides up to about 50 amino acids, can be made by chemical synthesis. Methods of chemical synthesis are known in the art and are commercially available. For example, an antibody could be produced by an automated polypeptide synthesizer employing the solid phase method. See also, U.S. Pat. Nos. 5,807,715; 4,816,567; and 6,331,415, all of the contents are incorporated in reference in their entirety.

[0158] In some embodiments, an antibody or an antigen-binding portion thereof that preferentially binds PD-1 can have a heavy chain complementarity determining region one (VHCDR1), a heavy chain complementarity determining region two (VHCDR2), and/or a heavy chain complementarity determining region three (VHCDR3) obtained from (e.g., derived, determined, or copied from) the heavy chain variable region of P1801 antibody (e.g., the sequence as recited in SEQ ID NO: 5; and a light chain complementarity determining region one (VLCDR1), a light chain complementarity determining region two (VLCDR2), and/or a light chain complementarity determining region three (VLCDR3) obtained from the light chain variable region of P1801 antibody (e.g., the sequence as recited in SEQ ID NO: 6). Given a heavy chain variable region sequence or a light chain variable region sequence, a skilled practitioner would be able to determine all six CDR sequences based on the methods described herein or known in the art.

[0159] In an embodiment, the analysis of P1801 antibody or an antigen-binding portion thereof that preferentially binds PD-1 shows P1801 includes a heavy chain complementarity determining region one (VHCDR1) including the amino acid sequence as recited in SEQ ID NO: 9, a heavy chain complementarity determining region two (VHCDR2) including the amino acid sequence as recited in SEQ ID NO: 10, and/or a

heavy chain complementarity determining region three (VHCDR3) including the amino acid sequence as recited in SEQ ID NO: 11; and a light chain complementarity determining region one (VLCDR1) including the amino acid sequence as recited in SEQ ID NO: 12, a light chain complementarity determining region two (VLCDR2) including the amino acid sequence as recited in SEQ ID NO: 13, and/or a light chain complementarity determining region three (VLCDR3) including the amino acid sequence as recited in and SEQ ID NO: 14, based on Kabat method.

[0160] In another embodiment, the analysis of P1801 antibody or an antigen-binding portion thereof that preferentially binds PD-1 shows P1801 includes a heavy chain complementarity determining region one (VHCDR1) including the amino acid sequence as recited in SEQ ID NO: 18, a heavy chain complementarity determining region two (VHCDR2) including the amino acid sequence as recited in SEQ ID NO: 10, and/or a heavy chain complementarity determining region three (VHCDR3) including the amino acid sequence as recited in SEQ ID NO: 11; and a light chain complementarity determining region one (VLCDR1) including the amino acid sequence as recited in SEQ ID NO: 12, a light chain complementarity determining region two (VLCDR2) including the amino acid sequence as recited in SEQ ID NO: 13, and/or a light chain complementarity determining region three (VLCDR3) including the amino acid sequence as recited in and SEQ ID NO: 14, based on Kabat method.

[0161] Yet in an embodiment, the analysis of P1801 antibody or an antigen-binding portion thereof that preferentially binds PD-1 shows P1801 includes a heavy chain complementarity determining region one (VHCDR1) including the amino acid sequence as recited in SEQ ID NO: 9, a heavy chain complementarity determining region two (VHCDR2) including the amino acid sequence as recited in SEQ ID NO: 10, and/or a heavy chain complementarity determining region three (VHCDR3) including the amino acid sequence as recited in SEQ ID NO: 20; and a light chain complementarity determining region one (VLCDR1) including the amino acid sequence as recited in SEQ ID NO: 12, a light chain complementarity determining region two (VLCDR2) including the amino acid sequence as recited in SEQ ID NO: 13, and/or a light chain complementarity determining region three (VLCDR3) including the amino acid sequence as recited in and SEQ ID NO: 14, based on Chothia method.

[0162] Yet in an embodiment, the analysis of P1801 antibody or an antigen-binding portion thereof that preferentially binds PD-1 shows P1801 includes a heavy chain complementarity determining region one (VHCDR1) including the amino acid sequence as recited in SEQ ID NO: 18, a heavy chain complementarity determining region two (VHCDR2) including the amino acid sequence as recited in SEQ ID NO: 10, and/or a heavy chain complementarity determining region three (VHCDR3) including the amino acid sequence as recited in SEQ ID NO: 20; and a light chain complementarity determining region one (VLCDR1) including the amino acid sequence as recited in SEQ ID NO: 12, a light chain complementarity determining region two (VLCDR2) including the amino acid sequence as recited in SEQ ID NO: 13, and/or a light chain complementarity determining region three (VLCDR3) including the amino acid sequence as recited in and SEQ ID NO: 14, based on Chothia method.

[0163] In an embodiment, the analysis of P1801 antibody or an antigen-binding portion thereof that preferentially binds PD-1 shows P1801 includes a heavy chain complementarity determining region one (VHCDR1) including the amino acid sequence as recited in SEQ ID NO: 19, a heavy chain complementarity determining region two (VHCDR2) including the amino acid sequence as recited in SEQ ID NO: 10, and/or a heavy chain complementarity determining region three (VHCDR3) including the amino acid sequence as recited in SEQ ID NO: 20; and a light chain complementarity determining region one (VLCDR1) including the amino acid sequence as recited in SEQ ID NO: 12, a light chain complementarity determining region two (VLCDR2) including the amino acid sequence as recited in SEQ ID NO: 13, and/or a light chain complementarity determining region three (VLCDR3) including the amino acid sequence as recited in and SEQ ID NO: 14, based on Chothia method.

[0164] In another embodiment, the analysis of P1801 antibody or an antigen-binding portion thereof that preferentially binds PD-1 shows P1801 includes a heavy chain complementarity determining region one (VHCDR1) including the amino acid sequence as recited in SEQ ID NO: 21, a heavy chain complementarity determining region two (VHCDR2) including the amino acid sequence as recited in SEQ ID NO: 10, and/or a heavy chain complementarity determining region three (VHCDR3) including the amino acid sequence as recited in SEQ ID NO: 20; and a light chain complementarity determining region one (VLCDR1) including the amino acid sequence as recited in SEQ ID NO: 12, a light chain complementarity determining region two (VLCDR2) including the amino acid sequence as recited in SEQ ID NO: 13, and/or a light chain complementarity determining region three (VLCDR3) including the amino acid sequence as recited in and SEQ ID NO: 14, based on Chothia method.

[0165] In an embodiment, the analysis of P1801 antibody or an antigen-binding portion thereof that preferentially binds PD-1 shows P1801 includes a heavy chain complementarity determining region one (VHCDR1) including the amino acid sequence as recited in SEQ ID NO: 19, a heavy chain complementarity determining region two (VHCDR2) including the amino acid sequence as recited in SEQ ID NO: 10, and/or a heavy chain complementarity determining region three (VHCDR3) including the amino acid sequence as recited in SEQ ID NO: 11; and a light chain complementarity determining region one (VLCDR1) including the amino acid sequence as recited in SEQ ID NO: 12, a light chain complementarity determining region two (VLCDR2) including the amino acid sequence as recited in SEQ ID NO: 13, and/or a light chain complementarity determining region three (VLCDR3) including the amino acid sequence as recited in and SEQ ID NO: 14, based on Chothia method.

[0166] In another embodiment, the analysis of P1801 antibody or an antigen-binding portion thereof that preferentially binds PD-1 shows P1801 includes a heavy chain complementarity determining region one (VHCDR1) including the amino acid sequence as recited in SEQ ID NO: 21, a heavy chain complementarity determining region two (VHCDR2) including the amino acid sequence as recited in SEQ ID NO: 10, and/or a heavy chain complementarity determining region three (VHCDR3) including the amino acid sequence as recited in SEQ ID NO: 11; and a light chain complementarity determining region one (VLCDR1) including

the amino acid sequence as recited in SEQ ID NO: 12, a light chain complementarity determining region two (VLCDR2) including the amino acid sequence as recited in SEQ ID NO: 13, and/or a light chain complementarity determining region three (VLCDR3) including the amino acid sequence as recited in and SEQ ID NO: 14, based on Chothia method.

iv. Properties

[0167] At least two known FDA approved anti-PD1 human monoclonal antibodies have been approved, namely pembrolizumab (trade name Keytruda), and nivolumab (trade named OPDIVO). Both of which can treat various diseases, such as certain types of cancer. In addition, both pembrolizumab and nivolumab's epitopes on hPD1 are known in the art.

[0168] In an embodiment, as denote in Figure 8, for instance, the present disclosure's P1801 binds to the same epitope of PD1 as commercially available anti-PD1 monoclonal antibody such as pembrolizumab and/or nivolumab with complete overlap. In another embodiment, the P1801 binds to substantially the same or have substantially overlap region of epitope of PD1 as commercially available anti-PD1 monoclonal antibody such as pembrolizumab and/or nivolumab. Yet in another embodiment, the P1801 partially binds to the same region of epitope PD1 as commercially available anti-PD1 monoclonal antibody such as pembrolizumab and/or nivolumab. Surprisingly and unexpectedly, even though P1801 partially overlaps the PD1 epitope as compared to pembrolizumab and/or nivolumab, which would suggest weaker characteristics, the kinetic performance of P1801 is substantially the same, if not better than pembrolizumab and/or nivolumab.

[0169] Yet in an embodiment, P1801 only partially covers PD1 but unexpected performs at least equivalent to existing commercial anti-PD1 monoclonal antibody.

[0170] In one embodiment, the present disclosure discloses an anti-human PD1 monoclonal antibody (P1801) with properties substantially similar to commercially available anti-PD1 monoclonal antibody (e.g., pembrolizumab and/or nivolumab) in various aspects such as molecular properties and/or cancer treatment efficacy. Exemplary characteristics and properties include, but not limited to binding strength against PD1 as measured in KD, stability/shelf-life, and/or ability to treat cancer. Figure 3 denotes one example in mouse model.

[0171] In another embodiment, the present disclosure discloses an anti-human PD1 monoclonal antibody (P1801) with properties substantially better than commercially available anti-PD1 monoclonal antibody in various aspects such as molecular properties and/or cancer treatment efficacy. Exemplary characteristics and properties include, but not limited to binding strength against PD1 as measured in KD, stability/shelf-life, and/or ability to treat various types of cancer.

[0172] Yet in another embodiment, the present disclosure discloses a combination of P1101 with an anti-human PD1 monoclonal antibody (P1801) with properties substantially better and/or having synergetic effects as compared to commercially available anti-PD1 monoclonal antibody treatment alone in various aspects such as molecular properties and/or cancer treatment efficacy. Exemplary characteristics and properties include, but

not limited to binding strength against PD1 as measured in KD, stability/shelf-life, ability to treat cancer and/or shrink tumor size.

[0173] Alterations to the variable region notwithstanding, those skilled in the art can modify the present disclosure's anti-PD1 antibodies or antigen-binding fragments thereof will comprise antibodies (e.g., full-length antibodies or immunoreactive fragments thereof) in which at least a fraction of one or more of the constant region domains has been deleted or otherwise altered so as to provide desired biochemical characteristics such as increased tumor localization or reduced serum half-life when compared with an antibody of approximately the same immunogenicity comprising a native or unaltered constant region. In some aspects, the constant region of the modified antibodies will comprise a human constant region. Modifications to the constant region compatible with this the anti-PD1 molecules disclosed herein comprise additions, deletions or substitutions of one or more amino acids in one or more domains. That is, the modified antibodies disclosed herein can comprise alterations or modifications to one or more of the three heavy chain constant domains (CH1, CH2 or CH3) and/or to the light chain constant domain (CL). In some aspects, modified constant regions wherein one or more domains are partially or entirely deleted are contemplated. In some aspects, the modified antibodies will comprise domain deleted constructs or variants wherein the entire CH2 domain has been removed (Δ CH2 constructs). In some aspects, the omitted constant region domain can be replaced by a short amino acid spacer (e.g., 1, 2, 3, 4, 5, 6, 7, 8, 9, or 10 residues) that provides some of the molecular flexibility typically imparted by the absent constant region.

[0174] The anti-PD1 antibody or antigen-binding fragment thereof can be further modified to contain additional chemical moieties not normally part of the protein. Those derivatized moieties can improve the solubility, the biological half-life or absorption of the protein. The moieties can also reduce or eliminate any desirable side effects of the proteins and the like. The moieties may include one or more detectable labels or tags (e.g., biotin, reporter enzymes, colorimetric or fluorescent tags, solid support or radioactive labels) to facilitate detection of the antibody or PD-1 in a sample.

[0175] In an embodiment, the paratope(s) of the present disclosure's anti-PD1 antibodies or antigen-binding fragments thereof may involve amino acid residues in all of the VH and VL CDRs or only some of the CDRs (e.g., certain CDRs may not be involved in binding antigen). The paratope for a particular antigen can be defined, for example, by scanning mutagenesis (e.g., alanine scanning mutagenesis) of amino acid residues within the antibody, particularly the CDRs, that are thought to be surface exposed (e.g., as determined by crystallographic modeling) which may be involved in antigen binding. Evaluation of the binding of the mutants to the antigen then can determine whether the mutated amino acid position is involved in antigen binding and thus forms part of the paratope of the antibody.

[0176] In another embodiment, paratopes can be determined by high resolution cryo-electron microscopy which can generate molecule-to molecule density map to determine the binding distance between certain amino acid between one or more CDRs and the antigen.

[0177] In an embodiment, the present disclosure's anti-PD1 antibodies or antigen-binding fragments thereof

comprises a heavy chain paratope comprising CDR1, CDR2 and CDR3 sequences as shown in SEQ ID NOs: 9, 18, 19, or 21 (CDR1), 10 (CDR2) and 11 (CDR3). In another preferred embodiment, the present disclosure's anti-PD1 antibodies or antigen-binding fragments thereof comprises a light chain paratope comprising CDR1, CDR2 and CDR3 sequences as shown in SEQ ID NO: 12 (CDR1), 13 (CDR2) and 14 (CDR3). In an embodiment, the present disclosure's anti-PD1 antibodies or antigen-binding fragments thereof comprises a heavy chain paratope comprising CDR1, CDR2 and CDR3 sequences as shown in SEQ ID NOs: SEQ ID NOs: 9, 10 and 11, and a light chain paratope comprising CDR1, CDR2 and CDR3 sequences as shown in SEQ ID NOs: 12, 13 and 14. In another embodiment, the present disclosure's anti-PD1 antibodies or antigen-binding fragments thereof comprises a heavy chain paratope comprising CDR1, CDR2 and CDR3 sequences as shown in SEQ ID NOs: SEQ ID NOs: 18, 10 and 11, and a light chain paratope comprising CDR1, CDR2 and CDR3 sequences as shown in SEQ ID NOs: 12, 13 and 14. In another embodiment, the present disclosure's anti-PD1 antibodies or antigen-binding fragments thereof comprises a heavy chain paratope comprising CDR1, CDR2 and CDR3 sequences as shown in SEQ ID NOs: SEQ ID NOs: 19, 10 and 11, and a light chain paratope comprising CDR1, CDR2 and CDR3 sequences as shown in SEQ ID NOs: 12, 13 and 14. Yet in another embodiment, the present disclosure's anti-PD1 antibodies or antigen-binding fragments thereof comprises a heavy chain paratope comprising CDR1, CDR2 and CDR3 sequences as shown in SEQ ID NOs: SEQ ID NOs: 21, 10 and 11, and a light chain paratope comprising CDR1, CDR2 and CDR3 sequences as shown in SEQ ID NOs: 12, 13 and 14.

[0178] Substitutions of Non-Paratope Amino Acid Residues

[0179] Non-paratope amino acids can be substituted recombinantly to make a variable light or heavy domain with similar or altered properties compared to the germline variable domain. "Modified" variable domains also include amino acid deletions, as well as substitutions. For example, the N-terminal or C-terminal amino acid residue may be deleted in a modified variable domain.

[0180] Non-paratope amino acid substitutions can be made, for example, to increase the stability and/or decrease the tendency to aggregate. Poor stability can affect the ability of an antigen-binding fragment, for example, to fold properly when recombinantly expressed, resulting in a fraction of the expressed fragments being non-functional. Low stability antibodies or antigen-binding fragments thereof also may be prone to forming potentially immunogenic aggregates or may have impaired avidity or shelf-life. scFv polypeptides in particular may demonstrate problems with stability, solubility, expression, aggregation, breakdown products, and overall manufacturability in both bacterial and mammalian expression systems. Framework amino acid substitutions that are expected to increase the stability and/or decrease the tendency to aggregate of a VH and/or VL domain, e.g., in an scFv polypeptide, are disclosed in WO 2007/109254, for example. Substitutions in corresponding residues in the present VH and VL domains are expected similarly to increase stability and/or decrease the tendency to aggregate.

v. Pharmaceutically Acceptable Carriers

[0181] The composition used in the present disclosure, whether P1801 alone, P1101 alone, or any

combinations thereof can further comprise pharmaceutically acceptable carriers, excipients, or stabilizers, in the form of lyophilized formulations or aqueous solutions. Acceptable carriers, excipients, or stabilizers are nontoxic to recipients at the dosages and concentrations, and may comprise buffers such as phosphate, citrate, and other organic acids; antioxidants including ascorbic acid and methionine; preservatives (such as octadecyldimethylbenzyl ammonium chloride; hexamethonium chloride; benzalkonium chloride, benzethonium chloride; phenol, butyl or benzyl alcohol; alkyl parabens such as methyl or propyl paraben; catechol; resorcinol; cyclohexanol; 3-pentanol; and m-cresol); low molecular weight (less than about 10 residues) polypeptides; proteins, such as serum albumin, gelatin, or immunoglobulins; hydrophilic polymers such as polyvinylpyrrolidone; amino acids such as glycine, glutamine, asparagine, histidine, arginine, or lysine; monosaccharides, disaccharides, and other carbohydrates including glucose, mannose, or dextrans; chelating agents such as EDTA; sugars such as sucrose, mannitol, trehalose or sorbitol; salt-forming counter-ions such as sodium; metal complexes (e.g. Zn-protein complexes); and/or non-ionic surfactants such as TWEEN (trademarked), PLURONICS (trademarked) or polyethylene glycol (PEG).

vi. Dosage

[0182] In various embodiments, the anti-PD1 antibody is administered at a concentration of about 1 mg/kg, about 2 mg/kg, about 3 mg/kg, about 4 mg/kg, about 5 mg/kg, about 6 mg/kg, about 7 mg/kg, about 8 mg/kg, about 9 mg/kg, about 10 mg/kg, about 11 mg/kg, about 12 mg/kg, about 13 mg/kg, about 14 mg/kg, about 15 mg/kg, about 16 mg/kg, about 17 mg/kg, about 18 mg/kg, about 19 mg/kg, or about 20 mg/kg.

vii. Combinations

[0183] An anti-PD-1 antibody can also be used in conjunction with, or administered separately, simultaneously, and/or sequentially with other agents that serve to enhance and/or complement the effectiveness of the antibody to treat cancer. One such non-limiting example of an anti-PD1 monoclonal antibody is P1801, where the combination is P1801 + P1101. Either an anti-PD1 monoclonal antibody, such as P1801, or P1101 can be referred to as first or second molecule in a combination. Additionally, or alternatively, the P1801 can be further optionally combined with another anti-PD1 monoclonal antibody, for example, Pembrolizumab, Nivolumab, and/or Cemiplimab.

[0184] In an embodiment, murine anti-PD-1 antibody (e.g., RMP 1-14) can be used in place of P1801 in mouse models. Alternatively, or additionally, mP1101, which has mouse version of interferon, can be used for murine models.

viii. Serial and Combination Administration

[0185] P1101 can be administered using various regimens and dosages as previously reported in the art.

Serial administration

[0186] In addition, the length between serial administration of one or more doses of P1101 or mP1101 to a subject can be 1 day, 2 days, 3 days, 4 days, 5 days, 6 days, 7 days, 8 days, 9 days, 10 days, 11 days, 12 days, 13 days, 14 days, 15 days, 16 days, 17 days, 18 days, 19 days, 20 days, 21 days, 22 days, 23 days, 24 days, 25 days, 26 days, 27 days, 28 days, 29 days, 30 days, 31 days, 5 weeks, 6 weeks, 7 weeks, 8 weeks, 9 weeks,

and/or 10 weeks. In another embodiment, the aforementioned P1101 or mP1101 serial administration can be followed by P1801 serial administration with length between administration of one or more doses of P1801 to a subject be 1 day, 2 days, 3 days, 4 days, 5 days, 6 days, 7 days, 8 days, 9 days, 10 days, 11 days, 12 days, 13 days, 14 days, 15 days, 16 days, 17 days, 18 days, 19 days, 20 days, 21 days, 22 days, 23 days, 24 days, 25 days, 26 days, 27 days, 28 days, 29 days, 30 days, 31 days, 5 weeks, 6 weeks, 7 weeks, 8 weeks, 9 weeks, and/or 10 weeks.

[0187] The above serial administration in any order (P1101 first or P1801 first) can provide “synergy” and prove “synergistic”, i.e., the effect achieved when the active ingredients used together is greater than the sum of the effects that results from using the compounds separately.

Combination administration

[0188] A combination administration can also provide “synergy” and prove “synergistic”, i.e., the effect achieved when the active ingredients used together is greater than the sum of the effects that results from using the compounds separately. The present disclosure also includes a synergistic effect that can be attained when the active ingredients are: (1) co-formulated and administered or delivered simultaneously in a combined, unit dosage formulation; (2) delivered by alternation or in parallel as separate formulations; and/or (3) by some other regimen. When delivered in alternation therapy, a synergistic effect can be attained when the compounds are administered or delivered sequentially, e.g., by different injections in separate syringes. In general, during alternation therapy, an effective dosage of each active ingredient is administered sequentially, i.e., serially, whereas in combination therapy, effective dosages of two or more active ingredients are administered together.

[0189] In an embodiment, a first molecule and a second molecule can be administered to a subject at the same time by mixing the two and administered to a subject simultaneously. Alternatively, or additionally, the two large molecules can be administered to a subject in any given sequence immediately or in close timing one right after another in any given order. In another embodiment, one large molecule can be administered first in a given regimen, then the second large molecule can be administered in a given regimen. Yet in another embodiment, the regimen frequency and length can differ in any length, for example, 1 day, 2 days, 3 days, 4 days, 5 days, 6 days, 7 days, 8 days, 9 days, 10 days, 11 days, 12 days, 13 days, 14 days, 15 days, 16 days, 17 days, 18 days, 19 days, 20 days, 21 days, 22 days, 23 days, 24 days, 25 days, 26 days, 27 days, 28 days, 29 days, 30 days, and/or 31 days or more. The first molecule can be P1101, and the second molecule can be P1801, and vice versa.

[0190] In some embodiments, a first molecule can be administered following a first regimen over a first treatment period and a second molecule can be administered following a second, independent regimen over a second, independent treatment period, wherein the two regimens and/or treatment periods can be in sync, staggered, sequential, alternate, or timed in any manner. For example, in a situation where both molecules are administered once every 2 weeks, the two regimens may be initiated on the same day or different days (e.g., the second regimen is initiated before or after the first regimen starts). In yet another example, in a case where the first molecule is administered once every 5 days for 3 months and the second molecule is administered

once every two weeks for one year, the two regimens may be initiated on the same day or different days (e.g., the second regimen is initiated before or after the first regimen starts, during the treatment period of the first molecule on a day the first molecule is administered or on a day the first molecule is not administered, or after the end of the treatment period of the first molecule). The dose of each administration and/or time between each administration in a regimen can remain constant or be varied. The first molecule can be P1101, and the second molecule can be an anti-PD1 monoclonal antibody, such as P1801. Alternatively, the first molecule can be an anti-PD1 monoclonal antibody, such as P1801, and the second molecule can be P1101.

[0191] Yet in another embodiment, a first molecule and a second molecule can be administered in an alternate fashion in any given order. In an embodiment, a first molecule and a molecule can be administered in any fixed period order. Figure 4(a) illustrates one such example, where the solid arrows denote administration of a first molecule to a subject, and the dotted arrow denotes administration of a second molecule, wherein each administration is 3 days apart. In addition, vice versa can be done where solid arrow denotes administration of second molecule to a subject, and the dotted arrows denote administration of a first molecule, wherein each administration is 3 days apart. In another embodiment, an illustrative example is where the solid arrows denote administration of P1101 to a subject, and the dotted arrows denote administration of anti-PD1 monoclonal antibody, such as P1801. In addition, vice versa can be done where solid arrows denote administration of anti-PD1 monoclonal antibody, such as P1801 to a subject, and the dotted arrows denote administration of P1101.

[0192] In a non-limiting embodiment, two molecules can be administered sequentially, yet one molecule can be initially administered with a fewer number of doses (e.g., 3 doses), with a set period between each dose (e.g., 3 days,) following by administration of a second molecule with double, triple, or quadruple number of administration doses, with also with a set period between each dose (e.g., 3 days). A non-limiting example can be seen in the top portion of Figure 4(b). Another illustrative example is where the solid arrow denotes administration of P1101 to a subject, and the dotted arrows denote administration of anti-PD1 monoclonal antibody, such as P1801. The first molecule can be P1101, and the second molecule can be P1801, and vice versa.

[0193] In a non-limiting embodiment, two molecules can be administered sequentially, yet one molecule can be initially administered with the same number of doses (e.g., 6 doses), with a set period between each dose (e.g., 3 days), following by administration of a second molecule administered with the same number of doses (e.g., 6 doses), also with a set period between each dose (e.g., 3 days). Figure 4(b) bottom denotes such non-limiting example. Briefly, the solid arrows denote administration of P1101 to a subject, and the dotted arrows denote administration of anti-PD1 monoclonal antibody, such as P1801. Alternatively, the solid arrows denote administration of anti-PD1 monoclonal antibody, such as P1801 to a subject, and the dotted arrows denote administration of P1101.

[0194] Yet in another embodiment, a first molecule and a second molecule can be administered in an alternate fashion for one dose each, but with 1 or 2 days in-between the two administrations. This regiment can be repeated for a fixed period (e.g., every 3, 4, 5, or 6 days), for two, three, four, or five times. Alternatively, or

additionally, this can be followed by a single administration of either molecule at a single dosage with a fixed period (e.g., 3, 4, 5, or 6 days). Figure 4(c) top illustrates such non-limiting example. In an embodiment, the solid arrows denote administration of P1101 to a subject, and the dotted arrows denote administration of anti-PD1 monoclonal antibody, such as P1801. Alternatively, the solid arrows denote administration of anti-PD1 monoclonal antibody, such as P1801 to a subject, and the dotted arrows denote administration of P1101.

[0195] In an embodiment, a first molecule and a second molecule can be administered in an alternate fashion for one dose each, but with 1 or 2 days in-between the two administrations. This regiment can be repeated for a fixed period (e.g., every 3, 4, 5, or 6 days), for two, three, four, or five times. Figure 4(c) Bottom illustrates such non-limiting example. In an embodiment, the solid arrows denote administration of P1101 to a subject, and the dotted arrows denote administration of anti-PD1 monoclonal antibody, such as P1801. Alternatively, the solid arrows denotes administration of anti-PD1 monoclonal antibody, such as P1801 to a subject, and the dotted arrows denote administration of P1101.

[0196] In a non-limiting embodiment, two molecules are administered at the same time or on the same day, yet one molecule can be initially administered with a fewer amount of doses, follow by double, triple, or quadruple amount of doses in second, third, fourth, or 5th administrations, while a second molecule is administered with a constant amount of doses. In addition, the vice versa can be done, where a first molecule is administered with a constant amount of doses, while the second molecule can be initially administered with a fewer amount of doses, follow by double, triple, or quadruple amount of doses in second, third, fourth, or 5th administrations. In an embodiment, the first molecule administration can be P1101, and the second molecule can be anti-PD1 monoclonal antibody, such as P1801. Alternatively, the first molecule can be any anti-PD1 monoclonal antibody, such as P1801, and the second molecule can be P1101.

[0197] In another embodiment, a first molecule can be administered in a fixed dosage every day for the first 13 days, and the second molecule can start to be administered on day 6, with 5 more administrations, all being 3 days apart. In addition, the first 4 administrations of the second molecules can have the same dosage, followed by the 5th administrations having double the dosage than the first 4. Future the 6th administration can have dosage that is quadrupled than the first 4 administrations. A non-limiting example can be shown in Figure 5. In an embodiment, the first molecule administration can be P1101, and the second molecule can be anti-PD1 monoclonal antibody, such as P1801. Alternatively, the first molecule can be anti-PD1 monoclonal antibody, such as P1801, and the second molecule can be P1101.

[0198] Yet in another embodiment, a first molecule can be administered either via i.p. or s.c. at 6, 7, or 8 days apart (QW x 4 from days 1-22), while the second molecules can be administered either via i.p. or s.c. at day 11, 13, 18, 20, 25 and 27 (BIW x2, day 11 to day 27). An example is denoted in Figure 6. In an embodiment, the first molecule administration can be P1101, and the second molecule can be anti-PD1 monoclonal antibody, such as P1801. Alternatively, the first molecule can be anti-PD1 monoclonal antibody, such as P1801, and the second molecule can be P1101.

[0199] In an embodiment, the length between administration of a single dose of P1801 or a single dose of

P1101 to a subject can be 1 day, 2 days, 3 days, 4 days, 5 days, 6 days, 7 days, 8 days, 9 days, 10 days, 11 days, 12 days, 13 days, 14 days, 15 days, 16 days, 17 days, 18 days, 19 days, 20 days, 21 days, 22 days, 23 days, 24 days, 25 days, 26 days, 27 days, 28 days, 29 days, 30 days, 31 days, 5 weeks, 6 weeks, 7 weeks, 8 weeks, 9 weeks, and/or 10 weeks or longer.

[0200] In another embodiment, the length between administration of a single regiment of P1801 and a single regiment of P1101 to a subject can be 1 day, 2 days, 3 days, 4 days, 5 days, 6 days, 7 days, 8 days, 9 days, 10 days, 11 days, 12 days, 13 days, 14 days, 15 days, 16 days, 17 days, 18 days, 19 days, 20 days, 21 days, 22 days, 23 days, 24 days, 25 days, 26 days, 27 days, 28 days, 29 days, 30 days, 31 days, 5 weeks, 6 weeks, 7 weeks, 8 weeks, 9 weeks, and/or 10 weeks or longer.

ix. Uses

[0201] In one embodiment, the use of anti-PD1 monoclonal antibody in the present disclosure enhances activation of antigen specific CD4⁺ or CD8⁺ T cells, thereby enhance a subject's immunity for treating, suppressing and/or preventing cancer, such as a solid tumor. Non-limiting examples of solid tumors include melanoma, Hepatocellular carcinoma (HCC), and/or kidney tumor (renal cell carcinoma or RCC).

[0202] In another embodiment, the combination of P1801 and P1101 can further enhance a subject's immunity for treating, suppressing and/or preventing cancer, such as a solid tumor, as compared to P1801 alone. Non-limiting examples include melanoma, Hepatocellular carcinoma (HCC), Colorectal Cancer (CRC), Melanoma, and/or kidney tumor (Renal Cell Carcinoma (RCC)).

[0203] In an embodiment, the presently disclosed P1801 and/or the combination of P1801 with P1101 can be used for treating, suppressing and/or preventing the following:

Advanced Melanoma or Adjuvant Therapy for Melanoma

[0204] P1801 and/or the combination of P1801 with another molecule (e.g., P1101) can be used for the treatment of subjects with unresectable or metastatic melanoma. The treatment can further optionally combine with pembrolizumab and/or nivolumab.

Advanced Hepatocellular Carcinoma (HCC)

[0205] P1801 and/or the combination of P1801 with another molecule (e.g., P1101) can be used for the treatment of subjects with hepatocellular carcinoma (HCC). The subject can be previously treated with sorafenib. The treatment can optionally further combine Cemiplimab, pembrolizumab and/or nivolumab.

Treatment for RCC or Advanced Renal Cell Carcinoma (RCC)

[0206] P1801 and/or the combination of P1801 with another molecule (e.g., P1101) can be used for the adjuvant treatment of subjects with renal cell carcinoma (RCC) at intermediate-high or high risk of recurrence following nephrectomy or following nephrectomy and resection of metastatic lesions. The treatment can optionally further combine Cemiplimab, pembrolizumab and/or nivolumab.

[0207] P1801 and/or the combination of P1801 with another molecule (e.g., P1101) can be used for the first-line treatment of adult subjects with advanced renal cell carcinoma (RCC). This treatment can further optionally combine with axitinib, and/or Cemiplimab, pembrolizumab and/or nivolumab.

For adults subjects with advanced melanoma

[0208] P1801, or combined with another molecule (e.g., P1101) can be used to treat people with a type of skin cancer called melanoma that has spread or cannot be removed by surgery (advanced melanoma). This therapy can further be optionally combined with Cemiplimab, nivolumab, pembrolizumab, and/or ipilimumab.

For adults subjects with melanoma after it and the affected lymph nodes have been removed by surgery to prevent it from coming back

[0209] P1801, or combined with another molecule (e.g., P1101) can be used to treat adult subjects with melanoma after it and the affected lymph nodes have been removed by surgery to prevent it from coming back. This therapy can further be optionally combined with Cemiplimab, pembrolizumab and/or nivolumab.

For certain subjects with advanced kidney cancer (renal cell carcinoma)

[0210] P1801, or combined with another molecule (e.g., P1101) can be used to treat certain subjects with kidney cancer when their cancer has spread (advanced renal cell carcinoma) and the subject has not already had treatment for the advanced RCC. This therapy can further be optionally combined with Cemiplimab, nivolumab, pembrolizumab, and/or ipilimumab.

For newly diagnosed subjects whose kidney cancer (renal cell carcinoma) has spread

[0211] P1801, or combined with another molecule (e.g., P1101) can be used to treat people with kidney cancer when the cancer has spread (advanced renal cell carcinoma) and the subject has not already had treatment for the advanced RCC. This therapy can further be optionally combined with Cemiplimab, nivolumab, pembrolizumab, and/or cabozantinib.

For subjects with previously treated advanced kidney cancer (renal cell carcinoma)

[0212] P1801, or combined with another molecule (e.g., P1101) can be used to treat people with kidney cancer (renal cell carcinoma) when the cancer has spread or grown after treatment with other cancer medications. This therapy can further be optionally combined with Cemiplimab, pembrolizumab and/or nivolumab.

For subjects with liver cancer (hepatocellular carcinoma) that have received treatment with sorafenib

[0213] P1801, or combined with another molecule (e.g., P1101) can be used to treat people with liver cancer (hepatocellular carcinoma) if the subject has previously received treatment with sorafenib. This therapy can further be optionally combined with Cemiplimab, nivolumab, pembrolizumab, and/or ipilimumab.

[0214] In addition, P1801, or combined with another molecule (e.g., P1101) can be used to treat adults and children 12 years of age and older with a type of colon or rectal cancer (colorectal cancer) that has spread to other parts of the body (metastatic), is microsatellite instability-high (MSI-H) or mismatch repair deficient (dMMR), and the subject has tried treatment with a fluoropyrimidine, oxaliplatin, and irinotecan, and it did not work or is no longer working. This therapy can further be optionally combined with Cemiplimab, pembrolizumab and/or nivolumab.

x. Administration Route, and Dosage

[0215] P1801, or combined with another molecule (e.g., P1101, nivolumab, and/or ipilimumab), can be formulated as injections for intravenous (IV) use. In one embodiment, nivolumab (about 10 mg/mL) and

ipilimumab (about 5 mg/mL) are injections for intravenous (IV) use. In an embodiment, the dosage of P1101 can be between about 50 to about 540 ug. Other non-limiting examples include sequential 6 doses of P1101 at 450 mcg, Q2W with P1801 at 0.3 mg/kg, Q2W; sequential 6 doses of P1101 at 450 mcg, Q2W with P1801 at 0.75 mg/kg, Q2W; sequential 6 doses of P1101 at 450 mcg, Q2W with P1801 at 1.5 mg/kg, Q2W; sequential 6 doses of P1101 at 450 mcg, Q2W with P1801 at 2.0 mg/kg, Q2W; or sequential 6 doses of P1101 at 450 mcg, Q2W with P1801 at 3.0 mg/kg, Q2W. The treatment period can be during a 28-day, 35-day, 42-day, 49-day, or 56-day treatment cycle.

EXAMPLES

Example 1

Binding affinity and inhibition of P1801

[0216] This example was performed to measure the binding affinity and inhibition ability of P1801 antibody to antigens. The direct binding ELISA was performed to measure the binding specificity and cross reactivity. The competitive ELISA and cell-based reporter gene assay were performed to measure the inhibition ability between P1801 PD1/PD-L1 and PD1/PD-L2.

[0217] All incubation should be performed at 25°C, except coating plate with antigen is performed at 4°C. The plate is kept away from drafts and other temperature fluctuations and covered with plate sealers. During all washing steps, add 300 µL/well of wash buffer then remove solutions from the plate by inverting and blotting the plate on paper towel. Repeat 3 times for each washing step.

[0218] Antigens were diluted to appropriate concentrations with 1X DPBS, 50 µL/well overnight, and blocked with 1 % BSA, 200 µL/well for 2 hr. Sample preparation and incubation: Dilute P1801 sample using 1 %BSA by 2.5-fold serial dilutions starting at 0.8 µg/mL for 8 points, then add 50 µL of the freshly prepared-serial diluted sample solutions to wells in duplicate. Seal the plate and incubate at 25°C without shaking for 1 hr. Secondary Ab incubation were performed by diluting Peroxidase-conjugated Goat Anti-Human IgG, Fe Fragment Specific Ab to 1: 10000 in 1 %BSA, then add 50 µL of the freshly prepared diluted Secondary Ab solution to wells of the plate. Seal the plate and incubate at 25°C without shaking for 40 min. Color development was done by adding 50 µL/well of TMB substrate solution, gently tap the plate to ensure thorough mixing, and allow color to develop for 2-5 min. After incubation. Stop the reaction directly with 50 uL/well of stop solution. Read the OD within 10 min and protect it from light. Ensure there are no bubbles in all wells.

Inhibition measurement of PD-1/PD-L1 PD-1/PD-L2 by competitive binding ELISA

[0219] Coating and Blocking were performed where antigens were diluted to appropriate concentrations with 1X DPBS, 50 µL/well overnight, and blocked with 1 % BSA, 200 µL/well for 2 hr. Sample preparation and incubation for competitor P1801: perform 2-fold serial dilution starting at 5 µg/mL for 10 points using 1 %BSA as dilution buffer. Then add 50 µL of sample dilutions to each appropriate well. For binder biotin-hPD-1-mlgG Fe, dilute to working solution for concentration of 0.25 µg/mL, then add 50 µL to each well. Incubate the plate with constant shaking (550 rpm) at 25°C for 2hr. Secondary Ab incubation were done by diluting anti-streptavidin HRP Ab to 1: 15000 in 1 %BSA, then add 50 µL of the freshly prepared diluted secondary Ab solution to wells of the plate. Seal the plate and incubate at 25°C with constant shaking (550 rpm) for 40 min.

Color development was done by adding 50 µL/well of TMB substrate solution, gently tap the plate to ensure thorough mixing, and allow color to develop for 30 min in dark. After incubation, stop the reaction directly with 50 µL/well of stop solution. Read the OD within 10 min and protect it from light. Ensure there are no bubbles in all wells.

[0220] The analytical method for a cell-based reporter gene assay for the inhibition measurement of PD-1/PD-L1 of P1801 anti-PD1 antibody was performed. Briefly, the PD-1 stably expressed Jurkat cell (Jurkat/PD1) expresses a luciferase reporter driven by NFAT response element (NFAT-RE) via TCR activation. However, while PD-1 on the Jurkat cell binds PD-L1, the interaction blocks the TCR-mediated luminescence. The luciferase is induced when the Jurkat cell is activated by TCR agonist and the PD-1/PD-L1 interaction is disrupted by anti-PD 1 (Figure 9). More the anti-PD1 blocks more interaction and induces more luciferase. The IC₅₀ can be determined by the dose-response curve of test sample.

In Vitro Binding Specificity of P1801 is shown in Table 2 below.

Table 2

Test System	Method of Administration	Dose (ng/mL)
Human, Protein Antigens	<i>In Vitro</i>	800, 320, 128, 51.2, 20.5, 8.2, 3.3, 1.3

Assay System: Direct Binding ELISA

Immobilized antigen	Antigen Concentration (ng/well)	Binding EC ₅₀ (nM)
Human PD-1-mIgG Fc	10	0.24
Human PD-L1	50	No binding at 1.3~800 ng/mL P1801
Human PD-L2	50	No binding at 1.3~800 ng/mL P1801
Human CTLA-4	50	No binding at 1.3~800 ng/mL P1801
Human CD28	50	No binding at 1.3~800 ng/mL P1801

Cross Reactivity of P1801 is reported below in Table 3 below:

Table 3

Test System	Method of Administration	Dose (ng/mL)		
Human, Monkey/Cynomolgus,	<i>In Vitro</i>	800, 320, 128, 51.2, 20.5, 8.2, 3.3, 1.3		

and Mouse Protein Antigens			
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Assay System: Direct Binding ELISA

Immobilized antigen	Antigen Concentration (ng/well)	Binding EC ₅₀ (nM)
Human PD-1-mIgG Fc	10	0.57
Cynomolgus PD-1	10	0.44
Mouse PD-1	50	No binding detected

Table 4 shows the inhibitory Activity of P1801.

Table 4

Test System	Method of Administration	Dose (ng/mL)
Human Protein Antigens	<i>In Vitro</i>	5000, 2500, 1250, 625, 312.5, 156.3, 78.1, 39.1, 19.5, 9.8

Assay System: Competitive Binding ELISA

Immobilized Antigen	Immobilized Antigen Concentration (ng/well)	Binder Antigen	Binder Antigen Concentration (ng/well)	Binding Inhibition IC ₅₀ (nM)
Human PD-L1	500	Human PD-1-mIgG Fc	12.5	0.58
Human PD-L2	50	Human PD-1-mIgG Fc	12.5	0.90

Assay System: Cell-based Reporter Gene Assay

Reporter Cell	Antigen Presenting Cell	Binding Inhibition IC ₅₀ (nM)
NFAT-Luc/PD-1 Jurkat	PD-L1/OKT3-CD14/CD80 CHO-K1	14.6

[0221] Briefly in summary, P1801 has a human PD 1 affinity (EC₅₀) of 0.57 nM and a cynomolgus PD-1 affinity (EC₅₀) of 0.44 nM. No significant difference was noted between the P1801 binding affinity of human and cynomolgus PD1. No cross reactivity was noted between P1801 and mouse-PD-1. The competition effect of human PD-L1/PD-L2 between P1801 and human PD-1 were observed both in competitive ELISA and cell-based assay. P1801 bound human PD-1 and cross-reacts with monkey PD-1, and binding efficacy (EC₅₀) was not significantly different between human and monkey PD-1. P1801 did not cross-react with mouse PD-1.

P1801 inhibited the binding of PD-1 to PD-L1 and PD-L2 in a competitive binding ELISA and a cell-based reporter gene assay. P1801 reacts with human and Cynomolgus PD-1, but not mouse PD-1.

Example 2

Epitope binding of P1801

[0222] P1801's epitope binding position was compared with two existing commercially available anti-PD1 mAb, namely pembrolizumab and nivolumab. BLI technology was used to determine the biomolecular mutual effect. This technique is an optical analytic approach analyzing the interference pattern of white light reverberated from two surfaces: an inner reference layer, and a layer of settled protein on the biosensor tip. A variation in the interference pattern is induced by any altering in the number of molecules combined with the biosensor tip. In this case, interactions can be gauged in real time, offering the capacity to supervise binding specificity, the ratio of association and dissociation, concentration, fidelity and accuracy. In addition, only molecules combining with or separating from the biosensor are capable of changing the interference pattern and produce a reaction profile on the BLI system. However, molecules without binding, vary in the refractive index of the ambient medium or vary in flow rate have no effect on the interference pattern.

[0223] Here, purified Anti-PD1 Monoclonal Antibodies P1801 were tested by Octet BMIA for epitope blocking of PD-1. PD1RB Purified Antibodies were preincubated with PD-1/His (10 ug/mL antibody; 0.336 ug/mL PD-1) for 1 hour. Anti-Human IgG (Fc) sensors were loaded with each benchmark antibody @ 3 ug/mL. Association of the preformed antibody + antigen complex was measured compared to association of antigen alone. High binding response indicates non-overlapping epitopes, while lower response indicates epitope overlap. The baseline was conducted for 30 seconds. Antibody loading on sensor was done at 4ug/ml for 700 second. Quench for 480 seconds, and baseline for 300 seconds. Association of blocking antibody at 10ug/ml + PD-1 at 0.336ug/ml for 600 seconds. Detail output can be seen in Figure 8(a) comparing nivolumab and P1801, and 8(b) for pembrolizumab. If P1801 binds to the same epitope than nivolumab or pembrolizumab, the line should overlap, but it did not. Instead, both of the lines marked with arrows do not overlap with any other lines. This indicates non-overlapping binding on the target, and denotes partial overlap.

[0224] For both benchmark antibodies, P1801 exhibits response ranked at 0.0453 against nivolumab and 0.0426 nm against pembrolizumab. Therefore, the results exhibited P1801 is a partial blocker to PD1 as compared to the two commercially available anti-PD1 mAb epitope binding sites, yet P1801 exhibits comparable other characteristics, which is surprising and unexpected.

Example 3

Binding Kinetics

[0225] This example was performed to measure the binding affinity of P1801 antibodies to antigens using Biacore 8K. First, immobilization of FcRn and C1q onto CM5 sensor chip were performed. The immobilization of FcRn and C1q was performed under 25 degrees Celsius while HBS EP was used as the running buffer. The sensor chip surface of flow cells 1 and 2 were activated by freshly mixed 50 mmol/L N Hydroxysuccinimide (NHS) and 200 mmol/L 1 ethyl 3 -(3 dimethylaminopropyl) carbodii mide

hydrochloride (EDC) for 200s (10 μ L/min). Afterwards, FcRn and C1q diluted in 30 mmol/L NaAC (pH 4.5) was injected into the flow cell 2 to achieve conjugation of appropriate Response Unit respectively, while flow cell 1 was set as blank. After the amine coupling reaction, the remaining active coupling sites on chip surface were blocked with 200 s injection of 1 mol/L ethanolamine hydrochloride. To measure affinity of FcRn and C1q, the assay was performed at 25°C and the running buffer was HBS EP (PH 6.0). Diluted P1801 antibodies were injected over the surface of flow cell 1 and 2 as association phase, followed by injecting running buffer as dissociation phase. Next, affinity of human PD1 was measured. The assay was performed at 25°C and the running buffer was HBS EP (PH 7.4). Diluted P1801 antibodies (2 μ g/ml) were injected over the surface as capture phase, human PD1 proteins were injected over the surface as association phase followed by injecting running buffer as dissociation phase. Finally, P1801 Fc-binding characterization was performed by SPR where kinetic data of P1801 antibodies to human PD1, FcRn and C1q were obtained through Affinity measurement. All the data was processed using the Biacore 8K Evaluation software version 1.1. Flow cell 1 and blank injection of buffer in each cycle were used as double reference for Response Units subtraction.

Data are listed in Table 5 below.

Table 5

Test System	Method of Administration	Dose (nM)
Human Protein Antigens	<i>In Vitro</i>	6.25 ~ 400 for FcRn 0.625 ~ 20 for C1q

Assay System: Surface Plasma Resonance (Biacore 8K)

Analyte	Analyte Concentration (nM)	Immobilized Ligand	Immobilized Ligand Concentration (RU)	K _a (1/Ms)	K _d (1/s)	KD (nM)	R _{max} (RU)
Human PD-1	1.5625, 3.125, 6.25, 12.5, 25, 50, 100	P1801 (K014)	~300	5.04E+05	1.26E-03	2.50	47.3
		P1801 (K015)		3.98E+05	1.25E-03	3.13	48.8
P1801	6.25, 12.5, 25, 50, 100, 200, 400	FcRn	~500	2.43E+05	6.16E-02	253	38.1
P1801	0.625, 1.25, 2.5, 5, 10, 20	C1q	844.2	1.00 E+05	5.68E-02	56.8	33.7

Reference Information

	FcRn KD (nM)	C1q KD (nM)
pembrolizumab (commercial anti-PD1 IgG ₄ 228P)	66 ± 15	39 ± 7
Wild type IgG ₄ (228S)	69 ± 11	47 ± 6
IgG ₁	64 ± 11	11 ± 5

[0226] Briefly, P1801 binds to human PD-1 with affinities (KD) of 2.50 and 3.13 nM with two drug lots, and P1801 binds to FcRn and C1q with an affinity (KD) that was comparable to pembrolizumab.

Example 4

Kinetic measurements against CD64

[0227] This example shows multi-cycle Kinetic Analysis of Human PD1, Cynomolgus PD1 and Human CD64 to Anti-PD1 Ab. In this example, the ligand anti-PD1 Ab was captured on the surface of a Sensor Chip Protein A. The multi-cycle kinetic analysis of human PD1, cynomolgus PD1 and human CD64 to anti-PD1 Ab was performed by using Biacore T200. Ligand anti-PD1 Ab capturing on the surface of a Sensor Chip Protein A. Anti-PD1 Ab was first diluted with 1xHBS-EP+ to obtain a final concentration of 3 µg/ml and captured by flow cell 2 of a Sensor Chip Protein A. The chip contains MabSelect SuRe ligand on the surface, allowing orientation-specific binding of the Fc region of an antibody. The assay was performed by using the Kinetic / Affinity wizard. The flow path was 2-1, as the ligand was injected and captured in flow cell 2 and flow cell 1 acted as a reference. Series of concentrations of analytes, human PD1, cynomolgus PD1 and human CD64 were injected, respectively, over the reference and the ligand surfaces consecutively as the association phase, with short dissociation phases in between by injecting running buffer. Then, regeneration solution was injected as the regeneration phase. All the resulting data were fitted to a 1:1 binding model by using Biacore T200 Evaluation Software version 3. Flow cell 1 and blank injection of running buffer were used as double references for subtraction. Results are summarized below in Table 6.

Table 6 P1801 Binding affinity to CD64 by SPR (IgG receptor Fc gamma RI)

Test System	Method of Administration	Dose ($\mu\text{g/mL}$)
Human and Monkey, Cynomolgus/Proteins	<i>In Vitro</i>	3

Assay System: Surface Plasma Resonance (Biacore T200)

Ligand (Fc-captured)	Analyte	Analyte Concentration (nM)	K _a (1/Ms)	K _d (1/s)	KD (nM)	R _{max} (RU)
P1801	Human PD-1	16, 8, 4, 2, 1	786370	0.001167	1.48	125.1451
P1801	Cynomolgus PD-1	16, 8, 4, 2, 1	739342	0.001198	1.62	131.3064
P1801	Human CD64 (Fc γ RI)	16, 8, 4, 2, 1	2959431	0.00875	0.296	229.6639

Reference Information

Fc γ RI KD (nM)	
pembrolizumab (commercial anti-PD1 IgG4 228P)	0.125 $\pm$ 0.007
Wild type IgG4 (228S)	0.123 $\pm$ 0.001
IgG1	0.023 $\pm$ 0.001

[0228] In brief sum, P1801 binds to human PD-1 with an affinity (KD) of 1.48 nM, and a cynomolgus PD1 affinity (KD) of 1.62nM. P1801 has a human CD64 (IgG receptor Fc gamma RI) affinity (KD) of 2.96x10⁻¹⁰ M.

Example 5

in vivo therapeutic efficacy

[0229] This Example is to show the in vivo therapeutic efficacy of test compound P1801 in the treatment of the HuCell MC38-hPDL1 model in female hPD-1 HuGEMM mice, as well as the effect of P1801 + mP1101. Each mouse will be inoculated subcutaneously in the right rear flank region with HuCell MC38 tumor cells (1 x 10⁶) in 0.1 ml of PBS for tumor development. Treatment was initiated when tumors reached a mean volume of approximately 65mm³. Treatment with test compounds P1801 resulted in significant decrease in the mean tumor volume as compared to the vehicle group. 30 mice were enrolled in the study. All animals were randomly allocated to 3 different study groups. The mean tumor size at randomization was approximately 65 mm³. The date of tumor randomization is denoted as day 0. After tumor cell inoculation, the animals were checked daily for morbidity and mortality. At the time of routine monitoring, the animals were checked for any effects of tumor growth and treatments on behavior such as mobility, food and water consumption, body weight gain/loss (body weight was measured twice per week after randomization), and any other abnormalities. Mortality and observed clinical signs were recorded for individual animals. Tumor volume was measured twice weekly in two dimensions using a caliper, and the volume was expressed in mm³ using the formula: $V = (L \times W \times W)/2$,

where V is tumor volume, L is tumor length (the longest tumor dimension) and W is tumor width (the longest tumor dimension perpendicular to L). Tumor weight was measured at the end of the study. Dosing as well as tumor and body weight measurement were conducted in a Laminar Flow Cabinet.

[0230] On day 23 post inoculation, the MTV of isotype control group reached 3489.56mm³, and the MTV of P1801 12mpk treatment group and Nivolumab 12mpk treatment group reached 39.39mm³ and 236.69 mm³ respectively. The test article P1801 compared with isotype control group showed significant tumor inhibitory effect in the subcutaneous HuCell MC38-hPDL1 model in female hPD-1 HuGEMM mice (TGI: 98.87%) ($p < 0.001$). In the follow-up observation, 6 mice were observed completely response (CR) in P1801 treatment group, and 8 mice was observed completely response (CR) in Nivolumab treatment group. From the survival result, Median survival time of P1801 treatment surpassed 50 days, showed significantly longer than isotype control group ($p = 0.002$). Median survival time of Nivolumab 12mpk positive control group also surpassed 50 days, showing significantly longer than isotype control group ($p < 0.001$). One administration regimen, for example, can be seen in Figure 6(a) and 6(b).

[0231] The tumor growth inhibition is summarized in Table 7 below:

Table 7

Test System		Method of Administration	Dose (mg/kg)	Dosing Frequency		
Mouse/ MC38- hPD-L1 Subcutaneous Xenograft in hPD-1 knock-in HuGEMM Mice		Intraperitoneal	12	BIW*3		
Group	N	Tumor size Day 23		Tumor Growth Inhibition		Median Survival
		mm ³ (Mean $\pm$ SEM)		%	P Value	Time Days P Value
Isotype Control (hIgG4)	10	3489.56 $\pm$ 333.14		N.A.	N.A.	23 N.A.
P1801	10	39.39 $\pm$ 26.80		99	<0.001	>50 0.002
Nivolumab	10	236.69 $\pm$ 199.59		93	<0.001	>50 <0.001
mP1101 + P1801	10	906.83 $\pm$ 289.53		74	0.02	41 0.017

[0232] The data denotes P1801 inhibited MC38-hPD-L1 tumor growth in hPD-1 mice compared to isotype control group at 12 mg/kg BIW*3. In addition, P1801 prolonged the survival of tumor bearing hPD-1 mice compared to isotype control group. The combination of mP1101 and P1801 also demonstrated efficacy.

Example 6IL2 inducement

[0233] This example contains the results of Whole Blood Cytokine Response Assay of P1801. The assay observed that IL-2 production of SEB1-stimulated whole blood would be enhanced by P1801 in a dose dependent fashion. Heparinized blood from healthy donor was pre-incubated with P1801 and subsequently treated with SEB to induce IL-2 release. IL-2 inducement can in turn regulate and/or active T-cell responses, thereby suppressing and/or treating cancer.

[0234] After 2 days of treatment, supernatants were collected, and IL-2 was quantified by ELISA. Whole blood was diluted ten-fold with culture medium and pre-incubated in the absence (Non-SEB induction and untreated) or presence of test articles and NC (0.04, 0.2, 1, 5 and 25 µg/mL) for 60 minutes in a 96-well plate at 37°C, 5% CO₂ incubator. Whole blood cells were subsequently stimulated with SEB (0.1 µg/mL) except control wells (Non-SEB induction) and then incubated at 37°C, 5% CO₂ incubator for 48 hours. After incubation, the supernatants were collected by centrifugation at 500 xg for 5 minutes, aliquoted and stored at -30°C until analyzed by ELISA. IL-2 was quantified by ELISA following the manufacturer's instructions. Briefly, standard and supernatants were serially diluted with Calibrator Diluent RD5-5. Add 100 µL of Assay Diluent RD1W to each well and subsequently add 100 µL of blank, standards (31.3 ~ 2000 pg/mL) and diluted supernatants (2-, 4-, 8- and 16-fold dilution) to indicated wells for two hours. Wash the wells with diluted wash buffer three times and then add 200 µL of Human IL-2 Conjugate to each well for two hours. Repeat wash step and add 200 µL of Substrate Solution to each well for 10~20 minutes. At last, add 50 µL of Stop Solution and determine the optical density (O.D.) using microplate reader set to 450 and 570 nm². Analyze the data using Gen5TM Software as follows (A) Average the duplicate readings for blank, standards and supernatants and then subtract the average O.D. of blank to obtain delta O.D. (B) Create the standard curve by delta O.D. and generate four parameter logistic (4-PL) curve fit. The IL-2 concentration of all supernatants read from the standard curve must be multiplied by the dilution factor. (C) Acceptance criteria a. If the delta O.D. of supernatant is below the lowest standard (LLOQ₃), the IL-2 value will be represented by the symbol "<LLOQ" and excluded from subsequent calculation. If the RPD 4 calculated from the IL-2 values of two indicated dilutions are higher than 20%, the concentration of lower dilution will be reported. Conversely, the average of the concentration from both dilutions will be reported.

The resulting Pharmacodynamics are summarized in the Table 8 below:

Table 8

[0235] SEB Induced IL-2 in Human Whole Blood

Test Article: P1801 Test System	Method of Administration	Dose (µg/mL)
Human Whole Blood	<i>Ex Vivo</i>	0.04, 0.2, 1, 5 and 25

Negative Control (NC) (Human IgG4)			P1801				Nivolumab		
Dose (µg/mL)	Mean (µg/mL)	SD	Mean (µg/mL)	SD	P value against NC	P value against comparator	Mean (µg/mL)	SD	P value against NC
0	397.6	293.7	-	-	-	-	-	-	-
0.04	440.4	247.8	445.5	248.8	0.392	0.057	490.3	248.8	0.053
0.2	411.9	237.8	517.0	278.5	0.019	0.047	588.6	286.6	<0.001
1	402.9	234.2	596.3	313.7	0.001	0.306	610.6	302.7	0.001
5	411.7	276.1	614.7	320.8	0.001	0.408	621.6	333.8	<0.001
25	480.3	253.6	633.2	373.8	0.015	0.074	590.3	344.9	0.031

[0236] Results show P1801 induced significant differences in IL-2 production at 0.2, 1, 5 and 25 $\mu\text{g/mL}$ in human whole blood compared to IgG4 isotype control. 2. P1801 induced IL-2 production was not significantly different from that induced by nivolumab at 0.04, 1, 5 and 25 $\mu\text{g/mL}$ (P values > 0.05).

N=8 for each dose level.

[0237] IL-2 was not detected in whole blood without SEB stimulation. At concentrations of 0.2, 1, 5 and 25 $\mu\text{g/mL}$, there were statistically significant differences in IL-2 production between P1801 and human IgG4 (p values of 0.019, 0.001, 0.001 and 0.015, respectively). At concentrations of 0.04, 1, 5 and 25 $\mu\text{g/mL}$, there were not statistically significant differences in IL-2 production between P1801 and nivolumab (p values > 0.05). Specific fold change over IgG4 was up to 1.49 in P1801 treated group and was comparable to nivolumab. IL-2 production in SEB-stimulated whole blood can be enhanced by P1801 in a dose dependent manner.

Example 7

[0238] This example evaluates the pharmacokinetics, anti-drug antibody (ADA), receptor occupancy (RO), and IL-2 release in Cynomolgus monkeys following a single intravenous infusion of P1801 at three dose levels. All cynomolgus monkeys assigned to the study were identified as healthy animals by regular blood tests before dosing. Actual body weights and dosing details following single intravenous injections to cynomolgus monkeys were measured. The weekly records of body weights were measured. Slight body weight changes due to blood collection and no abnormal was observed during the dosing to sample collection period. Blood was collected for IL-2 release analysis from all surviving animals at predose (-7 day, -1 day), and 24 hours post dose, and 168 (Day 7), 672 (Day 28) and 1334 (Day 56) hours post dose. IL-2 release from monkey white blood cells (predose, -7 day) was tested in naïve monkeys treated ex vivo with 0.04, 0.2, 1, 5, or 25 $\mu\text{g/mL}$ P1801, Nivolumab, or isotype (IgG4). In the SEB stimulation study, treatment with P1801 and Nivolumab promoted IL-2 release in a dose-dependent manner, while IgG4 treatment only led to a slight induction of IL-2 release that did not increase with increasing dose.

[0239] IL-2 release from monkey white blood cells was measured in P1801-treated monkeys intravenously infused with a single dose of 1, 5, or 20 mg/kg P1801. Monkey whole blood was collected at five time points (D-1, D1, D7, D28 and D56) of the pharmacokinetics study. Time-dose dependent IL-2 release was observed in the 5 and 20 mg/kg groups, while no significant P1801 dose-dependent IL-2 release was observed. A summary is provided in Tables 9 and 10 below.

Table 9

Test System		Method of Administration		Dose (µg/mL)						
Monkey, PBMC	Cynomolgus	Ex Vivo		0.04, 0.2, 1, 5 and 25						
SEB-induced IL-2 in PBMC from Naïve monkeys										
Negative Control (NC) (Human IgG4)				P1801				Nivolumab		
Dose (µg/mL)	Mean (mU/mL)	SD	P value against SEB only	Mean (mU/mL)	SD	P value against NC	P value against Nivolumab	Mean (mU/mL)	SD	P value against NC
No SEB	46.7	17.2	-	-	-	-	-	-	-	-
0 (SEB only)	98.2	31.9	-	-	-	-	-	-	-	-
0.04	67.6	34.6	0.024	95.7	40.5	0.159	0.072	124	69.9	<0.01
0.2	93.0	44.2	0.386	129	51.8	0.140	0.002	189	85.5	<0.01
1	85.8	43.0	0.291	148	60.4	0.046	0.002	216	102	<0.01
5	96.2	57.8	0.514	168	59.6	0.042	0.002	236	102	<0.01
25	93.5	46.2	0.603	177	68.4	0.012	0.001	258	119	<0.01

[0240] Briefly, Table 9 above shows that P1801 promoted significant IL-2 production in monkey PBMCs in a dose dependent manner, while IgG4 isotype control led to slight increases of IL-2 that did not increase with increasing dose. N=12 for each dose level.

Table 10

Test System		Method of Administration		Dose (mg/kg)					
Monkey, Cynomolgus Whole Blood		Intravenous Infusion		1, 5, 20					
Assay System: SEB-induced IL-2 in Whole Blood from P1801-Treated monkeys									
1mg/kg P1801				5 mg/kg P1801			20 mg/kg P1801		
Timepoint	Mean (mU/mL)	SD	P value1	Mean (mU/mL)	SD	P value1	Mean (mU/mL)	SD	P value1
Day -1	93.9	24.8	-	49.6	9.5	-	63.4	29.0	-
Day 1	152.4	51.3	0.104	198.1	4.6	<0.001	182.3	11.2	0.002
Day 7	105.2	25.5	0.551	120.3	9.9	<0.001	133.2	39.2	0.032
Day 28	83.8	13.4	0.507	74.5	16.5	0.050	87.5	20.1	0.228
Day 56	92.4	13.3	0.916	77.7	12.2	0.012	74.4	8.5	0.510

[0241] This example showed that P1801 promoted significant IL-2 production in monkey whole blood in a time dependent manner. No dose dependency was observed when P1801 is administered at 1, 5, or 20 mg/kg. N=4 (2 male and 2 female) for each dose level; 1P value against Day -1 (ANOVA/Kruskal-wallis & Wilcoxon rank sum test on group means after Bartlett test).

Example 8

in vitro PD-1 Receptor Occupancy (RO).

[0242] Human freshly isolated PBMCs were stimulated by the anti-CD3/CD28 coated Dynabeads for 2 days to induce PD-1 expression and was then incubated with P1801 at the indicated concentration. P1801 bound PD-1+ T cells were detected by using a secondary fluorophore-conjugated antibody (anti-human IgG4 Fc antibody) and analyzed on a flow cytometer. Human PBMCs were isolated from heparinized blood of healthy donors. Mouse Anti-Human IgG4 Fc Antibody with Fluorophore R-phycoerythrin (PE), and Mouse Anti-Human CD3 Antibody with Fluorophore Allophycocyanin (APC) were used. Negative control Ultra-LEAFTM Purified Human IgG4 Isotype Control Recombinant Antibody. T Cell Activator Dynabeads (trademarked) Human T-Activator CD3/CD28 were used. Human PBMCs were isolated using Ficoll-Paque (trademark) PLUS from heparinized whole blood of healthy donors not more than 6 hours after its collection. Human PBMC were stimulated to express PD-1 by Dynabeads (trademarked) Human T-Activator CD3/CD28 at 37°C, 5% CO₂ incubator for 2 days. After two-day activation, suspend PBMC and remove Dynabeads by magnet rack (DynaMag) (trademark). Incubate 2×10⁵ PBMC in the absence (untreated) or presence of P1801 (0.01, 0.1 and 1 µg/mL), comparator (0.01, 0.1 and 1 µg/mL) and negative control (1 µg/mL) in 100 µL of staining buffer for 30 minutes at room temperature in the dark. Washing PBMC twice with staining buffer prior staining. Dilute anti-hCD3 and anti-hIgG4 Fc antibodies in staining buffer and add 100 µL of diluted antibodies to the tubes that contain PBMC. Incubate for 20 minutes at room temperature in the dark.

[0243] PBMC were washed twice with 1 mL of staining buffer to remove unbound antibodies. Resuspend the cell pellet in 350 µL of staining buffer and analyze by flow cytometry as soon as possible. Data were analyzed using FCS Express Software as follows, (A) Gating CD3 positive region of FSC/CD3 dot plot for further analysis of PD-1 expression, including the percentage of PD-1+ cell (PD-1+ %) and the geometric mean fluorescence intensity (Geo MFI) of PD-1 on all CD3+ T cell. (B) The PD-1+ % is defined as the percentage of CD3+ T cells bound with anti-PD-1 antibody and calculated to subtract untreated from treated by Overton method. (C) The Geo MFI is defined as the geometric mean fluorescence intensity of PE channel, and it represents the binding level of anti-PD-1 antibody to PD-1 on the surface of all CD3+ T cell. Data in the summary table were expressed as the means ± SE3 of ten donors in eight independent experiments and analyzed by one-way ANOVA4 for P1801 and comparator. Results are summarized in the below Table 11.

Table 11

[0244] P1801 Receptor Occupancy in Human PBMCs

Test System	Method of Administration	Dose (µg/mL)
Human PBMC	<i>Ex Vivo</i>	0.01, 0.1, 1

Flow Cytometry Measured % P1801 bound T cells from Human Healthy Donors (N=10)

Groups (µg/mL)	Percent P1801-bound CD3+ T Cells	Fluorescence Intensity
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	%	SE	P value against nivolumab	Mean Geo MFI	SE	P value against nivolumab
Untreated	-	-	-	54.2	7.1	-
1 hIgG4 (Negative Control)	2.5	1.7	-	54.8	5.7	-
0.01 P1801	52	10.1	0.003	200.4	63.2	0.001
0.1 P1801	81.9	8.1	0.349	389.9	146.9	0.038
1 P1801	87.9	6.4	0.812	1050.7	449.2	0.782
0.01 Nivolumab	67.7	10	-	1705.9	808.2	-
0.1 Nivolumab	85.3	7.7	-	2238.8	1056.9	-
1 Nivolumab	87.2	7	-	2109.7	1003	-

[0245] Results show that the percent P1801 binding to human PBMCs was dose-dependent. The percent PD-1 Positive CD3+ T Cells of P1801 was not significantly different from that of nivolumab at 0.1 and 1 µg/mL. In addition, the PD-1 binding activity of P1801 showed dose dependent. At moderate and high concentration of 0.1 and 1 µg/mL, respectively, there were no statistically significant differences in PD-1+ % between P1801 and nivolumab ($p > 0.05$). At high concentration of 1 µg/mL, there were no statistically significant differences in Geo MFI between P1801 and nivolumab ($p > 0.05$).

Example 9

In Vivo Efficacy in the Treatment of Renca Subcutaneous Syngeneic Kidney Cancer Model in Female BALB/c Mice

[0246] To assess the anti-tumor efficacy of anti-PD1 antibody in combination of P1101 in mouse model, the murine version of both molecules was used, namely mP1101 where mice interferon was used instead of human version, and RMP 1-14, a known mouse anti-PD1 mAb, was used as anti-PD1 mAb. The study design includes: randomization into study groups; daily dosing, 7 days a week for 3 weeks; post-treatment observation period based on implantation site; subcutaneous or orthotopic administrations. During dosing and observation period, animals were examined daily, 5 days a week; body weight measured x2 weekly and tumor volume measured x2 weekly, if subcutaneous implantation.

[0247] Table 12 shows the design summary similar to Figure 4(a) to 4(c) show the administration of the combination with each molecule's frequency. In the Table 12 below, Q3D stands for one administration every 3 days. *6 means 6 time. S.C. stands for Subcutaneous administration. I.P. stands for Intraperitoneal injection. ROA stands for route of administration.

Table 12

Group	No. of mice	Treatment	Dose level (mg/kg)	Dosing Solution (mg/ml)	Dosing Volume (µL/g)	ROA	Dosing Frequency & Duration
1	8	Vehicle	-	-	10	i.p.	Q3D × 6
2	8	Anti-PD1	12	1.2	10	i.p.	Q3D × 6
3	8	mP1101	0.1	0.01	10	s.c.	Q3D × 6
4 (Combo)	8	mP1101	0.1	0.01	10	s.c.	Q3D × 3 (in

							different day)
		Anti-PD1	12	1.2	10	i.p.	Q3D × 6 (in different day)
5 (Combo)	8	mP1101	0.1	0.01	10	s.c.	Q3D × 6 (in different day)
		Anti-PD1	12	1.2	10	i.p.	Q3D × 6 (in different day)
6 (Sequential)	8	mP1101	0.1	0.01	10	s.c.	mP1011 Q3D × 3 following with anti-PD1 Q3D × 6
		Anti-PD1	12	1.2	10	i.p.	
7 (Sequential)	8	mP1101	0.1	0.01	10	s.c.	mP1011 Q3D × 6 following with anti-PD1 Q3D × 5
		Anti-PD1	12	1.2	10	i.p.	

Methods

[0248] The Renca (CL-00796) cells were maintained in vitro with DMEM medium supplemented with 10% fetal bovine serum at 37°C in an atmosphere of 5% CO₂ in air. The cells in exponential growth phase were harvested and quantitated by cell counter before tumor inoculation. For tumor Inoculation, each mouse was inoculated subcutaneously in the right lower flank region with Renca tumor cells (1×10^6) in 0.1 ml of PBS for tumor development. Randomization started when the mean tumor size reached approximately 70 mm³. 56 mice were enrolled in the study. All animals were randomly allocated to 7 study groups, 8 mice in each group. Randomization was performed based on “Matched distribution” method/ “Stratified” method (StudyDirector (trademarked) software, version 3.1.399.19). The date of randomization was denoted as day 0. The treatment was initiated on the same day of randomization (day 1) per study design.

Observation and Data Collection

[0249] After tumor cells inoculation, the animals were checked daily for morbidity and mortality. During routine monitoring, the animals were checked for any effects of tumor growth and treatments on behavior such as mobility, food and water consumption, body weight gain/loss (Body weights were measured twice per week after randomization), eye/hair matting and any other abnormalities. Mortality and observed clinical signs were recorded for individual animals in detail. Tumor volumes were measured twice per week after randomization in two dimensions using a caliper, and the volume was expressed in mm³ using the formula: “ $V = (L \times W \times W)/2$ ”, where V was tumor volume, L was tumor length (the longest tumor dimension) and W was tumor width (the longest tumor dimension perpendicular to L). Dosing as well as tumor and body weight measurements were conducted in a Laminar Flow Cabinet. The body weights and tumor volumes were also measured by using StudyDirector software.

[0250] The treatments were performed for 33 weeks. The study was terminated on day 33. Tumors was not

collected for euthanized mice before termination of the study. No sampling for mice found dead.

Statistical Analysis

[0251] Statistical Analysis Method was done by first test of homogeneity of variance by Bartlett's test. Second, if p-value of Bartlett's test is greater than 0.05, it indicates that there is not enough evidence to reject the null hypothesis of homogeneity of variance, which is one of the assumptions of ANOVA. Therefore, ANOVA was performed to test whether there exists any pair of groups with different group means, and ran the post hoc Tukey HSD for all pairwise comparisons or Dunnett's t test for each treatment against one control. If p-value of Bartlett's test is less than or equal to 0.05, it indicates that there is some evidence that variances may not be equal, or data do not follow normal distribution. Then we ran Kruskal-Wallis test to test if there exists any pair of groups with different group medians, and ran the post hoc Conover's non-parametric all-pairs comparison test for all pairwise comparisons, or Conover's non-parametric many-to-one comparison test for each treatment against one control. Both tests use single-step p-value adjustment. In addition, pairwise comparisons was performed without multiple comparison correction and report nominal/uncorrected p-values directly from Welch's t-test or Mann-Whitney U test. The criterion of choosing Welch's t-test or Mann-Whitney U test is the same as the two-group comparison.

[0252] All statistical analyses had been done in R language and environment for statistical computing and graphics (version 3.6.0). All tests were two-sided unless otherwise specified, and p-values of <0.05 are regarded as statistically significant. For survival analysis, Kaplan-Meier model with Log-rank method is applied.

Results

[0253] The Body Weight Growth Curves were first measured. The results of body weights and body weight changes at different time points. The tumor growth curves (mean tumor volume over time) of different groups are shown in Figure 10. Detailed measurement and statistics are shown below in Table 13.

Table 13

Group	Tumor (mm ³) ^a Day 1	Size on	Tumor (mm ³) ^a Day 22	Size on	TGI ^b (%)	T/C	P value ^c
1	70.73 3.02(8)	+/-	3486.36 193.39(5)	+/-	-	-	-
2	70.74 2.88(8)	+/-	3431.09 133.33(7)	+/-	1.59	0.98	1.00 ns
3	70.71 2.89(8)	+/-	1021.35 182.55(7)	+/-	70.70	0.29	5.30e-11 ***
4	70.71 2.86(8)	+/-	1237.43 119.99(7)	+/-	64.51	0.35	4.30e-10 ***
5	70.76 2.88(8)	+/-	711.74 43.56(3)	+/-	79.59	0.20	2.83e-10 ***
6	70.72 2.94(8)	+/-	1266.68 194.51(8)	+/-	63.67	0.36	2.53e-10 ***

7	70.76 2.99(8)	+/-	822.66 233.62(7)	+/-	76.40	0.24	2.11e-11 ***
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[0254] In this example, the mean tumor volume of Group 1 reached 3486.36 mm³ on day 22 post treatment initiation. Group 2 treated with Anti-PD1 12 mg/kg with TGI value of 1.59 % on day 22. Group 3 treated with mP1101 0.1 mg/kg with TGI value of 70.70 % on day 22. Group 4 treated with mP1101 0.1 mg/kg Q3D x 3 and Anti-PD1 12 mg/kg Q3D x 6 with TGI value of 64.51 % on day 22. Group 5 treated with mP1101 0.1 mg/kg Q3D x 6 and Anti-PD1 12 mg/kg Q3D x 6 with TGI value of 79.59 % on day 22. Group 6 treated with mP1101 0.1 mg/kg Q3D x 3 followed with Anti-PD1 12 mg/kg Q3D x 6 with TGI value of 63.67 % on day 22. Group 7 treated with mP1101 0.1 mg/kg Q3D x 6 followed with Anti-PD1 12 mg/kg Q3D x 6 with TGI value of 76.40 % on day 22.

[0255] The median survival time (MST) of each group was also investigated. Mice whose condition reached to the humane endpoints were euthanized. MST of vehicle control, Anti-PD1 12 mg/kg, mP1101 0.1 mg/kg, mP1101 0.1 mg/kg Q3D x 3 and Anti-PD1 12 mg/kg Q3D x 6, mP1101 0.1 mg/kg Q3D x 6 and Anti-PD1 12 mg/kg Q3D x 6, mP1101 0.1 mg/kg Q3D x 3 following with Anti-PD1 12 mg/kg Q3D x 6, mP1101 0.1 mg/kg Q3D x 6 following with Anti-PD1 12 mg/kg Q3D x 6 was respectively 23.00 days, 23.00 days (P>0.05 vs. vehicle control group), 31.50 days (P<0.01 vs. vehicle control group), 29.00 days (P<0.01 vs. vehicle control group), 16.50 days (P>0.05 vs. vehicle control group), 30.00 days (P<0.001 vs. vehicle control group) and 33.00 days (P<0.01 vs. vehicle control group).

[0256] Briefly in sum, in this example, the therapeutic efficacy of test agent mP1101 was evaluated in murine Renca model in female BALB/c mice. Compare with vehicle group, single agent mP1101 0.1 mg/kg (Group 3) and combination with Anti-PD1 12 mg/kg (Group 04, 05, 06 and 07) showed significant anti-tumor efficacy (P<0.001) In addition, single agent mP1101 0.1 mg/kg (Group 3) and combination with Anti-PD1 12 mg/kg (Group 04, 06 and 07) prolonged the survival of the mice.

Example 10

Therapeutic efficacy

[0257] To assess the anti-tumor efficacy of anti-PD1 mAb P1801, mammal subjects can be treated with P1801 in an effect amount to reduce, suppress and/or treat CRC, RCC and/or melanoma.

[0258] Each subject who has RCC or melanoma is treated. Treatments with P1801 result in decrease in the mean tumor volume as compared to the vehicle control group. 30 subjects are enrolled in the study. All subjects are randomly allocated to 3 different study groups. The mean tumor size at randomization initially is approximately 65 mm³. The date of tumor randomization is denoted as day 0. All subjects are checked daily. At the time of routine monitoring, the subjects are checked for any effects of tumor growth and treatments on behavior such as mobility, food and water consumption, body weight gain/loss, and any other abnormalities. Mortality and observed clinical signs are recorded for each individual. Tumor volume is measured twice weekly, and the volume is expressed in mm³ using the formula: $V = (L \times W \times W)/2$, where V is tumor volume, L is tumor length (the longest tumor dimension) and W is tumor width (the longest tumor dimension).

perpendicular to L). Tumor weight was measured at the end of the study.

[0259] The administration regimen can be seen in Figure 12. Subjects show reduce size in melanoma or RCC, as applicable.

Example 11

[0260] To assess the anti-tumor efficacy of anti-PD1 P1801 mAb which works in mammal subjects along, P1101 with human version of interferon along, and/or combination of anti-PD1 mAb P1801 + P1101, the human version of both molecules are used, namely P1101 having human interferon, and P1801 humanized version are used. The study design includes: randomization into study groups; daily dosing, 7 days a week for 3 weeks; post-treatment observation period based on implantation site; subcutaneous or orthotopic administrations. During dosing and observation period, human subjects are examined daily, 5 days a week; body weight measured x2 weekly and tumor volume measured x2 weekly, if subcutaneous implantation.

[0261] Table 13 shows the experimental design summary, and Figure 4(a) to 4(c) show the administration of the combination with each molecule's frequency. In Table 14 below, Q3D stands for one administration every 3 days. *6 means 6 time. S.C. stands for Subcutaneous administration. I.P. stands for Intraperitoneal injection.

Table 14

Test Article	Dosing Regimen	Route	Study Period	Index	Mouse number
P1801 alone	300mcg/subject (12mg/kg), Q3D *6	I.P	6-8 weeks	Tumor growth inhibition IHC analysis (Ki67) evaluation	8
P1101 alone	2.5mcg/subject (0.1mg/kg), Q3D*6	S.C			
P1101 + P1801 (sequential)	P1101 2.5mcg, Q3D*3 following P1801 300mcg, Q3D*6	S.C/I.P			
	P1101 2.5mcg, Q3D*6 following P1801 300mcg, Q3D*6				
P1101 + P1801 (combo)	P1101 2.5mcg, Q3D*3 + P1801 300mcg, Q3D*6 (in different day)				

	P1101 2.5mcg, Q3D*6 + P1801 300mcg, Q3D*6 (in different day)				
Vehicle	PBS, Q3D*6	I.P			

[0262] Results show that tumor size of RCC, CRC, and/or melanoma are reduced by all of the following: P1801 treatment along, P1101 treatment along, and the combination treatment of P1801 and P1101, in human subjects for treatment for RCC, CRC, and/or melanoma.

Example 12

Dose escalation of the serial administration in clinical trial

[0263] In order to check for toxicity/tolerance level of P1801 + P1101 in humans, a phase 1 trial in human is carried out. Starting dose of P1801 was determined based on the preclinical pharmacology and toxicology data. A NOAEL of 200 mg/kg was reported in the pre-clinical toxicity studies of cynomolgus monkey as the above example has shown. Considering a Conversion Factor (Monkey to Human) of 3.1 and a Safety Factor of 6 to introduce the safety margin, the maximum recommended starting dose of P1801 is 10.75 mg/kg (200 mg/kg divided by 3.1 then divided by 6). By imposing more stringent safety consideration, 0.3 mg/kg is selected for the starting dose of this example.

[0264] Initially, the screening will take place for subjects to meeting criteria such as age >18, who has solid tumor in RCC, melanoma, Hepatocellular carcinoma (HCC) and/or CRC, and some may be in advance phase. The subject would also have a life expectancy of greater than or equal to 3 months, with normal thyroid function and negative for HBV, HCV and HIV. In this Cohort 1-5, subjects with an advanced solid tumor including especially Renal Cell Carcinoma (RCC), Melanoma, Hepatocellular Carcinoma (HCC), and Colorectal Cancer (CRC) are treated by 6 doses of P1101 at 450 mcg Q2W and then followed by the treatment with P1801 Q2W at the pre-determined dose level by cohort during each 28-day treatment cycle.

[0265] For dose escalation, 5 different cohort are used as denoted in Figure 12. Once an effective dosage is determined, expansion cohort is carried out as denoted in Figure 13. PD-L1 expression is measured for all subjects but only subjects with a positive PD-L1 expression are enrolled in further Cohort as denoted in Figure 14. Note, in this example, P1101 and P1801 are administered sequentially, not interchangeably. Results are shown that an effective dosage denoted can be effective in treating CRC/HCC/RCC/Melanoma from this example.

Example 13

Paratope Determination

[0266] The structure and 3D model of the P1801/PD-1 was determined using a high-resolution Cryo-Electron Microscopy to provide atomic-level resolution structures of P1801/PD-1 to reveal the spatial arrangement of amino acids in the 3D structure and determination of the paratopes.

[0267] In order to determine the paratopes a grid was screened for sample PD1-AcroBio and the following dataset was acquired. One dataset was collected for sample PD1-AcroBio + PS00066 + PS00067. The dataset was processed in cryoSPARC 4.4. Micrographs with poor contrast transfer function fits were removed (CTF < 4 Å) and 5,306 micrographs were selected for further processing. A template-based particle picking was performed and about 4.0 million particles were extracted with a box size 320 pixel (~265 Å). Two rounds of 2D classification and select 2D classes were performed for an initial clean up, yielding ~1.5 million particles. The 2D class averages show discrete and well defined particles with different views and visible secondary structure features. The selected particles were used in one round of ab initio reconstruction followed by four heterogenous refinements with three decoys and a non-uniform refinement. The final 128 K particles generated a map with the highest resolution, and that map was selected for further refinement using CTF global refinement followed by local resolution refinement and reference-based motion correction. The estimated resolution is 2.9 Å based on gold standard Fourier shell correlation of 0.143 (Figures 16-17). The half-maps were further refined using DeepEMhancer a python package implemented in cryoSPARC. Models for the PD1 (Alpha-fold) and two FABs were docked into the available density using ChimeraX. The variable regions of the FABs were then mutated according to the sequence provided by the client for the PS00066 and PS00067. The constant regions were not shown. The resulting model underwent iterative rounds of manual rebuilding (using COOT) and computer-based refinement (Phenix). Glycans were built manually at position ASN 49 and ASN 58. The final model was validated against the half-maps and its quality assessed by MolProbity. The obtained structure provides the location of the protein-protein interface between the PD1 and PS00066/PS00067.

[0268] Sample and Grid Preparation for Cryo-EM. PD1-AcroBio + PS00066 + PS00067 was prepared by mixing sample PD1-AcroBio, sample PS00066, and sample PS00067, in a 1:1:1 molar ratio and incubating for 30 minutes rt, yielding a final protein concentration of 0.18 mg/mL for the complex. Sample PD1-AcroBio, sample PS00066, and sample PS00067, in a 1:1:1 molar ratio was diluted 17.5-fold in DPBS (provided by client), yielding a final sample concentration of 0.01 mg/mL prior to grid preparation and imaging. During traditional plunge-freezing, either the FEI Vitrobot instrument or manual plunger is used. In summary, a 3 µl drop of sample suspension is applied to an EM grid that has been plasma-cleaned using a Gatan Solarus (Pleasanton, California). After blotting the sample away with filter paper, grids are plunge-frozen in liquid ethane. Grids are stored under liquid nitrogen until transferred to the transmission electron microscope for imaging.

[0269] Cryo-EM Imaging using FEI Titan Krios Microscope. Electron microscopy is performed using an FEI Titan Krios (Hillsboro, Oregon) transmission electron microscope operated at 300kV and equipped with a Gatan Quantum 967 LS imaging filter, a Volta phase plate, and Gatan K3 Direct Detection Camera. Vitreous ice grids are clipped into cartridges, transferred into a cassette and then into the Krios autoloader, all while maintaining the grids at cryogenic temperature (below -170°C). Automated data-collection is carried out using Legion software, where high magnification movies are acquired by selecting targets at a lower magnification.

[0270] Pre-processing and 2D Classification Analysis (cryoSPARC, Relion). Dose-weighted movie frame alignment is done using MotionCor2 or Full-frame or Patch motion correction in cryoSPARC (Punjani et al, 2017) to account for stage drift and beam-induced motion. The contrast transfer function is estimated for each micrograph using CTFfind4, gCTF, or Patch CTF in cryoSPARC (Rohou et al., 2015; Zhang et al., 2016). Individual particles are selected using automated picking protocols and extracted into particle stacks in either Relion or cryoSPARC. The particles may then be submitted to reference-free 2D alignment and classification in either Relion or cryoSPARC.

[0271] CryoSPARC (Cryo-EM Single Particle Analysis Reconstruction and Classification) was used to streamline cryo-EM data processing, provide capabilities such as particle picking, 2D classification, 3D reconstruction, and refinement. Initially, grids were screened and the data complied. From the data generated from the cryoSPARC 4.4 micrograph data a map was generated. Figure 16 is an image generated with ChimeraX of the final 3D reconstruction of the PD1-AcroBio + PS 00066 + PS00067 at a nominal resolution of 2.9Å. The generated map with the highest resolution was selected for further refinement using CTF global refinement followed by local resolution refinement and reference-based motion correction. The estimated resolution is 2.9 Å based on gold standard Fourier shell correlation of 0.143. The maps were further refined using DeepEMhancer a python package implemented in cryoSPARC. Models for the PD1 (Alpha-fold) and two FABs were docked into the available density using ChimeraX. Figure 17 is an image generated with ChimeraX of the final 2.9Å map colored by the local resolution values calculated by cryoSPARC 4.4. The variable regions of the FABs were then mutated according to the sequence provided by the client for the PS00066 and PS00067. The resulting model underwent iterative rounds of manual rebuilding (using COOT) and computer-based refinement (Phenix). The final model was validated against the maps and its quality assessed by MolProbity. The obtained structure provided the location of the protein-protein interface between the PD1 and PS00066/PS00067. The model illustrated the hydrophobic and electrostatic interactions between PD1 and PS00067. The protein-protein contact appears to be primarily driven by hydrophobic interactions, with a cluster of aliphatic and aromatic residues such as Tyr 32, Pro 33, His 52, Tyr 54 (I:PS00067), Tyr 95 (M:PS00067), and Pro 39, Ala 40, Leu 41, Leu 42, Val 43, Thr 45, Thr 145 (PD1). Additionally, Arg 143 and Glu 141 (PD1) may be involved in hydrogen bonding with Glu 99 (I:PS00067) and Tyr 101 (M:PS00067) / His 104 (I:PS00067), respectively.

[0272] Figure 18 is an image of the structure of the P1801/PD1 complex solved by Cryo-Electron Microscopy. Figure 19 is an image of the structure of the P1801/PL1/PD1 complex solved by Cryo-Electron Microscopy showing an overlay of P1801/PD1 and PD-L1/PD1. Figure 20 is an image of the epitope mapping of the P1801 epitope showing binding to T59, S60, E61, S62, V64*, P83, E84*, D85, R86, L128*, and A129 of the heavy chain and S87, P130*, K131*, and A132* of the light chain, wherein * denotes sharing between epitopes. Figure 21 is an image of the epitope mapping of the PD-L1 epitope showing binding to V64*, N66, Y68, S73, N74, Q75, T76, D77, K78, E84*, G124, I126, L128*, P130*, K131*, A132*, I134, and E136, wherein * denotes sharing between epitopes. Figure 22 is a model of the P1801 CDRs at the PD-1 binding interface.

[0273] Figure 23 is a graph of epitope binding anti-PD-1 Antibodies. Figure 23 shows all antibodies can bind strongly to PD-1 in the presence of P1801, indicating their epitope is different. ISIS-clone19 was selected as non-competing Fa. Peresolimab is a humanized immunoglobulin G1 monoclonal antibody that stimulates human PD-1.

[0274] In one embodiment the P1801 Variable Region Heavy chain sequence with CDRs delineated is
EVQLVESGGGLVQPGGSLRLSCAASGFTFSSYDMSWVRQAPGKGLEWVATITGGGSYTYYP
DSVKGRFTISRDNKNSLYLQMNSLRAEDTAVYYCASPYNFYFDYWGQGLTVTVSS (SEQ
ID No:5).

Shown is VHCDR1 sequence SSYDMS (SEQ ID No:18); VHCDR2 sequence TITGGGSYTYYPDSVKG (SEQ ID No:10); and VHCDR3 sequence PYLNYFDY (SEQ ID No:11).

[0275] The P1801 Variable Region light chain sequence with CDRs delineated is
EIVLTQSPATLSLSPGERATLSCRASQSSNNLHWYQQKPGQAPRLLIYYASQSSIGIPARFSGS
GSGTDFTLTISLEPEDFAVYYCQQSNSWPLTFGQGQTKLEIK (SEQ ID No:6).

Shown is VLCDR1 sequence RASQSSNNLH (SEQ ID No:12); VLCDR2 sequence YASQSS (SEQ ID No:13); and VLCDR3 sequence QQSNSWPLT (SEQ ID No:14).

[0276] In another embodiment the P1801 Variable Region Heavy chain sequence with CDRs delineated is
EVQLVESGGGLVQPGGSLRLSCAASGFTFSSYDMSWVRQAPGKGLEWVATITGGGSYTYYP
DSVKGRFTISRDNKNSLYLQMNSLRAEDTAVYYCASPYNFYFDYWGQGLTVTVSS (SEQ
ID No:5).

Shown is VHCDR1 sequence GFTFSSYDMS (SEQ ID No:19); VHCDR2 sequence TITGGGSYTYYPDSVKG (SEQ ID No:10); and VHCDR3 sequence ASPYNFYFDY (SEQ ID No:20). The P1801 Variable Region light chain sequence with CDRs delineated is

EIVLTQSPATLSLSPGERATLSCRASQSSNNLHWYQQKPGQAPRLLIYYASQSSIGIPARFSGS
GSGTDFTLTISLEPEDFAVYYCQQSNSWPLTFGQGQTKLEIK (SEQ ID No:6).

Shown is VLCDR1 sequence RASQSSNNLH (SEQ ID No:12); VLCDR2 sequence YASQSS (SEQ ID No:13); and VLCDR3 sequence QQSNSWPLT (SEQ ID No:14).

[0277] In one embodiment the P1801 Variable Region Heavy chain sequence with CDRs delineated illustrating the paratopes is

EVQLVESGGGLVQPGGSLRLSCAASGFTFSSYDMSWVRQAPGKGLEWVATITGGGSYTYYP
DSVKGRFTISRDNKNSLYLQMNSLRAEDTAVYYCASPYNFYFDYWGQGLTVTVSS (SEQ
ID No:5).

[0278] Shown is the VHCDR1 sequence GFTFSSYDMS (SEQ ID No:19) can also be Gly Phe Thr Phe Ser Ser Tyr Asp Met Ser and numbered as Gly1 Phe2 Thr3 Phe4 Ser5 Ser6 Tyr7 Asp8 Met9 Ser10. The paratopes of the VHCDR1 sequence share Phe2 Ser5 Ser6 and Tyr7 over all paratopes. As such, Gly1 Thr3 Phe4 Asp8 Met9 and Ser10 may be individually substituted for any amino acid residue. For example, Gly1 may be independently substituted for Ala, Arg, Asn, Asp, Asx, Cys, Glu, Gln, Glx, His, Ile, Leu, Lys, Met, Phe, Pro,

Ser, Thr, Trp, Tyr, or Val; Thr3 may be independently substituted for Ala, Arg, Asn, Asp, Asx, Cys, Glu, Gln, Glx, Gly, His, Ile, Leu, Lys, Met, Phe, Pro, Ser, Trp, Tyr, or Val; Phe4 may be independently substituted for Ala, Arg, Asn, Asp, Asx, Cys, Glu, Gln, Glx, Gly, His, Ile, Leu, Lys, Met, Pro, Ser, Thr, Trp, Tyr, or Val; Asp8 may be independently substituted for Ala, Arg, Asn, Asx, Cys, Glu, Gln, Glx, Gly, His, Ile, Leu, Lys, Met, Phe, Pro, Ser, Thr, Trp, Tyr, or Val; Met9 may be independently substituted for Ala, Arg, Asn, Asp, Asx, Cys, Glu, Gln, Glx, Gly, His, Ile, Leu, Lys, Phe, Pro, Ser, Thr, Trp, Tyr, or Val; and Ser10 may be independently substituted for Ala, Arg, Asn, Asp, Asx, Cys, Glu, Gln, Glx, Gly, His, Ile, Leu, Lys, Met, Phe, Pro, Thr, Trp, Tyr, or Val.

[0279] VHCDR2 sequence TITGGGSYTYYPDSVKG (SEQ ID No:10) can be Thr Ile Thr Gly Gly Gly Ser Tyr Thr Tyr Tyr Pro Asp Ser Val Lys Gly and numbered as Thr11 Ile12 Thr13 Gly14 Gly15 Gly16 Ser17 Tyr18 Thr19 Tyr20 Tyr21 Pro22 Asp23 Ser24 Val25 Lys26 Gly27. The paratopes of the VHCDR2 sequence share Thr13 Gly14 Gly15 Gly16 Ser17 and Tyr18 over all paratopes. As such, Thr11, Ile12, Thr19, Tyr20, Tyr21, Pro22, Asp23, Ser24, Val25, Lys26, and Gly27 may be individually substituted for any amino acid residue. For example, Thr11 may be independently substituted for Ala, Arg, Asn, Asp, Asx, Cys, Glu, Gln, Glx, Gly, His, Ile, Leu, Lys, Met, Phe, Pro, Ser, Trp, Tyr, or Val; Ile12 may be independently substituted for for Ala, Arg, Asn, Asp, Asx, Cys, Glu, Gln, Glx, Gly, His, Leu, Lys, Met, Phe, Pro, Ser, Thr, Trp, Tyr, or Val; Thr19 may be independently substituted for for Ala, Arg, Asn, Asp, Asx, Cys, Glu, Gln, Glx, Gly, His, Ile, Leu, Lys, Met, Phe, Pro, Ser, Trp, Tyr, or Val; Tyr20 may be independently substituted for for Ala, Arg, Asn, Asp, Asx, Cys, Glu, Gln, Glx, Gly, His, Ile, Leu, Lys, Met, Phe, Pro, Ser, Thr, Trp, or Val; Tyr21 may be independently substituted for for Ala, Arg, Asn, Asp, Asx, Cys, Glu, Gln, Glx, Gly, His, Ile, Leu, Lys, Met, Phe, Pro, Ser, Thr, Trp, or Val; Pro22 may be independently substituted for for Ala, Arg, Asn, Asp, Asx, Cys, Glu, Gln, Glx, Gly, His, Ile, Leu, Lys, Met, Phe, Ser, Thr, Trp, Tyr, or Val; Asp23 may be independently substituted for for Ala, Arg, Asn, Asx, Cys, Glu, Gln, Glx, Gly, His, Ile, Leu, Lys, Met, Phe, Pro, Ser, Thr, Trp, Tyr, or Val; Ser24 may be independently substituted for for Ala, Arg, Asn, Asp, Asx, Cys, Glu, Gln, Glx, Gly, His, Ile, Leu, Lys, Met, Phe, Pro, Thr, Trp, Tyr, or Val; Val25 may be independently substituted for for Ala, Arg, Asn, Asp, Asx, Cys, Glu, Gln, Glx, Gly, His, Ile, Leu, Lys, Met, Phe, Pro, Ser, Thr, Trp, Tyr, or Val; Lys26 may be independently substituted for for Ala, Arg, Asn, Asp, Asx, Cys, Glu, Gln, Glx, Gly, His, Ile, Leu, Lys, Met, Phe, Pro, Ser, Thr, Trp, or Tyr; and Gly27 may be independently substituted for for Ala, Arg, Asn, Asp, Asx, Cys, Glu, Gln, Glx, Gly, His, Ile, Leu, Lys, Met, Phe, Pro, Ser, Thr, Trp, Tyr, or Val.

[0280] VHCDR3 sequence ASPYLNYFDY (SEQ ID No:20) can be Ala Ser Pro Tyr Leu Asn Tyr Phe Asp Tyr and numbered as Ala30 Ser31 Pro32 Tyr33 Leu34 Asn35 Tyr36 Phe37 Asp38 Tyr39. The paratopes of the VHCDR3 sequence share Ser31, Tyr33, Leu34, Asn35, Asp38, and Tyr39 over all paratopes. As such, Ala30, Pro32, Tyr36, and Phe37 may be individually substituted for any amino acid residue. For example, Ala30 may be independently substituted for Arg, Asn, Asp, Asx, Cys, Glu, Gln, Glx, Gly, His, Ile, Leu, Lys, Met, Phe, Pro, Ser, Thr, Trp, Tyr, or Val; Pro32 may be independently substituted for Ala, Arg, Asn, Asp, Asx, Cys, Glu, Gln, Glx, Gly, His, Ile, Leu, Lys, Met, Phe, Ser, Thr, Trp, Tyr, or Val; Tyr36 may be independently substituted for

Ala, Arg, Asn, Asp, Asx, Cys, Glu, Gln, Glx, Gly, His, Ile, Leu, Lys, Met, Phe, Pro, Ser, Thr, Trp, or Val; and Phe37 may be independently substituted for Ala, Arg, Asn, Asp, Asx, Cys, Glu, Gln, Glx, Gly, His, Ile, Leu, Lys, Met, Pro, Ser, Thr, Trp, Tyr, or Val.

[0281] In another embodiment the P1801 Variable Region Heavy chain alternative sequence with CDRs delineated illustrating the paratopes is

EVQLVESGGGLVQPGGSLRLSCAASGFTFSYDMSWVRQAPGKGLEWVATITGGGSYTYYPD
SVKGRFTISRDNKNSLYLQMNSLRAEDTAVYYCASPYNFYDWGQGTTLTVSS (SEQ ID
No:22)

Shown is the VHCDR1 alternative sequence GFTFSYDMS (SEQ ID No:21) can also be written as Gly Phe Thr Phe Ser Tyr Asp Met Ser and numbered as Gly1 Phe2 Thr3 Phe4 Ser5 Tyr6 Asp7 Met8 Ser9. The paratopes of the VHCDR1 sequence share Phe2, Ser5, and Tyr6 over all paratopes. As such, Gly1, Thr3, Phe4, Asp8, Met9, and Ser10 may be individually substituted for any amino acid residue. For example, Gly1 may be independently substituted for Ala, Arg, Asn, Asp, Asx, Cys, Glu, Gln, Glx, His, Ile, Leu, Lys, Met, Phe, Pro, Ser, Thr, Trp, Tyr, or Val; Thr3 may be independently substituted for Ala, Arg, Asn, Asp, Asx, Cys, Glu, Gln, Glx, Gly, His, Ile, Leu, Lys, Met, Phe, Pro, Ser, Trp, Tyr, or Val; Phe4 may be independently substituted for Ala, Arg, Asn, Asp, Asx, Cys, Glu, Gln, Glx, Gly, His, Ile, Leu, Lys, Met, Pro, Ser, Thr, Trp, Tyr, or Val; Asp7 may be independently substituted for Ala, Arg, Asn, Asp, Asx, Cys, Glu, Gln, Glx, Gly, His, Ile, Leu, Lys, Met, Phe, Pro, Ser, Thr, Trp, Tyr, or Val; Met8 may be independently substituted for Ala, Arg, Asn, Asp, Asx, Cys, Glu, Gln, Glx, Gly, His, Ile, Leu, Lys, Phe, Pro, Ser, Thr, Trp, Tyr, or Val; and Ser9 may be independently substituted for Ala, Arg, Asn, Asp, Asx, Cys, Glu, Gln, Glx, Gly, His, Ile, Leu, Lys, Met, Phe, Pro, Thr, Trp, Tyr, or Val.

[0282] VHCDR2 sequence TITGGGSYTYYPDSVKG (SEQ ID No:10) can be Thr Ile Thr Gly Gly Gly Ser Tyr Thr Tyr Tyr Pro Asp Ser Val Lys Gly and numbered as Thr11 Ile12 Thr13 Gly14 Gly15 Gly16 Ser17 Tyr18 Thr19 Tyr20 Tyr21 Pro22 Asp23 Ser24 Val25 Lys26 Gly27. The paratopes of the VHCDR2 sequence share Thr13, Gly14, Gly15, Gly16, Ser17, and Tyr18 over all paratopes. As such, Thr11, Ile12, Thr19, Tyr20, Tyr21, Pro22, Asp23, Ser24, Val25, Lys26, and Gly27 may be individually substituted for any amino acid residue. For example, Thr11 may be independently substituted for Ala, Arg, Asn, Asp, Asx, Cys, Glu, Gln, Glx, Gly, His, Ile, Leu, Lys, Met, Phe, Pro, Ser, Trp, Tyr, or Val; Ile12 may be independently substituted for for Ala, Arg, Asn, Asp, Asx, Cys, Glu, Gln, Glx, Gly, His, Leu, Lys, Met, Phe, Pro, Ser, Thr, Trp, Tyr, or Val; Thr19 may be independently substituted for for Ala, Arg, Asn, Asp, Asx, Cys, Glu, Gln, Glx, Gly, His, Ile, Leu, Lys, Met, Phe, Pro, Ser, Trp, Tyr, or Val; Tyr20 may be independently substituted for for Ala, Arg, Asn, Asp, Asx, Cys, Glu, Gln, Glx, Gly, His, Ile, Leu, Lys, Met, Phe, Pro, Ser, Thr, Trp, or Val; Tyr21 may be independently substituted for for Ala, Arg, Asn, Asp, Asx, Cys, Glu, Gln, Glx, Gly, His, Ile, Leu, Lys, Met, Phe, Pro, Ser, Thr, Trp, or Val; Pro22 may be independently substituted for for Ala, Arg, Asn, Asp, Asx, Cys, Glu, Gln, Glx, Gly, His, Ile, Leu, Lys, Met, Phe, Ser, Thr, Trp, Tyr, or Val; Asp23 may be independently substituted for for Ala, Arg, Asn, Asp, Asx, Cys, Glu, Gln, Glx, Gly, His, Ile, Leu, Lys, Met, Phe, Pro, Ser, Thr, Trp, Tyr, or Val; Ser24 may be independently substituted for for Ala, Arg, Asn, Asp, Asx, Cys, Glu, Gln, Glx, Gly, His, Ile, Leu, Lys, Met,

Phe, Pro, Thr, Trp, Tyr, or Val; Val25 may be independently substituted for for Ala, Arg, Asn, Asp, Asx, Cys, Glu, Gln, Glx, Gly, His, Ile, Leu, Lys, Met, Phe, Pro, Ser, Thr, Trp, Tyr, or Val; Lys26 may be independently substituted for for Ala, Arg, Asn, Asp, Asx, Cys, Glu, Gln, Glx, Gly, His, Ile, Leu, Lys, Met, Phe, Pro, Ser, Thr, Trp, or Tyr; and Gly27 may be independently substituted for for Ala, Arg, Asn, Asp, Asx, Cys, Glu, Gln, Glx, Gly, His, Ile, Leu, Lys, Met, Phe, Pro, Ser, Thr, Trp, Tyr, or Val.

[0283] VHCDR3 sequence ASPYLNIFYDY (SEQ ID No:20) can be Ala Ser Pro Tyr Leu Asn Tyr Phe Asp Tyr and numbered as Ala30 Ser31 Pro32 Tyr33 Leu34 Asn35 Tyr36 Phe37 Asp38 Tyr39. The paratopes of the VHCDR3 sequence share Ser31, Tyr33, Leu34, Asn35, Asp38, and Tyr39 over all paratopes. As such, Ala30, Pro32, Tyr36, and Phe37 may be individually substituted for any amino acid residue. For example, Ala30 may be independently substituted for Arg, Asn, Asp, Asx, Cys, Glu, Gln, Glx, Gly, His, Ile, Leu, Lys, Met, Phe, Pro, Ser, Thr, Trp, Tyr, or Val; Pro32 may be independently substituted for Ala, Arg, Asn, Asp, Asx, Cys, Glu, Gln, Glx, Gly, His, Ile, Leu, Lys, Met, Phe, Ser, Thr, Trp, Tyr, or Val; Tyr36 may be independently substituted for Ala, Arg, Asn, Asp, Asx, Cys, Glu, Gln, Glx, Gly, His, Ile, Leu, Lys, Met, Phe, Pro, Ser, Thr, Trp, or Val; and Phe37 may be independently substituted for Ala, Arg, Asn, Asp, Asx, Cys, Glu, Gln, Glx, Gly, His, Ile, Leu, Lys, Met, Pro, Ser, Thr, Trp, Tyr, or Val.

[0284] VHCDR3 sequence PYLNIFYDY (SEQ ID No:11) can be Pro Tyr Leu Asn Tyr Phe Asp Tyr and numbered as Pro32 Tyr33 Leu34 Asn35 Tyr36 Phe37 Asp38 Tyr39. The paratopes of the VHCDR3 sequence share Tyr33, Leu34, Asn35, Asp38, and Tyr39 over all paratopes. As such, Pro32, Tyr36, and Phe37 may be individually substituted for any amino acid residue. For example, Tyr36 may be independently substituted for Ala, Arg, Asn, Asp, Asx, Cys, Glu, Gln, Glx, Gly, His, Ile, Leu, Lys, Met, Phe, Pro, Ser, Thr, Trp, or Val; and Phe37 may be independently substituted for Ala, Arg, Asn, Asp, Asx, Cys, Glu, Gln, Glx, Gly, His, Ile, Leu, Lys, Met, Pro, Ser, Thr, Trp, Tyr, or Val.

[0285] VHCDR3 sequence SPYLNIFYDY (SEQ ID No:23) can be Ser Pro Tyr Leu Asn Tyr Phe Asp Tyr and numbered as Ser31 Pro32 Tyr33 Leu34 Asn35 Tyr36 Phe37 Asp38 Tyr39. The paratopes of the VHCDR3 sequence share Ser31, Tyr33, Leu34, Asn35, Asp38, and Tyr39 over all paratopes. As such, Pro32, Tyr36, and Phe37 may be individually substituted for any amino acid residue. For example, Pro32 may be independently substituted for Ala, Arg, Asn, Asp, Asx, Cys, Glu, Gln, Glx, Gly, His, Ile, Leu, Lys, Met, Phe, Ser, Thr, Trp, Tyr, or Val; Tyr36 may be independently substituted for Ala, Arg, Asn, Asp, Asx, Cys, Glu, Gln, Glx, Gly, His, Ile, Leu, Lys, Met, Phe, Pro, Ser, Thr, Trp, or Val; and Phe37 may be independently substituted for Ala, Arg, Asn, Asp, Asx, Cys, Glu, Gln, Glx, Gly, His, Ile, Leu, Lys, Met, Pro, Ser, Thr, Trp, Tyr, or Val.

[0286] In one embodiment the P1801 Variable Region light chain sequence with CDRs delineated illustrating the paratopes is

EIVLTQSPATLSLSPGERATLSCRASQSISNNLHWYQQKPGQAPRLLIYYASQSIGIPARFSGS
GSGTDFLTITISLEPEDFAVYYCQQSNSWPLTFGQGQTKLEIK (SEQ ID No:6).

Shown is VLCDR1 sequence RASQSISNNLH (SEQ ID No:12) can also be written as Arg Ala Ser Gln Ser Ile Ser Asn Asn Leu His and numbered as Arg1 Ala2 Ser3 Gln4 Ser5 Ile6 Ser7 Asn8 Asn9 Leu10 His11. The

paratopes of the VLCDR1 sequence share Asn9 over all paratopes. As such, Arg1, Ala2, Ser3, Gln4, Ser5, Ile6, Ser7, Asn8, Leu10, and His11 may be individually substituted for any amino acid residue. For example, Arg1 may be independently substituted for Ala, Asn, Asp, Asx, Cys, Glu, Gln, Glx, Gly, His, Ile, Leu, Lys, Met, Phe, Pro, Ser, Thr, Trp, Tyr, or Val; Ala2 may be independently substituted for Arg, Asn, Asp, Asx, Cys, Glu, Gln, Glx, Gly, His, Ile, Leu, Lys, Met, Phe, Pro, Ser, Thr, Trp, Tyr, or Val; Ser3 may be independently substituted for Ala, Arg, Asn, Asp, Asx, Cys, Glu, Gln, Glx, Gly, His, Ile, Leu, Lys, Met, Phe, Pro, Thr, Trp, Tyr, or Val; Gln4 may be independently substituted for Ala, Arg, Asn, Asp, Asx, Cys, Glu, Glx, Gly, His, Ile, Leu, Lys, Met, Phe, Pro, Ser, Thr, Trp, Tyr, or Val; Ser5 may be independently substituted for Ala, Arg, Asn, Asp, Asx, Cys, Glu, Gln, Glx, Gly, His, Ile, Leu, Lys, Met, Phe, Pro, Thr, Trp, Tyr, or Val; Ile6 may be independently substituted for Ala, Arg, Asn, Asp, Asx, Cys, Glu, Gln, Glx, Gly, His, Ile, Leu, Lys, Met, Phe, Pro, Ser, Thr, Trp, Tyr, or Val; Ser7 may be independently substituted for Ala, Arg, Asn, Asp, Asx, Cys, Glu, Gln, Glx, Gly, His, Ile, Leu, Lys, Met, Phe, Pro, Thr, Trp, Tyr, or Val; Asn8 may be independently substituted for Ala, Arg, Asp, Asx, Cys, Glu, Gln, Glx, Gly, His, Ile, Leu, Lys, Met, Phe, Pro, Ser, Thr, Trp, Tyr, or Val; and Leu10 may be independently substituted for Ala, Arg, Asn, Asp, Asx, Cys, Glu, Gln, Glx, Gly, His, Ile, Leu, Lys, Met, Phe, Pro, Ser, Thr, Trp, Tyr, or Val; and His11 may be independently substituted for Ala, Arg, Asn, Asp, Asx, Cys, Glu, Gln, Glx, Gly, Ile, Leu, Lys, Met, Phe, Pro, Ser, Thr, Trp, Tyr, or Val.

[0287] VLCDR2 sequence YASQSI (SEQ ID No:13) can be Tyr Ala Ser Gln Ser Ile Ser and numbered as Tyr12 Ala13 Ser14 Gln15 Ser16 Ile17 Ser18. The paratopes of the VLCDR2 sequence share Tyr12 and Ser18 over all paratopes. As such, Ala13, Ser14, Gln15, Ser16, and Ile17 may be individually substituted for any amino acid residue. For example, Ala13 may be independently substituted for Arg, Asn, Asp, Asx, Cys, Glu, Gln, Glx, Gly, His, Ile, Leu, Lys, Met, Phe, Pro, Ser, Thr, Trp, Tyr, or Val; Ser14 may be independently substituted for Ala, Arg, Asn, Asp, Asx, Cys, Glu, Gln, Glx, Gly, His, Ile, Leu, Lys, Met, Phe, Pro, Thr, Trp, Tyr, or Val; Gln15 may be independently substituted for Ala, Arg, Asn, Asp, Asx, Cys, Glu, Glx, Gly, His, Ile, Leu, Lys, Met, Phe, Pro, Ser, Thr, Trp, Tyr, or Val; Ser16 may be independently substituted for Ala, Arg, Asn, Asp, Asx, Cys, Glu, Gln, Glx, Gly, His, Ile, Leu, Lys, Met, Phe, Pro, Thr, Trp, Tyr, or Val; and Ile17 may be independently substituted for Ala, Arg, Asn, Asp, Asx, Cys, Glu, Gln, Glx, Gly, His, Ile, Leu, Lys, Met, Phe, Pro, Ser, Thr, Trp, Tyr, or Val.

[0288] VLCDR3 sequence QQSNWPLT (SEQ ID No:14) can be Gln Gln Ser Asn Ser Trp Pro Leu Thr and numbered as Gln20 Gln21 Ser22 Asn23 Ser24 Trp25 Pro26 Leu27 Thr28.

[0289] The paratopes of the VLCDR3 sequence share Ser22, Asn23, and Trp25 over all paratopes. As such, Gln20, Gln21, Ser24, Pro26, Leu27, and Thr28 may be individually substituted for any amino acid residue. For example, Gln20 may be independently substituted for Ala, Arg, Asn, Asp, Asx, Cys, Glu, Glx, Gly, His, Ile, Leu, Lys, Met, Phe, Pro, Ser, Thr, Trp, Tyr, or Val; Gln21 may be independently substituted for Ala, Arg, Asn, Asp, Asx, Cys, Glu, Glx, Gly, His, Ile, Leu, Lys, Met, Phe, Pro, Ser, Thr, Trp, Tyr, or Val; Ser24 may be independently substituted for Ala, Arg, Asn, Asp, Asx, Cys, Glu, Gln, Glx, Gly, His, Ile, Leu, Lys, Met, Phe, Pro, Thr, Trp, Tyr, or Val; Pro26 may be independently substituted for Ala, Arg, Asn, Asp, Asx, Cys, Glu, Gln,

Glx, Gly, His, Ile, Leu, Lys, Met, Phe, Ser, Thr, Trp, Tyr, or Val; Leu27 may be independently substituted for Ala, Arg, Asn, Asp, Asx, Cys, Glu, Gln, Glx, Gly, His, Ile, Lys, Met, Phe, Pro, Ser, Thr, Trp, Tyr, or Val; and Thr28 may be independently substituted for Ala, Arg, Asn, Asp, Asx, Cys, Glu, Gln, Glx, Gly, His, Ile, Leu, Lys, Met, Phe, Pro, Ser, Trp, Tyr, or Val.

Table 15

Name	Paratope Sequence
VHCDR1	<u>SY</u> DMS (SEQ ID No:9)
VHCDR1	<u>SSY</u> DMS (SEQ ID No:18)
VHCDR1	G <u>FTF</u> <u>SSY</u> DMS (SEQ ID No:19)
VHCDR1	G <u>FTF</u> <u>S</u> YDMS (SEQ ID No:21)
VHCDR2	TITGGGS <u>Y</u> TYYPDSVKG (SEQ ID No:10)
VHCDR3	P <u>Y</u> L <u>N</u> YFD <u>Y</u> (SEQ ID No:11)
VHCDR3	A <u>S</u> P <u>Y</u> L <u>N</u> YFD <u>Y</u> (SEQ ID No:20)
VHCDR3	<u>S</u> P <u>Y</u> L <u>N</u> YFD <u>Y</u> (SEQ ID No:23)
VLCDR1	<u>R</u> ASQSIS <u>N</u> <u>N</u> LH (SEQ ID No:12)
VLCDR2	<u>Y</u> ASQSIS (SEQ ID No:13)
VLCDR3	QQ <u>S</u> <u>N</u> <u>S</u> WPLT (SEQ ID No:14)

In the paratope sequences listed in Table 15 the amino acid residues presented in bold and underlined are constant over the various proteins. The remaining amino acid residues in the sequences may be individually substituted for any amino acid residue, e.g., Ala, Arg, Asn, Asp, Asx, Cys, Glu, Gln, Glx, Gly, His, Ile, Leu, Lys, Met, Phe, Pro, Ser, Thr, Trp, Tyr, and Val. Provided the bolded and underlined residues in the CDR's are maintained the remaining amino acid residues may be individually substituted while maintaining function. As such, the present disclosure envisions numerous variations in the sequence of the CDRs provided the bolded and underlined residues in the CDR's are maintained with one or more of the remaining amino acid residues being substituted. Given this functional definition the current inventors contemplate a plethora of different CDR sequences characterized by the bolded and underlined residues in the CDR, one more or substituted amino acids and the retention of the function of the sequence.

[0290] It is contemplated that any embodiment discussed in this specification can be implemented with respect to any method, kit, reagent, or composition of the disclosure, and vice versa. Furthermore, compositions of the disclosure can be used to achieve methods of the disclosure.

[0291] It will be understood that particular embodiments described herein are shown by way of illustration and not as limitations of the disclosure. The principal features of this disclosure can be employed in various embodiments without departing from the scope of the disclosure. Those skilled in the art will recognize, or be able to ascertain using no more than routine experimentation, numerous equivalents to the specific procedures described herein. Such equivalents are considered to be within the scope of this disclosure and are covered by

the claims.

[0292] All publications and patent applications mentioned in the specification are indicative of the level of skill of those skilled in the art to which this disclosure pertains. All publications and patent applications are herein incorporated by reference to the same extent as if each individual publication or patent application was specifically and individually indicated to be incorporated by reference.

[0293] The use of the word “a” or “an” when used in conjunction with the term “comprising” in the claims and/or the specification may mean “one,” but it is also consistent with the meaning of “one or more,” “at least one,” and “one or more than one.” The use of the term “or” in the claims is used to mean “and/or” unless explicitly indicated to refer to alternatives only or the alternatives are mutually exclusive, although the disclosure supports a definition that refers to only alternatives and “and/or.” Throughout this application, the term “about” is used to indicate that a value includes the inherent variation of error for the device, the method being employed to determine the value, or the variation that exists among the study subjects.

[0294] As used in this specification and claim(s), the words “comprising” (and any form of comprising, such as “comprise” and “comprises”), “having” (and any form of having, such as “have” and “has”), “including” (and any form of including, such as “includes” and “include”) or “containing” (and any form of containing, such as “contains” and “contain”) are inclusive or open-ended and do not exclude additional, unrecited elements or method steps.

[0295] The term “or combinations thereof” as used herein refers to all permutations and combinations of the listed items preceding the term. For example, “A, B, C, or combinations thereof” is intended to include at least one of: A, B, C, AB, AC, BC, or ABC, and if order is important in a particular context, also BA, CA, CB, CBA, BCA, ACB, BAC, or CAB. Continuing with this example, expressly included are combinations that contain repeats of one or more item or term, such as BB, AAA, AB, BBC, AAABCCCC, CBBAAA, CABABB, and so forth. The skilled artisan will understand that typically there is no limit on the number of items or terms in any combination, unless otherwise apparent from the context.

[0296] All of the compositions and/or methods disclosed and claimed herein can be made and executed without undue experimentation in light of the present disclosure. While the compositions and methods of this disclosure have been described in terms of preferred embodiments, it will be apparent to those of skill in the art that variations may be applied to the compositions and/or methods and in the steps or in the sequence of steps of the method described herein without departing from the concept, spirit and scope of the disclosure. All such similar substitutes and modifications apparent to those skilled in the art are deemed to be within the spirit, scope and concept of the disclosure as defined by the appended claims.

CLAIMS

1. An isolated antibody or an antigen-binding portion thereof that preferentially binds PD-1, comprising:
a heavy chain complementarity determining region one (VHCDR1) including the amino acid sequence as recited in SEQ ID NO: 9, SEQ ID NO: 18, SEQ ID NO: 19 or SEQ ID NO: 21, a heavy chain complementarity determining region two (VHCDR2) including the amino acid sequence as recited in SEQ ID NO: 10, and/or a heavy chain complementarity determining region three (VHCDR3) including the amino acid sequence as recited in SEQ ID NO: 11, SEQ ID NO:20 or SEQ ID NO:23; and
a light chain complementarity determining region one (VLCDR1) including the amino acid sequence as recited in SEQ ID NO: 12, a light chain complementarity determining region two (VLCDR2) including the amino acid sequence as recited in SEQ ID NO: 13, and/or a light chain complementarity determining region three (VLCDR3) including the amino acid sequence as recited in and SEQ ID NO: 14.
2. An isolated antibody or an antigen-binding portion thereof that preferentially binds PD-1 comprising one or more paratopes comprising:
at least one of the following residues: Phe2, Ser5, Ser6, and Tyr7 of a heavy chain variable sequence (VHCDR1) of SEQ ID NO: 19;
at least one of the following residues: Phe2, Ser5, and Tyr6 of a heavy chain variable sequence (VHCDR1) of SEQ ID NO: 21;
at least one of the following residues: Thr13, Gly14, Gly15, Gly16, Ser17, and Tyr18 of a heavy chain variable sequence (VHCDR2) of SEQ ID NO: 10;
at least one of the following residues: Tyr33, Leu34, Asn35, Asp38, and Tyr39 of a heavy chain variable sequence (VHCDR3) of SEQ ID NO: 11; or
at least one of the following residues: Ser31, Tyr33, Leu34, Asn35, Asp38, and Tyr39 of a heavy chain variable sequence (VHCDR3) of SEQ ID NO: 20.
3. An isolated antibody or an antigen-binding portion thereof that preferentially binds PD-1 comprising one or more paratopes comprising:
at least one of the following residues: Asn9 of a light chain variable sequence (VLCDR1) of SEQ ID NO: 12;
at least one of the following residues: Tyr12 and Ser18 of a heavy chain variable sequence (VLCDR2) of SEQ ID NO: 13; or
at least one of the following residues: Ser22, Asn23, and Trp25 of a heavy chain variable sequence (VLCDR3) of SEQ ID NO: 14.
4. An isolated antibody or an antigen-binding portion thereof that preferentially binds PD-1 comprising one or more paratopes comprising:

at least one of the following residues: Phe2, Ser5, Ser6, and Tyr7 of a heavy chain variable sequence (VHCDR1) of SEQ ID NO: 19;

at least one of the following residues: Phe2, Ser5, and Tyr6 of a heavy chain variable sequence (VHCDR1) of SEQ ID NO: 21;

at least one of the following residues: Thr13, Gly14, Gly15, Gly16, Ser17, and Tyr18 of a heavy chain variable sequence (VHCDR2) of SEQ ID NO: 10;

at least one of the following residues: Tyr33, Leu34, Asn35, Asp38, and Tyr39 of a heavy chain variable sequence (VHCDR3) of SEQ ID NO: 11;

at least one of the following residues: Ser31, Tyr33, Leu34, Asn35, Asp38, and Tyr39 of a heavy chain variable sequence (VHCDR3) of SEQ ID NO: 20;

at least one of the following residues: Asn9 of a light chain variable sequence (VLCDR1) of SEQ ID NO: 12;

at least one of the following residues: Tyr12 and Ser18 of a heavy chain variable sequence (VLCDR2) of SEQ ID NO: 13; or

at least one of the following residues: Ser22, Asn23, and Trp25 of a heavy chain variable sequence (VLCDR3) of SEQ ID NO: 14.

5. An isolated antibody or an antigen-binding portion thereof that preferentially binds PD-1 comprising:
 - (i) a heavy chain variable region (VH) comprising SEQ ID NO: 9 and a light chain variable region (VL) comprising SEQ ID NO: 6; or
 - (ii) a heavy chain variable region (VH) comprising SEQ ID NO: 18 and a light chain variable region (VL) comprising SEQ ID NO: 6; or
 - (iii) a heavy chain variable region (VH) comprising SEQ ID NO: 19 and a light chain variable region (VL) comprising SEQ ID NO: 6; or
 - (iv) a heavy chain variable region (VH) comprising SEQ ID NO: 21 and a light chain variable region (VL) comprising SEQ ID NO: 6.
6. An isolated antibody or an antigen-binding portion thereof preferentially binds PD-1 comprising
 - (i) a heavy chain variable region (VH) comprising SEQ ID NO: 5 or a heavy chain variable region (VH) comprising SEQ ID NO: 22; and
 - (ii) a light chain variable region (VL) comprising SEQ ID NO: 6.
7. An isolated antibody or an antigen-binding portion thereof that preferentially binds PD-1, comprising:
a heavy chain complementarity determining region one (VHCDR1), a heavy chain complementarity determining region two (VHCDR2), and/or a heavy chain complementarity determining region three (VHCDR3) obtained from a heavy chain variable region amino acid sequence as recited in SEQ ID NO: 5; and
a light chain complementarity determining region one (VLCDR1), a light chain complementarity

determining region two (VLCDR2), and/or a light chain complementarity determining region three (VLCDR3) obtained from a light chain variable region amino acid sequence as recited in SEQ ID NO: 6.

8. The antibody or the antigen-binding portion thereof in any one of claims 1 and 4-7, further comprising a heavy chain constant region (HCCR) including the amino acid sequence recited in SEQ ID NO: 7;

wherein the HCCR optionally includes one, two, or three conservatively modified amino acid substitutions.

9. The antibody or the antigen-binding portion thereof in any one of claims 1 and 4-7, further comprising a light chain constant region (LCCR) including the amino acid sequence of SEQ ID No: 8; wherein the LCCR optionally includes one, two, or three conservatively modified amino acid substitutions.

10. The antibody or the antigen-binding portion thereof in any one of claims 1 and 4-7, further comprising a heavy chain constant region (HCCR) including the amino acid sequence recited in SEQ ID NO: 7, wherein said HCCR optionally includes one, two, or three conservatively modified amino acid substitutions; and

a light chain constant region (LCCR) including the amino acid sequence of SEQ ID No:8, wherein the LCCR optionally includes one, two, or three conservatively modified amino acid substitutions.

11. The antibody or the antigen-binding portion thereof of claim 10, further comprising a heavy chain signal peptide (HCSP) including amino acids recited in SEQ ID NO: 15 on the N terminus of the VH.

12. The antibody or the antigen-binding portion thereof of claim 10, further comprising a light chain signal peptide (LCSP) including amino acids recited in SEQ ID NO: 16 on the N terminus of the VL.

13. The antibody or the antigen-binding portion thereof in any one of claims 1-7, wherein the antibody is human or humanized.

14. The antibody or the antigen-binding portion thereof in any one of claims 1-7, wherein the antibody is IgG4 isotype.

15. A pharmaceutical composition for treating, suppressing and/or inhibiting a solid tumor comprising
(i) the antibody or the antigen-binding portion thereof in any one of claims 1-7; and
(ii) a pharmaceutically accepted carrier.

16. A polynucleotide molecule encoding the antibody or the antigen-binding portion in any one of claims 1-7.

17. An expression vector comprising the polynucleotide molecule of claim 16, wherein said expression vector which encodes LCSP does not comprise an intron.

18. A host cell comprising the expression vector of claim 17.
19. A method of preventing, treating and/or suppressing a tumor in a subject, comprising the steps of:
administering an effective amount of the antibody or the antigen-binding portion of claims 1-7 to the subject.
20. The method of claim 19, wherein the tumor includes Hepatocellular carcinoma, or solid tumor comprising melanoma and/or renal cell carcinoma (RCC).
21. A method of regulating the activity of one or more T cells *in vitro*, *ex vivo*, and/or *in vivo* in a subject, the method comprises the steps of:
contacting the T cell with an antibody or an antigen-binding portion thereof which preferentially binds to human programmed death receptor 1 (hPD-1), wherein the antibody blocks and/or regulates the binding of human PD-L1 and/or human PD-L2 to hPD-1, and wherein said antibody or an antigen-binding portion thereof includes:
a heavy chain complementarity determining region one (VHCDR1) including the amino acid sequences as recited in SEQ ID NO: 9, SEQ ID NO: 18, SEQ ID NO: 19 or SEQ ID NO: 21, a heavy chain complementarity determining region two (VHCDR2) including the amino acid sequences as recited in SEQ ID NO: 10, and/or a heavy chain complementarity determining region three (VHCDR3) including the amino acid sequences as recited in SEQ ID NO: 11, SEQ ID NO:20 or SEQ ID NO:23; and
a light chain complementarity determining region one (VLCDR1) including the amino acid sequences as recited in SEQ ID NO: 12, a light chain complementarity determining region two (VLCDR2) including the amino acid sequences as recited in SEQ ID NO: 13, and/or a light chain complementarity determining region three (VLCDR3) including the amino acid sequences as recited in and SEQ ID NO: 14.
22. The method of claim 21, wherein said antibody or an antigen-binding portion thereof further comprises a heavy chain constant region (HCCR) including the amino acid sequence recited in SEQ ID NO: 7, and the HCCR optionally include one, two, or three conservatively modified amino acid substitutions.
23. The method of claim 21, wherein said antibody or an antigen-binding portion thereof further comprises a light chain constant region (LCCR) including the amino acid sequence of SEQ ID No: 8, and wherein the LCCR optionally include one, two, or three conservatively modified amino acid substitutions.
24. The method of claim 21, wherein said one or more T cells comprises one or more natural killer cells, one or more cytotoxic T cells, one or more helper T cells, one or more monocyte cells, one or more memory T cells, and/or one or more regulatory T cells.
25. The method of claim 21, wherein the antibody or antigen-binding portion blocks binding of human

PD-L1 and human PD-L2 to human PD-1 with an IC₅₀ of about 1 nM or lower.

26. A process for producing an antibody or an antigen-binding portion vector construct comprising culturing the host cell of claim 18 under conditions allowing the expression of said antibody or antigen-binding portion construct; and
purifying and/or recovering the produced antibody or antigen-binding portion vector construct from the culture.

27. An antibody or an antigen-binding portion thereof for use in the treatment of solid tumor which comprises:

- a. a heavy chain variable region (VH) comprising SEQ ID NO: 5 or a heavy chain variable region (VH) comprising SEQ ID NO: 22;
- b. a light chain variable region (VL) comprising SEQ ID NO: 6; and

optionally comprises another therapeutic agent.

28. The antibody or an antigen-binding portion thereof of claim 27, further comprising: a heavy chain constant region (HCCR) including the amino acid sequence recited in SEQ ID NO: 7, wherein the HCCR optionally includes one, two, or three conservatively modified amino acid substitutions; and a light chain constant region (LCCR) including the amino acid sequence of SEQ ID No: 8, and wherein the LCCR optionally includes one, two, or three conservatively modified amino acid substitutions; and
optionally comprises another therapeutic agent.

29. The antibody or an antigen-binding portion thereof of claim 27, wherein said another therapeutic agent comprises P1101, P1801, and/or another anti-human PD1 antibody.

30. The antibody or an antigen-binding portion thereof of claim 28, wherein said another therapeutic agent comprises P1101, P1801, and/or another anti-human PD1 monoclonal antibody.

31. The antibody or an antigen-binding portion thereof in any one of claims 28-30, wherein said solid tumor comprises Hepatocellular carcinoma, renal cell carcinoma and/or melanoma.

32. A combination for use in the treating, suppressing, preventing and/or inhibiting a solid tumor in a subject, wherein the combination comprises

- an antibody or an antigen-binding portion thereof; and
- another therapeutic agent,

wherein the antibody or an antigen-binding portion thereof includes:

- a. a heavy chain variable region (VH) comprising SEQ ID NO: 5 or a heavy chain variable region (VH) comprising SEQ ID NO: 22; and
- b. a light chain variable region (VL) comprising SEQ ID NO: 6.

33. The combination of claim 32, wherein said antibody or an antigen-binding portion thereof further comprises: a heavy chain constant region (HCCR) including the amino acid sequence recited in SEQ ID NO: 7, wherein the HCCR optionally includes one, two, or three conservatively modified amino acid substitutions; and a light chain constant region (LCCR) including the amino acid sequence of SEQ ID No: 8, and wherein the LCCR optionally includes one, two, or three conservatively modified amino acid substitutions.
34. The combination of any one of claims 32-33, wherein said therapeutic agent comprises P1101, P1801, and/or another anti-human PD1 monoclonal antibody.
35. The combination of claim 34, wherein said P1101, P1801, and/or another anti-human PD1 monoclonal antibody is administered serially and/or about simultaneously.
36. The combination of claim 35, wherein said P1101, and/or P1801, is administered before another anti-human PD1 monoclonal antibody.
37. The combination of claim 32 or 33, wherein said antibody or an antigen-binding portion thereof and another therapeutic agent are administered in a single composition or as separate compositions.
38. The combination of any one of claims 32-37, wherein said solid tumor includes Hepatocellular carcinoma, melanoma and/or renal cell carcinoma.
39. A recombinant protein which comprises
- (i) the antibody or an antigen-binding portion of claim 1; and
 - (ii) an optional tag sequence for expression detection and/or purification.
40. The isolated antibody or an antigen-binding portion thereof according to any one of the claims 1-2, 5-7 or 27 for use in a method of treating cancer, the method comprising administering the isolated antibody or an antigen-binding portion thereof to a subject.
41. The isolated antibody or an antigen-binding portion thereof according to claim 40, wherein said cancer comprises Hepatocellular carcinoma, melanoma and/or renal cell carcinoma.
42. The use of the isolated antibody or an antigen-binding portion thereof according to any one of the claims 1-2, 5-7 or 27 for the manufacture of a medicament for the therapeutic and/or prophylactic treatment of cancer.
43. The use of the isolated antibody or an antigen-binding portion thereof according to claim 42, wherein said cancer comprises Hepatocellular carcinoma, melanoma and/or renal cell carcinoma.