

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, a CDR2 comprising the amino acid sequence of SEQ ID NO: 142, and a CDR3 comprising the amino acid sequence of SEQ ID NO: 214; or
- (2) a CDR1 comprising the amino acid sequence of SEQ ID NO: 43, a CDR2 comprising the amino acid sequence of SEQ ID NO: 115, and a CDR3 comprising the amino acid sequence of SEQ ID NO: 187.

[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;

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, a CDR2 comprising the amino acid sequence of SEQ ID NO: 142, and a CDR3 comprising the amino acid sequence of SEQ ID NO: 214; or
 - (2) a CDR1 comprising the amino acid sequence of SEQ ID NO: 43, a CDR2 comprising the amino acid sequence of SEQ ID NO: 115, and a CDR3 comprising the amino acid sequence of SEQ ID NO: 187.
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 SEQ ID NOs: 295, 321-324, or a variant thereof having at least about 80% sequence identity to any one of SEQ ID NOs: 295, 321-324.
3. The isolated anti-PD-1 construct of claim 1 or 2, wherein the anti-PD-1 sdAb moiety is camelid, chimeric, human, partially humanized, or fully humanized.
4. The isolated anti-PD-1 construct of any one of claims 1-3, 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, an hIgG1 Fc comprising an effectorless mutation, 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.
5. The isolated anti-PD-1 construct of claim 4, wherein the HCAb comprises the amino acid sequence of any one of SEQ ID NOs: 331, 357-360.
6. The isolated anti-PD-1 construct of any one of claims 1-3, 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.
7. The isolated anti-PD-1 construct of claim 6, 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.
8. The isolated anti-PD-1 construct of claim 6 or 7, 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, an hIgG1 Fc comprising an effectorless mutation, hIgG4 Fc, or hIgG4 Fc (S228P).

9. The isolated anti-PD-1 construct of claim 8, 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 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 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 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 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 the first anti-PD-1 sdAb moiety is fused to the N-terminus of the first heavy chain of the full-length antibody, the C-terminus of the second anti-PD-1 sdAb moiety is fused to the N-terminus of the second heavy chain of the full-length antibody, the C-terminus of the third anti-PD-1 sdAb moiety is fused to the N-terminus of the first light chain of the full-length antibody, and the C-terminus of the fourth anti-PD-1 sdAb moiety is fused to the N-terminus of the second light chain of the full-length antibody;
 - (f) the isolated anti-PD-1 construct comprises four anti-PD-1 sdAb moieties, wherein the C-terminus of the first anti-PD-1 sdAb moiety is fused to the N-terminus of the second anti-PD-1 sdAb moiety, and the C-terminus of the second anti-PD-1 sdAb moiety is fused to the N-terminus of the first heavy chain of the full-length antibody, the C-terminus of the third anti-PD-1 sdAb moiety is fused to the N-terminus of the fourth anti-PD-1 sdAb moiety, and the C-terminus of the fourth anti-PD-1 sdAb moiety is fused to the N-terminus of the second heavy chain of the full-length antibody;
 - (g) the isolated anti-PD-1 construct comprises two anti-PD-1 sdAb moieties, wherein the N-terminus of the first anti-PD-1 sdAb moiety is fused to the C-terminus of the first heavy chain of the full-length antibody, and the N-terminus of the second anti-PD-1 sdAb moiety is fused to the C-terminus of the second heavy chain of the full-length antibody;
 - (h) the isolated anti-PD-1 construct comprises two anti-PD-1 sdAb moieties, wherein the C-terminus of the first anti-PD-1 sdAb moiety is fused to the N-terminus of the first heavy chain of the full-length antibody, and the C-terminus of the second anti-PD-1 sdAb moiety is fused to the N-terminus of the second heavy chain of the full-length antibody;

- (i) the isolated anti-PD-1 construct comprises two anti-PD-1 sdAb moieties, wherein the N-terminus of the first anti-PD-1 sdAb moiety is fused to the C-terminus of the first light chain of the full-length antibody, and the N-terminus of the second anti-PD-1 sdAb moiety is fused to the C-terminus of the second light chain of the full-length antibody; and
 - (j) the isolated anti-PD-1 construct comprises two anti-PD-1 sdAb moieties, wherein the C-terminus of the first anti-PD-1 sdAb moiety is fused to the N-terminus of the first light chain of the full-length antibody, and the C-terminus of the second anti-PD-1 sdAb moiety is fused to the N-terminus of the second light chain of the full-length antibody.
10. The isolated anti-PD-1 construct of claim 8 or 9, 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.
 11. The isolated anti-PD-1 construct of claim 8 or 9, 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.
 12. The isolated anti-PD-1 construct of claim 8 or 9, 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.
 13. The isolated anti-PD-1 construct of claim 8 or 9, 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.
14. The isolated anti-PD-1 construct of claim 8 or 9, 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.
15. The isolated anti-PD-1 construct of claim 6 or 7, 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.

16. A pharmaceutical composition comprising the isolated anti-PD-1 construct of any one of claims 1-15, and optionally a pharmaceutical acceptable carrier.
17. Use of an effective amount of the isolated anti-PD-1 construct of any one of claims 1-15 or the pharmaceutical composition of claim 16 in the manufacture of a medicament for treating a cancer, a pathogenic infection, or an immune-related disease, wherein the immune-related disease is selected from the group consisting of unresolved acute infection, chronic infection, and tumor immunity, preferably wherein the cancer is a solid tumor, more preferably wherein the cancer is a colon cancer.
18. An isolated nucleic acid encoding the isolated anti-PD-1 construct of any one of claims 1-15.
19. A vector comprising the isolated nucleic acid of claim 18.
20. An isolated host cell comprising the isolated nucleic acid of claim 18, or the vector of claim 19.