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 aspect, 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 embodiments 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

(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, there is proivded an isolated anti-CTLA-4 construct comprising a single-domain antibody (sdAb) moiety specifically recognizing CTLA-4, wherein the sdAb moiety comprises any one of the following:

(1) a CDR1 comprising the amino acid sequence of SEQ ID NO: 24, or a variant thereof comprising up to about 3 amino acid substitutions; a CDR2 comprising the amino acid sequence of SEQ ID NO: 56, or a variant thereof comprising up to about 3 amino acid substitutions; and a CDR3 comprising the amino acid sequence of SEQ ID NO: 88, or a variant thereof comprising up to about 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 amino acid substitutions; a CDR2 comprising the amino acid sequence of SEQ ID NO: 50, or a variant thereof comprising up to about 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 amino acid substitutions;

(3) a CDR1 comprising the amino acid sequence of SEQ ID NO: 26, or a variant thereof comprising up to about 3 amino acid substitutions; a CDR2 comprising the amino acid sequence of SEQ ID NO: 58, or a variant thereof comprising up to about 3 amino acid substitutions; and a CDR3 comprising the amino acid sequence of SEQ ID NO: 90, or a variant thereof comprising up to about 3 amino acid substitutions;

(4) a CDR1 comprising the amino acid sequence of SEQ ID NO: 213, or a variant thereof comprising up to about 3 amino acid substitutions; a CDR2 comprising the amino acid sequence of SEQ ID NO: 233, or a variant thereof comprising up to about 3 amino acid substitutions; and a CDR3 comprising the amino acid sequence of SEQ ID NO: 253, or a variant thereof comprising up to about 3 amino acid substitutions;

(5) a CDR1 comprising the amino acid sequence of SEQ ID NO: 17, or a variant thereof comprising up to about 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 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 amino acid substitutions;

(6) a CDR1 comprising the amino acid sequence of SEQ ID NO: 19, or a variant thereof comprising up to about 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 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 amino acid substitutions;

(7) a CDR1 comprising the amino acid sequence of SEQ ID NO: 20, or a variant thereof comprising up to about 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 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 amino acid substitutions;

(8) a CDR1 comprising the amino acid sequence of SEQ ID NO: 21, or a variant thereof comprising up to about 3 amino acid substitutions; a CDR2 comprising the amino acid sequence of SEQ ID NO: 53, or a variant thereof comprising up to about 3 amino acid substitutions; and a CDR3 comprising the amino acid sequence of SEQ ID NO: 85, or a variant thereof comprising up to about 3 amino acid substitutions;

(9) a CDR1 comprising the amino acid sequence of SEQ ID NO: 22, or a variant thereof comprising up to about 3 amino acid substitutions; a CDR2 comprising the amino acid sequence of SEQ ID NO: 54, or a variant thereof comprising up to about 3 amino acid substitutions; and a CDR3 comprising the amino acid sequence of SEQ ID NO: 86, or a variant thereof comprising up to about 3 amino acid substitutions;

(10) a CDR1 comprising the amino acid sequence of SEQ ID NO: 23, or a variant thereof comprising up to about 3 amino acid substitutions; a CDR2 comprising the amino acid sequence of SEQ ID NO: 55, or a variant thereof comprising up to about 3 amino acid substitutions; and a CDR3 comprising the amino acid sequence of SEQ ID NO: 87, or a variant thereof comprising up to about 3 amino acid substitutions;

(11) a CDR1 comprising the amino acid sequence of SEQ ID NO: 25, or a variant thereof comprising up to about 3 amino acid substitutions; a CDR2 comprising the amino acid sequence of SEQ ID NO: 57, or a variant thereof comprising up to about 3 amino acid substitutions; and a CDR3 comprising the amino acid sequence of SEQ ID NO: 89, or a variant thereof comprising up to about 3 amino acid substitutions;

(12) a CDR1 comprising the amino acid sequence of SEQ ID NO: 27, or a variant thereof comprising up to about 3 amino acid substitutions; a CDR2 comprising the amino acid sequence of SEQ ID NO: 59, or a variant thereof comprising up to about 3 amino acid substitutions; and a CDR3 comprising the amino acid sequence of SEQ ID NO: 91, or a variant thereof comprising up to about 3 amino acid substitutions;

(13) a CDR1 comprising the amino acid sequence of SEQ ID NO: 28, or a variant thereof comprising up to about 3 amino acid substitutions; a CDR2 comprising the amino acid sequence of SEQ ID NO: 60, or a variant thereof comprising up to about 3 amino

acid substitutions; and a CDR3 comprising the amino acid sequence of SEQ ID NO: 92, or a variant thereof comprising up to about 3 amino acid substitutions;

(14) a CDR1 comprising the amino acid sequence of SEQ ID NO: 29, or a variant thereof comprising up to about 3 amino acid substitutions; a CDR2 comprising the amino acid sequence of SEQ ID NO: 61, or a variant thereof comprising up to about 3 amino acid substitutions; and a CDR3 comprising the amino acid sequence of SEQ ID NO: 93, or a variant thereof comprising up to about 3 amino acid substitutions;

(15) a CDR1 comprising the amino acid sequence of SEQ ID NO: 30, or a variant thereof comprising up to about 3 amino acid substitutions; a CDR2 comprising the amino acid sequence of SEQ ID NO: 62, or a variant thereof comprising up to about 3 amino acid substitutions; and a CDR3 comprising the amino acid sequence of SEQ ID NO: 94, or a variant thereof comprising up to about 3 amino acid substitutions;

(16) a CDR1 comprising the amino acid sequence of SEQ ID NO: 31, or a variant thereof comprising up to about 3 amino acid substitutions; a CDR2 comprising the amino acid sequence of SEQ ID NO: 63, or a variant thereof comprising up to about 3 amino acid substitutions; and a CDR3 comprising the amino acid sequence of SEQ ID NO: 95, or a variant thereof comprising up to about 3 amino acid substitutions;

(17) a CDR1 comprising the amino acid sequence of SEQ ID NO: 32, or a variant thereof comprising up to about 3 amino acid substitutions; a CDR2 comprising the amino acid sequence of SEQ ID NO: 64, or a variant thereof comprising up to about 3 amino acid substitutions; and a CDR3 comprising the amino acid sequence of SEQ ID NO: 96, or a variant thereof comprising up to about 3 amino acid substitutions;

(18) a CDR1 comprising the amino acid sequence of SEQ ID NO: 214, or a variant thereof comprising up to about 3 amino acid substitutions; a CDR2 comprising the amino acid sequence of SEQ ID NO: 234, or a variant thereof comprising up to about 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 amino acid substitutions;

(19) a CDR1 comprising the amino acid sequence of SEQ ID NO: 215, or a variant thereof comprising up to about 3 amino acid substitutions; a CDR2 comprising the amino acid sequence of SEQ ID NO: 235, or a variant thereof comprising up to about 3 amino

acid substitutions; and a CDR3 comprising the amino acid sequence of SEQ ID NO: 255, or a variant thereof comprising up to about 3 amino acid substitutions;

(20) a CDR1 comprising the amino acid sequence of SEQ ID NO: 216, or a variant thereof comprising up to about 3 amino acid substitutions; a CDR2 comprising the amino acid sequence of SEQ ID NO: 236, or a variant thereof comprising up to about 3 amino acid substitutions; and a CDR3 comprising the amino acid sequence of SEQ ID NO: 256, or a variant thereof comprising up to about 3 amino acid substitutions;

(21) a CDR1 comprising the amino acid sequence of SEQ ID NO: 217, or a variant thereof comprising up to about 3 amino acid substitutions; a CDR2 comprising the amino acid sequence of SEQ ID NO: 237, or a variant thereof comprising up to about 3 amino acid substitutions; and a CDR3 comprising the amino acid sequence of SEQ ID NO: 257, or a variant thereof comprising up to about 3 amino acid substitutions;

(22) a CDR1 comprising the amino acid sequence of SEQ ID NO: 218, or a variant thereof comprising up to about 3 amino acid substitutions; a CDR2 comprising the amino acid sequence of SEQ ID NO: 238, or a variant thereof comprising up to about 3 amino acid substitutions; and a CDR3 comprising the amino acid sequence of SEQ ID NO: 258, or a variant thereof comprising up to about 3 amino acid substitutions;

(23) a CDR1 comprising the amino acid sequence of SEQ ID NO: 219, or a variant thereof comprising up to about 3 amino acid substitutions; a CDR2 comprising the amino acid sequence of SEQ ID NO: 239, or a variant thereof comprising up to about 3 amino acid substitutions; and a CDR3 comprising the amino acid sequence of SEQ ID NO: 259, or a variant thereof comprising up to about 3 amino acid substitutions;

(24) a CDR1 comprising the amino acid sequence of SEQ ID NO: 220, or a variant thereof comprising up to about 3 amino acid substitutions; a CDR2 comprising the amino acid sequence of SEQ ID NO: 240, or a variant thereof comprising up to about 3 amino acid substitutions; and a CDR3 comprising the amino acid sequence of SEQ ID NO: 260, or a variant thereof comprising up to about 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 amino acid substitutions; a CDR2 comprising the amino acid sequence of SEQ ID NO: 241, or a variant thereof comprising up to about 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 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 amino acid substitutions; a CDR2 comprising the amino acid sequence of SEQ ID NO: 242, or a variant thereof comprising up to about 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 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 amino acid substitutions; a CDR2 comprising the amino acid sequence of SEQ ID NO: 339, or a variant thereof comprising up to about 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 amino acid substitutions.

[0012] In some embodiments 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;

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

[0021] In some embodiments 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 aspect, 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, there is provided an isolated anti-CTLA-4 construct that specifically binds to CTLA-4 competitively with an sdAb moiety comprising CDR1, CDR2, and CDR3 of any one of SEQ ID NOs: 113-129, 200-202, 274-282, 341-344, 352, and 353.

[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, there is provided 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 embodiments, the CTLA-4 related disease is cancer. In some embodiments, the cancer is a solid tumor, such as a colon cancer. In some embodiments, 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 embodiments, the CTLA-4 related disease is a pathogenic infection. In some embodiments, the pharmaceutical composition is administered systemically, such as intravenously (i.v.) or intraperitoneally (i.p.). In some embodiments, the pharmaceutical composition is administered locally, such as intratumorally. In some embodiments, the individual is a human.

[0025a] In another aspect, there is provided use of an effective amount of the isolated anti-CTLA-4 construct as described herein in the manufacture of a medicament for treating a CTLA-4-related disease 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 embodiments, 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 embodiments, 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, wherein the sdAb moiety comprises any one of the following:

(1) a CDR1 comprising the amino acid sequence of SEQ ID NO: 24, or a variant thereof comprising up to about 3 amino acid substitutions; a CDR2 comprising the amino acid sequence of SEQ ID NO: 56, or a variant thereof comprising up to about 3 amino acid substitutions; and a CDR3 comprising the amino acid sequence of SEQ ID NO: 88, or a variant thereof comprising up to about 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 amino acid substitutions; a CDR2 comprising the amino acid sequence of SEQ ID NO: 50, or a variant thereof comprising up to about 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 amino acid substitutions;

(3) a CDR1 comprising the amino acid sequence of SEQ ID NO: 26, or a variant thereof comprising up to about 3 amino acid substitutions; a CDR2 comprising the amino acid sequence of SEQ ID NO: 58, or a variant thereof comprising up to about 3 amino acid substitutions; and a CDR3 comprising the amino acid sequence of SEQ ID NO: 90, or a variant thereof comprising up to about 3 amino acid substitutions;

(4) a CDR1 comprising the amino acid sequence of SEQ ID NO: 213, or a variant thereof comprising up to about 3 amino acid substitutions; a CDR2 comprising the amino acid sequence of SEQ ID NO: 233, or a variant thereof comprising up to about 3 amino acid substitutions; and a CDR3 comprising the amino acid sequence of SEQ ID NO: 253, or a variant thereof comprising up to about 3 amino acid substitutions;

(5) a CDR1 comprising the amino acid sequence of SEQ ID NO: 17, or a variant thereof comprising up to about 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 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 amino acid substitutions;

(6) a CDR1 comprising the amino acid sequence of SEQ ID NO: 19, or a variant thereof comprising up to about 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 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 amino acid substitutions;

(7) a CDR1 comprising the amino acid sequence of SEQ ID NO: 20, or a variant thereof comprising up to about 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 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 amino acid substitutions;

(8) a CDR1 comprising the amino acid sequence of SEQ ID NO: 21, or a variant thereof comprising up to about 3 amino acid substitutions; a CDR2 comprising the amino acid sequence of SEQ ID NO: 53, or a variant thereof comprising up to about 3 amino acid substitutions; and a CDR3 comprising the amino acid sequence of SEQ ID NO: 85, or a variant thereof comprising up to about 3 amino acid substitutions;

(9) a CDR1 comprising the amino acid sequence of SEQ ID NO: 22, or a variant thereof comprising up to about 3 amino acid substitutions; a CDR2 comprising the amino acid sequence of SEQ ID NO: 54, or a variant thereof comprising up to about 3 amino acid substitutions; and a CDR3 comprising the amino acid sequence of SEQ ID NO: 86, or a variant thereof comprising up to about 3 amino acid substitutions;

(10) a CDR1 comprising the amino acid sequence of SEQ ID NO: 23, or a variant thereof comprising up to about 3 amino acid substitutions; a CDR2 comprising the amino acid sequence of SEQ ID NO: 55, or a variant thereof comprising up to about 3 amino acid substitutions; and a CDR3 comprising the amino acid sequence of SEQ ID NO: 87, or a variant thereof comprising up to about 3 amino acid substitutions;

(11) a CDR1 comprising the amino acid sequence of SEQ ID NO: 25, or a variant thereof comprising up to about 3 amino acid substitutions; a CDR2 comprising the amino acid sequence of SEQ ID NO: 57, or a variant thereof comprising up to about 3 amino acid substitutions; and a CDR3 comprising the amino acid sequence of SEQ ID NO: 89, or a variant thereof comprising up to about 3 amino acid substitutions;

(12) a CDR1 comprising the amino acid sequence of SEQ ID NO: 27, or a variant thereof comprising up to about 3 amino acid substitutions; a CDR2 comprising the amino acid sequence of SEQ ID NO: 59, or a variant thereof comprising up to about 3 amino acid substitutions; and a CDR3 comprising the amino acid sequence of SEQ ID NO: 91, or a variant thereof comprising up to about 3 amino acid substitutions;

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

amino acid sequence of SEQ ID NO: 60, or a variant thereof comprising up to about 3 amino acid substitutions; and a CDR3 comprising the amino acid sequence of SEQ ID NO: 92, or a variant thereof comprising up to about 3 amino acid substitutions;

(14) a CDR1 comprising the amino acid sequence of SEQ ID NO: 29, or a variant thereof comprising up to about 3 amino acid substitutions; a CDR2 comprising the amino acid sequence of SEQ ID NO: 61, or a variant thereof comprising up to about 3 amino acid substitutions; and a CDR3 comprising the amino acid sequence of SEQ ID NO: 93, or a variant thereof comprising up to about 3 amino acid substitutions;

(15) a CDR1 comprising the amino acid sequence of SEQ ID NO: 30, or a variant thereof comprising up to about 3 amino acid substitutions; a CDR2 comprising the amino acid sequence of SEQ ID NO: 62, or a variant thereof comprising up to about 3 amino acid substitutions; and a CDR3 comprising the amino acid sequence of SEQ ID NO: 94, or a variant thereof comprising up to about 3 amino acid substitutions;

(16) a CDR1 comprising the amino acid sequence of SEQ ID NO: 31, or a variant thereof comprising up to about 3 amino acid substitutions; a CDR2 comprising the amino acid sequence of SEQ ID NO: 63, or a variant thereof comprising up to about 3 amino acid substitutions; and a CDR3 comprising the amino acid sequence of SEQ ID NO: 95, or a variant thereof comprising up to about 3 amino acid substitutions;

(17) a CDR1 comprising the amino acid sequence of SEQ ID NO: 32, or a variant thereof comprising up to about 3 amino acid substitutions; a CDR2 comprising the amino acid sequence of SEQ ID NO: 64, or a variant thereof comprising up to about 3 amino acid substitutions; and a CDR3 comprising the amino acid sequence of SEQ ID NO: 96, or a variant thereof comprising up to about 3 amino acid substitutions;

(18) a CDR1 comprising the amino acid sequence of SEQ ID NO: 214, or a variant thereof comprising up to about 3 amino acid substitutions; a CDR2 comprising the amino acid sequence of SEQ ID NO: 234, or a variant thereof comprising up to about 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 amino acid substitutions;

(19) a CDR1 comprising the amino acid sequence of SEQ ID NO: 215, or a variant thereof comprising up to about 3 amino acid substitutions; a CDR2 comprising the amino acid sequence of SEQ ID NO: 235, or a variant thereof comprising up to about 3 amino acid substitutions; and a CDR3 comprising the amino acid sequence of SEQ ID NO: 255, or a variant thereof comprising up to about 3 amino acid substitutions;

(20) a CDR1 comprising the amino acid sequence of SEQ ID NO: 216, or a variant thereof comprising up to about 3 amino acid substitutions; a CDR2 comprising the amino acid sequence of SEQ ID NO: 236, or a variant thereof comprising up to about 3 amino acid substitutions; and a CDR3 comprising the amino acid sequence of SEQ ID NO: 256, or a variant thereof comprising up to about 3 amino acid substitutions;

(21) a CDR1 comprising the amino acid sequence of SEQ ID NO: 217, or a variant thereof comprising up to about 3 amino acid substitutions; a CDR2 comprising the amino acid sequence of SEQ ID NO: 237, or a variant thereof comprising up to about 3 amino acid substitutions; and a CDR3 comprising the amino acid sequence of SEQ ID NO: 257, or a variant thereof comprising up to about 3 amino acid substitutions;

(22) a CDR1 comprising the amino acid sequence of SEQ ID NO: 218, or a variant thereof comprising up to about 3 amino acid substitutions; a CDR2 comprising the amino acid sequence of SEQ ID NO: 238, or a variant thereof comprising up to about 3 amino acid substitutions; and a CDR3 comprising the amino acid sequence of SEQ ID NO: 258, or a variant thereof comprising up to about 3 amino acid substitutions;

(23) a CDR1 comprising the amino acid sequence of SEQ ID NO: 219, or a variant thereof comprising up to about 3 amino acid substitutions; a CDR2 comprising the amino acid sequence of SEQ ID NO: 239, or a variant thereof comprising up to about 3 amino acid substitutions; and a CDR3 comprising the amino acid sequence of SEQ ID NO: 259, or a variant thereof comprising up to about 3 amino acid substitutions;

(24) a CDR1 comprising the amino acid sequence of SEQ ID NO: 220, or a variant thereof comprising up to about 3 amino acid substitutions; a CDR2 comprising the amino acid sequence of SEQ ID NO: 240, or a variant thereof comprising up to about 3 amino acid substitutions; and a CDR3 comprising the amino acid sequence of SEQ ID NO: 260, or a variant thereof comprising up to about 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 amino acid substitutions; a CDR2 comprising the amino acid sequence of SEQ ID NO: 241, or a variant thereof comprising up to about 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 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 amino acid substitutions; a CDR2 comprising the amino acid sequence of SEQ ID NO: 242, or a variant thereof comprising up to about

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 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 amino acid substitutions; a CDR2 comprising the amino acid sequence of SEQ ID NO: 339, or a variant thereof comprising up to about 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 amino acid substitutions.

2. 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.

3. The isolated anti-CTLA-4 construct of claim 1 or 2, wherein the 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.

4. The isolated anti-CTLA-4 construct of any one of claims 1-3, wherein the 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, or a variant thereof having at least about 80% sequence identify to any one of SEQ ID NOs: 113-129, 200-202, 274-282, 341-344, 352, and 353.
5. The isolated anti-CTLA-4 construct of any one of claims 1-4, wherein the 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.
6. The isolated anti-CTLA-4 construct of any one of claims 1-5, wherein the sdAb moiety specifically recognizing CTLA-4 is camelid, chimeric, human, partially humanized, or fully humanized.
7. The isolated anti-CTLA-4 construct of any one of claims 1-6, wherein the isolated anti-CTLA-4 construct is a heavy chain-only antibody (HCAb).
8. The isolated anti-CTLA-4 construct of claim 7, wherein the HCAb is monomeric or dimeric.
9. The isolated anti-CTLA-4 construct of claim 7 or 8, wherein the HCAb comprises the amino acid sequence of any one of SEQ ID NOs: 130-133, 283-291, and 366-371.
10. The isolated anti-CTLA-4 construct of any one of claims 1-6, wherein the isolated anti-CTLA-4 construct further comprises a second antibody moiety specifically recognizing a second epitope.
11. The isolated anti-CTLA-4 construct of claim 10, 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.
12. The isolated anti-CTLA-4 construct of claim 10 or 11, wherein the sdAb moiety specifically recognizing CTLA-4 and the second antibody moiety are connected by a peptide linker.
13. The isolated anti-CTLA-4 construct of claim 12, wherein the peptide linker comprises the amino acid sequence of SEQ ID NO: 162, 163, 307, or 365.
14. The isolated anti-CTLA-4 construct of any one of claims 10-13, wherein the second antibody moiety is a full-length antibody consisting of two heavy chains and two light chains.

15. The isolated anti-CTLA-4 construct of claim 14, comprising a configuration selected from the group consisting of:

(1) 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

(2) 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;

(3) 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;

(4) 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;

(5) the anti-CTLA-4 construct comprises four sdAb moieties specifically recognizing CTLA-4, wherein the C-terminus of each sdAb moiety specifically recognizing CTLA-4 is fused to the N-terminus of each chain of the full-length antibody; and

(6) the anti-CTLA-4 construct comprises four sdAb moieties specifically recognizing CTLA-4, wherein two of the four sdAb moieties specifically recognizing CTLA-4 are fused together, which is further fused to the N-terminus of each heavy chain of the full-length antibody.

16. The isolated anti-CTLA-4 construct of claim 14 or 15, wherein the full-length antibody specifically recognizes PD-1.

17. The isolated anti-CTLA-4 construct of claim 16, 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.

18. The isolated anti-CTLA-4 construct of claim 16 or 17, 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.

19. The isolated anti-CTLA-4 construct of any one of claims 16-18, 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.

20. The isolated anti-CTLA-4 construct of claim 14 or 15, wherein the full-length antibody specifically recognizes PD-L1.

21. The isolated anti-CTLA-4 construct of claim 20, 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.

22. The isolated anti-CTLA-4 construct of claim 20 or 21, 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.

23. 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.

24. An isolated anti-CTLA-4 construct that specifically binds to CTLA-4 competitively with an sdAb moiety comprising CDR1, CDR2, and CDR3 of any one of SEQ ID NOs: 113-129, 200-202, 274-282, 341-344, 352, and 353.

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

26. Use of an effective amount of the isolated anti-CTLA-4 construct of any one of claims 1-24 in the manufacture of a medicament for treating a CTLA-4-related disease in an individual.

27. The use of claim 26, wherein the CTLA-4 related disease is cancer.

28. The use of claim 27, wherein the cancer is a colon cancer.

29. The use of any one of claims 26-28, wherein the medicament is to be administered systemically or locally.