effects which can be life-threatening and are often dose and treatment duration-limiting (Weber, J.S., et al., 2008. Phase I/II study of ipilimumab for patients with metastatic melanoma. Journal of Clinical Oncology 26:5950-5956). These include enterocolitis, dermatitis, hypophysitis, uveitis, hepatitis, nephritis and death. Enterocolitis is the most common major toxicity (affecting approximately 20% of patients). The severe safety risks related to immune-mediated adverse reactions prompted the FDA to approve ipilimumab with a Risk Evaluation and Mitigation Strategy (REMS). Recently, coadministration of ipilimumab and a second immune checkpoint modulator targeting PD1 (e.g., nivolumab) has been shown to significantly increase efficacy of immunotherapy of melanoma when compared to ipilimumab alone. This gain, however, was associated with increased frequencies of grade 3/4 adverse effects, which affected more than 50% of patients receiving combination treatment (Wolchok, J.D., et al. 2013. Nivolumab plus Ipilimumab in Advanced Melanoma. N Engl J Med).

[0005] These findings illustrate the need for developing anti-CTLA4 protein therapeutics that effectively target tumors without the side effects associated with certain anti-CTLA4 antibodies such as ipilimumab. Provided herein are anti-CTLA binding proteins, compositions thereof and methods of use thereof for addressing this need.

[0006] All references cited herein, including patent applications, patent publications, and scientific literature, are herein incorporated by reference in their entirety, as if each individual reference were specifically and individually indicated to be incorporated by reference.

SUMMARY

[0007] In one aspect, the present disclosure provides an anti-CTLA4 antibody or antigen-binding fragment thereof comprising a light chain variable (VL) domain and a heavy chain variable (VH) domain, wherein: a) the VL domain comprises (i) a CDR-L1 comprising the amino acid sequence of SEQ ID NO: 1, (ii) a CDR-L2 comprising the amino acid sequence of SEQ ID NO: 2, and (iii) a CDR-L3 comprising the amino acid sequence of SEQ ID NO: 3; and/or the VH domain comprises (i) a CDR-H1 comprising the amino acid sequence of SEQ ID NO: 4, (ii) a CDR-H2 comprising the amino acid sequence of SEQ ID NO: 5, and (iii) a CDR-H3 comprising the amino acid sequence of SEQ ID NO: 6; or b) the VL domain comprises (i) a CDR-L1 comprising the amino acid sequence of SEQ ID NO: 13, (ii) a CDR-L2 comprising the amino acid sequence of

SEQ ID NO: 14, and (iii) a CDR-L3 comprising the amino acid sequence of SEQ ID NO: 15; and/or the VH domain comprises (i) a CDR-H1 comprising the amino acid sequence of SEQ ID NO: 16, (ii) a CDR-H2 comprising the amino acid sequence of SEQ ID NO: 17, and (iii) a CDR-H3 comprising the amino acid sequence of SEQ ID NO: 18; or c) the VL domain comprises (i) a CDR-L1 comprising the amino acid sequence of SEQ ID NO: 7, (ii) a CDR-L2 comprising the amino acid sequence of SEQ ID NO: 8, and (iii) a CDR-L3 comprising the amino acid sequence of SEQ ID NO: 9; and/or the VH domain comprises (i) a CDR-H1 comprising the amino acid sequence of SEQ ID NO: 10, (ii) a CDR-H2 comprising the amino acid sequence of SEQ ID NO: 11, and (iii) a CDR-H3 comprising the amino acid sequence of SEQ ID NO: 12; or d) the VL domain comprises (i) a CDR-L1 comprising the amino acid sequence of SEQ ID NO: 19, (ii) a CDR-L2 comprising the amino acid sequence of SEQ ID NO: 20, and (iii) a CDR-L3 comprising the amino acid sequence of SEQ ID NO: 21; and/or the VH domain comprises (i) a CDR-H1 comprising the amino acid sequence of SEQ ID NO: 22, (ii) a CDR-H2 comprising the amino acid sequence of SEQ ID NO: 23, and (iii) a CDR-H3 comprising the amino acid sequence of SEQ ID NO: 24; and wherein the antibody or antigen-binding fragment thereof comprises a heavy chain constant domain (CH), wherein the CH comprises amino acid substitutions S239D and I332E, wherein the amino acid residues are numbered according to the EU index as in Kabat.

[0007a] In another aspect, the present disclosure provides a bispecific antibody or antigen-binding fragment thereof comprising the anti-CTLA4 antibody or antigen-binding fragment thereof as described herein. Provided herein are anti-cytotoxic T-lymphocyte-associated protein 4 (CTLA4) binding proteins, compositions comprising thereof, and methods of using the same.

[0008] Provided herein are anti-CTLA4 antibodies or antigen-binding fragment thereof comprising a light chain variable (VL) domain and a heavy chain variable (VH) domain, wherein: the VL domain comprises a CDR-L1 comprising the amino acid sequence of SEQ ID NO: 1, a CDR-L2 comprising the amino acid sequence of SEQ ID NO: 2, and a CDR-L3 comprising the amino acid sequence of SEQ ID NO: 3; and/or the VH domain comprises a CDR-H1 comprising the amino acid sequence of SEQ ID NO: 4, a CDR-H2 comprising the

amino acid sequence of SEQ ID NO: 5, and a CDR-H3 comprising the amino acid sequence of SEQ ID NO: 6; or the VL domain comprises a CDR-L1 comprising the amino acid sequence of SEQ ID NO: 13, a CDR-L2 comprising the amino acid sequence of SEQ ID NO: 14, and a CDR-L3 comprising the amino acid sequence of SEQ ID NO: 15; and/or the VH domain comprises a CDR-H1 comprising the amino acid sequence of SEQ ID NO: 16, a CDR-H2 comprising the amino acid sequence of SEQ ID NO: 17, and a CDR-H3 comprising the amino acid sequence of SEQ ID NO: 18; or the VL domain comprises a CDR-L1 comprising the amino acid sequence of SEQ ID NO: 7, a CDR-L2 comprising the amino acid sequence of SEQ ID NO: 8, and a CDR-L3 comprising the amino acid sequence of SEQ ID NO: 9; and/or the VH domain comprises a CDR-H1 comprising the amino acid sequence of SEQ ID NO: 10, a CDR- H2 comprising the amino acid sequence of SEQ ID NO: 11, and a CDR-H3 comprising the amino acid sequence of SEQ ID NO: 12; or the VL domain comprises a CDR-L1 comprising the amino acid sequence of SEQ ID NO: 19, a CDR-L2 comprising the amino acid sequence of SEQ ID NO: 20, and a CDR-L3 comprising the amino acid sequence of SEQ ID NO: 21; and/or the VH domain comprises a CDR-H1 comprising the amino acid sequence of SEQ ID NO: 22, a CDR-H2 comprising the amino acid sequence of SEQ ID NO: 23, and a CDR-H3 comprising the amino acid sequence of SEQ ID NO: 24.

[0009] Provided herein are anti-CTLA4 antibodies or antigen-binding fragment thereof comprising a light chain variable (VL) domain and a heavy chain variable (VH) domain, wherein the VL domain comprises a CDR-L1 comprising the amino acid sequence of SEQ ID NO: 1, a CDR-L2 comprising the amino acid sequence of SEQ ID NO: 2, and a CDR-L3 comprising the amino acid sequence of SEQ ID NO: 3; and/or the VH domain comprises a CDR-H1 comprising the amino acid sequence of SEQ ID NO: 4, a CDR-H2 comprising the amino acid sequence of SEQ ID NO: 5, and a CDR-H3 comprising the amino acid sequence of SEQ ID NO: 6.

[0010] Provided herein are anti-CTLA4 antibodies or antigen-binding fragment thereof comprising a light chain variable (VL) domain and a heavy chain variable (VH) domain, wherein the VL domain comprises a CDR-L1 comprising the amino acid sequence of SEQ ID

NO: 13, a CDR-L2 comprising the amino acid sequence of SEQ ID NO: 14, and a CDR-L3 comprising the amino acid sequence of SEQ ID NO: 15; and/or the VH domain comprises a CDR-H1 comprising the amino acid sequence of SEQ ID NO: 16, a CDR-H2 comprising the amino acid sequence of SEQ ID NO: 17, and a CDR-H3 comprising the amino acid sequence of SEQ ID NO: 18.

[0011] Provided herein are anti-CTLA4 antibodies or antigen-binding fragment thereof comprising a light chain variable (VL) domain and a heavy chain variable (VH) domain, wherein the VL domain comprises a CDR-L1 comprising the amino acid sequence of SEQ ID NO: 7, a CDR-L2 comprising the amino acid sequence of SEQ ID NO: 8, and a CDR-L3 comprising the amino acid sequence of SEQ ID NO: 9; and/or the VH domain comprises a CDR-H1 comprising the amino acid sequence of SEQ ID NO: 10, a CDR-H2 comprising the amino acid sequence of SEQ ID NO: 11, and a CDR-H3 comprising the amino acid sequence of SEQ ID NO: 12.

[0012] Provided herein are anti-CTLA4 antibodies or antigen-binding fragment thereof comprising a light chain variable (VL) domain and a heavy chain variable (VH) domain, wherein the VL domain comprises a CDR-L1 comprising the amino acid sequence of SEQ ID NO: 19, a CDR-L2 comprising the amino acid sequence of SEQ ID NO: 20, and a CDR-L3 comprising the amino acid sequence of SEQ ID NO: 21; and/or the VH domain comprises a CDR-H1 comprising the amino acid sequence of SEQ ID NO: 22, a CDR-H2 comprising the amino acid sequence of SEQ ID NO: 23, and a CDR-H3 comprising the amino acid sequence of SEQ ID NO: 24.

[0013] In some of any of the provided of the provided examples, the VL domain comprises an amino acid sequence that has at least about 90% sequence identity to the amino acid sequence of SEQ ID NO: 25, and the VH domain comprises an amino acid sequence that has at least about 90% sequence identity to the amino acid sequence of SEQ ID NO: 26; or the VL domain comprises an amino acid sequence that has at least about 90% sequence identity to the amino acid sequence of SEQ ID NO: 30, and the VH domain comprises an amino acid

sequence that has at least about 90% sequence identity to the amino acid sequence of SEQ ID NO: 31.

[0014] In some of any of the provided examples, the VL domain comprises an amino acid sequence that has at least about 90% sequence identity to the amino acid sequence of SEQ ID NO: 25, and the VH domain comprises an amino acid sequence that has at least about 90% sequence identity to the amino acid sequence of SEQ ID NO: 26. In some of any of the provided examples, the VL domain comprises an amino acid sequence that has at least about 90% sequence identity to the amino acid sequence of SEQ ID NO: 30, and the VH domain comprises an amino acid sequence that has at least about 90% sequence identity to the amino acid sequence of SEQ ID NO: 31.

[0015] In some of any of the provided examples, the VL domain comprises the amino acid sequence of SEQ ID NO: 25, and the VH domain comprises the amino acid sequence of SEQ ID NO: 26; or the VL domain comprises the amino acid sequence of SEQ ID NO: 30, and the VH domain comprises the amino acid sequence of SEQ ID NO: 31.

[0016] Provided herein are anti-CTLA4 antibodies or antigen-binding fragment thereof comprising a light chain variable (VL) domain and a heavy chain variable (VH) domain, wherein: the VL domain comprises the amino acid sequence of SEQ ID NO: 25 and/or the VH domain comprises the amino acid sequence of SEQ ID NO: 26; or the VL domain comprises the amino acid sequence of SEQ ID NO: 30 and/or the VH domain comprises the amino acid sequence of SEQ ID NO: 31.

[0017] In some of any of the provided examples, the VL domain comprises the amino acid sequence of SEQ ID NO: 25, and the VH domain comprises the amino acid sequence of SEQ ID NO: 26. In some of any of the provided examples, the VL domain comprises the amino acid sequence of SEQ ID NO: 30, and the VH domain comprises the amino acid sequence of SEQ ID NO: 31.

[0018] In some of any of the provided examples, the antibody or antigen-binding fragment thereof comprises a heavy chain constant domain (CH). In some of any of the provided examples, the antibody or antigen-binding fragment thereof comprises a CH sequence

selected from the group consisting of SEQ ID NOS: 35-38. In some of any of the provided examples, the CH comprises amino acid substitutions S239D or I332E or both, wherein the amino acid residues are numbered according to the EU index as in Kabat. In some of any of the provided examples, the antibody or antigen-binding fragment thereof comprises a CH sequence of SEQ ID NO: 38.

[0019] In some of any examples, the antibody or antigen-binding fragment thereof comprises a light chain constant domain (CL). In some of any examples, the antibody or antigen-binding fragment thereof comprises a CL sequence of SEQ ID NO:39.

[0020] In some of any examples, the light chain comprises the amino acid sequence of SEQ ID NO: 27, and the heavy chain comprises the amino acid sequence of SEQ ID NO: 29; or the light chain comprises the amino acid sequence of SEQ ID NO: 32, and the heavy chain comprises the amino acid sequence of SEQ ID NO: 34.

[0021] Provided herein are anti-CTLA4 antibodies or antigen-binding fragment thereof comprising a light chain comprising the amino acid sequence of SEQ ID NO: 27 and a heavy chain comprising the amino acid sequence of SEQ ID NO: 29.

[0022] Provided herein are anti-CTLA4 antibodies or antigen-binding fragment thereof comprising a light chain comprising the amino acid sequence of SEQ ID NO: 32 and a heavy chain comprising the amino acid sequence of SEQ ID NO: 34.

[0023] In some of any examples, the antibody or antigen-binding fragment thereof is afucosylated or fucose-deficient.

[0024] In some of any examples, the anti-CTLA4 antibody or antigen-binding fragment thereof is conjugated to an agent. In some of any examples, the agent is an inhibitor of tubulin polymerization, a DNA damaging agent, or a DNA synthesis inhibitor. In some of any examples, the agent is a maytansinoid, an auristatin, a pyrrolobenzodiazepine (PBD) dimer, a calicheamicin, a duocarmycin, a indo-linobenzodiazepine dimer, or exatecan derivative Dxd.

[0025] Provided herein are bispecific antibodies or an antigen-binding fragment thereof, comprising a light chain and a heavy chain of a first pair that specifically binds to CTLA4; a

light chain and a heavy chain of a second pair that specifically binds to an antigen; wherein the light chain of the first pair comprises a VL domain, and the heavy chain of the first pair comprises a VH domain and wherein: the VL domain comprises a CDR-L1 comprising the amino acid sequence of SEQ ID NO: 1, a CDR-L2 comprising the amino acid sequence of SEQ ID NO: 2, and a CDR-L3 comprising the amino acid sequence of SEQ ID NO: 3; and/or the VH domain comprises a CDR-H1 comprising the amino acid sequence of SEQ ID NO: 4, a CDR-H2 comprising the amino acid sequence of SEQ ID NO: 5, and a CDR-H3 comprising the amino acid sequence of SEQ ID NO: 6; or the VL domain comprises a CDR-L1 comprising the amino acid sequence of SEQ ID NO: 13, a CDR-L2 comprising the amino acid sequence of SEQ ID NO: 14, and a CDR-L3 comprising the amino acid sequence of SEQ ID NO: 15; and/or the VH domain comprises a CDR-H1 comprising the amino acid sequence of SEQ ID NO: 16, a CDR- H2 comprising the amino acid sequence of SEQ ID NO: 17, and a CDR-H3 comprising the amino acid sequence of SEQ ID NO: 18; or the VL domain comprises a CDR-L1 comprising the amino acid sequence of SEQ ID NO: 7, a CDR-L2 comprising the amino acid sequence of SEQ ID NO: 8, and a CDR-L3 comprising the amino acid sequence of SEQ ID NO: 9; and/or the VH domain comprises a CDR-H1 comprising the amino acid sequence of SEQ ID NO: 10, a CDR- H2 comprising the amino acid sequence of SEQ ID NO: 11, and a CDR-H3 comprising the amino acid sequence of SEQ ID NO: 12; or the VL domain comprises a CDR-L1 comprising the amino acid sequence of SEQ ID NO: 19, a CDR-L2 comprising the amino acid sequence of SEQ ID NO: 20, and a CDR-L3 comprising the amino acid sequence of SEQ ID NO: 21; and/or the VH domain comprises a CDR-H1 comprising the amino acid sequence of SEQ ID NO: 22, a CDR-H2 comprising the amino acid sequence of SEQ ID NO: 23, and a CDR-H3 comprising the amino acid sequence of SEQ ID NO: 24.

[0026] Provided herein are bispecific antibodies or an antigen-binding fragment thereof, comprising a light chain and a heavy chain of a first pair that specifically binds to CTLA4; a light chain and a heavy chain of a second pair that specifically binds to an antigen; wherein the light chain of the first pair comprises a VL domain, and the heavy chain of the first pair comprises a VH domain and wherein the VL domain comprises a CDR-L1 comprising the amino acid sequence of SEQ ID NO: 1, a CDR-L2 comprising the amino acid sequence of SEQ

ID NO: 2, and a CDR-L3 comprising the amino acid sequence of SEQ ID NO: 3; and/or the VH domain comprises a CDR-H1 comprising the amino acid sequence of SEQ ID NO: 4, a CDR-H2 comprising the amino acid sequence of SEQ ID NO: 5, and a CDR-H3 comprising the amino acid sequence of SEQ ID NO: 6.

[0027] Provided herein are bispecific antibodies or an antigen-binding fragment thereof, comprising a light chain and a heavy chain of a first pair that specifically binds to CTLA4; a light chain and a heavy chain of a second pair that specifically binds to an antigen; wherein the light chain of the first pair comprises a VL domain, and the heavy chain of the first pair comprises a VH domain and wherein the VL domain comprises a CDR-L1 comprising the amino acid sequence of SEQ ID NO: 13, a CDR-L2 comprising the amino acid sequence of SEQ ID NO: 14, and a CDR-L3 comprising the amino acid sequence of SEQ ID NO: 15; and/or the VH domain comprises a CDR-H1 comprising the amino acid sequence of SEQ ID NO: 16, a CDR-H2 comprising the amino acid sequence of SEQ ID NO: 17, and a CDR-H3 comprising the amino acid sequence of SEQ ID NO: 18.

[0028] Provided herein are bispecific antibodies or an antigen-binding fragment thereof, comprising a light chain and a heavy chain of a first pair that specifically binds to CTLA4; a light chain and a heavy chain of a second pair that specifically binds to an antigen; wherein the light chain of the first pair comprises a VL domain, and the heavy chain of the first pair comprises a VH domain and wherein the VL domain comprises a CDR-L1 comprising the amino acid sequence of SEQ ID NO: 7, a CDR-L2 comprising the amino acid sequence of SEQ ID NO: 8, and a CDR-L3 comprising the amino acid sequence of SEQ ID NO: 9; and/or the VH domain comprises a CDR-H1 comprising the amino acid sequence of SEQ ID NO: 10, a CDR- H2 comprising the amino acid sequence of SEQ ID NO: 11, and a CDR-H3 comprising the amino acid sequence of SEQ ID NO: 12.

[0029] Provided herein are bispecific antibodies or an antigen-binding fragment thereof, comprising a light chain and a heavy chain of a first pair that specifically binds to CTLA4; a light chain and a heavy chain of a second pair that specifically binds to an antigen; wherein the light chain of the first pair comprises a VL domain, and the heavy chain of the first pair

comprises a VH domain and wherein the VL domain comprises a CDR-L1 comprising the amino acid sequence of SEQ ID NO: 19, a CDR-L2 comprising the amino acid sequence of SEQ ID NO: 20, and a CDR-L3 comprising the amino acid sequence of SEQ ID NO: 21; and/or the VH domain comprises a CDR-H1 comprising the amino acid sequence of SEQ ID NO: 22, a CDR-H2 comprising the amino acid sequence of SEQ ID NO: 23, and a CDR-H3 comprising the amino acid sequence of SEQ ID NO: 24.

[0030] In some of any examples, the VL domain comprises an amino acid sequence that has at least about 90% sequence identity to the amino acid sequence of SEQ ID NO: 25, and the VH domain comprises an amino acid sequence that has at least about 90% sequence identity to the amino acid sequence of SEQ ID NO: 26; or the VL domain comprises an amino acid sequence that has at least about 90% sequence identity to the amino acid sequence of SEQ ID NO: 30, and the VH domain comprises an amino acid sequence that has at least about 90% sequence identity to the amino acid sequence of SEQ ID NO: 31.

[0031] Provided herein are bispecific antibodies or an antigen-binding fragment thereof, comprising a light chain and a heavy chain of a first pair that specifically binds to CTLA4; a light chain and a heavy chain of a second pair that specifically binds to an antigen; wherein the light chain of the first pair comprises a VL domain, and the heavy chain of the first pair comprises a VH domain and wherein: the VL domain comprises the amino acid sequence of SEQ ID NO: 25 and/or the VH domain comprises the amino acid sequence of SEQ ID NO: 26; or the VL domain comprises the amino acid sequence of SEQ ID NO: 30 and/or the VH domain comprises the amino acid sequence of SEQ ID NO: 31.

[0032] In some of any examples, the VL domain comprises the amino acid sequence of SEQ ID NO: 25, and the VH domain comprises the amino acid sequence of SEQ ID NO: 26; or the VL domain comprises the amino acid sequence of SEQ ID NO: 30, and the VH domain comprises the amino acid sequence of SEQ ID NO: 31.

[0033] In some of any examples, the bispecific antibody or antigen-binding fragment thereof comprises a heavy chain constant domain (CH). In some of any examples, the bispecific antibody or antigen-binding fragment thereof comprises a CH sequence selected from

the group consisting of SEQ ID NOS: 35-38. In some of any examples, the CH comprises amino acid substitutions S239D or I332E or both, wherein the amino acid residues are numbered according to the EU index as in Kabat. In some of any examples, the bispecific antibody or antigen-binding fragment thereof comprises a CH sequence of SEQ ID NO: 38.

[0034] In some of any examples, the bispecific antibody or antigen-binding fragment thereof comprises a light chain constant domain (CL). In some of any examples, the bispecific antibody or antigen-binding fragment thereof comprises a CL sequence of SEQ ID NO:39.

[0035] In some of any examples, the light chain comprises the amino acid sequence of SEQ ID NO: 27, and the heavy chain comprises the amino acid sequence of SEQ ID NO: 29; or the light chain comprises the amino acid sequence of SEQ ID NO: 32, and the heavy chain comprises the amino acid sequence of SEQ ID NO: 34.

[0036] Provided herein are bispecific antibodies or an antigen-binding fragment thereof, comprising a light chain and a heavy chain of a first pair that specifically binds to CTLA4; a light chain and a heavy chain of a second pair that specifically binds to an antigen; wherein the first pair comprises a light chain comprising the amino acid sequence of SEQ ID NO: 27 and a heavy chain comprising the amino acid sequence of SEQ ID NO: 29.

[0037] Provided herein are bispecific antibodies or an antigen-binding fragment thereof, comprising a light chain and a heavy chain of a first pair that specifically binds to CTLA4; a light chain and a heavy chain of a second pair that specifically binds to an antigen; wherein the first pair comprises a light chain comprising the amino acid sequence of SEQ ID NO: 32 and a heavy chain comprising the amino acid sequence of SEQ ID NO: 34.

[0038] In some of any examples, the bispecific antibody or antigen-binding fragment thereof is afucosylated or fucose-deficient.

[0039] In some of any examples, the bispecific antibody or antigen-binding fragment thereof is conjugated to an agent. In some of any examples, the agent is an inhibitor of tubulin polymerization, a DNA damaging agent, or a DNA synthesis inhibitor. In some of any

examples, the agent is a maytansinoid, an auristatin, a pyrrolobenzodiazepine (PBD) dimer, a calicheamicin, a duocarmycin, a indo-linobenzodiazepine dimer, or exatecan derivative Dxd.

[0040] In another aspect, also provided herein are nucleic acids encoding any of the provided anti-CTLA4 antibody or antigen-binding fragment thereof or any of the provided bispecific antibody or antigen-binding fragment thereof.

[0041] In another aspect, also provided are vectors comprising any of the nucleic acids provided herein.

[0042] In another aspect, also provided are host cells comprising any of the provided anti-CTLA4 antibody or antigen-binding fragment thereof, any of the provided bispecific antibody or antigen-binding fragment thereof, or any of the nucleic acids provided herein.

[0043] In some of any such examples, the host cell is capable of producing an antibody or antigen-binding fragment thereof that is afucosylated or fucose-deficient. In some of any such examples, the host cell has an alpha1,6-fucosyltransferase (Fut8) knockout. In some of any such examples, the host cell overexpresses β1,4-N-acetylglycosminyltransferase III (GnT- III). In some of any such examples, the host cell overexpresses Golgi μ-mannosidase I (ManII).

[0044] In yet another aspect, the present disclosure provides a method of producing an antibody or antigen-binding fragment thereof, comprising culturing the host cell as described herein under conditions that produce the antibody or antigen-binding fragment thereof or the bispecific antibody or antigen-binding fragment thereof. Also provided are methods of producing an antibody or antigen-binding fragment thereof, that involve culturing any of the provided host cells under conditions that produce the antibody or antigen-binding fragment thereof.

[0045] In yet another aspect, also provided are methods of producing an afucosylated or fucose-deficient antibody or antigen-binding fragment thereof, that involve culturing any of the provided host cells under conditions that produce the antibody or antigen-binding fragment thereof.

[0046] In some of any such examples, the methods also involve recovering the antibody or antigen-binding fragment thereof produced by the host cell.

[0047] In yet another aspect, also provided are antibodies or antigen-binding fragment thereof produced by any of the methods of producing antibodies or antigen-binding fragment thereof provided herein.

[0048] In yet another aspect, also provided are compositions comprising any of the provided anti-CTLA4 antibody or antigen-binding fragment thereof, any of the provided bispecific antibody or

antigen-binding fragment thereof or any of the provided antibody or antigen-binding fragment thereof.

[0049] In yet another aspect, also provided are pharmaceutical compositions comprising any of the provided anti- CTLA4 antibody or antigen-binding fragment thereof, any of the provided bispecific antibody or antigen-binding fragment thereof or any of the provided antibody or antigen-binding fragment thereof and a pharmaceutically acceptable carrier.

[0050] In another aspect, the present disclosure provides a pharmaceutical composition comprising the anti-CTLA4 antibody or antigen-binding fragment thereof as described herein, the bispecific antibody or antigen-binding fragment thereof as described herein or the antibody or antigen-binding fragment thereof as described herein and a pharmaceutically acceptable carrier, for use in therapy. Also provided are pharmaceutical compositions comprising any of the provided anti- CTLA4 antibody or antigen-binding fragment thereof, any of the provided bispecific antibody or antigen-binding fragment thereof or any of the provided antibody or antigen-binding fragment thereof and a pharmaceutically acceptable carrier, for use in treating or preventing a neoplastic disease in a subject.

[0051] In another aspect, also provided are uses of a pharmaceutical composition comprising any of the provided anti-CTLA4 antibody or antigen-binding fragment thereof, any of the provided bispecific antibody or antigen-binding fragment thereof or any of the provided antibody or antigen-binding fragment thereof and a pharmaceutically acceptable carrier, in the manufacture of a medicament for treating or preventing a neoplastic disease in a subject.

[0052] In another aspect, also provided are kits comprising any of the provided anti-CTLA4 antibody or antigen-binding fragment thereof, any of the provided bispecific antibody or antigen-binding fragment thereof or any of the provided antibody or antigen-binding fragment thereof.

[0053] Also provided are method of treating or preventing a neoplastic disease in a subject, the method involving administering to a subject an effective amount any of the provided anti- CTLA4 antibody or antigen-binding fragment thereof, any of the provided bispecific antibody or antigen-binding fragment thereof, any of the provided antibody or antigen-binding fragment thereof or any of the provided compositions.

[0054] It is to be understood that one, some, or all of the properties of the various examples described herein may be combined to form other examples of the present disclosure. These and other examples of the disclosure will become apparent to one of skill in the

art. These and other examples of the disclosure are further described by the detailed description that follows.

BRIEF DESCRIPTION OF THE DRAWINGS

[0055] **FIGS. 1A-1B** are graphs showing the binding of anti-CTLA4 antibodies to human CTLA4-Fc across a range of antibody concentrations. **FIG. 1A** shows the binding of forms of Antibody 1 (Antibody 1-1 and Antibody 1-2) to human CTLA4-Fc across a range of antibody concentrations, which demonstrated similar binding among the antibodies shown. **FIG. 1B** shows the binding of forms of Antibody 2 (Antibody 2-1, Antibody 2-2, Antibody 2-3, Antibody 2-4, and Antibody 2-5) to human CTLA4-Fc across a range of antibody concentrations, which demonstrated similar binding among the antibodies shown. **FIGS. 1C-1E** show the results of binding of human CTLA-Fc and the humanized anti-CTLA4 antibodies and variants (e.g., containing mutations in the Fc region or afucosylated versions) of Antibody 1 (**FIG. 1C**), Antibody 2 (**FIG. 1D**), or ipilimumab (**FIG. 1E**), as assessed by ELISA. As shown, all variants of each of the antibodies bound to the human CTLA4-Fc similarly.

[0056] **FIG. 2A** shows results from an SPR analysis demonstrating binding between Antibody 2-6 (a version of Antibody 2 having a S239D mutation, an I332E mutation in the Fc region) or ipilimumab and human CTLA4 (huCTLA4) at 32 nM, 16 nM, 8 nM, 4 nM, and 2 nM. **FIG. 2B** provides the association rate constant (ka), the dissociation rate constant (kd), and the equilibrium dissociation constant (K_D), and the fold difference between Antibody 2-6 and ipilimumab, for the results shown in **FIG. 2A**. **FIG. 2C** shows results from an SPR analysis demonstrating binding between a Fab of ipilimumab (ipilimumab-Fab) or a Fab of Antibody 2 (Antibody 2-Fab) and rhCTLA4-Fc at 32 nM, 16 nM, 8 nM, 4 nM, and 2 nM. **FIG. 2D** provides the association rate constant (ka), the dissociation rate constant (kd), the equilibrium dissociation constant (K_D), and the Chi^2 value, for the results shown in **FIG. 2C**. **FIG. 2E** shows results from an SPR analysis demonstrating binding between a Fab of ipilimumab (ipilimumab-Fab) or a Fab of Antibody 2 (Antibody 2-Fab) and cynoCTLA4-Fc at 32 nM, 16 nM, 8 nM, 4 nM, and 2 nM. **FIG. 2F** provides the association rate constant (ka), the dissociation rate constant (kd), the equilibrium dissociation constant (K_D), and the Chi^2 value, for the results shown in **FIG. 2E**.

CLAIMS

1. An anti-CTLA4 antibody or antigen-binding fragment thereof comprising a light chain variable (VL) domain and a heavy chain variable (VH) domain, wherein:

a) the VL domain comprises (i) a CDR-L1 comprising the amino acid sequence of SEQ ID NO: 1, (ii) a CDR-L2 comprising the amino acid sequence of SEQ ID NO: 2, and (iii) a CDR-L3 comprising the amino acid sequence of SEQ ID NO: 3; and/or the VH domain comprises (i) a CDR-H1 comprising the amino acid sequence of SEQ ID NO: 4, (ii) a CDR-H2 comprising the amino acid sequence of SEQ ID NO: 5, and (iii) a CDR-H3 comprising the amino acid sequence of SEQ ID NO: 6; or

b) the VL domain comprises (i) a CDR-L1 comprising the amino acid sequence of SEQ ID NO: 13, (ii) a CDR-L2 comprising the amino acid sequence of SEQ ID NO: 14, and (iii) a CDR-L3 comprising the amino acid sequence of SEQ ID NO: 15; and/or the VH domain comprises (i) a CDR-H1 comprising the amino acid sequence of SEQ ID NO: 16, (ii) a CDR-H2 comprising the amino acid sequence of SEQ ID NO: 17, and (iii) a CDR-H3 comprising the amino acid sequence of SEQ ID NO: 18; or

c) the VL domain comprises (i) a CDR-L1 comprising the amino acid sequence of SEQ ID NO: 7, (ii) a CDR-L2 comprising the amino acid sequence of SEQ ID NO: 8, and (iii) a CDR-L3 comprising the amino acid sequence of SEQ ID NO: 9; and/or the VH domain comprises (i) a CDR-H1 comprising the amino acid sequence of SEQ ID NO: 10, (ii) a CDR-H2 comprising the amino acid sequence of SEQ ID NO: 11, and (iii) a CDR-H3 comprising the amino acid sequence of SEQ ID NO: 12; or

d) the VL domain comprises (i) a CDR-L1 comprising the amino acid sequence of SEQ ID NO: 19, (ii) a CDR-L2 comprising the amino acid sequence of SEQ ID NO: 20, and (iii) a CDR-L3 comprising the amino acid sequence of SEQ ID NO: 21; and/or the VH domain comprises (i) a CDR-H1 comprising the amino acid sequence of SEQ ID NO: 22, (ii) a CDR-H2 comprising the amino acid sequence of SEQ ID NO: 23, and (iii) a CDR-H3 comprising the amino acid sequence of SEQ ID NO: 24; and

wherein the antibody or antigen-binding fragment thereof comprises a heavy chain constant domain (CH), wherein the CH comprises amino acid substitutions S239D and I332E, wherein the amino acid residues are numbered according to the EU index as in Kabat.

2. The anti-CTLA4 antibody or antigen-binding fragment thereof of claim 1, wherein:

a) the VL domain comprises an amino acid sequence that has at least about 90% sequence identity to the amino acid sequence of SEQ ID NO: 25, and the VH domain comprises an amino acid sequence that has at least about 90% sequence identity to the amino acid sequence of SEQ ID NO: 26; or

b) the VL domain comprises an amino acid sequence that has at least about 90% sequence identity to the amino acid sequence of SEQ ID NO: 30, and the VH domain comprises an amino acid sequence that has at least about 90% sequence identity to the amino acid sequence of SEQ ID NO: 31.

3. The anti-CTLA4 antibody or antigen-binding fragment thereof of claim 1 or 2, wherein:

a) the VL domain comprises the amino acid sequence of SEQ ID NO: 25, and the VH domain comprises the amino acid sequence of SEQ ID NO: 26; or

b) the VL domain comprises the amino acid sequence of SEQ ID NO: 30, and the VH domain comprises the amino acid sequence of SEQ ID NO: 31.

4. The anti-CTLA4 antibody or antigen-binding fragment thereof of any one of claims 1 to 3, wherein the antibody or antigen-binding fragment thereof comprises a heavy chain constant domain (CH), and optionally

wherein the antibody or antigen-binding fragment thereof comprises a CH sequence selected from the group consisting of SEQ ID NOS: 35-38.

5. The anti-CTLA4 antibody or antigen-binding fragment thereof any one of claims 1 to 4, wherein the antibody or antigen-binding fragment thereof comprises a CH sequence of SEQ ID NO: 38.

6. The anti-CTLA4 antibody or antigen-binding fragment thereof of any one of claims 1 to 5, wherein the antibody or antigen-binding fragment thereof comprises a light chain constant domain (CL), and, optionally

wherein the antibody or antigen-binding fragment thereof comprises a CL sequence of SEQ ID NO: 39.

7. The anti-CTLA4 antibody or antigen-binding fragment thereof of any one of claims 1 to 6, wherein:

a) the light chain comprises the amino acid sequence of SEQ ID NO: 27, and the heavy chain comprises the amino acid sequence of SEQ ID NO: 29; or

b) the light chain comprises the amino acid sequence of SEQ ID NO: 32, and the heavy chain comprises the amino acid sequence of SEQ ID NO: 34.

8. The anti-CTLA4 antibody or antigen-binding fragment thereof any one of claims 1 to 7, wherein the antibody or antigen-binding fragment thereof is afucosylated or fucose-deficient.

9. The anti-CTLA4 antibody or antigen-binding fragment thereof any one of claims 1 to 8, wherein the anti-CTLA4 antibody or antigen-binding fragment thereof is conjugated to an agent, optionally

wherein the agent is an inhibitor of tubulin polymerization, a DNA damaging agent, or a DNA synthesis inhibitor, or

wherein the agent is a maytansinoid, an auristatin, a pyrrolobenzodiazepine (PBD) dimer, a calicheamicin, a duocarmycin, an indo-linobenzodiazepine dimer, or exatecan derivative Dxd.

10. A bispecific antibody or antigen-binding fragment thereof comprising the anti-CTLA4 antibody or antigen-binding fragment thereof of any one of claims 1 to 9.

11. A nucleic acid encoding the anti-CTLA4 antibody or antigen-binding fragment thereof of any one of claims 1 to 9 or the bispecific antibody or antigen-binding fragment thereof of claim 10.

12. A vector comprising the nucleic acid of claim 11.

13. A host cell comprising the anti-CTLA4 antibody or antigen-binding fragment thereof of any one of claims 1 to 9, the bispecific antibody or antigen-binding fragment thereof of claim 10, or the nucleic acid of claim 11.

14. The host cell of claim 13, wherein the host cell is capable of producing an antibody or antigen-binding fragment thereof that is afucosylated or fucose-deficient and/or

wherein the host cell has an alpha1,6- fucosyltransferase (Fut8) knockout.

15. The host cell of claim 13 or claim 14, wherein the host cell overexpresses β1,4-N-acetylglycosminyltransferase III (GnT-III), and/or

wherein the host cell overexpresses Golgi μ-mannosidase I (ManII).

16. A method of producing an antibody or antigen-binding fragment thereof, comprising culturing the host cell of any one of claims 13 to 15 under conditions that produce the antibody or antigen-binding fragment thereof or the bispecific antibody or antigen-binding fragment thereof.

17. A method of producing an afucosylated or fucose-deficient antibody or antigen-binding fragment thereof, comprising culturing the host cell of claim 14 or claim 15 under conditions that produce the antibody or antigen-binding fragment thereof.

18. The method of claim 16 or claim 17, further comprising recovering the antibody or antigen-binding fragment thereof produced by the host cell.

19. An antibody or antigen-binding fragment thereof produced by the method of any one of claims 16 to 18.

20. A composition comprising the anti-CTLA4 antibody or antigen-binding fragment thereof of any one of claims 1 to 9, the bispecific antibody or antigen-binding fragment thereof of claim 10 or the antibody or antigen-binding fragment thereof of claim 19.

21. A pharmaceutical composition comprising the anti-CTLA4 antibody or antigen-binding fragment thereof of any one of claims 1 to 9, the bispecific antibody or antigen-binding fragment thereof of claim 10 or the antibody or antigen-binding fragment thereof of claim 19 and a pharmaceutically acceptable carrier.

22. A pharmaceutical composition comprising the anti-CTLA4 antibody or antigen-binding fragment thereof of any one of claims 1 to 9, the bispecific antibody or antigen-binding fragment thereof of claim 10 or the antibody or antigen-binding fragment thereof of claim 19 and a pharmaceutically acceptable carrier, for use in therapy.

23. Use of a pharmaceutical composition comprising the anti-CTLA4 antibody or antigen-binding fragment thereof of any one of claims 1 to 9, the bispecific antibody or antigen-binding fragment thereof of claim 10 or the antibody or antigen-binding fragment thereof of claim 19 and a pharmaceutically acceptable carrier, in the manufacture of a medicament for treating or preventing a neoplastic disease in a subject.

24. A kit comprising the anti-CTLA4 antibody or antigen-binding fragment thereof of any one of claims 1 to 9, the bispecific antibody or antigen-binding fragment thereof of claim 10 or the antibody or antigen-binding fragment thereof of claim 19.