

[6] Antibody-mediated blockade of PD-1/PD-L1 interaction has entered clinical trials in the treatment of refractory solid tumors, including melanoma, renal cell carcinoma, colorectal cancer, non-small cell lung cancer, and hematologic malignancies. However, there remains a need of an optimal therapy for treating, stabilizing, preventing, and/or delaying the development of various cancers, especially in view of the resistance or relapse upon PD-1/PD-L1 blockade.

[7] The disclosures of all publications, patents, patent applications and published patent applications referred to herein are hereby incorporated herein by reference in their entirety.

SUMMARY

[8] The present disclosure relates to anti-PD-1 constructs comprising an sdAb moiety that specifically recognizes PD-1 (hereinafter referred to as “anti-PD-1 sdAb”), such as anti-PD-1 sdAb, anti-PD-1 HCAb e.g., anti-PD-1 sdAb-Fc fusion protein comprising an anti-PD-1 sdAb fused to a crystalline fragment (Fc) fragment of human immunoglobulin G (IgG), and multispecific (such as bispecific) antigen binding proteins comprising an anti-PD-1 sdAb fused to, for example, other sdAbs, a full-length four-chain antibody or antigen binding fragments thereof (e.g., Fab or scFv), and methods of making and using thereof.

[9] One example of the present application provides an isolated anti-PD-1 construct comprising an sdAb moiety specifically recognizing PD-1, wherein the sdAb moiety comprises a CDR1 comprising the amino acid sequence of any one of SEQ ID NOs: 37-72, or a variant thereof comprising up to about 3 (such as about any of 1, 2, or 3) amino acid substitutions; a CDR2 comprising the amino acid sequence of any one of SEQ ID NOs: 109-144, or a variant thereof comprising up to about 3 (such as about any of 1, 2, or 3) amino acid substitutions; and a CDR3 comprising the amino acid sequence of any one of SEQ ID NOs: 181-216, or a variant thereof comprising up to about 3 (such as about any of 1, 2, or 3) amino acid substitutions. In some examples, the isolated anti-PD-1 construct comprises an sdAb moiety specifically recognizing PD-1, wherein the sdAb moiety comprises a CDR1 comprising the amino acid sequence of any one of SEQ ID NOs: 37-72, or a variant thereof comprising up to about 3 (such as about any of 1, 2, or 3) amino acid substitutions; a CDR2 comprising the amino acid sequence of any one of SEQ ID NOs: 109-144, or a variant thereof comprising up to about 3 (such as about any of 1, 2, or 3) amino acid substitutions; and a CDR3 comprising the amino acid sequence of any one of SEQ ID NOs: 181-216. In some examples, the sdAb moiety specifically recognizing PD-1 comprises a CDR1 comprising the amino acid sequence of any one of SEQ ID NOs: 37-72; a CDR2 comprising the amino acid sequence of any one of SEQ ID NOs: 109-144; and a CDR3 comprising the amino acid sequence of

any one of SEQ ID NOs: 181-216; or a variant thereof comprising up to about 3 (such as about any of 1, 2, or 3) amino acid substitutions in the CDR regions. In some examples, the amino acid substitutions are in CDR1 and/or CDR2, wherein CDR3 comprises the amino acid sequence of any one of SEQ ID NOs: 181-216. In some examples, the sdAb moiety specifically recognizing PD-1 comprises a CDR1 comprising the amino acid sequence of any one of SEQ ID NOs: 37-72; a CDR2 comprising the amino acid sequence of any one of SEQ ID NOs: 109-144; and a CDR3 comprising the amino acid sequence of any one of SEQ ID NOs: 181-216.

[10] In some examples according to any one of the isolated anti-PD-1 constructs described above, the sdAb moiety specifically recognizing PD-1 comprises any one of the following:

(1) a CDR1 comprising the amino acid sequence of SEQ ID NO: 37, or a variant thereof comprising up to about 3 (such as about any of 1, 2, or 3) amino acid substitutions; a CDR2 comprising the amino acid sequence of SEQ ID NO: 109, or a variant thereof comprising up to about 3 (such as about any of 1, 2, or 3) amino acid substitutions; and a CDR3 comprising the amino acid sequence of SEQ ID NO: 181, or a variant thereof comprising up to about 3 (such as about any of 1, 2, or 3) amino acid substitutions;

(2) a CDR1 comprising the amino acid sequence of SEQ ID NO: 38, or a variant thereof comprising up to about 3 (such as about any of 1, 2, or 3) amino acid substitutions; a CDR2 comprising the amino acid sequence of SEQ ID NO: 110, or a variant thereof comprising up to about 3 (such as about any of 1, 2, or 3) amino acid substitutions; and a CDR3 comprising the amino acid sequence of SEQ ID NO: 182, or a variant thereof comprising up to about 3 (such as about any of 1, 2, or 3) amino acid substitutions;

(3) a CDR1 comprising the amino acid sequence of SEQ ID NO: 39, or a variant thereof comprising up to about 3 (such as about any of 1, 2, or 3) amino acid substitutions; a CDR2 comprising the amino acid sequence of SEQ ID NO: 111, or a variant thereof comprising up to about 3 (such as about any of 1, 2, or 3) amino acid substitutions; and a CDR3 comprising the amino acid sequence of SEQ ID NO: 183, or a variant thereof comprising up to about 3 (such as about any of 1, 2, or 3) amino acid substitutions;

(4) a CDR1 comprising the amino acid sequence of SEQ ID NO: 40, or a variant thereof comprising up to about 3 (such as about any of 1, 2, or 3) amino acid substitutions; a CDR2 comprising the amino acid sequence of SEQ ID NO: 112, or a variant thereof comprising up to about 3 (such as about any of 1, 2, or 3) amino acid substitutions; and a CDR3 comprising the amino acid sequence of SEQ ID NO: 184, or a variant thereof comprising up to about 3 (such as about any of 1, 2, or 3) amino acid substitutions;

(5) a CDR1 comprising the amino acid sequence of SEQ ID NO: 41, or a variant thereof comprising up to about 3 (such as about any of 1, 2, or 3) amino acid substitutions; a CDR2 comprising the amino acid

sequence of SEQ ID NO: 143, or a variant thereof comprising up to about 3 (such as about any of 1, 2, or 3) amino acid substitutions; and a CDR3 comprising the amino acid sequence of SEQ ID NO: 215, or a variant thereof comprising up to about 3 (such as about any of 1, 2, or 3) amino acid substitutions; or

(35) a CDR1 comprising the amino acid sequence of SEQ ID NO: 72, or a variant thereof comprising up to about 3 (such as about any of 1, 2, or 3) amino acid substitutions; a CDR2 comprising the amino acid sequence of SEQ ID NO: 144, or a variant thereof comprising up to about 3 (such as about any of 1, 2, or 3) amino acid substitutions; and a CDR3 comprising the amino acid sequence of SEQ ID NO: 216, or a variant thereof comprising up to about 3 (such as about any of 1, 2, or 3) amino acid substitutions.

[10a] In one aspect, the present disclosure refers to an isolated anti-PD-1 construct comprising a single-domain antibody (sdAb) moiety specifically recognizing PD-1 (“anti-PD-1 sdAb moiety”), wherein the anti-PD-1 sdAb moiety comprises any one of the following:

- (1) a CDR1 comprising the amino acid sequence of SEQ ID NO: 70, or a variant thereof comprising up to about 3 amino acid substitutions; a CDR2 comprising the amino acid sequence of SEQ ID NO: 142, or a variant thereof comprising up to about 3 amino acid substitutions; and a CDR3 comprising the amino acid sequence of SEQ ID NO: 214, or a variant thereof comprising up to about 3 amino acid substitutions;
- (2) a CDR1 comprising the amino acid sequence of SEQ ID NO: 43, or a variant thereof comprising up to about 3 amino acid substitutions; a CDR2 comprising the amino acid sequence of SEQ ID NO: 115, or a variant thereof comprising up to about 3 amino acid substitutions; and a CDR3 comprising the amino acid sequence of SEQ ID NO: 187, or a variant thereof comprising up to about 3 amino acid substitutions;
- (3) a CDR1 comprising the amino acid sequence of SEQ ID NO: 42, or a variant thereof comprising up to about 3 amino acid substitutions; a CDR2 comprising the amino acid sequence of SEQ ID NO: 114, or a variant thereof comprising up to about 3 amino acid substitutions; and a CDR3 comprising the amino acid sequence of SEQ ID NO: 186, or a variant thereof comprising up to about 3 amino acid substitutions;
- (4) a CDR1 comprising the amino acid sequence of SEQ ID NO: 40, or a variant thereof comprising up to about 3 amino acid substitutions; a CDR2 comprising the amino acid sequence of SEQ ID NO: 112, or a variant thereof comprising up to about 3 amino acid substitutions; and a CDR3 comprising the amino acid sequence of SEQ ID NO: 184, or a variant thereof comprising up to about 3 amino acid substitutions;

- (5) a CDR1 comprising the amino acid sequence of SEQ ID NO: 48, or a variant thereof comprising up to about 3 amino acid substitutions; a CDR2 comprising the amino acid sequence of SEQ ID NO: 120, or a variant thereof comprising up to about 3 amino acid substitutions; and a CDR3 comprising the amino acid sequence of SEQ ID NO: 192, or a variant thereof comprising up to about 3 amino acid substitutions;
- (6) a CDR1 comprising the amino acid sequence of SEQ ID NO: 45, or a variant thereof comprising up to about 3 amino acid substitutions; a CDR2 comprising the amino acid sequence of SEQ ID NO: 117, or a variant thereof comprising up to about 3 amino acid substitutions; and a CDR3 comprising the amino acid sequence of SEQ ID NO: 189, or a variant thereof comprising up to about 3 amino acid substitutions;
- (7) a CDR1 comprising the amino acid sequence of SEQ ID NO: 62, or a variant thereof comprising up to about 3 amino acid substitutions; a CDR2 comprising the amino acid sequence of SEQ ID NO: 134, or a variant thereof comprising up to about 3 amino acid substitutions; and a CDR3 comprising the amino acid sequence of SEQ ID NO: 206, or a variant thereof comprising up to about 3 amino acid substitutions;
- (8) a CDR1 comprising the amino acid sequence of SEQ ID NO: 47, or a variant thereof comprising up to about 3 amino acid substitutions; a CDR2 comprising the amino acid sequence of SEQ ID NO: 119, or a variant thereof comprising up to about 3 amino acid substitutions; and a CDR3 comprising the amino acid sequence of SEQ ID NO: 191, or a variant thereof comprising up to about 3 amino acid substitutions;
- (9) a CDR1 comprising the amino acid sequence of SEQ ID NO: 46, or a variant thereof comprising up to about 3 amino acid substitutions; a CDR2 comprising the amino acid sequence of SEQ ID NO: 118, or a variant thereof comprising up to about 3 amino acid substitutions; and a CDR3 comprising the amino acid sequence of SEQ ID NO: 190, or a variant thereof comprising up to about 3 amino acid substitutions;
- (10) a CDR1 comprising the amino acid sequence of SEQ ID NO: 38, or a variant thereof comprising up to about 3 amino acid substitutions; a CDR2 comprising the amino acid sequence of SEQ ID NO: 110, or a variant thereof comprising up to about 3 amino acid substitutions; and a CDR3 comprising the amino acid sequence of SEQ ID NO: 182, or a variant thereof comprising up to about 3 amino acid substitutions;
- (11) a CDR1 comprising the amino acid sequence of SEQ ID NO: 41, or a variant thereof comprising up to about 3 amino acid substitutions; a CDR2 comprising the amino acid sequence of SEQ ID NO: 113, or a variant thereof comprising up to about 3 amino acid substitutions; and a CDR3

- comprising the amino acid sequence of SEQ ID NO: 185, or a variant thereof comprising up to about 3 amino acid substitutions;
- (12) a CDR1 comprising the amino acid sequence of SEQ ID NO: 37, or a variant thereof comprising up to about 3 amino acid substitutions; a CDR2 comprising the amino acid sequence of SEQ ID NO: 109, or a variant thereof comprising up to about 3 amino acid substitutions; and a CDR3 comprising the amino acid sequence of SEQ ID NO: 181, or a variant thereof comprising up to about 3 amino acid substitutions;
- (13) a CDR1 comprising the amino acid sequence of SEQ ID NO: 44, or a variant thereof comprising up to about 3 amino acid substitutions; a CDR2 comprising the amino acid sequence of SEQ ID NO: 116, or a variant thereof comprising up to about 3 amino acid substitutions; and a CDR3 comprising the amino acid sequence of SEQ ID NO: 188, or a variant thereof comprising up to about 3 amino acid substitutions;
- (14) a CDR1 comprising the amino acid sequence of SEQ ID NO: 39, or a variant thereof comprising up to about 3 amino acid substitutions; a CDR2 comprising the amino acid sequence of SEQ ID NO: 111, or a variant thereof comprising up to about 3 amino acid substitutions; and a CDR3 comprising the amino acid sequence of SEQ ID NO: 183, or a variant thereof comprising up to about 3 amino acid substitutions;
- (15) a CDR1 comprising the amino acid sequence of SEQ ID NO: 49, or a variant thereof comprising up to about 3 amino acid substitutions; a CDR2 comprising the amino acid sequence of SEQ ID NO: 121, or a variant thereof comprising up to about 3 amino acid substitutions; and a CDR3 comprising the amino acid sequence of SEQ ID NO: 193, or a variant thereof comprising up to about 3 amino acid substitutions;
- (16) a CDR1 comprising the amino acid sequence of SEQ ID NO: 50, or a variant thereof comprising up to about 3 amino acid substitutions; a CDR2 comprising the amino acid sequence of SEQ ID NO: 122, or a variant thereof comprising up to about 3 amino acid substitutions; and a CDR3 comprising the amino acid sequence of SEQ ID NO: 194, or a variant thereof comprising up to about 3 amino acid substitutions;
- (17) a CDR1 comprising the amino acid sequence of SEQ ID NO: 51, or a variant thereof comprising up to about 3 amino acid substitutions; a CDR2 comprising the amino acid sequence of SEQ ID NO: 123, or a variant thereof comprising up to about 3 amino acid substitutions; and a CDR3 comprising the amino acid sequence of SEQ ID NO: 195, or a variant thereof comprising up to about 3 amino acid substitutions;

- [illegible]

comprising the amino acid sequence of SEQ ID NO: 202, or a variant thereof comprising up to about 3 amino acid substitutions;

- (25) a CDR1 comprising the amino acid sequence of SEQ ID NO: 59, or a variant thereof comprising up to about 3 amino acid substitutions; a CDR2 comprising the amino acid sequence of SEQ ID NO: 131, or a variant thereof comprising up to about 3 amino acid substitutions; and a CDR3 comprising the amino acid sequence of SEQ ID NO: 203, or a variant thereof comprising up to about 3 amino acid substitutions;
- (26) a CDR1 comprising the amino acid sequence of SEQ ID NO: 60, or a variant thereof comprising up to about 3 amino acid substitutions; a CDR2 comprising the amino acid sequence of SEQ ID NO: 132, or a variant thereof comprising up to about 3 amino acid substitutions; and a CDR3 comprising the amino acid sequence of SEQ ID NO: 204, or a variant thereof comprising up to about 3 amino acid substitutions;
- (27) a CDR1 comprising the amino acid sequence of SEQ ID NO: 61, or a variant thereof comprising up to about 3 amino acid substitutions; a CDR2 comprising the amino acid sequence of SEQ ID NO: 133, or a variant thereof comprising up to about 3 amino acid substitutions; and a CDR3 comprising the amino acid sequence of SEQ ID NO: 205, or a variant thereof comprising up to about 3 amino acid substitutions;
- (28) a CDR1 comprising the amino acid sequence of SEQ ID NO: 63, or a variant thereof comprising up to about 3 amino acid substitutions; a CDR2 comprising the amino acid sequence of SEQ ID NO: 135, or a variant thereof comprising up to about 3 amino acid substitutions; and a CDR3 comprising the amino acid sequence of SEQ ID NO: 207, or a variant thereof comprising up to about 3 amino acid substitutions;
- (29) a CDR1 comprising the amino acid sequence of SEQ ID NO: 64, or a variant thereof comprising up to about 3 amino acid substitutions; a CDR2 comprising the amino acid sequence of SEQ ID NO: 136, or a variant thereof comprising up to about 3 amino acid substitutions; and a CDR3 comprising the amino acid sequence of SEQ ID NO: 208, or a variant thereof comprising up to about 3 amino acid substitutions;
- (30) a CDR1 comprising the amino acid sequence of SEQ ID NO: 65, or a variant thereof comprising up to about 3 amino acid substitutions; a CDR2 comprising the amino acid sequence of SEQ ID NO: 137, or a variant thereof comprising up to about 3 amino acid substitutions; and a CDR3 comprising the amino acid sequence of SEQ ID NO: 209, or a variant thereof comprising up to about 3 amino acid substitutions;

- (31) a CDR1 comprising the amino acid sequence of SEQ ID NO: 66, or a variant thereof comprising up to about 3 amino acid substitutions; a CDR2 comprising the amino acid sequence of SEQ ID NO: 138, or a variant thereof comprising up to about 3 amino acid substitutions; and a CDR3 comprising the amino acid sequence of SEQ ID NO: 210, or a variant thereof comprising up to about 3 amino acid substitutions;
- (32) a CDR1 comprising the amino acid sequence of SEQ ID NO: 67, or a variant thereof comprising up to about 3 amino acid substitutions; a CDR2 comprising the amino acid sequence of SEQ ID NO: 139, or a variant thereof comprising up to about 3 amino acid substitutions; and a CDR3 comprising the amino acid sequence of SEQ ID NO: 211, or a variant thereof comprising up to about 3 amino acid substitutions; or
- (33) a CDR1 comprising the amino acid sequence of SEQ ID NO: 68, or a variant thereof comprising up to about 3 amino acid substitutions; a CDR2 comprising the amino acid sequence of SEQ ID NO: 140, or a variant thereof comprising up to about 3 amino acid substitutions; and a CDR3 comprising the amino acid sequence of SEQ ID NO: 212, or a variant thereof comprising up to about 3 amino acid substitutions.

[11] In some examples according to any one of the isolated anti-PD-1 constructs described above, the sdAb moiety specifically recognizing PD-1 comprises any one of the following:

- (1) a CDR1 comprising the amino acid sequence of SEQ ID NO: 37; a CDR2 comprising the amino acid sequence of SEQ ID NO: 109; and a CDR3 comprising the amino acid sequence of SEQ ID NO: 181; or a variant thereof comprising up to about 3 (such as about any of 1, 2, or 3) amino acid substitutions in the CDR regions;
- (2) a CDR1 comprising the amino acid sequence of SEQ ID NO: 38; a CDR2 comprising the amino acid sequence of SEQ ID NO: 110; and a CDR3 comprising the amino acid sequence of SEQ ID NO: 182; or a variant thereof comprising up to about 3 (such as about any of 1, 2, or 3) amino acid substitutions in the CDR regions;
- (3) a CDR1 comprising the amino acid sequence of SEQ ID NO: 39; a CDR2 comprising the amino acid sequence of SEQ ID NO: 111; and a CDR3 comprising the amino acid sequence of SEQ ID NO: 183; or a variant thereof comprising up to about 3 (such as about any of 1, 2, or 3) amino acid substitutions in the CDR regions;

(4) a CDR1 comprising the amino acid sequence of SEQ ID NO: 40; a CDR2 comprising the amino acid sequence of SEQ ID NO: 112; and a CDR3 comprising the amino acid sequence of SEQ ID NO: 184; or a variant thereof comprising up to about 3 (such as about any of 1, 2, or 3) amino acid substitutions in the CDR regions;

(5) a CDR1 comprising the amino acid sequence of SEQ ID NO: 41; a CDR2 comprising the amino acid sequence of SEQ ID NO: 113; and a CDR3 comprising the amino acid sequence of SEQ ID NO: 185; or a variant thereof comprising up to about 3 (such as about any of 1, 2, or 3) amino acid substitutions in the CDR regions;

(36) a CDR1 comprising the amino acid sequence of SEQ ID NO: 72; a CDR2 comprising the amino acid sequence of SEQ ID NO: 144; and a CDR3 comprising the amino acid sequence of SEQ ID NO: 216; or a variant thereof comprising up to about 3 (such as about any of 1, 2, or 3) amino acid substitutions in the CDR regions.

[12] In some examples according to any one of the isolated anti-PD-1 constructs described above, the sdAb moiety specifically recognizing PD-1 comprises a V_HH domain comprising the amino acid sequence of any one of the following: a-1) the amino acid residue at position 37 is selected from the group consisting of F, Y, L, I, and V (such as F, Y or V, or such as F); a-2) the amino acid residue at position 44 is selected from the group consisting of A, G, E, D, G, Q, R, S, and L (such as E, Q, or G, or such as E); a-3) the amino acid residue at position 45 is selected from the group consisting of L, R, and C (such as L or R); a-4) the amino acid residue at position 103 is selected from the group consisting of W, R, G, and S (such as W, G, or R, or such as W); and a-5) the amino acid residue at position 108 is Q; or b-1) the amino acid residue at position 37 is selected from the group consisting of F, Y, L, I, and V (such as F, V or Y, or such as F); b-2) the amino acid residue at position 44 is selected from the group consisting of E, Q, and G; b-3) the amino acid residue at position 45 is R; b-4) the amino acid residue at position 103 is selected from the group consisting of W, R, and S (such as W); and b-5) the amino acid residue at position 108 is selected from the group consisting of Q and L (such as Q); wherein the amino acid position is according to Kabat numbering, and wherein position 108 can be optionally humanized to L when position 108 is Q.

[13] In some examples according to any one of the isolated anti-PD-1 constructs described above, the sdAb moiety specifically recognizing PD-1 comprises a V_HH domain comprising the amino acid sequence of any one of SEQ ID NOs: 289-324, or a variant thereof having at least about 80% (such as at least about any of 80%, 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, or 99%) sequence identity to any one of SEQ ID NOs: 289-324. In some examples, the sdAb moiety specifically recognizing PD-1 comprises a V_HH domain comprising the amino acid sequence of any one of SEQ ID NOs: 289-324, or a variant thereof comprising up to about 3 (such as about any of 1, 2, or 3) amino acid substitutions in the V_HH domain. In some examples, the amino acid substitutions are in the CDRs, such as the CDR1, and/or the CDR2, and/or the CDR3 of any one of SEQ ID NOs: 289-324. In some examples, the amino acid substitutions are in the FRs, such as the FR1, and/or the FR2, and/or the FR3, and/or the FR4 of any one of SEQ ID NOs: 289-324. In some examples, the amino acid substitutions are in both CDRs and FRs. In some examples, the sdAb moiety specifically recognizing PD-1 comprises a V_HH domain comprising the amino acid sequence of any one of SEQ ID NOs: 289-324.

[14] In some examples according to any one of the isolated anti-PD-1 constructs described above, the K_d of the binding between the sdAb moiety specifically recognizing PD-1 and PD-1 is about 10^{-5} M to about 10^{-12} M (such as about 10^{-5} M to about 10^{-12} M, about 10^{-7} M to about 10^{-12} M, or about 10^{-8} M to about 10^{-12} M).

[15] In some examples according to any one of the isolated anti-PD-1 constructs described above, the sdAb moiety specifically recognizing PD-1 is camelid, chimeric, human, partially humanized, or fully humanized.

[16] In some examples according to any one of the isolated anti-PD-1 constructs described above, the isolated anti-PD-1 construct is a heavy chain-only antibody (HCAb) comprising the sdAb moiety specifically recognizing PD-1 fused to an Fc fragment via an optional linker. In some examples, HCAb is monomeric. In some examples, the HCAb is dimeric. In some examples, the Fc fragment is a human IgG1 (hIgG1) Fc, effectorless (inert) hIgG1 Fc, hIgG4 Fc, or hIgG4 Fc (S228P). In some examples, the Fc fragment comprises the amino acid sequence of any one of SEQ ID NOs: 363-365. In some examples, the Fc fragment is hIgG4 Fc (S228P). In some examples, the optional linker comprises the amino acid sequence of any one of SEQ ID NOs: 367-376. In some examples, the HCAb comprises the amino acid sequence of any one of SEQ ID NOs: 325-360.

[17] In some examples according to any one of the isolated anti-PD-1 construct described above, the isolated anti-PD-1 construct further comprises a second antibody moiety specifically recognizing a second epitope. In some examples, the second antibody moiety is a full-length antibody, a Fab, a Fab', a (Fab')₂, an Fv, a single chain Fv (scFv), an scFv-scFv, a minibody, a diabody, or an sdAb. In some examples, the anti-PD-1 construct is monospecific. In some examples, the anti-PD-1 construct is multispecific (such as bispecific). In some examples, the second epitope is not from PD-1. In some examples, the second epitope is from PD-1 but different from that specifically recognized by the anti-PD-1 sdAb moiety. In some examples, the second epitope is the same as that specifically recognized by the anti-PD-1 sdAb moiety. In some examples, the sdAb moiety specifically recognizing PD-1 and the second antibody moiety are optionally connected by a peptide linker, such as peptide linker comprising the amino acid sequence of any one of SEQ ID NOs: 367-376. In some examples, the second antibody moiety is an sdAb, such as an sdAb specifically recognizing PD-1 or CTLA-4. In some examples, the second antibody moiety is a Fab. In some examples, the second antibody moiety is an scFv. In some examples, the second antibody moiety is a full-length antibody consisting of two heavy chains and two light chains. In some examples, the Fc fragment of the heavy chain is IgG1 Fc, effectorless IgG1 Fc, IgG2 Fc, IgG4 Fc, or IgG4 Fc (S228P), such as any of SEQ ID NOs: 363-365. In some examples, the N-terminus of the sdAb moiety specifically recognizing PD-1 is fused to the C-

terminus of at least one of the heavy chains of the full-length antibody. In some examples, the C-terminus of the sdAb moiety specifically recognizing PD-1 is fused to the N-terminus of at least one of the heavy chains of the full-length antibody. In some examples, the N-terminus of the sdAb moiety specifically recognizing PD-1 is fused to the C-terminus of at least one of the light chains of the full-length antibody. In some examples, the C-terminus of the sdAb moiety specifically recognizing PD-1 is fused to the N-terminus of at least one of the light chains of the full-length antibody. In some examples, the isolated anti-PD-1 construct comprises four identical sdAb moieties specifically recognizing PD-1 as described above, the C-terminus of each anti-PD-1 sdAb moiety is fused to the N-terminus of each chain of the full-length antibody via an optional peptide linker. In some examples, the isolated anti-PD-1 construct comprises four identical sdAb moieties specifically recognizing PD-1 as described above, two anti-PD-1 sdAb moieties are fused to each other via an optional peptide linker, the other two anti-PD-1 sdAb moieties are fused to each other via an optional peptide linker, and the C-terminus of each of the anti-PD-1 sdAb moiety fusion polypeptide is fused to the N-terminus of each heavy chain of the full-length antibody via an optional peptide linker. In some examples the isolated anti-PD-1 construct consists of four polypeptide chains with structures from the N-terminus to the C-terminus as follows: (1) V_L-C_L ; (2) anti-PD-1 sdAb- $V_H-C_{H1}-C_{H2}-C_{H3}$; (3) anti-PD-1 sdAb- $V_H-C_{H1}-C_{H2}-C_{H3}$; and (4) V_L-C_L , wherein V_H and V_L of polypeptide chains (1) and (2) forms an antigen binding site that specifically binds a first copy of the second epitope (*e.g.*, TIGIT, LAG-3, TIM-3, CTLA-4, PD-1 (such as a second PD-1 epitope different from that recognized by the anti-PD-1 sdAb moiety described herein)), V_H and V_L of polypeptide chains (3) and (4) forms an antigen binding site that specifically binds a second copy of the second epitope (*e.g.*, TIGIT, LAG-3, TIM-3, CTLA-4, PD-1 (such as a second PD-1 epitope different from that recognized by the anti-PD-1 sdAb moiety described herein)), and each anti-PD-1 sdAb specifically binds a copy of PD-1. In some examples the isolated anti-PD-1 construct consists of four polypeptide chains with structures from the N-terminus to the C-terminus as follows: (1) V_L-C_L ; (2) $V_H-C_{H1}-C_{H2}-C_{H3}$ -anti-PD-1 sdAb; (3) $V_H-C_{H1}-C_{H2}-C_{H3}$ -anti-PD-1 sdAb; and (4) V_L-C_L , wherein V_H and V_L of polypeptide chains (1) and (2) forms an antigen binding site that specifically binds a first copy of the second epitope (*e.g.*, TIGIT, LAG-3, TIM-3, CTLA-4, PD-1 (such as a second PD-1 epitope different from that recognized by the anti-PD-1 sdAb moiety described herein)), V_H and V_L of polypeptide chains (3) and (4) forms an antigen binding site that specifically binds a second copy of the second epitope (*e.g.*, TIGIT, LAG-3, TIM-3, CTLA-4, PD-1 (such as a second PD-1 epitope different from that recognized by the anti-PD-1 sdAb moiety described herein)), and each anti-PD-1 sdAb specifically binds a copy of PD-1. In some examples the isolated anti-PD-1 construct consists of four polypeptide chains with structures from the N-terminus to the C-terminus as follows: (1) anti-PD-1 sdAb- V_L-C_L ; (2) $V_H-C_{H1}-C_{H2}-C_{H3}$; (3) $V_H-C_{H1}-C_{H2}-C_{H3}$; and (4) anti-PD-1 sdAb- V_L-C_L , wherein V_H and V_L

of polypeptide chains (1) and (2) forms an antigen binding site that specifically binds a first copy of the second epitope (*e.g.*, TIGIT, LAG-3, TIM-3, CTLA-4, PD-1 (such as a second PD-1 epitope different from that recognized by the anti-PD-1 sdAb moiety described herein)), V_H and V_L of polypeptide chains (3) and (4) forms an antigen binding site that specifically binds a second copy of the second epitope (*e.g.*, TIGIT, LAG-3, TIM-3, CTLA-4, PD-1 (such as a second PD-1 epitope different from that recognized by the anti-PD-1 sdAb moiety described herein)), and each anti-PD-1 sdAb specifically binds a copy of PD-1. In some examples the isolated anti-PD-1 construct consists of four polypeptide chains with structures from the N-terminus to the C-terminus as follows: (1) V_L - C_L -anti-PD-1 sdAb; (2) V_H - C_{H1} - C_{H2} - C_{H3} ; (3) V_H - C_{H1} - C_{H2} - C_{H3} ; and (4) V_L - C_L -anti-PD-1 sdAb, wherein V_H and V_L of polypeptide chains (1) and (2) forms an antigen binding site that specifically binds a first copy of the second epitope (*e.g.*, TIGIT, LAG-3, TIM-3, CTLA-4, PD-1 (such as a second PD-1 epitope different from that recognized by the anti-PD-1 sdAb moiety described herein)), V_H and V_L of polypeptide chains (3) and (4) forms an antigen binding site that specifically binds a second copy of the second epitope (*e.g.*, TIGIT, LAG-3, TIM-3, CTLA-4, PD-1 (such as a second PD-1 epitope different from that recognized by the anti-PD-1 sdAb moiety described herein)), and each anti-PD-1 sdAb specifically binds a copy of PD-1. In some examples the isolated anti-PD-1 construct consists of four polypeptide chains with structures from the N-terminus to the C-terminus as follows: (1) anti-PD-1 sdAb- V_L - C_L ; (2) anti-PD-1 sdAb- V_H - C_{H1} - C_{H2} - C_{H3} ; (3) anti-PD-1 sdAb- V_H - C_{H1} - C_{H2} - C_{H3} ; and (4) anti-PD-1 sdAb- V_L - C_L , wherein V_H and V_L of polypeptide chains (1) and (2) forms an antigen binding site that specifically binds a first copy of the second epitope (*e.g.*, TIGIT, LAG-3, TIM-3, CTLA-4, PD-1 (such as a second PD-1 epitope different from that recognized by the anti-PD-1 sdAb moiety described herein)), V_H and V_L of polypeptide chains (3) and (4) forms an antigen binding site that specifically binds a second copy of the second epitope (*e.g.*, TIGIT, LAG-3, TIM-3, CTLA-4, PD-1 (such as a second PD-1 epitope different from that recognized by the anti-PD-1 sdAb moiety described herein)), and each anti-PD-1 sdAb specifically binds a copy of PD-1. In some examples the isolated anti-PD-1 construct consists of four polypeptide chains with structures from the N-terminus to the C-terminus as follows: (1) V_L - C_L ; (2) anti-PD-1 sdAb-anti-PD-1 sdAb- V_H - C_{H1} - C_{H2} - C_{H3} ; (3) anti-PD-1 sdAb-anti-PD-1 sdAb- V_H - C_{H1} - C_{H2} - C_{H3} ; and (4) V_L - C_L , wherein V_H and V_L of polypeptide chains (1) and (2) forms an antigen binding site that specifically binds a first copy of the second epitope (*e.g.*, TIGIT, LAG-3, TIM-3, CTLA-4, PD-1 (such as a second PD-1 epitope different from that recognized by the anti-PD-1 sdAb moiety described herein)), V_H and V_L of polypeptide chains (3) and (4) forms an antigen binding site that specifically binds a second copy of the second epitope (*e.g.*, TIGIT, LAG-3, TIM-3, CTLA-4, PD-1 (such as a second PD-1 epitope different from that recognized by the anti-PD-1 sdAb moiety described herein)), and each anti-PD-1 sdAb specifically binds a copy of PD-1. In some examples the isolated anti-PD-1 construct consists of four polypeptide chains with structures

from the N-terminus to the C-terminus as follows: (1) V_L - C_L ; (2) V_H - C_H1 -anti-PD-1 sdAb- C_H2 - C_H3 ; (3) V_H - C_H1 -anti-PD-1 sdAb- C_H2 - C_H3 ; and (4) V_L - C_L , wherein V_H and V_L of polypeptide chains (1) and (2) forms an antigen binding site that specifically binds a first copy of the second epitope (*e.g.*, TIGIT, LAG-3, TIM-3, CTLA-4, PD-1 (such as a second PD-1 epitope different from that recognized by the anti-PD-1 sdAb moiety described herein)), V_H and V_L of polypeptide chains (3) and (4) forms an antigen binding site that specifically binds a second copy of the second epitope (*e.g.*, TIGIT, LAG-3, TIM-3, CTLA-4, PD-1 (such as a second PD-1 epitope different from that recognized by the anti-PD-1 sdAb moiety described herein)), and each anti-PD-1 sdAb specifically binds a copy of PD-1. In some examples the isolated anti-PD-1 construct consists of two polypeptide chains each with a structure from the N-terminus to the C-terminus as follows: V_L - V_H -anti-PD-1 sdAb- C_H2 - C_H3 , wherein V_H and V_L of each polypeptide chain forms a scFv domain that specifically binds a copy of the second epitope (*e.g.*, TIGIT, LAG-3, TIM-3, CTLA-4, PD-1 (such as a second PD-1 epitope different from that recognized by the anti-PD-1 sdAb moiety described herein)), and each anti-PD-1 sdAb specifically binds a copy of PD-1. In some examples the isolated anti-PD-1 construct consists of four polypeptide chains with structures from the N-terminus to the C-terminus as follows: (1) V_L - C_L -anti-PD-1 sdAb- C_L ; (2) V_H - C_H1 -anti-PD-1 sdAb- C_H1 - C_H2 - C_H3 ; (3) V_H - C_H1 -anti-PD-1 sdAb- C_H1 - C_H2 - C_H3 ; and (4) V_L - C_L -anti-PD-1 sdAb- C_L , wherein V_H and V_L of polypeptide chains (1) and (2) forms an antigen binding site that specifically binds a first copy of the second epitope (*e.g.*, TIGIT, LAG-3, TIM-3, CTLA-4, PD-1 (such as a second PD-1 epitope different from that recognized by the anti-PD-1 sdAb moiety described herein)), V_H and V_L of polypeptide chains (3) and (4) forms an antigen binding site that specifically binds a second copy of the second epitope (*e.g.*, TIGIT, LAG-3, TIM-3, CTLA-4, PD-1 (such as a second PD-1 epitope different from that recognized by the anti-PD-1 sdAb moiety described herein)), and each anti-PD-1 sdAb specifically binds a copy of PD-1. In some examples the isolated anti-PD-1 construct consists of four polypeptide chains with structures from the N-terminus to the C-terminus as follows: (1) anti-PD-1 sdAb- C_L ; (2) V_L - V_H -anti-PD-1 sdAb- C_H1 - C_H2 - C_H3 ; (3) V_L - V_H -anti-PD-1 sdAb- C_H1 - C_H2 - C_H3 ; and (4) anti-PD-1 sdAb- C_L , wherein V_H and V_L of polypeptide chains (2) and (3) each forms an scFv that specifically binds a copy of the second epitope (*e.g.*, TIGIT, LAG-3, TIM-3, CTLA-4, PD-1 (such as a second PD-1 epitope different from that recognized by the anti-PD-1 sdAb moiety described herein)), and each anti-PD-1 sdAb specifically binds a copy of PD-1. In some examples, the full-length antibody (or antigen binding portion comprising a V_H and a V_L) specifically recognizes TIGIT. In some examples, the anti-TIGIT full-length antibody (or antigen binding portion comprising a V_H and a V_L) comprises a V_H comprising HC-CDR1, HC-CDR2, and HC-CDR3 of a heavy chain comprising the amino acid sequence of SEQ ID NO: 377, and a V_L comprising LC-CDR1, LC-CDR2, and LC-CDR3 of a light chain comprising the amino acid sequence of SEQ ID NO: 378. In some examples, the anti-TIGIT full-

length antibody comprises a heavy chain comprising the amino acid sequence of SEQ ID NO: 377, and a light chain comprising the amino acid sequence of SEQ ID NO: 378. In some examples, the full-length antibody (or antigen binding portion comprising a V_H and a V_L) specifically recognizing TIGIT is derived from tiragolumab. In some examples, the full-length antibody (or antigen binding portion comprising a V_H and a V_L) specifically recognizes LAG-3. In some examples, the anti-LAG-3 full-length antibody (or antigen binding portion comprising a V_H and a V_L) comprises a V_H comprising HC-CDR1, HC-CDR2, and HC-CDR3 of a heavy chain comprising the amino acid sequence of SEQ ID NO: 379, and a V_L comprising LC-CDR1, LC-CDR2, and LC-CDR3 of a light chain comprising the amino acid sequence of SEQ ID NO: 380. In some examples, the anti-LAG-3 full-length antibody comprises a heavy chain comprising the amino acid sequence of SEQ ID NO: 379, and a light chain comprising the amino acid sequence of SEQ ID NO: 380. In some examples, the full-length antibody (or antigen binding portion comprising a V_H and a V_L) specifically recognizing LAG-3 is derived from relatlimab. In some examples, the full-length antibody (or antigen binding portion comprising a V_H and a V_L) specifically recognizes TIM-3. In some examples, the anti-TIM-3 full-length antibody (or antigen binding portion comprising a V_H and a V_L) comprises a V_H comprising HC-CDR1, HC-CDR2, and HC-CDR3 of a heavy chain comprising the amino acid sequence of SEQ ID NO: 381, and a V_L comprising LC-CDR1, LC-CDR2, and LC-CDR3 of a light chain comprising the amino acid sequence of SEQ ID NO: 382. In some examples, the anti-TIM-3 full-length antibody comprises a heavy chain comprising the amino acid sequence of SEQ ID NO: 381, and a light chain comprising the amino acid sequence of SEQ ID NO: 382. In some examples, the full-length antibody (or antigen binding portion comprising a V_H and a V_L) specifically recognizing TIM-3 is derived from MBG453. In some examples, the full-length antibody (or antigen binding portion comprising a V_H and a V_L) specifically recognizes CTLA-4. In some examples, the anti-CTLA-4 full-length antibody (or antigen binding portion comprising a V_H and a V_L) comprises a V_H comprising HC-CDR1, HC-CDR2, and HC-CDR3 of a heavy chain comprising the amino acid sequence of SEQ ID NO: 383, and a V_L comprising LC-CDR1, LC-CDR2, and LC-CDR3 of a light chain comprising the amino acid sequence of SEQ ID NO: 384. In some examples, the anti-CTLA-4 full-length antibody comprises a heavy chain comprising the amino acid sequence of SEQ ID NO: 383, and a light chain comprising the amino acid sequence of SEQ ID NO: 384. In some examples, the full-length antibody (or antigen binding portion comprising a V_H and a V_L) specifically recognizing CTLA-4 is derived from ipilimumab (*e.g.*, Yervoy®). In some examples, the full-length antibody (or antigen binding portion comprising a V_H and a V_L) specifically recognizes PD-1 (such as a second PD-1 epitope different from that recognized by the anti-PD-1 sdAb moiety described herein). In some examples, the anti-PD-1 full-length antibody (or antigen binding portion comprising a V_H and a V_L) comprises a V_H comprising HC-CDR1, HC-CDR2, and HC-CDR3 of a heavy chain comprising the amino

acid sequence of SEQ ID NO: 385, and a V_L comprising LC-CDR1, LC-CDR2, and LC-CDR3 of a light chain comprising the amino acid sequence of SEQ ID NO: 386. In some examples, the anti-PD-1 full-length antibody comprises a heavy chain comprising the amino acid sequence of SEQ ID NO: 385, and a light chain comprising the amino acid sequence of SEQ ID NO: 386. In some examples, the full-length antibody (or antigen binding portion comprising a V_H and a V_L) specifically recognizing PD-1 is derived from pembrolizumab (*e.g.*, Keytruda®) or nivolumab (*e.g.*, Opdivo®).

[18] In some examples according to any one of the isolated anti-PD-1 constructs described above, the isolated anti-PD-1 construct further comprises a biologically active protein or fragments thereof.

[19] Further provided is an isolated anti-PD-1 construct comprising an sdAb moiety specifically recognizing PD-1, wherein the sdAb moiety comprises CDR1, CDR2, and CDR3 of any one of SEQ ID NOs: 289-324.

[20] Further provided is an isolated anti-PD-1 construct (*e.g.*, anti-PD-1 sdAb, anti-PD-1 HCAb (*e.g.*, anti-PD-1 sdAb-Fc fusion), PD-1×TIGIT BABP, PD-1×LAG-3 BABP, PD-1×TIM-3 BABP, PD-1×CTLA-4 BABP, or PD-1×PD-1 BABP) that specifically binds to PD-1 competitively with the any of the isolated anti-PD-1 constructs described above.

[20a] In another aspect, the present disclosure refers to an isolated anti-PD-1 construct that specifically binds to PD-1 competitively with the isolated anti-PD-1 construct as disclosed herein.

[21] Further provided is a pharmaceutical composition comprising any one of the isolated anti-PD-1 constructs described above, and optionally a pharmaceutical acceptable carrier.

[21a] In another aspect, the present disclosure refers to a pharmaceutical composition comprising the isolated anti-PD-1 construct as disclosed herein, and optionally a pharmaceutical acceptable carrier.

[22] Another example of the present application provides a method of treating an individual having a PD-1-related disease (such as cancer, or immune-related disease), comprising administering to the individual an effective amount of any one of the pharmaceutical compositions described above. In some examples, the PD-1-related disease is cancer. In some examples, the cancer is a solid tumor, such as a colon cancer. In some examples, the PD-1-related disease is an immune-related disease. In some examples, immune-related disease is associated with a T cell dysfunctional disorder. In some examples, the T cell dysfunctional disorder is characterized by T cell anergy or decreased ability to secrete cytokines, proliferate or execute cytolytic activity. In some examples, the T cell dysfunctional disorder is characterized by T cell exhaustion. In some examples, the immune-related disease is selected from the group consisting of unresolved acute infection, chronic infection, and tumor immunity. In some examples, the PD-1 related disease is a pathogenic infection. In some examples, the method further comprises administering to the individual an additional therapy (*e.g.*, cancer therapy), such as surgery, radiation, chemotherapy, immunotherapy, hormone therapy, or a combination thereof. In some examples, the additional therapy is immunotherapy. In some examples, the immunotherapy

comprises administering to the individual an effective amount of a second pharmaceutical composition comprising an immunomodulator, such as an immune checkpoint inhibitor (e.g., antibody specifically recognizing TIGIT, LAG-3, TIM-3, CTLA-4, PD-1, or PD-L1). In some examples, the pharmaceutical composition is administered systemically, such as intravenously (i.v.) or intraperitoneally (i.p.). In some examples, the pharmaceutical composition is administered locally, such as intratumorally. In some examples, the individual is a human.

[22a] In another aspect, the present disclosure refers to use of an effective amount of the isolated anti-PD-1 construct as disclosed herein or the pharmaceutical composition as disclosed herein in the manufacture of a medicament for treating a cancer, an immune-related disease, or a pathogenic infection, preferably wherein the cancer is a solid tumor, more preferably wherein the cancer is a colon cancer.

[23] Further provided is an isolated nucleic acid encoding any one of the isolated anti-PD-1 constructs described above. In some examples, the isolated nucleic acid comprises the nucleic acid sequence of any one of SEQ ID NOs: 253-288.

[23a] In another aspect, the present disclosure refers to an isolated nucleic acid encoding the isolated anti-PD-1 construct as disclosed herein.

[24] Further provided is a vector comprising any one of the isolated nucleic acids described above.

[24a] In another aspect, the present disclosure refers to a vector comprising the isolated nucleic acid as disclosed herein.

[25] Further provided is an isolated host cell comprising any one of the isolated nucleic acid or vector described above.

[25a] In another aspect, the present disclosure refers to an isolated host cell comprising the isolated nucleic acid as disclosed herein, or the vector as disclosed herein.

[26] Further provided is a kit comprising any one of the isolated anti-PD-1 construct, isolated nucleic acid, vector, or isolated host cell described above.

[27] Another example of the present application provides a method of producing any one of isolated anti-PD-1 constructs described above, comprising culturing a host cell comprising any one of the isolated nucleic acid or vector described above, or culturing any one of the isolated host cell described above, under conditions effective to express the encoded anti-PD-1 construct; and obtaining the expressed anti-PD-1 construct from said host cell. In some examples, the method further comprises producing a host cell comprising any one of the isolated nucleic acid or vector described above.

BRIEF DESCRIPTION OF THE DRAWINGS

[28] FIGs. 1A-1B depict immune response evaluation of the first camel after PD-1 immunization. FIG. 1A depicts immune response evaluation of pre-immune serum and immune serum of the first camel after the 6th immunization. FIG. 1B depicts immune response evaluation of heavy chain antibodies (IgG2 and IgG3) after the 6th immunization (terminal bleed). Heavy chain antibodies fractionated from pre-immune serum were used as negative controls.

[29] FIGs. 2A-2B depict immune response evaluation of the second camel after PD-1 immunization. FIG. 2A depicts immune response evaluation of pre-immune serum and immune serum of the 2nd camel after the 6th immunization. FIG. 2B depicts immune response evaluation of heavy chain antibodies (IgG2 and IgG3) after the 6th immunization (terminal bleed). Heavy chain antibodies fractionated from pre-immune serum were used as negative controls.

CLAIMS

What is claimed is:

1. An isolated anti-PD-1 construct comprising a single-domain antibody (sdAb) moiety specifically recognizing PD-1 (“anti-PD-1 sdAb moiety”), wherein the anti-PD-1 sdAb moiety comprises any one of the following:
 - (1) a CDR1 comprising the amino acid sequence of SEQ ID NO: 70, or a variant thereof comprising up to about 3 amino acid substitutions; a CDR2 comprising the amino acid sequence of SEQ ID NO: 142, or a variant thereof comprising up to about 3 amino acid substitutions; and a CDR3 comprising the amino acid sequence of SEQ ID NO: 214, or a variant thereof comprising up to about 3 amino acid substitutions;
 - (2) a CDR1 comprising the amino acid sequence of SEQ ID NO: 43, or a variant thereof comprising up to about 3 amino acid substitutions; a CDR2 comprising the amino acid sequence of SEQ ID NO: 115, or a variant thereof comprising up to about 3 amino acid substitutions; and a CDR3 comprising the amino acid sequence of SEQ ID NO: 187, or a variant thereof comprising up to about 3 amino acid substitutions;
 - (3) a CDR1 comprising the amino acid sequence of SEQ ID NO: 42, or a variant thereof comprising up to about 3 amino acid substitutions; a CDR2 comprising the amino acid sequence of SEQ ID NO: 114, or a variant thereof comprising up to about 3 amino acid substitutions; and a CDR3 comprising the amino acid sequence of SEQ ID NO: 186, or a variant thereof comprising up to about 3 amino acid substitutions;
 - (4) a CDR1 comprising the amino acid sequence of SEQ ID NO: 40, or a variant thereof comprising up to about 3 amino acid substitutions; a CDR2 comprising the amino acid sequence of SEQ ID NO: 112, or a variant thereof comprising up to about 3 amino acid substitutions; and a CDR3 comprising the amino acid sequence of SEQ ID NO: 184, or a variant thereof comprising up to about 3 amino acid substitutions;
 - (5) a CDR1 comprising the amino acid sequence of SEQ ID NO: 48, or a variant thereof comprising up to about 3 amino acid substitutions; a CDR2 comprising the amino acid sequence of SEQ ID NO: 120, or a variant thereof comprising up to about 3 amino acid substitutions; and a CDR3 comprising the amino acid sequence of SEQ ID NO: 192, or a variant thereof comprising up to about 3 amino acid substitutions;
 - (6) a CDR1 comprising the amino acid sequence of SEQ ID NO: 45, or a variant thereof comprising up to about 3 amino acid substitutions; a CDR2 comprising the amino acid sequence of SEQ ID NO: 117, or a variant thereof comprising up to about 3 amino acid substitutions; and a CDR3 comprising

the amino acid sequence of SEQ ID NO: 189, or a variant thereof comprising up to about 3 amino acid substitutions;

(7) a CDR1 comprising the amino acid sequence of SEQ ID NO: 62, or a variant thereof comprising up to about 3 amino acid substitutions; a CDR2 comprising the amino acid sequence of SEQ ID NO: 134, or a variant thereof comprising up to about 3 amino acid substitutions; and a CDR3 comprising the amino acid sequence of SEQ ID NO: 206, or a variant thereof comprising up to about 3 amino acid substitutions;

(8) a CDR1 comprising the amino acid sequence of SEQ ID NO: 47, or a variant thereof comprising up to about 3 amino acid substitutions; a CDR2 comprising the amino acid sequence of SEQ ID NO: 119, or a variant thereof comprising up to about 3 amino acid substitutions; and a CDR3 comprising the amino acid sequence of SEQ ID NO: 191, or a variant thereof comprising up to about 3 amino acid substitutions;

(9) a CDR1 comprising the amino acid sequence of SEQ ID NO: 46, or a variant thereof comprising up to about 3 amino acid substitutions; a CDR2 comprising the amino acid sequence of SEQ ID NO: 118, or a variant thereof comprising up to about 3 amino acid substitutions; and a CDR3 comprising the amino acid sequence of SEQ ID NO: 190, or a variant thereof comprising up to about 3 amino acid substitutions;

(10) a CDR1 comprising the amino acid sequence of SEQ ID NO: 38, or a variant thereof comprising up to about 3 amino acid substitutions; a CDR2 comprising the amino acid sequence of SEQ ID NO: 110, or a variant thereof comprising up to about 3 amino acid substitutions; and a CDR3 comprising the amino acid sequence of SEQ ID NO: 182, or a variant thereof comprising up to about 3 amino acid substitutions;

(11) a CDR1 comprising the amino acid sequence of SEQ ID NO: 41, or a variant thereof comprising up to about 3 amino acid substitutions; a CDR2 comprising the amino acid sequence of SEQ ID NO: 113, or a variant thereof comprising up to about 3 amino acid substitutions; and a CDR3 comprising the amino acid sequence of SEQ ID NO: 185, or a variant thereof comprising up to about 3 amino acid substitutions;

(12) a CDR1 comprising the amino acid sequence of SEQ ID NO: 37, or a variant thereof comprising up to about 3 amino acid substitutions; a CDR2 comprising the amino acid sequence of SEQ ID NO: 109, or a variant thereof comprising up to about 3 amino acid substitutions; and a CDR3 comprising the amino acid sequence of SEQ ID NO: 181, or a variant thereof comprising up to about 3 amino acid substitutions;

(13) a CDR1 comprising the amino acid sequence of SEQ ID NO: 44, or a variant thereof comprising up to about 3 amino acid substitutions; a CDR2 comprising the amino acid sequence of SEQ ID NO:

116, or a variant thereof comprising up to about 3 amino acid substitutions; and a CDR3 comprising the amino acid sequence of SEQ ID NO: 188, or a variant thereof comprising up to about 3 amino acid substitutions;

(14) a CDR1 comprising the amino acid sequence of SEQ ID NO: 39, or a variant thereof comprising up to about 3 amino acid substitutions; a CDR2 comprising the amino acid sequence of SEQ ID NO: 111, or a variant thereof comprising up to about 3 amino acid substitutions; and a CDR3 comprising the amino acid sequence of SEQ ID NO: 183, or a variant thereof comprising up to about 3 amino acid substitutions;

(15) a CDR1 comprising the amino acid sequence of SEQ ID NO: 49, or a variant thereof comprising up to about 3 amino acid substitutions; a CDR2 comprising the amino acid sequence of SEQ ID NO: 121, or a variant thereof comprising up to about 3 amino acid substitutions; and a CDR3 comprising the amino acid sequence of SEQ ID NO: 193, or a variant thereof comprising up to about 3 amino acid substitutions;

(16) a CDR1 comprising the amino acid sequence of SEQ ID NO: 50, or a variant thereof comprising up to about 3 amino acid substitutions; a CDR2 comprising the amino acid sequence of SEQ ID NO: 122, or a variant thereof comprising up to about 3 amino acid substitutions; and a CDR3 comprising the amino acid sequence of SEQ ID NO: 194, or a variant thereof comprising up to about 3 amino acid substitutions;

(17) a CDR1 comprising the amino acid sequence of SEQ ID NO: 51, or a variant thereof comprising up to about 3 amino acid substitutions; a CDR2 comprising the amino acid sequence of SEQ ID NO: 123, or a variant thereof comprising up to about 3 amino acid substitutions; and a CDR3 comprising the amino acid sequence of SEQ ID NO: 195, or a variant thereof comprising up to about 3 amino acid substitutions;

(18) a CDR1 comprising the amino acid sequence of SEQ ID NO: 52, or a variant thereof comprising up to about 3 amino acid substitutions; a CDR2 comprising the amino acid sequence of SEQ ID NO: 124, or a variant thereof comprising up to about 3 amino acid substitutions; and a CDR3 comprising the amino acid sequence of SEQ ID NO: 196, or a variant thereof comprising up to about 3 amino acid substitutions;

(19) a CDR1 comprising the amino acid sequence of SEQ ID NO: 53, or a variant thereof comprising up to about 3 amino acid substitutions; a CDR2 comprising the amino acid sequence of SEQ ID NO: 125, or a variant thereof comprising up to about 3 amino acid substitutions; and a CDR3 comprising the amino acid sequence of SEQ ID NO: 197, or a variant thereof comprising up to about 3 amino acid substitutions;

the amino acid sequence of SEQ ID NO: 204, or a variant thereof comprising up to about 3 amino acid substitutions;

(27) a CDR1 comprising the amino acid sequence of SEQ ID NO: 61, or a variant thereof comprising up to about 3 amino acid substitutions; a CDR2 comprising the amino acid sequence of SEQ ID NO: 133, or a variant thereof comprising up to about 3 amino acid substitutions; and a CDR3 comprising the amino acid sequence of SEQ ID NO: 205, or a variant thereof comprising up to about 3 amino acid substitutions;

(28) a CDR1 comprising the amino acid sequence of SEQ ID NO: 63, or a variant thereof comprising up to about 3 amino acid substitutions; a CDR2 comprising the amino acid sequence of SEQ ID NO: 135, or a variant thereof comprising up to about 3 amino acid substitutions; and a CDR3 comprising the amino acid sequence of SEQ ID NO: 207, or a variant thereof comprising up to about 3 amino acid substitutions;

(29) a CDR1 comprising the amino acid sequence of SEQ ID NO: 64, or a variant thereof comprising up to about 3 amino acid substitutions; a CDR2 comprising the amino acid sequence of SEQ ID NO: 136, or a variant thereof comprising up to about 3 amino acid substitutions; and a CDR3 comprising the amino acid sequence of SEQ ID NO: 208, or a variant thereof comprising up to about 3 amino acid substitutions;

(30) a CDR1 comprising the amino acid sequence of SEQ ID NO: 65, or a variant thereof comprising up to about 3 amino acid substitutions; a CDR2 comprising the amino acid sequence of SEQ ID NO: 137, or a variant thereof comprising up to about 3 amino acid substitutions; and a CDR3 comprising the amino acid sequence of SEQ ID NO: 209, or a variant thereof comprising up to about 3 amino acid substitutions;

(31) a CDR1 comprising the amino acid sequence of SEQ ID NO: 66, or a variant thereof comprising up to about 3 amino acid substitutions; a CDR2 comprising the amino acid sequence of SEQ ID NO: 138, or a variant thereof comprising up to about 3 amino acid substitutions; and a CDR3 comprising the amino acid sequence of SEQ ID NO: 210, or a variant thereof comprising up to about 3 amino acid substitutions;

(32) a CDR1 comprising the amino acid sequence of SEQ ID NO: 67, or a variant thereof comprising up to about 3 amino acid substitutions; a CDR2 comprising the amino acid sequence of SEQ ID NO: 139, or a variant thereof comprising up to about 3 amino acid substitutions; and a CDR3 comprising the amino acid sequence of SEQ ID NO: 211, or a variant thereof comprising up to about 3 amino acid substitutions; or

(33) a CDR1 comprising the amino acid sequence of SEQ ID NO: 68, or a variant thereof comprising up to about 3 amino acid substitutions; a CDR2 comprising the amino acid sequence of SEQ ID NO:

140, or a variant thereof comprising up to about 3 amino acid substitutions; and a CDR3 comprising the amino acid sequence of SEQ ID NO: 212, or a variant thereof comprising up to about 3 amino acid substitutions.

2. The isolated anti-PD-1 construct of claim 1, wherein the anti-PD-1 sdAb moiety comprises a V_HH domain comprising the amino acid sequence of any one of the following:
 - a-1) the amino acid residue at position 37 is selected from the group consisting of F, Y, L, I, and V;
 - a-2) the amino acid residue at position 44 is selected from the group consisting of A, G, E, D, G, Q, R, S, and L;
 - a-3) the amino acid residue at position 45 is selected from the group consisting of L, R, and C;
 - a-4) the amino acid residue at position 103 is selected from the group consisting of W, R, G, and S; and
 - a-5) the amino acid residue at position 108 is Q; or
 - b-1) the amino acid residue at position 37 is selected from the group consisting of F, Y, L, I, and V;
 - b-2) the amino acid residue at position 44 is selected from the group consisting of E, Q, and G;
 - b-3) the amino acid residue at position 45 is R;
 - b-4) the amino acid residue at position 103 is selected from the group consisting of W, R, and S; and
 - b-5) the amino acid residue at position 108 is selected from the group consisting of Q and L;
 wherein the amino acid position is according to Kabat numbering, and wherein position 108 can be optionally humanized to L when position 108 is Q.
3. The isolated anti-PD-1 construct of claim 1 or 2, wherein the anti-PD-1 sdAb moiety comprises a V_HH domain comprising the amino acid sequence of any one of SEQ ID NOs: 289-324, or a variant thereof having at least about 80% sequence identity to any one of SEQ ID NOs: 289-324.
4. The isolated anti-PD-1 construct of any one of claims 1-3, wherein the anti-PD-1 sdAb moiety is camelid, chimeric, human, partially humanized, or fully humanized.
5. The isolated anti-PD-1 construct of any one of claims 1-4, wherein the isolated anti-PD-1 construct is a heavy chain-only antibody (HCAb) comprising the anti-PD-1 sdAb moiety fused to an Fc fragment via an optional linker; optionally wherein the HCAb is monomeric or dimeric; optionally wherein the Fc fragment is a human IgG1 (hIgG1) Fc, effectorless (inert) hIgG1 Fc, hIgG4 Fc, or hIgG4 Fc (S228P); and optionally wherein the optional linker comprises the amino acid sequence of any one of SEQ ID NOs: 367-376.
6. The isolated anti-PD-1 construct of claim 5, wherein the HCAb comprises the amino acid sequence of any one of SEQ ID NOs: 325-360.

7. The isolated anti-PD-1 construct of any one of claims 1-4, wherein the isolated anti-PD-1 construct further comprises a second antibody moiety specifically recognizing a second epitope, optionally wherein the second antibody moiety is a full-length antibody, a Fab, a Fab', a (Fab')₂, an Fv, a single chain Fv (scFv), an scFv-scFv, a minibody, a diabody, or an sdAb.
8. The isolated anti-PD-1 construct of claim 7, wherein the anti-PD-1 sdAb moiety and the second antibody moiety are connected by an optional peptide linker, optionally wherein the peptide linker comprises the amino acid sequence of any one of SEQ ID NOs: 367-376.
9. The isolated anti-PD-1 construct of claim 7 or 8, wherein the second antibody moiety is a full-length antibody consisting of two heavy chains and two light chains, optionally wherein the Fc fragment of the heavy chain is human IgG1 (hIgG1) Fc, effectorless (inert) hIgG1 Fc, hIgG4 Fc, or hIgG4 Fc (S228P).
10. The isolated anti-PD-1 construct of claim 9, wherein the isolated anti-PD-1 construct comprises a configuration selected from the group consisting of:
 - (a) the N-terminus of the anti-PD-1 sdAb moiety is fused to the C-terminus of at least one of the heavy chains of the full-length antibody;
 - (b) the C-terminus of the anti-PD-1 sdAb moiety is fused to the N-terminus of at least one of the heavy chains of the full-length antibody;
 - (c) the N-terminus of the anti-PD-1 sdAb moiety is fused to the C-terminus of at least one of the light chains of the full-length antibody;
 - (d) the C-terminus of the anti-PD-1 sdAb moiety is fused to the N-terminus of at least one of the light chains of the full-length antibody;
 - (e) the isolated anti-PD-1 construct comprises four anti-PD-1 sdAb moieties, wherein the C-terminus of each anti-PD-1 sdAb moiety is fused to the N-terminus of each chain of the full-length antibody; and
 - (f) the isolated anti-PD-1 construct comprises four anti-PD-1 sdAb moieties, wherein two of the four anti-PD-1 sdAb moieties are fused together, which is further fused to the N-terminus of each heavy chain of the full-length antibody.
11. The isolated anti-PD-1 construct of claim 9 or 10, wherein the full-length antibody specifically recognizes TIGIT, optionally wherein
 - (a) the full-length antibody comprises HC-CDR1, HC-CDR2, and HC-CDR3 of a heavy chain comprising the amino acid sequence of SEQ ID NO: 377, and LC-CDR1, LC-CDR2, and LC-CDR3 of a light chain comprising the amino acid sequence of SEQ ID NO: 378; or
 - (b) the full-length antibody comprises a heavy chain comprising the amino acid sequence of SEQ ID NO: 377, and a light chain comprising the amino acid sequence of SEQ ID NO: 378.

12. The isolated anti-PD-1 construct of claim 9 or 10, wherein the full-length antibody specifically recognizes LAG-3, optionally wherein
 - (a) the full-length antibody comprises HC-CDR1, HC-CDR2, and HC-CDR3 of a heavy chain comprising the amino acid sequence of SEQ ID NO: 379, and LC-CDR1, LC-CDR2, and LC-CDR3 of a light chain comprising the amino acid sequence of SEQ ID NO: 380; or
 - (b) the full-length antibody comprises a heavy chain comprising the amino acid sequence of SEQ ID NO: 379, and a light chain comprising the amino acid sequence of SEQ ID NO: 380.
13. The isolated anti-PD-1 construct of claim 9 or 10, wherein the full-length antibody specifically recognizes TIM-3, optionally wherein
 - (a) the full-length antibody comprises HC-CDR1, HC-CDR2, and HC-CDR3 of a heavy chain comprising the amino acid sequence of SEQ ID NO: 381, and LC-CDR1, LC-CDR2, and LC-CDR3 of a light chain comprising the amino acid sequence of SEQ ID NO: 382; or
 - (b) the full-length antibody comprises a heavy chain comprising the amino acid sequence of SEQ ID NO: 381, and a light chain comprising the amino acid sequence of SEQ ID NO: 382.
14. The isolated anti-PD-1 construct of claim 9 or 10, wherein the full-length antibody specifically recognizes CTLA-4, optionally wherein
 - (a) the full-length antibody comprises HC-CDR1, HC-CDR2, and HC-CDR3 of a heavy chain comprising the amino acid sequence of SEQ ID NO: 383, and LC-CDR1, LC-CDR2, and LC-CDR3 of a light chain comprising the amino acid sequence of SEQ ID NO: 384; or
 - (b) the full-length antibody comprises a heavy chain comprising the amino acid sequence of SEQ ID NO: 383, and a light chain comprising the amino acid sequence of SEQ ID NO: 384.
15. The isolated anti-PD-1 construct of claim 9 or 10, wherein the full-length antibody specifically recognizes PD-1, optionally wherein
 - (a) the full-length antibody comprises HC-CDR1, HC-CDR2, and HC-CDR3 of a heavy chain comprising the amino acid sequence of SEQ ID NO: 385, and LC-CDR1, LC-CDR2, and LC-CDR3 of a light chain comprising the amino acid sequence of SEQ ID NO: 386; or
 - (b) the full-length antibody comprises a heavy chain comprising the amino acid sequence of SEQ ID NO: 385, and a light chain comprising the amino acid sequence of SEQ ID NO: 386.
16. The isolated anti-PD-1 construct of claim 7 or 8, comprising a configuration selected from the group consisting of:
 - (a) the isolated anti-PD-1 construct comprises four polypeptides from N-terminus to C-terminus: (i) V_L-C_L, (ii) V_H-C_{H1}-optional peptide linker-first anti-PD-1 sdAb moiety-C_{H2}-C_{H3}, (iii) V_H-C_{H1}-optional peptide linker-second anti-PD-1 sdAb moiety-C_{H2}-C_{H3}, and (iv) V_L-C_L; wherein V_L-C_L of polypeptide (i) and V_H-C_{H1} of polypeptide (ii) form a second antibody moiety specifically

recognizing a second epitope, and V_L-C_L of polypeptide (iv) and V_H-C_H1 of polypeptide (iii) form a third antibody moiety specifically recognizing a third epitope;

(b) the isolated anti-PD-1 construct comprises two polypeptides from N-terminus to C-terminus: (i) first scFv specifically recognizing a second epitope-optional peptide linker-first anti-PD-1 sdAb moiety- C_H2-C_H3 , and (ii) second scFv specifically recognizing a third epitope-optional peptide linker-second anti-PD-1 sdAb moiety- C_H2-C_H3 ;

(c) the isolated anti-PD-1 construct comprises four polypeptides from N-terminus to C-terminus: (i) V_L-C_L -optional peptide linker-first anti-PD-1 sdAb moiety- C_L , (ii) V_H-C_H1 -optional peptide linker-second anti-PD-1 sdAb moiety- $C_H1-C_H2-C_H3$, (iii) V_H-C_H1 -optional peptide linker-third anti-PD-1 sdAb moiety- $C_H1-C_H2-C_H3$, and (iv) V_L-C_L -optional peptide linker-fourth anti-PD-1 sdAb moiety- C_L ; wherein V_L-C_L of polypeptide (i) and V_H-C_H1 of polypeptide (ii) form a second antibody moiety specifically recognizing a second epitope, and V_L-C_L of polypeptide (iv) and V_H-C_H1 of polypeptide (iii) form a third antibody moiety specifically recognizing a third epitope; and

(d) the isolated anti-PD-1 construct comprises four polypeptides from N-terminus to C-terminus: (i) first anti-PD-1 sdAb moiety- C_L , (ii) first scFv specifically recognizing a second epitope-optional peptide linker-second anti-PD-1 sdAb moiety- $C_H1-C_H2-C_H3$, (iii) second scFv specifically recognizing a third epitope-optional peptide linker-third anti-PD-1 sdAb moiety- $C_H1-C_H2-C_H3$, and (iv) fourth anti-PD-1 sdAb moiety- C_L .

17. An isolated anti-PD-1 construct that specifically binds to PD-1 competitively with the isolated anti-PD-1 construct of any one of claims 1-16.
18. A pharmaceutical composition comprising the isolated anti-PD-1 construct of any one of claims 1-17, and optionally a pharmaceutical acceptable carrier.
19. Use of an effective amount of the isolated anti-PD-1 construct of any one of claims 1-17 or the pharmaceutical composition of claim 18 in the manufacture of a medicament for treating a cancer, an immune-related disease, or a pathogenic infection, preferably wherein the cancer is a solid tumor, more preferably wherein the cancer is a colon cancer.
20. An isolated nucleic acid encoding the isolated anti-PD-1 construct of any one of claims 1-17.
21. A vector comprising the isolated nucleic acid of claim 20.
22. An isolated host cell comprising the isolated nucleic acid of claim 20, or the vector of claim 21.