

(25) a CDR1 comprising the amino acid sequence of SEQ ID NO: 221, 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: 241, 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: 261, or a variant thereof comprising up to about 3 (such as about any of 1, 2, or 3) amino acid substitutions;

(26) a CDR1 comprising the amino acid sequence of SEQ ID NO: 222, 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: 242, 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: 262, or a variant thereof comprising up to about 3 (such as about any of 1, 2, or 3) amino acid substitutions; or

(27) a CDR1 comprising the amino acid sequence of SEQ ID NO: 214, 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: 339, 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: 254, or a variant thereof comprising up to about 3 (such as about any of 1, 2, or 3) amino acid substitutions.

[0011a] In another aspect, the present disclosure refers to an isolated anti-cytotoxic T-lymphocyte-associated protein 4 (anti-CTLA-4) construct comprising a single-domain antibody (sdAb) moiety specifically recognizing CTLA-4 (“anti-CTLA-4 sdAb moiety”), wherein the anti-CTLA-4 sdAb moiety comprises any one of the following:

- (8) a CDR1 comprising the amino acid sequence of SEQ ID NO: 21; a CDR2 comprising the amino acid sequence of SEQ ID NO: 53; and a CDR3 comprising the amino acid sequence of SEQ ID NO: 85;
- (9) a CDR1 comprising the amino acid sequence of SEQ ID NO: 22; a CDR2 comprising the amino acid sequence of SEQ ID NO: 54; and a CDR3 comprising the amino acid sequence of SEQ ID NO: 86;
- (10) a CDR1 comprising the amino acid sequence of SEQ ID NO: 23; a CDR2 comprising the amino acid sequence of SEQ ID NO: 55; and a CDR3 comprising the amino acid sequence of SEQ ID NO: 87;
- ~~(11) a CDR1 comprising the amino acid sequence of SEQ ID NO: 25; a CDR2 comprising the amino acid sequence of SEQ ID NO: 57; and a CDR3 comprising the amino acid sequence of SEQ ID NO: 89;~~
- ~~(12)~~(11) a CDR1 comprising the amino acid sequence of SEQ ID NO: 27; a CDR2 comprising the amino acid sequence of SEQ ID NO: 59; and a CDR3 comprising the amino acid sequence of SEQ ID NO: 91;
- ~~(13)~~(12) a CDR1 comprising the amino acid sequence of SEQ ID NO: 28; a CDR2 comprising the amino acid sequence of SEQ ID NO: 60; and a CDR3 comprising the amino acid sequence of SEQ ID NO: 92;
- ~~(14)~~(13) a CDR1 comprising the amino acid sequence of SEQ ID NO: 29; a CDR2 comprising the amino acid sequence of SEQ ID NO: 61; and a CDR3 comprising the amino acid sequence of SEQ ID NO: 93;

~~(15)~~(14) a CDR1 comprising the amino acid sequence of SEQ ID NO: 30; a CDR2 comprising the amino acid sequence of SEQ ID NO: 62; and a CDR3 comprising the amino acid sequence of SEQ ID NO: 94;

~~(16)~~(15) a CDR1 comprising the amino acid sequence of SEQ ID NO: 31; a CDR2 comprising the amino acid sequence of SEQ ID NO: 63; and a CDR3 comprising the amino acid sequence of SEQ ID NO: 95;

~~(17)~~(16) a CDR1 comprising the amino acid sequence of SEQ ID NO: 32; a CDR2 comprising the amino acid sequence of SEQ ID NO: 64; and a CDR3 comprising the amino acid sequence of SEQ ID NO: 96;

~~(18)~~(17) a CDR1 comprising the amino acid sequence of SEQ ID NO: 214; a CDR2 comprising the amino acid sequence of SEQ ID NO: 234; and a CDR3 comprising the amino acid sequence of SEQ ID NO: 254;

~~(19)~~(18) a CDR1 comprising the amino acid sequence of SEQ ID NO: 215; a CDR2 comprising the amino acid sequence of SEQ ID NO: 235; and a CDR3 comprising the amino acid sequence of SEQ ID NO: 255;

~~(20)~~(19) a CDR1 comprising the amino acid sequence of SEQ ID NO: 216; a CDR2 comprising the amino acid sequence of SEQ ID NO: 236; and a CDR3 comprising the amino acid sequence of SEQ ID NO: 256;

~~(21)~~(20) a CDR1 comprising the amino acid sequence of SEQ ID NO: 217; a CDR2 comprising the amino acid sequence of SEQ ID NO: 237; and a CDR3 comprising the amino acid sequence of SEQ ID NO: 257;

~~(22)~~(21) a CDR1 comprising the amino acid sequence of SEQ ID NO: 218; a CDR2 comprising the amino acid sequence of SEQ ID NO: 238; and a CDR3 comprising the amino acid sequence of SEQ ID NO: 258;

~~(23)~~(22) a CDR1 comprising the amino acid sequence of SEQ ID NO: 219; a CDR2 comprising the amino acid sequence of SEQ ID NO: 239; and a CDR3 comprising the amino acid sequence of SEQ ID NO: 259;

~~(24)~~(23) a CDR1 comprising the amino acid sequence of SEQ ID NO: 220; a CDR2 comprising the amino acid sequence of SEQ ID NO: 240; and a CDR3 comprising the amino acid sequence of SEQ ID NO: 260;

~~(25)~~(24) a CDR1 comprising the amino acid sequence of SEQ ID NO: 221; a CDR2 comprising the amino acid sequence of SEQ ID NO: 241; and a CDR3 comprising the amino acid sequence of SEQ ID NO: 261;

~~(26)~~(25) a CDR1 comprising the amino acid sequence of SEQ ID NO: 222; a CDR2 comprising the amino acid sequence of SEQ ID NO: 242; and a CDR3 comprising the amino acid sequence of SEQ ID NO: 262; or

(267) a CDR1 comprising the amino acid sequence of SEQ ID NO: 214; a CDR2 comprising the amino acid sequence of SEQ ID NO: 339; and a CDR3 comprising the amino acid sequence of SEQ ID NO: 254.

[0012] In some examples according to any one of the isolated anti-CTLA-4 constructs described above, the sdAb moiety specifically recognizing CTLA-4 comprises any one of the following:

(1) a CDR1 comprising the amino acid sequence of SEQ ID NO: 17; a CDR2 comprising the amino acid sequence of SEQ ID NO: 49; and a CDR3 comprising the amino acid sequence of

SEQ ID NOs: 183-194, and two copies of light chains comprising the amino acid sequence of SEQ ID NO: 198.

[0021] In some examples according to any one of the isolated anti-CTLA-4 constructs described above, the isolated anti-CTLA-4 construct further comprises a biologically active protein or fragments thereof.

[0022] Further provided is an isolated anti-CTLA-4 construct comprising a sdAb moiety specifically recognizing CTLA-4, wherein the sdAb moiety comprises CDR1, CDR2, and CDR3 of any one of SEQ ID NOs: 113-129, 200-202, 274-282, 341-344, 352, and 353.

[0022a] In another example, there is provided an isolated anti-CTLA-4 construct comprising an sdAb moiety specifically recognizing CTLA-4, wherein the sdAb moiety comprises CDR1, CDR2, and CDR3 of any one of SEQ ID NOs: 113-129, 200-202, 274-282, 341-344, 352, and 353.

[0023] Further provided is an isolated anti-CTLA-4 construct that specifically binds to CTLA-4 competitively with any one of the isolated anti-CTLA-4 constructs described above.

[0023a] In another aspect example, the present disclosure refers to an isolated anti-CTLA-4 construct that specifically binds to CTLA-4 competitively with the isolated anti-CTLA-4 construct as disclosed herein.

[0024] Further provided is a pharmaceutical composition comprising any one of the isolated anti-CTLA-4 constructs described above, and a pharmaceutical acceptable carrier.

[0024a] In another aspect, the present disclosure refers to a pharmaceutical composition comprising the isolated anti-CTLA-4 construct as described herein, and a pharmaceutical acceptable carrier.

[0025] Disclosed is a method of treating an individual having a CTLA-4-related disease, comprising administering to the individual an effective amount of any one of the pharmaceutical composition described above. In some examples, the CTLA-4 related disease is cancer. In some examples, the cancer is a solid tumor, such as a colon cancer. In some examples, the method further comprises administering to the individual an additional cancer therapy, such as surgery, radiation, chemotherapy, immunotherapy, hormone therapy, or a combination thereof. In some examples, the CTLA-4 related disease is a pathogenic infection. 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.

[0025a] In another aspect, the present disclosure refers to use of an effective amount of the isolated anti-CTLA-4 construct as described herein or the pharmaceutical composition disclosed herein in the manufacture of a medicament for treating a cancer or a pathogenic-viral infection in an individual.

[0026] Further provided is an isolated nucleic acid encoding any one of the isolated anti-CTLA-4 construct described above. In some examples, the isolated nucleic acid comprises the nucleic acid sequence of any one of SEQ ID NOs: 97-112 and 264-273.

[0027] Further provided is a vector comprising any one of the isolated nucleic acid described above.

CLAIMS

What is claimed is:

1. An isolated anti-cytotoxic T-lymphocyte-associated protein 4 (anti-CTLA-4) construct comprising a single-domain antibody (sdAb) moiety specifically recognizing CTLA-4 (“anti-CTLA-4 sdAb moiety”), wherein the anti-CTLA-4 sdAb moiety comprises any one of the following:
 - (1) a CDR1 comprising the amino acid sequence of SEQ ID NO: 24; a CDR2 comprising the amino acid sequence of SEQ ID NO: 56; and a CDR3 comprising the amino acid sequence of SEQ ID NO: 88;
 - (2) a CDR1 comprising the amino acid sequence of SEQ ID NO: 18; a CDR2 comprising the amino acid sequence of SEQ ID NO: 50; and a CDR3 comprising the amino acid sequence of SEQ ID NO: 82;
 - (3) a CDR1 comprising the amino acid sequence of SEQ ID NO: 26; a CDR2 comprising the amino acid sequence of SEQ ID NO: 58; and a CDR3 comprising the amino acid sequence of SEQ ID NO: 90;
 - (4) a CDR1 comprising the amino acid sequence of SEQ ID NO: 213; a CDR2 comprising the amino acid sequence of SEQ ID NO: 233; and a CDR3 comprising the amino acid sequence of SEQ ID NO: 253;
 - (5) a CDR1 comprising the amino acid sequence of SEQ ID NO: 17; a CDR2 comprising the amino acid sequence of SEQ ID NO: 49; and a CDR3 comprising the amino acid sequence of SEQ ID NO: 81;
 - (6) a CDR1 comprising the amino acid sequence of SEQ ID NO: 19; a CDR2 comprising the amino acid sequence of SEQ ID NO: 51; and a CDR3 comprising the amino acid sequence of SEQ ID NO: 83;
 - (7) a CDR1 comprising the amino acid sequence of SEQ ID NO: 20; a CDR2 comprising the amino acid sequence of SEQ ID NO: 52; and a CDR3 comprising the amino acid sequence of SEQ ID NO: 84;
 - (8) a CDR1 comprising the amino acid sequence of SEQ ID NO: 21; a CDR2 comprising the amino acid sequence of SEQ ID NO: 53; and a CDR3 comprising the amino acid sequence of SEQ ID NO: 85;
 - (9) a CDR1 comprising the amino acid sequence of SEQ ID NO: 22; a CDR2 comprising the amino acid sequence of SEQ ID NO: 54; and a CDR3 comprising the amino acid sequence of SEQ ID NO: 86;

- (10) a CDR1 comprising the amino acid sequence of SEQ ID NO: 23; a CDR2 comprising the amino acid sequence of SEQ ID NO: 55; and a CDR3 comprising the amino acid sequence of SEQ ID NO: 87;
- ~~(11) a CDR1 comprising the amino acid sequence of SEQ ID NO: 25; a CDR2 comprising the amino acid sequence of SEQ ID NO: 57; and a CDR3 comprising the amino acid sequence of SEQ ID NO: 89;~~
- ~~(12)~~(11) a CDR1 comprising the amino acid sequence of SEQ ID NO: 27; a CDR2 comprising the amino acid sequence of SEQ ID NO: 59; and a CDR3 comprising the amino acid sequence of SEQ ID NO: 91;
- ~~(13)~~(12) a CDR1 comprising the amino acid sequence of SEQ ID NO: 28; a CDR2 comprising the amino acid sequence of SEQ ID NO: 60; and a CDR3 comprising the amino acid sequence of SEQ ID NO: 92;
- ~~(14)~~(13) a CDR1 comprising the amino acid sequence of SEQ ID NO: 29; a CDR2 comprising the amino acid sequence of SEQ ID NO: 61; and a CDR3 comprising the amino acid sequence of SEQ ID NO: 93;
- ~~(15)~~(14) a CDR1 comprising the amino acid sequence of SEQ ID NO: 30; a CDR2 comprising the amino acid sequence of SEQ ID NO: 62; and a CDR3 comprising the amino acid sequence of SEQ ID NO: 94;
- ~~(16)~~(15) a CDR1 comprising the amino acid sequence of SEQ ID NO: 31; a CDR2 comprising the amino acid sequence of SEQ ID NO: 63; and a CDR3 comprising the amino acid sequence of SEQ ID NO: 95;
- ~~(17)~~(16) a CDR1 comprising the amino acid sequence of SEQ ID NO: 32; a CDR2 comprising the amino acid sequence of SEQ ID NO: 64; and a CDR3 comprising the amino acid sequence of SEQ ID NO: 96;
- ~~(18)~~(17) a CDR1 comprising the amino acid sequence of SEQ ID NO: 214; a CDR2 comprising the amino acid sequence of SEQ ID NO: 234; and a CDR3 comprising the amino acid sequence of SEQ ID NO: 254;
- ~~(19)~~(18) a CDR1 comprising the amino acid sequence of SEQ ID NO: 215; a CDR2 comprising the amino acid sequence of SEQ ID NO: 235; and a CDR3 comprising the amino acid sequence of SEQ ID NO: 255;
- ~~(20)~~(19) a CDR1 comprising the amino acid sequence of SEQ ID NO: 216; a CDR2 comprising the amino acid sequence of SEQ ID NO: 236; and a CDR3 comprising the amino acid sequence of SEQ ID NO: 256;

- ~~(24)~~(20) a CDR1 comprising the amino acid sequence of SEQ ID NO: 217; a CDR2 comprising the amino acid sequence of SEQ ID NO: 237; and a CDR3 comprising the amino acid sequence of SEQ ID NO: 257;
- ~~(22)~~(21) a CDR1 comprising the amino acid sequence of SEQ ID NO: 218; a CDR2 comprising the amino acid sequence of SEQ ID NO: 238; and a CDR3 comprising the amino acid sequence of SEQ ID NO: 258;
- ~~(23)~~(22) a CDR1 comprising the amino acid sequence of SEQ ID NO: 219; a CDR2 comprising the amino acid sequence of SEQ ID NO: 239; and a CDR3 comprising the amino acid sequence of SEQ ID NO: 259;
- ~~(24)~~(23) a CDR1 comprising the amino acid sequence of SEQ ID NO: 220; a CDR2 comprising the amino acid sequence of SEQ ID NO: 240; and a CDR3 comprising the amino acid sequence of SEQ ID NO: 260;
- ~~(25)~~(24) a CDR1 comprising the amino acid sequence of SEQ ID NO: 221; a CDR2 comprising the amino acid sequence of SEQ ID NO: 241; and a CDR3 comprising the amino acid sequence of SEQ ID NO: 261;
- ~~(26)~~(25) a CDR1 comprising the amino acid sequence of SEQ ID NO: 222; a CDR2 comprising the amino acid sequence of SEQ ID NO: 242; and a CDR3 comprising the amino acid sequence of SEQ ID NO: 262; or
- ~~(27)~~(26) a CDR1 comprising the amino acid sequence of SEQ ID NO: 214; a CDR2 comprising the amino acid sequence of SEQ ID NO: 339; and a CDR3 comprising the amino acid sequence of SEQ ID NO: 254.
2. The isolated anti-CTLA-4 construct of claim 1, wherein the anti-CTLA-4 sdAb moiety comprises a V_HH domain comprising the amino acid sequence of any one of SEQ ID NOs: 113-120, 122-129, 200-202, 274-282, 341-344, 352, and 353.
 3. The isolated anti-CTLA-4 construct of claim 1 or 2, wherein the anti-CTLA-4 sdAb moiety is camelid, chimeric, ~~human~~, partially humanized, or fully humanized.
 4. The isolated anti-CTLA-4 construct of any one of claims 1-3, wherein the isolated anti-CTLA-4 construct is a heavy chain-only antibody (HCAb).
 5. The isolated anti-CTLA-4 construct of claim 4, wherein the HCAb is monomeric or dimeric.
 6. The isolated anti-CTLA-4 construct of claim 4 or 5, wherein the HCAb comprises the amino acid sequence of any one of SEQ ID NOs: 130-133, 283-291, and 366-371.

7. The isolated anti-CTLA-4 construct of any one of claims 1-3, wherein the isolated anti-CTLA-4 construct further comprises a second antibody moiety specifically recognizing a second epitope.
8. The isolated anti-CTLA-4 construct of claim 7, 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.
9. The isolated anti-CTLA-4 construct of claim 7 or 8, wherein the anti-CTLA-4 sdAb moiety and the second antibody moiety are connected by a peptide linker.
10. The isolated anti-CTLA-4 construct of claim 9, wherein the peptide linker comprises the amino acid sequence of SEQ ID NO: 162, 163, 307, or 365.
11. The isolated anti-CTLA-4 construct of any one of claims 7-10, wherein the second antibody moiety is a full-length antibody consisting of two heavy chains and two light chains.
12. The isolated anti-CTLA-4 construct of claim 11, comprising a configuration selected from the group consisting of:
 - (1) the N-terminus of the anti-CTLA-4 sdAb moiety is fused to the C-terminus of at least one of the heavy chains of the full-length antibody;
 - (2) the C-terminus of the anti-CTLA-4 sdAb moiety is fused to the N-terminus of at least one of the heavy chains of the full-length antibody;
 - (3) the N-terminus of the anti-CTLA-4 sdAb moiety is fused to the C-terminus of at least one of the light chains of the full-length antibody;
 - (4) the C-terminus of the anti-CTLA-4 sdAb moiety is fused to the N-terminus of at least one of the light chains of the full-length antibody;
 - (5) the isolated anti-CTLA-4 construct comprises four anti-CTLA-4 sdAb moieties, wherein the C-terminus of each anti-CTLA-4 sdAb moiety is fused to the N-terminus of each chain of the full-length antibody; and
 - (6) the isolated anti-CTLA-4 construct comprises four anti-CTLA-4 sdAb moieties, wherein the C-terminus of the first anti-CTLA-4 sdAb moiety is fused to the N-terminus of the second anti-CTLA-4 sdAb moiety, wherein the C-terminus of the second anti-CTLA-4 sdAb moiety is fused to the N-terminus of the first heavy chain of the full-length antibody, wherein the C-terminus of the third anti-CTLA-4 sdAb moiety is fused to the N-terminus of the fourth anti-CTLA-4 sdAb moiety, and wherein the C-terminus of the fourth anti-CTLA-4 sdAb moiety is fused to the N-terminus of the second heavy chain of the full-length antibody~~two of the four~~

~~anti-CTLA-4 sdAb moieties are fused together, which is further fused to the N-terminus of each heavy chain of the full-length antibody.~~

13. The isolated anti-CTLA-4 construct of claim 11 or 12, wherein the full-length antibody specifically recognizes programmed cell death protein 1 (PD-1) (anti-PD-1 full-length antibody).
14. The isolated anti-CTLA-4 construct of claim 13, wherein the anti-PD-1 full-length antibody comprises heavy chain complementarity-determining regions (CDRs) and light chain CDRs of:
 - (1) a heavy chain comprising the amino acid sequence of SEQ ID NO: 158, and a light chain comprising the amino acid sequence of SEQ ID NO: 159;
 - (2) a heavy chain comprising the amino acid sequence of SEQ ID NO: 160, and a light chain comprising the amino acid sequence of SEQ ID NO: 161; or
 - (3) a heavy chain comprising the amino acid sequence of SEQ ID NO: 308, and a light chain comprising the amino acid sequence of SEQ ID NO: 309.
15. The isolated anti-CTLA-4 construct of claim 13 or 14,
 - (i) wherein the isolated anti-CTLA-4 construct comprises a heavy chain fusion polypeptide comprising the amino acid sequence of any one of SEQ ID NOs: 134-157, 292-301, 310-337, 356, and 358; and/or
 - (ii) wherein the isolated anti-CTLA-4 construct comprises a light chain fusion polypeptide comprising the amino acid sequence of any one of SEQ ID NOs: 354, 355, and 357.
- ~~16. The isolated anti-CTLA-4 construct of any one of claims 13-15, wherein the isolated anti-CTLA-4 construct comprises a light chain fusion polypeptide comprising the amino acid sequence of any one of SEQ ID NOs: 354, 355, and 357.~~
- ~~17.~~16. The isolated anti-CTLA-4 construct of any one of claims 13-15~~6~~, wherein:
 - (a) the anti-PD-1 full-length antibody comprises ~~two~~a heavy chains each comprising the amino acid sequence of SEQ ID NO: 158, and ~~two~~a light chains each comprising the amino acid sequence of SEQ ID NO: 159, wherein at least one of the heavy chains of the anti-PD-1 full-length antibody is fused to the anti-CTLA-4 sdAb moiety, and wherein the heavy chain fusion polypeptide comprises the amino acid sequence of any one of SEQ ID NOs: 134-145, 292-296, and 319-323;
 - (b) the anti-PD-1 full-length antibody comprises ~~two~~a heavy chains each comprising the amino acid sequence of SEQ ID NO: 160 and ~~two~~a light chains each comprising the amino acid sequence of SEQ ID NO: 161, wherein at least one of the heavy chains of the anti-PD-1

full-length antibody is fused to the anti-CTLA-4 sdAb moiety, and wherein the heavy chain fusion polypeptide comprises the amino acid sequence of any one of SEQ ID NOs: 146-157, 297-301, and 324-328;

(c) the anti-PD-1 full-length antibody comprises two heavy chains each comprising the amino acid sequence of SEQ ID NO: 308 and two light chains each comprising the amino acid sequence of SEQ ID NO: 309, wherein at least one of the heavy chains of the anti-PD-1 full-length antibody is fused to the anti-CTLA-4 sdAb moiety, and wherein the heavy chain fusion polypeptide comprises the amino acid sequence of any one of SEQ ID NOs: 310-318 and 329-337;

(d) the anti-PD-1 full-length antibody comprises two heavy chains each comprising the amino acid sequence of SEQ ID NO: 158, and two light chains each comprising the amino acid sequence of SEQ ID NO: 159, wherein the isolated anti-CTLA-4 construct comprises four anti-CTLA-4 sdAb moieties, wherein the C-terminus of each anti-CTLA-4 sdAb moiety is fused to the N-terminus of each chain of the anti-PD-1 full-length antibody, wherein the heavy chain fusion polypeptides each comprises the amino acid sequence of SEQ ID NO: 356, and the light chain fusion polypeptides each comprises the amino acid sequence of SEQ ID NO: 357;

(e) the anti-PD-1 full-length antibody comprises two heavy chains each comprising the amino acid sequence of SEQ ID NO: 158 and two light chains each comprising the amino acid sequence of SEQ ID NO: 159, wherein the isolated anti-CTLA-4 construct comprises four anti-CTLA-4 sdAb moieties, wherein the C-terminus of the first anti-CTLA-4 sdAb moiety is fused to the N-terminus of the second anti-CTLA-4 sdAb moiety, wherein the C-terminus of the second anti-CTLA-4 sdAb moiety is fused to the N-terminus of the first heavy chain of the anti-PD-1 full-length antibody, wherein the C-terminus of the third anti-CTLA-4 sdAb moiety is fused to the N-terminus of the fourth anti-CTLA-4 sdAb moiety, wherein the C-terminus of the fourth anti-CTLA-4 sdAb moiety is fused to the N-terminus of the second ~~two of the four anti-CTLA-4 sdAb moieties are fused together, which is further fused to the N-terminus of each~~ heavy chain of the anti-PD-1 full-length antibody, ~~wherein the full-length antibody comprises a heavy chain comprising the amino acid sequence of SEQ ID NO: 158 and a light chain comprising the amino acid sequence of SEQ ID NO: 159,~~ and wherein the heavy chain fusion polypeptides each comprises the amino acid sequence of SEQ ID NO: 358; or

(f) the anti-PD-1 full-length antibody comprises two heavy chains each comprising the amino acid sequence of SEQ ID NO: 158 and two light chains each comprising the amino acid sequence of SEQ ID NO: 159, wherein at least one of the light chains of the anti-PD-1

full-length antibody is fused to the anti-CTLA-4 sdAb moiety, and wherein the light chain fusion polypeptide comprises the amino acid sequence of SEQ ID NO: 354 or 355.

17. The isolated anti-CTLA-4 construct of any one of claims 13-16, wherein each heavy chain of the anti-PD-1 full-length antibody is fused to an anti-CTLA-4 sdAb moiety,
- (i) wherein the anti-PD-1 full-length antibody comprises two heavy chains each comprising the amino acid sequence of SEQ ID NO: 158, and two light chains each comprising the amino acid sequence of SEQ ID NO: 159, and wherein each heavy chain fusion polypeptide comprises the amino acid sequence of SEQ ID NO: 292;
 - (ii) wherein the anti-PD-1 full-length antibody comprises two heavy chains each comprising the amino acid sequence of SEQ ID NO: 158, and two light chains each comprising the amino acid sequence of SEQ ID NO: 159, and wherein each heavy chain fusion polypeptide comprises the amino acid sequence of SEQ ID NO: 293;
 - (iii) wherein the anti-PD-1 full-length antibody comprises two heavy chains each comprising the amino acid sequence of SEQ ID NO: 158, and two light chains each comprising the amino acid sequence of SEQ ID NO: 159, and wherein each heavy chain fusion polypeptide comprises the amino acid sequence of SEQ ID NO: 294;
 - (iv) wherein the anti-PD-1 full-length antibody comprises two heavy chains each comprising the amino acid sequence of SEQ ID NO: 158, and two light chains each comprising the amino acid sequence of SEQ ID NO: 159, and wherein each heavy chain fusion polypeptide comprises the amino acid sequence of SEQ ID NO: 295;
 - (v) wherein the anti-PD-1 full-length antibody comprises two heavy chains each comprising the amino acid sequence of SEQ ID NO: 158, and two light chains each comprising the amino acid sequence of SEQ ID NO: 159, and wherein each heavy chain fusion polypeptide comprises the amino acid sequence of SEQ ID NO: 296;
 - (vi) wherein the anti-PD-1 full-length antibody comprises two heavy chains each comprising the amino acid sequence of SEQ ID NO: 160, and two light chains each comprising the amino acid sequence of SEQ ID NO: 161, and wherein each heavy chain fusion polypeptide comprises the amino acid sequence of SEQ ID NO: 297;
 - (vii) wherein the anti-PD-1 full-length antibody comprises two heavy chains each comprising the amino acid sequence of SEQ ID NO: 160, and two light chains each comprising the amino acid sequence of SEQ ID NO: 161, and wherein each heavy chain fusion polypeptide comprises the amino acid sequence of SEQ ID NO: 298; or
 - (viii) wherein the anti-PD-1 full-length antibody comprises two heavy chains each comprising the amino acid sequence of SEQ ID NO: 160, and two light chains each

comprising the amino acid sequence of SEQ ID NO: 161, and wherein each heavy chain fusion polypeptide comprises the amino acid sequence of SEQ ID NO: 299.

18. The isolated anti-CTLA-4 construct of claim 11 or 12, wherein the full-length antibody specifically recognizes programmed death-ligand 1 (PD-L1) (anti-PD-L1 full-length antibody).
19. The isolated anti-CTLA-4 construct of claim 18, wherein the anti-PD-L1 full-length antibody comprises heavy chain CDRs and light chain CDRs of:
- (1) a heavy chain comprising the amino acid sequence of SEQ ID NO: 195, and a light chain comprising the amino acid sequence of SEQ ID NO: 196; or
 - (2) a heavy chain comprising the amino acid sequence of SEQ ID NO: 197, and a light chain comprising the amino acid sequence of SEQ ID NO: 198.

20. The isolated anti-CTLA-4 construct of claim 18 or 19, wherein the isolated anti-CTLA-4 construct comprises a heavy chain fusion polypeptide comprising the amino acid sequence of any one of SEQ ID NOs: 171-194, 302-306, and 345-349.

~~20.21.~~ The isolated anti-CTLA-4 construct of any one of claims 18-20 ~~or 19~~, wherein:

(a) the anti-PD-L1 full-length antibody comprises two heavy chains each comprising the amino acid sequence of SEQ ID NO: 195 and two light chains each comprising the amino acid sequence of SEQ ID NO: 196, wherein at least one of the heavy chains of the anti-PD-L1 full-length antibody is fused to the anti-CTLA-4 sdAb moiety, and wherein the heavy chain fusion polypeptide comprises the amino acid sequence of any one of SEQ ID NOs: 171-182, 302-306, and 345-349; or

(b) the anti-PD-L1 full-length antibody comprises two heavy chains each comprising the amino acid sequence of SEQ ID NO: 197 and two light chains each comprising the amino acid sequence of SEQ ID NO: 198, wherein at least one of the heavy chains of the anti-PD-L1 full-length antibody is fused to the anti-CTLA-4 sdAb moiety, and wherein the heavy chain fusion polypeptide comprises the amino acid sequence of any one of SEQ ID NOs: 183-194; ~~or~~

~~(c) the isolated anti-CTLA-4 construct comprises a heavy chain fusion polypeptide comprising the amino acid sequence of any one of SEQ ID NOs: 171-194, 302-306, and 345-349.~~

21.22. The isolated anti-CTLA-4 construct of any one of claims 7-10, comprising a configuration selected from the group consisting of:

(a) the isolated anti-CTLA-4 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-CTLA-4 sdAb moiety-C_{H2}-

C_H3, (iii) V_H-C_H1-optional peptide linker-second anti-CTLA-4 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-CTLA-4 construct comprises two polypeptides from N-terminus to C-terminus: (i) first scFv specifically recognizing a second epitope-optional peptide linker-first anti-CTLA-4 sdAb moiety-C_H2-C_H3, and (ii) second scFv specifically recognizing a third epitope-optional peptide linker-second anti-CTLA-4 sdAb moiety-C_H2-C_H3;

(c) the isolated anti-CTLA-4 construct comprises four polypeptides from N-terminus to C-terminus: (i) V_L-C_L-optional peptide linker-first anti-CTLA-4 sdAb moiety-C_L, (ii) V_H-C_H1-optional peptide linker-second anti-CTLA-4 sdAb moiety-C_H1-C_H2-C_H3, (iii) V_H-C_H1-optional peptide linker-third anti-CTLA-4 sdAb moiety-C_H1-C_H2-C_H3, and (iv) V_L-C_L-optional peptide linker-fourth anti-CTLA-4 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-CTLA-4 construct comprises four polypeptides from N-terminus to C-terminus: (i) first anti-CTLA-4 sdAb moiety-C_L, (ii) first scFv specifically recognizing a second epitope-optional peptide linker-second anti-CTLA-4 sdAb moiety-C_H1-C_H2-C_H3, (iii) second scFv specifically recognizing a third epitope-optional peptide linker-third anti-CTLA-4 sdAb moiety-C_H1-C_H2-C_H3, and (iv) fourth anti-CTLA-4 sdAb moiety-C_L.

~~22.23.~~ The isolated anti-CTLA-4 construct of any one of claims 7-10 and ~~22~~²¹, wherein the isolated anti-CTLA-4 construct comprises any of:

(a) two identical polypeptides comprising the amino acid sequence of SEQ ID NO: 359, and two identical polypeptides comprising the amino acid sequence of SEQ ID NO: 159;

(b) two identical polypeptides comprising the amino acid sequence of SEQ ID NO: 360;

(c) two identical polypeptides comprising the amino acid sequence of SEQ ID NO: 361, and two identical polypeptides comprising the amino acid sequence of SEQ ID NO: 362; and

(d) two identical polypeptides comprising the amino acid sequence of SEQ ID NO: 363, and two identical polypeptides comprising the amino acid sequence of SEQ ID NO: 364.

~~23. An isolated anti-CTLA-4 construct that specifically binds to CTLA-4 competitively with the isolated anti-CTLA-4 construct of any one of claims 1-22.~~

24. A pharmaceutical composition comprising the isolated anti-CTLA-4 construct of any one of claims 1-23, and a pharmaceutical acceptable carrier.

25. Use of an effective amount of the isolated anti-CTLA-4 construct of any one of claims 1-23 or the pharmaceutical composition of claim 24 in the manufacture of a medicament for treating a cancer or a pathogenic-viral infection in an individual.
- ~~26. The use of claim 25, wherein the pathogenic infection is a viral infection.~~
- ~~27.~~26. The use of claim 25, wherein the cancer is a colon cancer.
- ~~28.~~27. The use of any one of claims 25 or 26-27, wherein the medicament is to be administered systemically or locally.