between B7 and CTLA-4 dampens T cell activation, which constitutes an important mechanism of tumor immune escape. Anti-CTLA-4 antibody therapy has shown promise in a number of cancers, such as melanoma.

[0006] Single-chain antibodies (sdAbs) are different from conventional 4-chain antibodies by having a single monomeric antibody variable domain. For example, camelids and sharks produce single-domain antibodies named heavy chain-only antibodies (HCAbs), which naturally lack light chains. The antigen-binding fragment in each arm of the camelid HCAb has a single heavy chain variable domain (V_HH), which can exhibit high affinity to an antigen without the aid of a light chain. Camelid V_HH is known as the smallest functional antigen-binding fragment with a molecular weight of approximately 15 kD.

[0007] 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

[0008] The present disclosure relates to constructs comprising a single-domain antibody (sdAb) moiety that specifically recognizes CTLA-4, and methods of making and using thereof.

[0009] Disclosed is an isolated anti-CTLA-4 construct comprising a sdAb moiety specifically recognizing CTLA-4, wherein the sdAb moiety comprises a CDR1 comprising the amino acid sequence of any one of SEQ ID NOs: 17-32 and 213-222, 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 any one of SEQ ID NOs: 49-64, 233-242, and 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 any one of SEQ ID NOs: 81-96 and 253-262, or a variant thereof comprising up to about 3 (such as about any of 1, 2, or 3) amino acid substitutions.

[0009a] In one example, there is provided an isolated anti-CTLA-4 construct comprising a sdAb moiety specifically recognizing CTLA-4, wherein the sdAb moiety comprises a CDR1 comprising the amino acid sequence of any one of SEQ ID NOs: 24, 18, 26, 213, 17, 19, 20, 21, 22, 23, 25, 27, 28, 29, 30, 31, 32, 214, 215, 216, 217, 218, 219, 220, 221 and 222, or a variant thereof comprising up to about 3 amino acid substitutions; a CDR2 comprising the amino acid sequence of any one of SEQ ID NOs: 56, 50, 58, 233, 49, 51, 52, 53, 54, 55, 57, 59, 60, 61, 62, 63, 64, 234, 235, 236, 237, 238, 239, 240, 241, 242, and 339, or a variant thereof comprising up to about 3 amino acid substitutions; and a CDR3 comprising the amino acid sequence of any one of SEQ ID NOs: 88, 82, 90, 253, 81, 83, 84, 85, 86, 87, 89, 91, 92, 93, 94, 95, 96, 254, 255, 256, 257, 258, 259, 260, 261, and 262, or a variant thereof comprising up to about 3 amino acid substitutions.

[0010] In some examples according to any one of the isolated anti-CTLA-4 constructs described above, the sdAb moiety specifically recognizing CTLA-4 comprises a CDR1 comprising the amino acid sequence of any one of SEQ ID NOs: 17-32 and 213-222; a CDR2 comprising the amino acid sequence of any one of SEQ ID NOs: 49-64, 233-242, and 339; and a

CDR3 comprising the amino acid sequence of any one of SEQ ID NOs: 81-96 and 253-262; 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.

[0011] 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, 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: 49, 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: 81, 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: 18, 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: 50, 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: 82, 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: 19, 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: 51, 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: 83, 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: 20, 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: 52, 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: 84, or a variant thereof comprising up to about 3 (such as about any of 1, 2, or 3) amino acid substitutions;

(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-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) 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) 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) 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) 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) 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) 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) 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) 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) 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) 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) 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) 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) 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) 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) 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) 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 NO: 81; 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: 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; 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;

(24) 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; 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;

(25) 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; 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;

(26) 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 a variant thereof comprising up to about 3 (such as about any of 1, 2, or 3) amino acid substitutions in the CDR regions; or

(27) 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; 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.

[0013] In some examples according to any one of the isolated anti-CTLA-4 constructs described above, the sdAb moiety specifically recognizing CTLA-4 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, V, L, A, H, S, I, W, C, N, G, D, T, and P (such as F, Y, L, I, or V, such as F or Y, or such as F); a-2) the amino acid residue at position 44 is selected from the group consisting of E, Q, G, D, A, K, R, L, P, S, V, H, T, N, W, M, and I (such as A, G, E, D, Q, R, S, or L, or such as G, E, or Q); a-3) the amino acid residue at position 45 is selected from the group consisting of L, R, P, H, F, G, Q, S, E, T, Y, C, I, D, and V (such as L, C, or R, or such as L or R); a-4) the amino acid residue at position 103 is selected from the group consisting of W, R, G, S, K, A, M, Y, I, F, T, N, V, Q, P, E, and C (such as W, G, or R, or such as W); and a-5) the amino acid residue at position 108 is selected from the group consisting of Q, L, R, P, E, K, S, T, M, A, and H (such as 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 or Y, or such as F); b-2) the amino acid residue at position 44 is selected from the group consisting of E and Q; b-3) the

amino acid residue at position 45 is selected from the group consisting of L and R (such as R); b-4) the amino acid residue at position 103 is selected from the group consisting of G, 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); or c-1) the amino acid residue at position 37 is selected from the group consisting of F, Y, L, I, and V (such as F or Y, or such as F); c-2) the amino acid residue at position 44 is selected from the group consisting of A, G, E, D, Q, R, S and L (such as G, E, or Q); c-3) the amino acid residue at position 45 is selected from the group consisting of L, R and C (such as L or R); c-4) the amino acid residue at position 103 is selected from the group consisting of P, R and S (such as R or S); and c-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. In some examples, position 108 can be optionally humanized to L when position 108 is Q.

[0014] In some examples according to any one of the isolated anti-CTLA-4 constructs described above, the sdAb moiety specifically recognizing CTLA-4 comprises a V_HH domain comprising the amino acid sequence of any one of SEQ ID NOs: 113-129, 200-202, 274-282, 341-344, 352, and 353, 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 identify to any one of SEQ ID NOs: 113-129, 200-202, 274-282, 341-344, 352, and 353. In some examples, the sdAb moiety specifically recognizing CTLA-4 comprises a V_HH domain comprising the amino acid sequence of any one of SEQ ID NOs: 113-129, 200-202, 274-282, 341-344, 352, and 353, 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 sdAb moiety specifically recognizing CTLA-4 comprises a V_HH domain comprising the amino acid sequence of any one of SEQ ID NOs: 113-129, 200-202, 274-282, 341-344, 352, and 353. In some examples, the sdAb moiety specifically recognizing CTLA-4 comprises a V_HH domain comprising the amino acid sequence of any one of SEQ ID NOs: 114, 129, 201, 202, 274-282, 341-344, 352, and 353.

[0015] In some examples according to any one of the isolated anti-CTLA-4 constructs described above, the K_d of the binding between the sdAb moiety specifically recognizing CTLA-4 and CTLA-4 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).

[0016] In some examples according to any one of the isolated anti-CTLA-4 constructs described above, the sdAb moiety specifically recognizing CTLA-4 is camelid, chimeric, human, partially humanized, or fully humanized.

[0017] In some examples according to any one of the isolated anti-CTLA-4 constructs described above, the isolated anti-CTLA-4 construct is a heavy chain-only antibody (HCAb). In some examples, the sdAb moiety specifically recognizing CTLA-4 is fused to a human IgG1 Fc. In some examples, the HCAb is monomeric or dimeric. In some examples, the HCAb comprises the amino acid sequence of any one of SEQ ID NOs: 130-133, 283-291, and 366-371.

[0018] In some examples according to any one of the isolated anti-CTLA-4 construct described above, the isolated anti-CTLA-4 construct further comprises a second antibody moiety specifically recognizing a second antigen (or 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-CTLA-4 construct is monospecific. In some examples, the anti-CTLA-4 construct is multispecific (such as bispecific). In some examples, the sdAb moiety specifically recognizing CTLA-4 is fused to the N-terminus and/or C-terminus of the second antibody moiety. In some examples, the sdAb moiety specifically recognizing CTLA-4 and the second antibody moiety are optionally connected by a peptide linker (such as SEQ ID NO: 162, 163, 307, or 365). In some examples, the second antibody moiety is an sdAb. In some examples, the second epitope is from CTLA-4. In some examples, the second epitope is not from CTLA-4. In some examples, the isolated anti-CTLA-4 construct comprises two or more sdAbs that specifically recognize CTLA-4 (such as same or different epitopes of CTLA-4). In some examples, the second epitope is from human serum albumin (HSA).

[0019] In some examples according to any one of the isolated anti-CTLA-4 construct described above, the isolated anti-CTLA-4 construct further comprises a second antibody moiety specifically recognizing a second antigen, wherein the second antibody moiety is a full-length antibody consisting of two heavy chains and two light chains. In some examples, the anti-CTLA-4 construct is monospecific. In some examples, the anti-CTLA-4 construct is multispecific (such as bispecific). In some examples, the sdAb moiety specifically recognizing CTLA-4 is fused to the N-terminus and/or C-terminus of the second antibody moiety.

In some examples, the sdAb moiety specifically recognizing CTLA-4 and the second antibody moiety are optionally connected by a peptide linker (such as SEQ ID NO: 162, 163, 307, or 365). In some examples, the N-terminus of the sdAb moiety specifically recognizing CTLA-4 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 CTLA-4 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 CTLA-4 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 CTLA-4 is fused to the N-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 CTLA-4 is fused to the N-terminus of both heavy and light chains of the full-length antibody. In some examples, the full-length antibody specifically recognizes PD-1. In some examples, 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. In some examples, the full-length antibody comprises 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. In some examples, the full-length antibody comprises 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. In some examples, 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, wherein at least one of the heavy chains of the full-length antibody is fused to the anti-CTLA-4 sdAb, 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. In some examples, the full-length antibody comprises 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, wherein at least one of the heavy chains of the full-length antibody is fused to the anti-CTLA-4 sdAb, 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. In some examples, the full-length antibody comprises 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, wherein at

least one of the heavy chains of the full-length antibody is fused to the anti-CTLA-4 sdAb, and wherein the heavy chain fusion polypeptide comprises the amino acid sequence of any one of SEQ ID NOs: 310-318 and 329-337. In some examples, 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, wherein at least one of the light chains of the full-length antibody is fused to the anti-CTLA-4 sdAb, and wherein the light chain fusion polypeptide comprises the amino acid sequence of any one of SEQ ID NOs: 354 and 355. In some examples, 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, wherein the anti-CTLA-4 sdAb is fused to the N-terminus of both heavy and light chains of the full-length antibody, and wherein the heavy chain fusion polypeptide comprises the amino acid sequence of SEQ ID NO: 356, and the light chain fusion polypeptide comprises the amino acid sequence of SEQ ID NO: 357. In some examples, the anti-CTLA-4 construct comprises four identical sdAbs specifically recognizing CTLA-4, wherein fused to the N-terminus of each heavy chain of the full-length antibody are two identical sdAbs, wherein the two identical sdAbs are fused to each other via an optional peptide linker, and wherein the two identical sdAbs are fused to the N-terminus of each heavy chain of the full-length antibody via an optional peptide linker. In some examples, 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 polypeptide comprises the amino acid sequence of SEQ ID NO: 358. In some examples, the full-length antibody specifically recognizes PD-L1. In some examples, the full-length antibody comprises 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. In some examples, the full-length antibody comprises 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. In some examples, the full-length antibody comprises 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, wherein at least one of the heavy chains of the full-length antibody is fused to the anti-CTLA-4 sdAb, 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. In some examples,

the full-length antibody comprises 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, wherein at least one of the heavy chains of the full-length antibody is fused to the anti-CTLA-4 sdAb, and wherein the heavy chain fusion polypeptide comprises the amino acid sequence of any one of SEQ ID NOs: 183-194. the anti-CTLA-4 construct comprises four identical sdAbs specifically recognizing CTLA-4, wherein fused to the N-terminus of each heavy chain of the full-length antibody are two identical sdAbs, wherein the two identical sdAbs are fused to each other via an optional peptide linker, and wherein the two identical sdAbs are fused to the N-terminus of each heavy chain of the full-length antibody via an optional peptide linker.

[0020] In some examples according to any one of the isolated anti-CTLA-4 constructs described above, the anti-CTLA-4 construct comprises: (a) a first antigen binding portion comprising any of the sdAb moiety specifically recognizing CTLA-4 described above, and (b) a second antigen binding portion comprising a heavy chain variable domain (V_H) and a light chain variable domain (V_L), wherein the V_H and V_L together form an antigen-binding site that specifically binds a second epitope, wherein the first antigen binding portion and the second antigen binding portion are fused to each other. In some examples, the second epitope is from CTLA-4. In some examples, the second epitope is not from CTLA-4. In some examples, the anti-CTLA-4 construct is bispecific. In some examples, the second antigen binding portion is a full-length antibody consisting of two heavy chains and two light chains. In some examples, the second antigen binding portion is an antibody fragment comprising a heavy chain comprising the V_H and a light chain comprising the V_L (such as a Fab). In some examples, the first antigen binding portion comprises a single polypeptide chain comprising the sdAb moiety specifically recognizing CTLA-4. In some examples, the first antigen binding portion comprises two identical sdAb moieties specifically recognizing CTLA-4 fused together via an optional peptide linker. In some examples, the C-terminus of the first antigen binding portion is fused to the N-terminus of at least one heavy chain of the second antigen binding portion. In some examples, the C-terminus of the first antigen binding portion is fused to the N-terminus of at least one light chain of the second antigen binding portion. In some examples, the N-terminus of the first antigen binding portion is fused to the C-terminus of at least one heavy chain of the second antigen binding portion. In some examples, the N-terminus of the first antigen binding portion is fused to the C-terminus of at least one light chain

of the second antigen binding portion. In some examples, the C-terminus of the first antigen binding portion is fused to the N-terminus of both heavy and light chains of the second antigen binding portion. In some examples, the second antigen binding portion is an scFv. In some examples, the C-terminus of the first antigen binding portion is fused to the N-terminus of the second antigen binding portion. In some examples, the N-terminus of the first antigen binding portion is fused to the C-terminus of the second antigen binding portion. In some examples, the first antigen binding portion is a Fab-like domain comprising a first polypeptide chain comprising a first sdAb moiety specifically recognizing CTLA-4 fused to a C_H1 domain, and a second polypeptide chain comprising a second sdAb moiety specifically recognizing CTLA-4 fused to a C_L domain. In some examples, the first antigen binding portion is fused to the N-terminus of the second antigen binding portion. In some examples, the first antigen binding portion is fused to the C-terminus of the second antigen binding portion. In some examples, the first and/or second antigen binding portion comprises a human, humanized, human, or chimeric antibody or antigen binding fragment thereof. In some examples, the second antigen binding portion comprises an Fc region. In some examples, the first antigen binding portion is fused to the N-terminus of the Fc region. In some examples, the Fc region is an IgG1 Fc. In some examples, the Fc region is an IgG4 Fc having an S228P mutation. In some examples, the first antigen binding portion and the second antigen binding portion are fused to each other via an optional peptide linker (such as peptide linker comprising the amino acid sequence of SEQ ID NO: 162, 163, 307, or 365). In some examples, the second epitope is from an immune checkpoint molecule, such as PD-1, PD-L1. In some examples, the second antigen binding portion is an anti-PD-1 antibody or antigen binding fragment thereof. In some examples, the anti-PD-1 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. In some examples, the anti-PD-1 antibody comprises 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. In some examples, the full-length antibody comprises 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. In some examples, the anti-CTLA-4 construct comprises two copies of heavy chain fusion polypeptide comprising the amino acid sequence of any one of SEQ ID NOs: 134-145, 292-296,

319-323, 358, and 359, and two copies of light chains comprising the amino acid sequence of SEQ ID NO: 159. In some examples, the anti-CTLA-4 construct comprises two copies of heavy chains comprising the amino acid sequence of SEQ ID NO: 158, and two copies of light chain fusion polypeptide comprising the amino acid sequence of SEQ ID NO: 354 or 355. In some examples, the anti-CTLA-4 construct comprises two copies of heavy chain fusion polypeptide comprising the amino acid sequence of SEQ ID NO: 356, and two copies of light chain fusion polypeptide comprising the amino acid sequence of SEQ ID NO: 357. In some examples, the anti-CTLA-4 construct comprises two copies of heavy chain fusion polypeptide comprising the amino acid sequence of SEQ ID NO: 361, and two copies of light chain fusion polypeptide comprising the amino acid sequence of SEQ ID NO: 362. In some examples, the anti-CTLA-4 construct comprises two copies of heavy chain fusion polypeptide comprising the amino acid sequence of SEQ ID NO: 363, and two copies of light chain fusion polypeptide comprising the amino acid sequence of SEQ ID NO: 364. In some examples, the anti-CTLA-4 construct comprises two copies of polypeptide comprising the amino acid sequence of SEQ ID NO: 360. In some examples, the anti-CTLA-4 construct comprises two copies of heavy chain fusion polypeptide comprising the amino acid sequence of any one of SEQ ID NOs: 146-157, 297-301, and 324-328, and two copies of light chains comprising the amino acid sequence of SEQ ID NO: 161. In some examples, the anti-CTLA-4 construct comprises two copies of heavy chain fusion polypeptide comprising the amino acid sequence of any one of SEQ ID NOs: 310-318 and 329-337, and two copies of light chains comprising the amino acid sequence of SEQ ID NO: 309. In some examples, the second antigen binding portion is an anti-PD-L1 antibody or antigen binding fragment thereof. In some examples, the anti-PD-L1 antibody comprises 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. In some examples, the anti-PD-L1 antibody comprises 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. In some examples, the anti-CTLA-4 construct comprises two copies of heavy chain fusion polypeptide comprising the amino acid sequence of any one of SEQ ID NOs: 171-182, 302-306, and 345-349, and two copies of light chains comprising the amino acid sequence of SEQ ID NO: 196. In some examples, the anti-CTLA-4 construct comprises two copies of heavy chain fusion polypeptide comprising the amino acid sequence of any one 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, 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 in the manufacture of a medicament for treating a cancer or a pathogenic 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.

[0028] Further provided is an isolated host cell comprising any one of the isolated nucleic acid or vector described above.

[0029] Further provided is a kit comprising any one of the isolated anti-CTLA-4 construct, isolated nucleic acid, vector, or isolated host cell described above.

[0030] Disclosed is a method of producing any one of isolated anti-CTLA-4 construct 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-CTLA-4 construct; and obtaining the expressed anti-CTLA-4 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

[0031] FIG. 1 depicts immune response evaluation of pre-immune serum and immune serum after the 4^{th}, 5^{th}, and 6^{th} immunization.

[0032] FIG. 2 depicts the immune response evaluation of heavy chain antibodies (IgG2 and IgG3) after the 6^{th} immunization (terminal bleed). Heavy chain antibodies fractionated from pre-immune serum were used as negative controls.

[0033] FIGS. 3A-3B depict functional activity evaluation of sdAbs (FIG. 3A) and their heavy chain antibodies (FIG. 3B) by FACS-based ligand competition assay using B7-1 expressing stable cell lines and biotin-labeled hCTLA-4/Fc protein. Yervoy® was used as a positive anti-CTLA-4 antibody control.

[0034] FIG. 4 depicts functional activity evaluation of purified sdAbs by CTLA-4-based blockade assay. Yervoy® was used as a positive anti-CTLA-4 antibody control.

[0035] FIGS. 5A-5F depict functional activity evaluation of purified top HCAbs (FIGS. 5B-5E) by CTLA-4-based blockade assay. Yervoy® served as a positive anti-CTLA-4 antibody control (FIG. 5A). EC_{50} from all assays was summarized in FIG. 5F.

CLAIMS

What is claimed is:

1. An isolated 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) 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) 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) 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) 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) 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) 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) 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) 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) 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) 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) 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) 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) 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) 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) 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) 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: 113-129, 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 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 PD-1.
14. The isolated anti-CTLA-4 construct of claim 13, wherein the 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: 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, 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.

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. The isolated anti-CTLA-4 construct of any one of claims 13-16, wherein:

(a) 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, wherein at least one of the heavy chains of the 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 full-length antibody comprises 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, wherein at least one of the heavy chains of the 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 full-length antibody comprises 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, wherein at least one of the heavy chains of the 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 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, wherein the heavy chain fusion polypeptide comprises the amino acid sequence of SEQ ID NO: 356, and the light chain fusion polypeptide comprises the amino acid sequence of SEQ ID NO: 357;

(e) the isolated anti-CTLA-4 construct comprises four anti-CTLA-4 sdAb moieties, wherein 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, 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 polypeptide comprises the amino acid sequence of SEQ ID NO: 358; or

(f) 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, wherein at least one of the light chains of the 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.

18. The isolated anti-CTLA-4 construct of claim 11 or 12, wherein the full-length antibody specifically recognizes PD-L1.

19. The isolated anti-CTLA-4 construct of claim 18, wherein the 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:
(a) the full-length antibody comprises 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, wherein at least one of the heavy chains of the 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;

(b) the full-length antibody comprises 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, wherein at least one of the heavy chains of the 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. 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. The isolated anti-CTLA-4 construct of any one of claims 7-10 and 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 in the manufacture of a medicament for treating a cancer or a pathogenic infection in an individual.
26. The use of claim 25, wherein the pathogenic infection is a viral infection.
27. The use of claim 25, wherein the cancer is a colon cancer.
28. The use of any one of claims 25-27, wherein the medicament is to be administered systemically or locally.