

al., *Science*, 355: 1428-1433 (2017)). PD-1 deficiency can lead to autoimmunity. For example, C57BL/6 PD-1 knockout mice have been shown to develop a lupus-like syndrome (see, e.g., Nishimura et al., *Immunity*, 11: 141-151 (1999)). In humans, a single nucleotide polymorphism in the PD-1 gene is associated with higher incidences of systemic lupus erythematosus, type 1 diabetes, rheumatoid arthritis, and progression of multiple sclerosis (see, e.g., Nielsen et al., *Tissue Antigens*, 62(6): 492-497 (2003); Bertsias et al., *Arthritis Rheum.*, 60(1): 207-218 (2009); Ni et al., *Hum. Genet.*, 121(2): 223-232 (2007); Tahoori et al., *Clin. Exp. Rheumatol.*, 29(5): 763-767 (2011); and Kroner et al., *Ann. Neurol.*, 58(1): 50-57 (2005)).

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

BRIEF SUMMARY ~~OF THE INVENTION~~ DISCLOSURE

[0006] The ~~invention~~ disclosure provides an agonistic PD-1 binding agent. In one aspect, the present disclosure provides an anti-PD-1 binding agent comprising (a) an immunoglobulin heavy chain variable region of any one of SEQ ID NOs: 43-47 or 61-63, or at least the CDRs thereof, and (b) an immunoglobulin light chain variable region of any one of SEQ ID NOs: 48-50, or at least the CDRs thereof. In one ~~embodiment~~ example, the PD-1 binding agent comprises an immunoglobulin heavy chain variable region and an immunoglobulin light chain variable region, wherein the immunoglobulin heavy chain variable region comprises: a CDR1 comprising SEQ ID NO: 1; a CDR2 comprising SEQ ID NO: 2; and a CDR3 comprising SEQ ID NO: 3; and the immunoglobulin light chain variable region comprises a CDR1 comprising SEQ ID NO: 4; a CDR2 comprising SEQ ID NO: 5; and a CDR3 comprising SEQ ID NO: 6.

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

[0008] In another aspect, the PD-1 binding agent comprises an immunoglobulin heavy chain variable region and an immunoglobulin light chain variable region, wherein the immunoglobulin heavy chain variable region comprises: a CDR1 comprising SEQ ID NO: 7; a CDR2 comprising SEQ ID NO: 8; and a CDR3 comprising SEQ ID NO: 9; and the immunoglobulin light chain variable region comprises a CDR1 comprising SEQ ID NO: 10; a CDR2 comprising SEQ ID NO: 11; and a CDR3 comprising SEQ ID NO: 12.

[0008a] In another aspect, the present disclosure provides a pharmaceutical composition comprising (a) anti-PD-1 binding agent as described herein, and (b) a pharmaceutically acceptable carrier. In another aspect, the present disclosure provides a nucleic acid encoding the immunoglobulin heavy chain and/or immunoglobulin light chain of the anti-PD-1 binding agent as described herein, optionally in a vector. In yet another aspect, the present disclosure provides a cell that expresses the anti-PD-1 binding agent as described herein.

[0008b] In a further aspect, the present disclosure provides a method of preparing the anti-PD-1 binding agent as described herein, the method comprising expressing in a cell *in vitro* a nucleic acid sequence encoding the immunoglobulin heavy chain and a nucleic acid sequence encoding the immunoglobulin light chain of the anti-PD-1 binding agent as described herein.

[0008c] In a further aspect, the present disclosure provides a use of the anti-PD-1 binding agent as described herein, or the pharmaceutical composition as described herein in the manufacture of a medicament for inhibiting an immune response in a mammal. In a further aspect, the present disclosure provides a use of the anti-PD-1 binding agent as described herein, or the pharmaceutical composition as described herein in the manufacture of a medicament for treating an inflammatory or autoimmune disorder in a mammal.

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

[0010] In addition, the ~~invention~~disclosure provides isolated or purified nucleic acid sequences encoding the foregoing immunoglobulin polypeptides, vectors comprising such nucleic acid sequences, isolated PD-1-binding agents comprising the foregoing immunoglobulin polypeptides, nucleic acid sequences encoding such PD-1-binding agents, vectors comprising such nucleic acid sequences, isolated cells comprising such vectors,

compositions comprising such PD-1-binding agents or such vectors with a pharmaceutically acceptable carrier, and methods inhibiting an immune response and treating inflammatory or autoimmune disorders in mammals by administering effective amounts of such compositions to mammals.

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

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

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

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

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

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

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

CLAIMS

1. An anti-PD-1 binding agent comprising (a) an immunoglobulin heavy chain variable region of any one of SEQ ID NOs: 43-47 or 61-63, or at least the CDRs thereof, and (b) an immunoglobulin light chain variable region of any of any one of SEQ ID NOs: 48-50, or at least the CDRs thereof.

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

(a) the immunoglobulin heavy chain variable region comprises:

_____ a CDR1 comprising SEQ ID NO: 1;

_____ a CDR2 comprising SEQ ID NO: 2; and

_____ a CDR3 comprising SEQ ID NO: 3;

~~and the immunoglobulin light chain variable region comprises~~

_____ a CDR1 comprising SEQ ID NO: 4;

_____ a CDR2 comprising SEQ ID NO: 5; and

_____ a CDR3 comprising SEQ ID NO: 6;

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

~~(c) the immunoglobulin heavy chain variable region comprises:~~

a CDR1 comprising SEQ ID NO: 7;

a CDR2 comprising SEQ ID NO: 8; and

a CDR3 comprising SEQ ID NO: 9;

and

(b) the immunoglobulin light chain variable region comprises

a CDR1 comprising SEQ ID NO: 10;

a CDR2 comprising SEQ ID NO: 11; and

a CDR3 comprising SEQ ID NO: 12;

or

~~(d) — the immunoglobulin heavy chain variable region comprises at least 80% sequence identity to any one of SEQ ID NOs: 43-47 or 61-63, and/or comprises at least the CDR regions of any one of SEQ ID NOs: 43-47 or 61-63, and the immunoglobulin light chain variable region comprises at least 80% sequence identity to SEQ ID NOs: 48-50, and/or comprises at least the CDR regions of any one of SEQ ID NOs: 48-50.~~

3. The anti-PD-1 binding agent of claim 1 or 2, wherein the immunoglobulin heavy chain variable region comprises at least 90% sequence identity to any one of SEQ ID NOs: 43-47 or 61-63.

4. The anti-PD-1 binding agent of any of claims 1-3, wherein the immunoglobulin light chain variable region comprises at least 90% sequence identity to SEQ ID NOs: 48-50.

5. The anti-PD-1 binding agent of any of claims 1-4, wherein the immunoglobulin heavy chain variable region comprises any one of SEQ ID NOs: 43-47 or 61-63.

6. The anti-PD-1 binding agent of any of claims 1-5, wherein the immunoglobulin light chain variable region comprises any one of SEQ ID NOs: 48-50.

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

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

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

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

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

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

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

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

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

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

~~—— a CDR1 comprising SEQ ID NO: 15;~~
~~—— a CDR2 comprising SEQ ID NO: 2; and~~
~~—— a CDR3 comprising SEQ ID NO: 20;~~
~~and the immunoglobulin light chain variable region comprises~~
~~—— a CDR1 comprising SEQ ID NO: 23;~~
~~—— a CDR2 comprising SEQ ID NO: 5; and~~
~~—— a CDR3 comprising SEQ ID NO: 6;~~

~~or~~

~~wherein the immunoglobulin heavy chain variable region comprises:~~

~~—— a CDR1 comprising SEQ ID NO: 15;~~
~~—— a CDR2 comprising SEQ ID NO: 2; and~~
~~—— a CDR3 comprising SEQ ID NO: 21;~~
~~and the immunoglobulin light chain variable region comprises~~
~~—— a CDR1 comprising SEQ ID NO: 23;~~

~~—— a CDR2 comprising SEQ ID NO: 5; and~~

~~—— a CDR3 comprising SEQ ID NO: 6;~~

~~or~~

~~wherein the immunoglobulin heavy chain variable region comprises:~~

~~—— a CDR1 comprising SEQ ID NO: 13;~~

~~—— a CDR2 comprising SEQ ID NO: 2; and~~

~~—— a CDR3 comprising SEQ ID NO: 19;~~

~~and the immunoglobulin light chain variable region comprises~~

~~—— a CDR1 comprising SEQ ID NO: 23;~~

~~—— a CDR2 comprising SEQ ID NO: 5; and~~

~~—— a CDR3 comprising SEQ ID NO: 6.~~

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

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

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

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

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

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

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

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

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

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

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

~~——a CDR1 comprising SEQ ID NO: 7;~~

~~——a CDR2 comprising SEQ ID NO: 8; and~~

~~——a CDR3 comprising SEQ ID NO: 9;~~

~~and the immunoglobulin light chain variable region comprises~~

~~——a CDR1 comprising SEQ ID NO: 10;~~

~~——a CDR2 comprising SEQ ID NO: 11; and~~

~~——a CDR3 comprising SEQ ID NO: 12.~~

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

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

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

~~—— a CDR1 comprising SEQ ID NO: 57;~~
~~—— a CDR2 comprising SEQ ID NO: 42; and~~
~~—— a CDR3 comprising SEQ ID NO: 9;~~
~~and the immunoglobulin light chain variable region comprises~~
~~—— a CDR1 comprising SEQ ID NO: 10;~~
~~—— a CDR2 comprising SEQ ID NO: 11; and~~
~~—— a CDR3 comprising SEQ ID NO: 12;~~

~~or~~

~~wherein the immunoglobulin heavy chain variable region comprises:~~

~~—— a CDR1 comprising SEQ ID NO: 57;~~
~~—— a CDR2 comprising SEQ ID NO: 41; and~~
~~—— a CDR3 comprising SEQ ID NO: 9;~~
~~and the immunoglobulin light chain variable region comprises~~
~~—— a CDR1 comprising SEQ ID NO: 10;~~
~~—— a CDR2 comprising SEQ ID NO: 11; and~~
~~—— a CDR3 comprising SEQ ID NO: 12.~~

217. The anti-PD-1 binding agent of any of claims 1-6, wherein the immunoglobulin heavy chain variable region comprises at least 90% sequence identity to SEQ ID NO: 47, or comprises at least the CDR regions of SEQ ID NO: 47;

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

8. The anti-PD-1 binding agent of any of claims 1-6, ~~or~~ wherein the immunoglobulin heavy chain variable region comprises at least 90% sequence identity to SEQ ID NO: 46, or comprises at least the CDR regions of SEQ ID NO: 46;

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

229. The anti-PD-1 binding agent of any of claims 1-6~~claim 21~~, comprising the immunoglobulin heavy chain variable region of SEQ ID NO: 47, and the immunoglobulin light chain variable region of SEQ ID NO: 49;~~;~~

10. The anti-PD-1 binding agent of any of claims 1-6, or comprising the immunoglobulin heavy chain variable region of SEQ ID NO: 46, and the immunoglobulin light chain variable region of SEQ ID NO: 50.

1123. The anti-PD-1 binding agent of any one of claims 1-1022, wherein the anti-PD-1 binding agent is an antibody, an antibody conjugate, or an antigen-binding antibody fragment ~~thereof~~.

1224. The anti-PD-1 binding agent of any one of claims 1-1023, wherein the anti-PD-1 binding agent is a F(ab')₂, Fab', Fab, Fv, scFv, dsFv, or a diabody~~single chain binding polypeptide~~.

1325. The anti-PD-1 binding agent of any one of claims 1-1224, wherein the anti-PD-1 binding agent comprises an IgG Fc region that binds an Fc receptor on an antigen presenting cell.

1426. The anti-PD-1 binding agent of any one of claims 1-1225, wherein the anti-PD-1 binding agent comprises an Fc region of IgG1 or other Fc region that binds FcγR.

1527. The anti-PD-1 binding agent of claim 1 or 2, comprising an immunoglobulin heavy chain comprising SEQ ID NO: 36 and an immunoglobulin light chain comprising SEQ ID NO: 37.

1628. The anti-PD-1 binding agent of claim 1 or 2, comprising an immunoglobulin heavy chain comprising SEQ ID NO: 51 and an immunoglobulin light chain comprising SEQ ID NO: 52.

1729. A pharmaceutical composition comprising (a) anti-PD-1 binding agent of any one of claims 1-1628, and (b) a pharmaceutically acceptable carrier.

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

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

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

1833. A nucleic acid encoding the immunoglobulin heavy chain and/or immunoglobulin light chain of the anti-PD-1 binding agent of any of claims 1-1628, optionally in a vector.

1934. A cell that expresses the anti-PD-1 binding agent of any of claims 1-1628.

2035. A method of preparing the anti-PD-1 binding agent of any one of claims 1-1628, the method comprising expressing in a cell in vitro a nucleic acid sequence encoding the immunoglobulin heavy chain and a nucleic acid sequence encoding the immunoglobulin light chain of the anti-PD-1 binding agent of any of claims 1-1628.

2136. Use of tThe anti-PD-1 binding agent of any of claims 1-1628, or the pharmaceutical composition of claim 17 in the manufacture of a medicament~~29~~, for inhibiting an immune response in a mammal.

2237. Use of tThe anti-PD-1 binding agent of any of claims 1-1628, or the pharmaceutical composition of claim 17 in the manufacture of a medicament~~29~~, for treating an inflammatory or autoimmune disorder in a mammal.

2332. The use anti-PD-1 binding agent or pharmaceutical composition of claim 2237, wherein the inflammatory or autoimmune disorder is Primary Biliary Cholangitis (PBC), Graft vs Host Disease (GvHD), Vitiligo, ANCA Vasculitis, Type 1 Diabetes, or Noninfectious Uveitis.