ANTI-CTLA-4 ANTIBODIES AND METHODS OF USE THEREOF

1. RELATED APPLICATIONS

[0001] This application claims the benefit of U.S. Provisional Application No: 62/431,272, filed December 7, 2016, which is incorporated by reference herein in its entirety.

2. FIELD

[0002] The instant disclosure relates to antibodies that specifically bind to CTLA-4 (*e.g.*, human CTLA-4) and methods of using the same.

3. BACKGROUND

[0003] T-lymphocytes are central to the adaptive immune response to antigen. At least two signals are required for full activation of naive T-cells (Bretscher 1999, Proc Natl Acad Sci USA 96:185-90). A first, antigen-specific signal is provided by interaction of the T-cell receptor (TCR) with MHC/peptide complex on an antigen-presenting cell (APC). A second, co-stimulatory signal is provided by the interactions between receptors on the T-cell and their ligands on an antigen presenting cell (APC). Engagement of both TCR/MHC and co-stimulatory interactions leads to T-cell activation via a number of intracellular pathways, including calcium-calcineurin and RAS mitogen-activated protein kinase, and subsequent activation of transcription factors for a number of effector compounds, including cytokines such as IL-2. These events lead to T-cell proliferation, generation of a CD4+ helper T-cell (TH) pool, and expansion of activated CD8+ cytotoxic T-cells. Not only is co-stimulation critical for full T-cell activation, its absence during TCR/MHC engagement results in anergy and/or apoptosis.

[0004] Multiple positive and negative co-stimulatory pathways are involved in T-cell regulation; however, the most critical are between CD28 on T-cells and B7-1 (CD80) and B7-2 (CD86) on APCs. CD28 promotes T-cell differentiation into TH1 phenotype cells and enhances antibody production by B cells and activation of T-cells. B7-1 and B7-2, expressed on APCs such as dendritic cells (DC) and B cells, have overlapping but distinct functions. B7-2 is constitutively expressed and is rapidly upregulated on APCs coincident with TCR/MHC engagement (signal 1). B7-1 expression is very low on the resting cell, but is typically induced after prolonged T-cell stimulation. These differences suggest that while B7-2 may be important in initialization of T-cell activation, B7-1 may play a greater role in perpetuating the immune response.

[0005] After T-cell activation, the negative regulatory receptor Cytotoxic T-Lymphocyte

Antigen 4 (CTLA-4) is upregulated on T-cells (Alegre et al., 2001, Nat Rev Immunol 1:220-8). CTLA-4 is structurally homologous to CD28 but binds more tightly to both B7-1 and B7-2 ligands. CTLA-4 inhibits the immune response in several ways: it competes with CD28 for the B7 ligands and thus blocks co-stimulation; it negatively signals to inhibit T-cell activation; and it can capture CD80 and CD86 from opposing cells by trans-endocytosis, resulting in impaired costimulation via CD28 (Krummel and Allison, 1995, J Exp Med 182:459-465; Walunas et al., 1994, Immunity 1:405-413; Qureshi et al., 2011, Science 332:600-603).

[0006] Given the critical role of the B7 co-stimulatory pathway in promoting and maintaining an immune response, therapeutic agents designed to antagonize this pathway are promising for the treatment of autoimmune diseases and disorders.

4. SUMMARY

[0007] The instant disclosure provides antibodies that specifically bind to human CTLA-4 and antagonize CTLA-4 function, *e.g.*, CTLA-4-mediated immune suppression. Also provided are pharmaceutical compositions comprising these antibodies, nucleic acids encoding these antibodies, expression vectors and host cells for making these antibodies, and methods of treating a subject using these antibodies. The antibodies described herein are particularly useful for increasing T-cell activation in response to an antigen (*e.g.*, a tumor antigen or an infectious disease antigen) and/or decreasing Treg-mediated immune suppression, and hence for treating cancer in a subject or for treating or preventing an infectious disease in a subject.

[0008] Accordingly, in one aspect the instant disclosure provides an isolated antibody comprising a heavy chain variable region comprising complementarity determining regions CDRH1, CDRH2, and CDRH3 and a light chain variable region comprising complementarity determining regions CDRL1, CDRL2, and CDRL3, wherein:

(a) CDRH1 comprises the amino acid sequence of SYSMN (SEQ ID NO: 10);

(b) CDRH2 comprises the amino acid sequence of SISSSSSYIYYAXSVKG (SEQ ID NO: 18), wherein X is E or D;

(c) CDRH3 comprises the amino acid sequence of VGLXGPFDI (SEQ ID NO: 19), wherein X is F or M;

(d) CDRL1 comprises the amino acid sequence of RASQSVSRYLG (SEQ ID NO: 15);

(e) CDRL2 comprises the amino acid sequence of GASTRAT (SEQ ID NO: 16); and

(f) CDRL3 comprises the amino acid sequence of QQYGSSPWT (SEQ ID NO: 17),

and wherein the CDRH1, CDRH2, and CDRH3 sequences of the antibody are not SEQ ID NOs: 10, 11, and 13, respectively.

[0009] In certain embodiments, the CDRH2 comprises the amino acid sequence of SEQ ID NO: 11. In certain embodiments, the CDRH2 comprises the amino acid sequence of SEQ ID NO: 12. In certain embodiments, the CDRH3 comprises the amino acid sequence of SEQ ID NO: 13. In certain embodiments, the CDRH3 comprises the amino acid sequence of SEQ ID NO: 14. In certain embodiments, CDRH1, CDRH2, and CDRH3 comprise the CDRH1, CDRH2, and CDRH3 amino acid sequences set forth in SEQ ID NOs: 10, 11, and 14; 10, 12, and 13; or 10, 12, and 14, respectively. In certain embodiments, CDRH1, CDRH2, and CDRH3 comprise the CDRH1, CDRH2, and CDRH3 amino acid sequences set forth in SEQ ID NOs: 10, 12, and 14, respectively. In certain embodiments, CDRH1, CDRH2, CDRH3, CDRL1, CDRL2, and CDRL3 comprise the amino acid sequences set forth in SEQ ID NOs: 10, 11, 14, 15, 16, and 17; 10, 12, 13, 15, 16, and 17; or 10, 12, 14, 15, 16, and 17, respectively. In certain embodiments, CDRH1, CDRH2, CDRH3, CDRL1, CDRL2, and CDRL3 comprise the amino acid sequences set forth in SEQ ID NOs: 10, 12, 14, 15, 16, and 17, respectively.

[0010] In another aspect, the instant disclosure provides an isolated antibody that specifically binds to human CTLA-4, comprising a heavy chain variable region comprising complementarity determining regions CDRH1, CDRH2, and CDRH3 and a light chain variable region comprising complementarity determining regions CDRL1, CDRL2, and CDRL3, wherein:

(a) CDRH1 comprises the amino acid sequence of SYSMN (SEQ ID NO: 10);

(b) CDRH2 comprises the amino acid sequence of SISSSSSYIYYAXSVKG (SEQ ID NO: 18), wherein X is E or D;

(c) CDRH3 comprises the amino acid sequence of VGLXGPFDI (SEQ ID NO: 19), wherein X is F or M;

(d) CDRL1 comprises the amino acid sequence of RASQSVSRYLG (SEQ ID NO: 15);

(e) CDRL2 comprises the amino acid sequence of GASTRAT (SEQ ID NO: 16); and

(f) CDRL3 comprises the amino acid sequence of QQYGSSPWT (SEQ ID NO: 17),

and wherein the CDRH1, CDRH2, and CDRH3 sequences of the antibody are not SEQ ID NOs: 10, 11, and 13, respectively.

[0011] In certain embodiments, the CDRH2 comprises the amino acid sequence of SEQ ID NO: 11. In certain embodiments, the CDRH2 comprises the amino acid sequence of SEQ ID NO: 12. In certain embodiments, the CDRH3 comprises the amino acid sequence of SEQ

ID NO: 13. In certain embodiments, the CDRH3 comprises the amino acid sequence of SEQ ID NO: 14. In certain embodiments, CDRH1, CDRH2, and CDRH3 comprise the CDRH1, CDRH2, and CDRH3 amino acid sequences set forth in SEQ ID NOs: 10, 11, and 14; 10, 12, and 13; or 10, 12, and 14, respectively. In certain embodiments, CDRH1, CDRH2, and CDRH3 comprise the CDRH1, CDRH2, and CDRH3 amino acid sequences set forth in SEQ ID NOs: 10, 12, and 14, respectively. In certain embodiments, CDRH1, CDRH2, CDRH3, CDRL1, CDRL2, and CDRL3 comprise the amino acid sequences set forth in SEQ ID NOs: 10, 11, 14, 15, 16, and 17; 10, 12, 13, 15, 16, and 17; or 10, 12, 14, 15, 16, and 17, respectively. In certain embodiments, CDRH1, CDRH2, CDRH3, CDRL1, CDRL2, and CDRL3 comprise the amino acid sequences set forth in SEQ ID NOs: 10, 12, 14, 15, 16, and 17, respectively.

[0012] In another aspect, the instant disclosure provides an isolated antibody that specifically binds to human CTLA-4, comprising a heavy chain variable region comprising complementarity determining regions CDRH1, CDRH2, and CDRH3, and a light chain variable region comprising complementarity determining regions CDRL1, CDRL2, and CDRL3, wherein CDRH1, CDRH2, CDRH3, CDRL1, CDRL2, and CDRL3 comprise the amino acid sequences set forth in SEQ ID NOs: 10, 12, 14, 15, 16, and 17, respectively.

[0013] In certain embodiments, the antibody comprises a heavy chain variable region comprising the amino acid sequence of SEQ ID NO: 20. In certain embodiments, the antibody comprises a heavy chain variable region comprising an amino acid sequence which is at least 75%, 80%, 85%, 90%, 95%, 99%, or 100% identical to an amino acid sequence selected from the group consisting of SEQ ID NOs: 2 and 4-8. In certain embodiments, the antibody comprises a heavy chain variable region comprising an amino acid sequence which is at least 75%, 80%, 85%, 90%, 95%, 99%, or 100% identical to an amino acid sequence selected from the group consisting of SEQ ID NOs: 3. In certain embodiments, the heavy chain variable region comprises an amino acid sequence selected from the group consisting of SEQ ID NOs: 2 and 4-8. In certain embodiments, the heavy chain variable region comprises the amino acid sequence of SEQ ID NO: 8. In certain embodiments, the heavy chain variable region comprises the amino acid sequence of SEQ ID NO: 3. In certain embodiments, the antibody comprises a heavy chain comprising the amino acid sequence of SEQ ID NO: 23. In certain embodiments, the antibody comprises a heavy chain comprising the amino acid sequence of SEQ ID NO: 24. In certain embodiments, the antibody comprises a heavy chain comprising the amino acid sequence of SEQ ID NO: 25. In certain embodiments, the antibody comprises a heavy chain comprising the amino acid sequence of

SEQ ID NO: 26. In certain embodiments, the antibody comprises a heavy chain variable region having an amino acid sequence derived from a human IGHV3-21 germline sequence (e.g., IGHV3-21*01, e.g., having amino acid sequence of SEQ ID NO: 21).

[0014] In certain embodiments, the antibody comprises a light chain variable region comprising an amino acid sequence which is at least 75%, 80%, 85%, 90%, 95%, 99%, or 100% identical to the amino acid sequence of SEQ ID NO: 9. In certain embodiments, the antibody comprises a light chain variable region comprising the amino acid sequence of SEQ ID NO: 9. In certain embodiments, the antibody comprises a light chain comprising the amino acid sequence of SEQ ID NO: 27. In certain embodiments, the antibody comprises a light chain variable region having an amino acid sequence derived from a human IGKV3-20 germline sequence (e.g., IGKV3-20*01, e.g., having amino acid sequence of SEQ ID NO: 22).

[0015] In certain embodiments, the instant disclosure provides an isolated antibody that specifically binds to human CTLA-4, the antibody comprising a heavy chain variable region having an amino acid sequence derived from a human IGHV3-21 germline sequence (e.g., IGHV3-21*01, e.g., having amino acid sequence of SEQ ID NO: 21), wherein the heavy chain variable region comprises the amino acid sequence set forth in SEQ ID NO: 14. In certain embodiments, the antibody comprises a light chain variable region having an amino acid sequence derived from a human IGKV3-20 germline sequence (e.g., IGKV3-20*01, e.g., having amino acid sequence of SEQ ID NO: 22).

[0016] In another aspect, the instant disclosure provides an isolated antibody that specifically binds to human CTLA-4, comprising a heavy chain variable region comprising an amino acid sequence selected from the group consisting of SEQ ID NOs: 2-8. In certain embodiments, the antibody comprises a heavy chain variable region comprising the amino acid sequence of SEQ ID NO: 8. In certain embodiments, the antibody comprises a heavy chain comprising an amino acid sequence selected from the group consisting of SEQ ID NOs: 23-26. In certain embodiments, the antibody comprises a heavy chain comprising the amino acid sequence of SEQ ID NO: 23. In certain embodiments, the antibody comprises a heavy chain comprising the amino acid sequence of SEQ ID NO: 24. In certain embodiments, the antibody comprises a heavy chain comprising the amino acid sequence of SEQ ID NO: 25. In certain embodiments, the antibody comprises a heavy chain comprising the amino acid sequence of SEQ ID NO: 26.

[0017] In another aspect, the instant disclosure provides an isolated antibody that specifically binds to human CTLA-4, comprising a heavy chain variable region and a light

chain variable region, wherein the heavy chain variable region and the light chain variable region comprise the amino acid sequences set forth in SEQ ID NOs: 2 and 9; 3 and 9; 4 and 9; 5 and 9; 6 and 9; 7 and 9; or 8 and 9, respectively. In certain embodiments, the antibody comprises a heavy chain variable region comprising the amino acid sequence of SEQ ID NO: 8 and a light chain variable region comprising the amino acid sequence of SEQ ID NO: 9. In certain embodiments, the antibody comprises a heavy chain comprising the amino acid sequence of SEQ ID NO: 23; and a light chain comprising the amino acid sequence of SEQ ID NO: 27. In certain embodiments, the antibody comprises a heavy chain comprising the amino acid sequence of SEQ ID NO: 24; and a light chain comprising the amino acid sequence of SEQ ID NO: 27. In certain embodiments, the antibody comprises a heavy chain comprising the amino acid sequence of SEQ ID NO: 25; and a light chain comprising the amino acid sequence of SEQ ID NO: 27. In certain embodiments, the antibody comprises a heavy chain comprising the amino acid sequence of SEQ ID NO: 26; and a light chain comprising the amino acid sequence of SEQ ID NO: 27.

[0018] In another aspect, the instant disclosure provides an isolated antibody that specifically binds to human CTLA-4, comprising a heavy chain variable region having an amino acid sequence derived from a human IGHV3-21*01 germline sequence (e.g., IGHV3-21*01, e.g., having amino acid sequence of SEQ ID NO: 21); and a light chain variable region having an amino acid sequence derived from a human IGKV3-20*01 germline sequence (e.g., IGKV3-20*01, e.g., having amino acid sequence of SEQ ID NO: 22).

[0019] In certain embodiments, the antibody comprises a heavy chain constant region selected from the group consisting of human IgG_1, IgG_2, IgG_3, IgG_4, IgA_1, and IgA_2. In certain embodiments, the heavy chain constant region is IgG_1. In certain embodiments, the heavy chain constant region is IgG_2. In certain embodiments, the antibody comprises a light chain constant region selected from the group consisting of human Igκ and Igλ.

[0020] In certain embodiments, the antibody comprises an IgG_1 heavy chain constant region. In certain embodiments, the antibody comprises a heavy chain constant region comprising the amino acid sequence of SEQ ID NO: 28. In certain embodiment, the amino acid sequence of the IgG_1 heavy chain constant region comprises S239D/I332E mutations, numbered according to the EU numbering system. In certain embodiments, the antibody comprises a heavy chain constant region comprising the amino acid sequence of SEQ ID NO: 29. In certain embodiments, the amino acid sequence of the IgG_1 heavy chain constant region comprises S239D/A330L/I332E mutations, numbered according to the EU numbering system. In certain embodiments, the antibody comprises a heavy chain constant region

comprising the amino acid sequence of SEQ ID NO: 30. In certain embodiments, the amino acid sequence of the IgG_1 heavy chain constant region comprises L235V/F243L/R292P/Y300L/P396L mutations, numbered according to the EU numbering system. In certain embodiments, the antibody comprises a heavy chain constant region comprising the amino acid sequence of SEQ ID NO: 31. In certain embodiments, the IgG_1 heavy chain constant region is afucosylated IgG_1.

[0021] In certain embodiments, the antibody comprises a human IgG heavy chain constant region that is a variant of a wild type human IgG heavy chain constant region, **wherein the variant human IgG heavy chain constant region binds to FcγRIIIA with a higher affinity than the wild type human IgG heavy chain constant region binds to FcγRIIIA. In** certain embodiments, the variant human IgG heavy chain constant region is a variant human IgG_1 heavy chain constant region.

[0022] In certain embodiments, the antibody comprises a light chain constant region **selected from the group consisting of human Igκ and Igλ. In certain embodiments, the antibody comprises an Igκ light chain constant region. In certain embodiments, the antibody** comprises a light chain constant region comprising the amino acid sequence of SEQ ID NO: 32.

[0023] In another aspect, the instant disclosure provides an isolated antibody that cross-competes for binding to human CTLA-4 with an antibody described herein. In another aspect, the instant disclosure provides an isolated antibody that cross-competes for binding to human CTLA-4 with an antibody comprising the heavy and light chain variable region amino acid sequences set forth in SEQ ID NOs: 8 and 9, respectively.

[0024] In another aspect, the instant disclosure provides an isolated antibody that binds to the same epitope on human CTLA-4 as an antibody described herein. In another aspect, the instant disclosure provides an isolated antibody that binds to the same epitope on human CTLA-4 as an antibody comprising the heavy and light chain variable region amino acid sequences set forth in SEQ ID NOs: 8 and 9, respectively.

[0025] In another aspect, the instant disclosure provides an isolated antibody that binds, *e.g.*, specifically binds, to an epitope of human CTLA-4. In certain embodiments, the antibody binds to an epitope located within a region of human CTLA-4 consisting of an amino acid sequence selected from the group consisting of SEQ ID NOs: 34-39. In certain embodiments, the antibody binds to an epitope located within a region of human CTLA-4 consisting of the amino acid sequence of SEQ ID NO: 37. In certain embodiments, the antibody binds to an epitope located within a region of human CTLA-4 consisting of the

amino acid sequence of SEQ ID NO: 36. In certain embodiments, the antibody binds to an epitope located within a region of human CTLA-4 consisting of the amino acid sequence of SEQ ID NO: 35. In certain embodiments, the antibody binds to an epitope located within a region of human CTLA-4 consisting of the amino acid sequence of SEQ ID NO: 34. In certain embodiments, the antibody binds to an epitope located within a region of human CTLA-4 consisting of the amino acid sequence of SEQ ID NO: 38. In certain embodiments, the antibody binds to an epitope located within a region of human CTLA-4 consisting of the amino acid sequence of SEQ ID NO: 39.

[0026] In another aspect, the instant disclosure provides an antibody or isolated antibody that specifically binds to the same epitope of human CTLA-4 as any antibody of the present invention. In certain embodiments, the antibody binds to an epitope located within a region of human CTLA-4 consisting of an amino acid sequence selected from the group consisting of SEQ ID NOs: 34-39. In certain embodiments, the antibody binds to an epitope located within a region of human CTLA-4 consisting of the amino acid sequence of SEQ ID NO: 37. In certain embodiments, the antibody binds to an epitope located within a region of human CTLA-4 consisting of the amino acid sequence of SEQ ID NO: 36. In certain embodiments, the antibody binds to an epitope located within a region of human CTLA-4 consisting of the amino acid sequence of SEQ ID NO: 35. In certain embodiments, the antibody binds to an epitope located within a region of human CTLA-4 consisting of the amino acid sequence of SEQ ID NO: 34. In certain embodiments, the antibody binds to an epitope located within a region of human CTLA-4 consisting of the amino acid sequence of SEQ ID NO: 38. In certain embodiments, the antibody binds to an epitope located within a region of human CTLA-4 consisting of the amino acid sequence of SEQ ID NO: 39.

[0027] In another aspect, the instant disclosure provides an antibody that, when bound to a human CTLA-4 protein or fragment thereof, *e.g.*, comprising the amino acid sequence of residues 37-162 of SEQ ID NO: 33, reduces hydrogen/deuterium exchange in a region consisting of the amino acid sequence set forth in SEQ ID NO: 34 relative to hydrogen/deuterium exchange in the region consisting of the amino acid sequence set forth in SEQ ID NO: 34 in the absence of the antibody, as determined by a hydrogen/deuterium assay. In another aspect, the instant disclosure provides an antibody that, when bound to a human CTLA-4 protein or fragment thereof, *e.g.*, comprising the amino acid sequence of residues 37-162 of SEQ ID NO: 33, reduces hydrogen/deuterium exchange in a region consisting of the amino acid sequence set forth in SEQ ID NO: 35 relative to hydrogen/deuterium exchange in the region consisting of the amino acid sequence set forth in

SEQ ID NO: 35 in the absence of the antibody, as determined by a hydrogen/deuterium assay. In another aspect, the instant disclosure provides an antibody that, when bound to a human CTLA-4 protein or fragment thereof, *e.g.*, comprising the amino acid sequence of residues 37-162 of SEQ ID NO: 33, reduces hydrogen/deuterium exchange in a region consisting of the amino acid sequence set forth in SEQ ID NO: 36 relative to hydrogen/deuterium exchange in the region consisting of the amino acid sequence set forth in SEQ ID NO: 36 in the absence of the antibody, as determined by a hydrogen/deuterium assay. In another aspect, the instant disclosure provides an antibody that, when bound to a human CTLA-4 protein or fragment thereof, *e.g.*, comprising the amino acid sequence of residues 37-162 of SEQ ID NO: 33, reduces hydrogen/deuterium exchange in a region consisting of the amino acid sequence set forth in SEQ ID NO: 37 relative to hydrogen/deuterium exchange in the region consisting of the amino acid sequence set forth in SEQ ID NO: 37 in the absence of the antibody, as determined by a hydrogen/deuterium assay. In another aspect, the instant disclosure provides an antibody that, when bound to a human CTLA-4 protein or fragment thereof, *e.g.*, comprising the amino acid sequence of residues 37-162 of SEQ ID NO: 33, reduces hydrogen/deuterium exchange in a region consisting of the amino acid sequence set forth in SEQ ID NO: 38 relative to hydrogen/deuterium exchange in the region consisting of the amino acid sequence set forth in SEQ ID NO: 38 in the absence of the antibody, as determined by a hydrogen/deuterium assay. In another aspect, the instant disclosure provides an antibody that, when bound to a human CTLA-4 protein or fragment thereof, *e.g.*, comprising the amino acid sequence of residues 37-162 of SEQ ID NO: 33, reduces hydrogen/deuterium exchange in a region consisting of the amino acid sequence set forth in SEQ ID NO: 39 relative to hydrogen/deuterium exchange in the region consisting of the amino acid sequence set forth in SEQ ID NO: 39 in the absence of the antibody, as determined by a hydrogen/deuterium assay.

[0028] In another aspect, the instant disclosure provides an antibody or isolated antibody that specifically binds to the same epitope of human CTLA-4 as any antibody of the present invention. In certain embodiments, the antibody, when bound to a human CTLA-4 protein or fragment thereof, *e.g.*, comprising the amino acid sequence of residues 37-162 of SEQ ID NO: 33, reduces hydrogen/deuterium exchange in a region consisting of the amino acid sequence set forth in SEQ ID NO: 34 relative to hydrogen/deuterium exchange in the region consisting of the amino acid sequence set forth in SEQ ID NO: 34 in the absence of the antibody, as determined by a hydrogen/deuterium assay. In certain embodiments, the

antibody, when bound to a human CTLA-4 protein or fragment thereof, *e.g.*, comprising the amino acid sequence of residues 37-162 of SEQ ID NO: 33, reduces hydrogen/deuterium exchange in a region consisting of the amino acid sequence set forth in SEQ ID NO: 35 relative to hydrogen/deuterium exchange in the region consisting of the amino acid sequence set forth in SEQ ID NO: 35 in the absence of the antibody, as determined by a hydrogen/deuterium assay. In certain embodiments, the antibody, when bound to a human CTLA-4 protein or fragment thereof, *e.g.*, comprising the amino acid sequence of residues 37-162 of SEQ ID NO: 33, reduces hydrogen/deuterium exchange in a region consisting of the amino acid sequence set forth in SEQ ID NO: 36 relative to hydrogen/deuterium exchange in the region consisting of the amino acid sequence set forth in SEQ ID NO: 36 in the absence of the antibody, as determined by a hydrogen/deuterium assay. In certain embodiments, the antibody, when bound to a human CTLA-4 protein or fragment thereof, *e.g.*, comprising the amino acid sequence of residues 37-162 of SEQ ID NO: 33, reduces hydrogen/deuterium exchange in a region consisting of the amino acid sequence set forth in SEQ ID NO: 37 relative to hydrogen/deuterium exchange in the region consisting of the amino acid sequence set forth in SEQ ID NO: 37 in the absence of the antibody, as determined by a hydrogen/deuterium assay. In certain embodiments, the antibody, when bound to a human CTLA-4 protein or fragment thereof, *e.g.*, comprising the amino acid sequence of residues 37-162 of SEQ ID NO: 33, reduces hydrogen/deuterium exchange in a region consisting of the amino acid sequence set forth in SEQ ID NO: 38 relative to hydrogen/deuterium exchange in the region consisting of the amino acid sequence set forth in SEQ ID NO: 38 in the absence of the antibody, as determined by a hydrogen/deuterium assay. In certain embodiments, the antibody, when bound to a human CTLA-4 protein or fragment thereof, *e.g.*, comprising the amino acid sequence of residues 37-162 of SEQ ID NO: 33, reduces hydrogen/deuterium exchange in a region consisting of the amino acid sequence set forth in SEQ ID NO: 39 relative to hydrogen/deuterium exchange in the region consisting of the amino acid sequence set forth in SEQ ID NO: 39 in the absence of the antibody, as determined by a hydrogen/deuterium assay.

[0029] In certain embodiments, the antibody is a human antibody. In certain embodiment, the antibody is a bispecific antibody.

[0030] In certain embodiments, the antibody is antagonistic to human CTLA-4. In certain embodiments, the antibody deactivates, reduces, or inhibits an activity of human CTLA-4. In certain embodiments, the antibody inhibits binding of human CTLA-4 to human CD80 or human CD86. In certain embodiments, the antibody induces IL-2 production by

peripheral blood mononuclear cells (PBMCs) stimulated with staphylococcal enterotoxin A (SEA).

[0031] In certain embodiments, the antibody is conjugated to a cytotoxic agent, cytostatic agent, toxin, radionuclide, or detectable label.

[0032] In certain embodiments, the N-terminal amino acid residue of the heavy chain variable region and/or the light chain variable region of the antibody has been converted to pyroglutamate.

[0033] In one embodiment, the present invention relates to an antibody of the present invention for use as a medicament.

[0034] In one embodiment, the present invention relates to use of an antibody of the present invention for preparing pharmaceutical compositions or medicaments for immunotherapy. In certain embodiments, the immunotherapy is for increasing T-cell activation in response to an antigen in a subject, optionally for treating cancer, or treating or preventing infectious diseases.

[0035] In one embodiment, the present invention relates to an antibody of the present invention for use as a diagnostic.

[0036] In one embodiment, the present invention relates to the use of an antibody of the present invention for *in vitro* detection of human CTLA-4 in a biological sample.

[0037] In another aspect, the instant disclosure provides a pharmaceutical composition comprising an anti-CTLA-4 antibody described herein and a pharmaceutically acceptable carrier or excipient.

[0038] In another aspect, the instant disclosure provides an isolated polynucleotide encoding a heavy and/or light chain of an antibody described herein. In another aspect, the instant disclosure provides a vector comprising the polynucleotide. In another aspect, the instant disclosure provides a recombinant host cell comprising the polynucleotide or the vector. In another aspect, the instant disclosure provides a method of producing an antibody that binds to human CTLA-4, the method comprising culturing the host cell so that the polynucleotide is expressed and the antibody is produced.

[0039] In another aspect, the instant disclosure provides a method of increasing T-cell activation in response to an antigen in a subject, the method comprising administering to the subject an effective amount of an anti-CTLA-4 antibody or pharmaceutical composition described herein. In another aspect, the instant disclosure provides a method of treating cancer in a subject, the method comprising administering to the subject an effective amount of an anti-CTLA-4 antibody or pharmaceutical composition described herein.

[0040] In certain embodiments, the subject has cancer. In certain embodiments, the subject has a metastatic or locally advanced tumor (e.g., solid tumor). In certain embodiments, the cancer is treated in accordance with a method described herein as a first cancer therapy after diagnosis of the metastatic or locally advanced tumor (e.g., within 1, 2, 3, 4, 5, or 6 days; 1, 2, 3, 4, 6, 8, or 12 weeks; or, 1, 2, 3, 4, 6, 8, or 12 months after diagnosis). In certain embodiments, the cancer is treated in accordance with a method described herein as the first cancer therapy after diagnosis of tumor progression (e.g., within 1, 2, 3, 4, 5, or 6 days; 1, 2, 3, 4, 6, 8, or 12 weeks; or, 1, 2, 3, 4, 6, 8, or 12 months after diagnosis of tumor progression) that has occurred despite previous treatment of the tumor with a different cancer therapy, optionally wherein the method described herein is provided as the second cancer therapy administered. In certain embodiments, the cancer is treated in accordance with a method described herein as the first cancer therapy after diagnosis of toxicity of a different cancer therapy (e.g., within 1, 2, 3, 4, 5, or 6 days; 1, 2, 3, 4, 6, 8, or 12 weeks; or, 1, 2, 3, 4, 6, 8, or 12 months after diagnosis of toxicity of the different cancer therapy), optionally wherein the method described herein is provided as the second cancer therapy administered. In certain embodiments, the cancer treated in accordance with the methods described herein is a metastatic or locally advanced cancer (e.g., solid tumor) for which no standard therapy is available. In other embodiments, the cancer treated in accordance with the methods described herein is a metastatic or locally advanced cancer (e.g., solid tumor) for which a standard therapy has failed (*i.e.*, the cancer has progressed after the standard therapy). In certain embodiments, a therapy fails if the cancer is refractory to the therapy. In certain embodiments, a therapy fails if the cancer relapses after responding, fully or partially, to the therapy. In certain embodiments, metastatic or locally advanced cancer (e.g., solid tumor) has been confirmed histologically or cytologically.

[0041] In certain embodiments, the cancer expresses PD-L1. In certain embodiments, the percentage of tumor cells in a sample of the cancer that exhibit detectable membrane expression (e.g., partial or complete membrane expression) of PD-L1 is at least 1% (e.g., at least 2%, 3%, 4%, 5%, 10%, 15%, 20%, 25%, 30%, 35%, 40%, 45%, 50%, 60%, 70%, 80%, or 90%). In certain embodiments, the percentage of tumor cells in a sample of the cancer that exhibit detectable membrane expression (e.g., partial or complete membrane expression) of PD-L1 is at least 1%. In certain embodiments, the percentage of tumor cells in a sample of the cancer that exhibit detectable membrane expression (e.g., partial or complete membrane expression) of PD-L1 is at least 5%. In certain embodiments, the percentage of tumor cells in a sample of the cancer that exhibit detectable membrane expression (e.g., partial or complete

membrane expression) of PD-L1 is at least 25%. In certain embodiments, the percentage of tumor cells in a sample of the cancer that exhibit detectable membrane expression (e.g., partial or complete membrane expression) of PD-L1 is at least 50%.

[0042] In certain embodiments, the metastatic or locally advanced tumor expresses PD-L1. In certain embodiments, the percentage of tumor cells in a sample of the metastatic or locally advanced tumor that exhibit detectable membrane expression (e.g., partial or complete membrane expression) of PD-L1 is at least 1% (e.g., at least 2%, 3%, 4%, 5%, 10%, 15%, 20%, 25%, 30%, 35%, 40%, 45%, 50%, 60%, 70%, 80%, or 90%). In certain embodiments, the percentage of tumor cells in a sample of the metastatic or locally advanced tumor that exhibit detectable membrane expression (e.g., partial or complete membrane expression) of PD-L1 is at least 1%. In certain embodiments, the percentage of tumor cells in a sample of the metastatic or locally advanced tumor that exhibit detectable membrane expression (e.g., partial or complete membrane expression) of PD-L1 is at least 5%. In certain embodiments, the percentage of tumor cells in a sample of the metastatic or locally advanced tumor that exhibit detectable membrane expression (e.g., partial or complete membrane expression) of PD-L1 is at least 25%. In certain embodiments, the percentage of tumor cells in a sample of the metastatic or locally advanced tumor that exhibit detectable membrane expression (e.g., partial or complete membrane expression) of PD-L1 is at least 50%.

[0043] In certain embodiments, the cancer is a cervical cancer. In certain embodiments, the cancer is a metastatic or locally advanced cancer (e.g., solid tumor). In certain embodiments, the metastatic or locally advanced cancer (e.g., solid tumor) is a metastatic or locally advanced, unresectable squamous cell carcinoma, adenosquamous carcinoma, or adenocarcinoma of the cervix. In certain embodiments, no standard therapy is available for the cancer (e.g., cervical cancer) or metastatic or locally advanced tumor (e.g., solid tumor). In certain embodiments, the cancer (e.g., cervical cancer) or metastatic or locally advanced tumor (e.g., solid tumor) is refractory to a standard therapy. In certain embodiments, the cancer (e.g., cervical cancer) or metastatic or locally advanced tumor (e.g., solid tumor) has relapsed after a standard therapy. In certain embodiments, the standard therapy comprises a platinum-containing chemotherapy. In certain embodiments, the standard therapy is a platinum-containing doublet. In certain embodiments, the cancer (e.g., cervical cancer) is a metastatic or locally advanced, unresectable squamous cell carcinoma, adenosquamous carcinoma, or adenocarcinoma of the cervix that has relapsed after a platinum-containing doublet administered for treatment of advanced (recurrent, unresectable, or metastatic) disease. In certain embodiments, the cancer (e.g., cervical cancer) or metastatic or locally

advanced tumor is HPV positive. In certain embodiments, the cancer or metastatic or locally advanced solid tumor is head and neck cancer, melanoma, renal cell carcinoma, urothelial carcinoma, or endometrial carcinoma. In certain embodiments, the cancer (e.g., cervical cancer) or metastatic or locally advanced solid tumor is associated with microsatellite instability.

[0044] In certain embodiments, the subject has cervical cancer (e.g., a metastatic or locally advanced, unresectable squamous cell carcinoma, adenosquamous carcinoma, or adenocarcinoma of the cervix), and the method comprises administering to the subject an effective amount of an anti-CTLA-4 antibody described herein, e.g., AGEN1884.H3 (IgG_1 S239D/A330L/I332E), or pharmaceutical composition comprising such anti-CTLA-4 antibody as a first cancer therapy after diagnosis of the cervical cancer (e.g., within 1, 2, 3, 4, 5, or 6 days; 1, 2, 3, 4, 6, 8, or 12 weeks; or, 1, 2, 3, 4, 6, 8, or 12 months after diagnosis), optionally wherein the anti-CTLA-4 antibody described herein, e.g., AGEN1884.H3 (IgG_1 S239D/A330L/I332E), or pharmaceutical composition comprising such anti-CTLA-4 antibody is administered at the dosage and frequency selected from the group consisting of 0.3 mg/kg every four weeks, 1 mg/kg every four weeks, 3 mg/kg every four weeks, 0.3 mg/kg every six weeks, 1 mg/kg every six weeks, 3 mg/kg every six weeks, 0.3 mg/kg every twelve weeks, 1 mg/kg every twelve weeks, and 3 mg/kg every twelve weeks. In certain embodiments, the subject has cervical cancer (e.g., a metastatic or locally advanced, unresectable squamous cell carcinoma, adenosquamous carcinoma, or adenocarcinoma of the cervix), and the method comprises administering to the subject an effective amount of a therapeutic combination comprising an anti-CTLA-4 antibody described herein, e.g., AGEN1884.H3 (IgG_1 S239D/A330L/I332E), or pharmaceutical composition comprising such anti-CTLA-4 antibody, and pembrolizumab as a first cancer therapy after diagnosis of the cervical cancer (e.g., within 1, 2, 3, 4, 5, or 6 days; 1, 2, 3, 4, 6, 8, or 12 weeks; or, 1, 2, 3, 4, 6, 8, or 12 months after diagnosis), optionally wherein the anti-CTLA-4 antibody described herein, e.g., AGEN1884.H3 (IgG_1 S239D/A330L/I332E), or pharmaceutical composition comprising such anti-CTLA-4 antibody, is administered at the dosage and frequency selected from the group consisting of 0.3 mg/kg every four weeks, 1 mg/kg every four weeks, 3 mg/kg every four weeks, 0.3 mg/kg every six weeks, 1 mg/kg every six weeks, 3 mg/kg every six weeks, 0.3 mg/kg every twelve weeks, 1 mg/kg every twelve weeks, and 3 mg/kg every twelve weeks, and pembrolizumab is administered at 200 mg every three weeks.

[0045] In certain embodiments, the cancer is a non-small cell lung cancer (NSCLC). In certain embodiments, the NSCLC is a Stage IV NSCLC. In certain embodiments, the

percentage of tumor cells in a sample of the NSCLC that exhibit detectable membrane expression (e.g., partial or complete membrane expression) of PD-L1 is at least 1% (e.g., at least 2%, 3%, 4%, 5%, 10%, 15%, 20%, 25%, 30%, 35%, 40%, 45%, 50%, 60%, 70%, 80%, or 90%). In certain embodiments, the percentage of tumor cells in a sample of the NSCLC that exhibit detectable membrane expression (e.g., partial or complete membrane expression) of PD-L1 is at least 1%. In certain embodiments, the percentage of tumor cells in a sample of the NSCLC that exhibit detectable membrane expression (e.g., partial or complete membrane expression) of PD-L1 is at least 5%. In certain embodiments, the percentage of tumor cells in a sample of the NSCLC that exhibit detectable membrane expression (e.g., partial or complete membrane expression) of PD-L1 is at least 25%. In certain embodiments, the percentage of tumor cells in a sample of the NSCLC that exhibit detectable membrane expression (e.g., partial or complete membrane expression) of PD-L1 is at least 50%. In certain embodiments, the NSCLC has no EGFR or ALK genomic tumor aberrations. In certain embodiments, the metastatic or locally advanced NSCLC has no EGFR sensitizing mutation (e.g., mutation that is amenable to treatment with a tyrosine kinase inhibitor including erlotinib, gefitinib, or afatanib) or ALK translocation. In certain embodiments, the subject has received no prior systemic chemotherapy treatment for NSCLC.

[0046] In certain embodiments, the metastatic or locally advanced solid tumor is a metastatic or locally advanced non-small cell lung cancer (NSCLC). In certain embodiments, the metastatic or locally advanced solid tumor is a metastatic non-small cell lung cancer (NSCLC). In certain embodiments, the metastatic or locally advanced solid tumor is a Stage IV, metastatic or locally advanced NSCLC. In certain embodiments, the metastatic or locally advanced solid tumor is a Stage IV, metastatic NSCLC. In certain embodiments, the percentage of tumor cells in a sample of the metastatic or locally advanced NSCLC that exhibit detectable membrane expression (e.g., partial or complete membrane expression) of PD-L1 is at least 1% (e.g., at least 2%, 3%, 4%, 5%, 10%, 15%, 20%, 25%, 30%, 35%, 40%, 45%, 50%, 60%, 70%, 80%, or 90%). In certain embodiments, the percentage of tumor cells in a sample of the metastatic or locally advanced NSCLC that exhibit detectable membrane expression (e.g., partial or complete membrane expression) of PD-L1 is at least 1%. In certain embodiments, the percentage of tumor cells in a sample of the metastatic or locally advanced NSCLC that exhibit detectable membrane expression (e.g., partial or complete membrane expression) of PD-L1 is at least 5%. In certain embodiments, the percentage of tumor cells in a sample of the metastatic or locally advanced NSCLC that exhibit detectable membrane expression (e.g., partial or complete membrane expression) of PD-L1 is at least

25%. In certain embodiments, the percentage of tumor cells in a sample of the metastatic or locally advanced NSCLC that exhibit detectable membrane expression (e.g., partial or complete membrane expression) of PD-L1 is at least 50%. In certain embodiments, the metastatic or locally advanced NSCLC has no EGFR or ALK genomic tumor aberrations. In certain embodiments, the subject has received no prior systemic chemotherapy treatment for metastatic or locally advanced NSCLC.

[0047] In certain embodiments, the subject has NSCLC (e.g., Stage IV, metastatic, or locally advanced NSCLC), optionally wherein the percentage of tumor cells in a sample of the NSCLC that exhibit detectable expression (e.g., membrane expression, partial or complete membrane expression) of PD-L1 is at least 1%, 2%, 3%, 4%, 5%, 10%, 15%, 20%, 25%, 30%, 35%, 40%, 45%, 50%, 60%, 70%, 80%, or 90%, and the method comprises administering to the subject an effective amount of an anti-CTLA-4 antibody described herein, e.g., AGEN1884.H3 (IgG_1 S239D/A330L/I332E), or pharmaceutical composition comprising such anti-CTLA-4 antibody, as a first cancer therapy after diagnosis of the cervical cancer (e.g., within 1, 2, 3, 4, 5, or 6 days; 1, 2, 3, 4, 6, 8, or 12 weeks; or, 1, 2, 3, 4, 6, 8, or 12 months after diagnosis), optionally wherein the anti-CTLA-4 antibody described herein, e.g., AGEN1884.H3 (IgG_1 S239D/A330L/I332E), or pharmaceutical composition comprising such anti-CTLA-4 antibody, is administered at the dosage and frequency selected from the group consisting of 0.3 mg/kg every four weeks, 1 mg/kg every four weeks, 3 mg/kg every four weeks, 0.3 mg/kg every six weeks, 1 mg/kg every six weeks, 3 mg/kg every six weeks, 0.3 mg/kg every twelve weeks, 1 mg/kg every twelve weeks, and 3 mg/kg every twelve weeks. In certain embodiments, the subject has NSCLC (e.g., Stage IV, metastatic, or locally advanced NSCLC), optionally wherein the percentage of tumor cells in a sample of the NSCLC that exhibit detectable expression (e.g., membrane expression, partial or complete membrane expression) of PD-L1 is at least 1%, 2%, 3%, 4%, 5%, 10%, 15%, 20%, 25%, 30%, 35%, 40%, 45%, 50%, 60%, 70%, 80%, or 90%, and the method comprises administering to the subject a therapeutic combination comprising an anti-CTLA-4 antibody described herein, e.g., AGEN1884.H3 (IgG_1 S239D/A330L/I332E), or pharmaceutical composition comprising such anti-CTLA-4 antibody, and pembrolizumab as a first cancer therapy after diagnosis of the cervical cancer (e.g., within 1, 2, 3, 4, 5, or 6 days; 1, 2, 3, 4, 6, 8, or 12 weeks; or, 1, 2, 3, 4, 6, 8, or 12 months after diagnosis), optionally wherein the anti-CTLA-4 antibody described herein, e.g., AGEN1884.H3 (IgG_1 S239D/A330L/I332E), or pharmaceutical composition comprising such anti-CTLA-4 antibody, is administered at the dosage and frequency selected from the group consisting of 0.3 mg/kg every four weeks, 1

mg/kg every four weeks, 3 mg/kg every four weeks, 0.3 mg/kg every six weeks, 1 mg/kg every six weeks, 3 mg/kg every six weeks, 0.3 mg/kg every twelve weeks, 1 mg/kg every twelve weeks, and 3 mg/kg every twelve weeks, and pembrolizumab is administered at 200 mg every three weeks.

[0048] In certain embodiments, the cancer is a cutaneous squamous-cell carcinoma (cSCC). In certain embodiments, the metastatic or locally advanced solid tumor is a Stage IV cutaneous squamous-cell carcinoma (cSCC). In certain embodiments, the cSCC is diagnosed histologically or cytologically according to the eighth edition of the American Joint Committee on Cancer staging manual. In certain embodiments, the cSCC is not curable with radiation therapy. In certain embodiments, the subject has cSCC (e.g., Stage IV cSCC), and the method comprises administering to the subject an effective amount of an anti-CTLA-4 antibody described herein, e.g., AGEN1884.H3 (IgG_1 S239D/A330L/I332E), or pharmaceutical composition comprising such anti-CTLA-4 antibody, as a first cancer therapy after diagnosis of the cSCC (e.g., within 1, 2, 3, 4, 5, or 6 days; 1, 2, 3, 4, 6, 8, or 12 weeks; or, 1, 2, 3, 4, 6, 8, or 12 months after diagnosis), optionally wherein the anti-CTLA-4 antibody described herein, e.g., AGEN1884.H3 (IgG_1 S239D/A330L/I332E), or pharmaceutical composition comprising such anti-CTLA-4 antibody, is administered at the dosage and frequency selected from the group consisting of 0.3 mg/kg every four weeks, 1 mg/kg every four weeks, 3 mg/kg every four weeks, 0.3 mg/kg every six weeks, 1 mg/kg every six weeks, 3 mg/kg every six weeks, 0.3 mg/kg every twelve weeks, 1 mg/kg every twelve weeks, and 3 mg/kg every twelve weeks. In certain embodiments, the subject has cSCC (e.g., Stage IV cSCC), and the method comprises administering to the subject an effective amount of a therapeutic combination comprising an anti-CTLA-4 antibody described herein, e.g., AGEN1884.H3 (IgG_1 S239D/A330L/I332E), or pharmaceutical composition comprising such anti-CTLA-4 antibody, and pembrolizumab as a first cancer therapy after diagnosis of the cSCC (e.g., within 1, 2, 3, 4, 5, or 6 days; 1, 2, 3, 4, 6, 8, or 12 weeks; or, 1, 2, 3, 4, 6, 8, or 12 months after diagnosis), optionally wherein the anti-CTLA-4 antibody described herein, e.g., AGEN1884.H3 (IgG_1 S239D/A330L/I332E), or pharmaceutical composition comprising such anti-CTLA-4 antibody, is administered at the dosage and frequency selected from the group consisting of 0.3 mg/kg every four weeks, 1 mg/kg every four weeks, 3 mg/kg every four weeks, 0.3 mg/kg every six weeks, 1 mg/kg every six weeks, 3 mg/kg every six weeks, 0.3 mg/kg every twelve weeks, 1 mg/kg every twelve weeks, and 3 mg/kg every twelve weeks, and pembrolizumab is administered at 200 mg every three weeks.

[0049] In certain embodiments, the anti-CTLA-4 antibody or pharmaceutical composition described herein is administered intravenously. In certain embodiments, the anti-CTLA-4 antibody or pharmaceutical composition described herein is administered intravenously at 0.01 mg/kg, 0.03 mg/kg, 0.1 mg/kg, 0.3 mg/kg, 1 mg/kg, 3 mg/kg, 6 mg/kg, or 10 mg/kg, optionally at an interval of once every two weeks. In certain embodiments, the anti-CTLA-4 antibody or pharmaceutical composition described herein is administered intravenously at 0.01 mg/kg, 0.03 mg/kg, 0.1 mg/kg, 0.3 mg/kg, 1 mg/kg, 3 mg/kg, 6 mg/kg, or 10 mg/kg, optionally at an interval of once every three weeks. In certain embodiments, the anti-CTLA-4 antibody or pharmaceutical composition described herein is administered intravenously at 0.01 mg/kg, 0.03 mg/kg, 0.1 mg/kg, 0.3 mg/kg, 1 mg/kg, 3 mg/kg, 6 mg/kg, or 10 mg/kg, optionally at an interval of once every four weeks. In certain embodiments, the anti-CTLA-4 antibody or pharmaceutical composition described herein is administered intravenously at 0.01 mg/kg, 0.03 mg/kg, 0.1 mg/kg, 0.3 mg/kg, 1 mg/kg, 3 mg/kg, 6 mg/kg, or 10 mg/kg, optionally at an interval of once every six weeks. In certain embodiments, the anti-CTLA-4 antibody or pharmaceutical composition described herein is administered intravenously at 0.01 mg/kg, 0.03 mg/kg, 0.1 mg/kg, 0.3 mg/kg, 1 mg/kg, 3 mg/kg, 6 mg/kg, or 10 mg/kg, optionally at an interval of once every twelve weeks. In certain embodiments, the anti-CTLA-4 antibody or pharmaceutical composition described herein is administered intratumorally. In certain embodiments, the anti-CTLA-4 antibody or pharmaceutical composition described herein is administered intratumorally at 0.01 mg/kg, 0.03 mg/kg, 0.1 mg/kg, 0.3 mg/kg, 1 mg/kg, or 3 mg/kg, optionally at an interval of once every three weeks. In certain embodiments, the anti-CTLA-4 antibody or pharmaceutical composition described herein is administered intratumorally at 0.03 mg/kg, 0.1 mg/kg, or 0.3 mg/kg, optionally at an interval of once every three weeks. In certain embodiments, the anti-CTLA-4 antibody or pharmaceutical composition described herein is administered intratumorally at a dose that is up to 5-fold, 10-fold, 20-fold, 30-fold, 40-fold, 50-fold, 60-fold, 70-fold, 80-fold, 90-fold, 100-fold, or 200-fold lower than a dose given by systemic administration. In certain embodiments, the anti-CTLA-4 antibody or pharmaceutical composition described herein is administered intratumorally at a dose that is up to 10-fold lower than a dose given by systemic administration. In certain embodiments, the anti-CTLA-4 antibody or pharmaceutical composition described herein is administered intratumorally at a dose that is up to 100-fold lower than a dose given by systemic administration. In certain embodiments, the anti-CTLA-4 antibody or pharmaceutical composition described herein is administered (e.g., intratumorally or systemically) as a monotherapy. In certain embodiments, the anti-

CTLA-4 antibody or pharmaceutical composition described herein is administered intratumorally and the method further comprises administering an additional therapeutic agent to the subject. In certain embodiments, the additional therapeutic agent is administered systemically. In certain embodiments, the subject has a solid tumor and the additional therapeutic agent is an anti-PD-1 antibody. In certain embodiments, the anti-PD-1 antibody is pembrolizumab or nivolumab. In certain embodiments, the pembrolizumab is administered at a dose of 200 mg every three weeks. In certain embodiments, the subject has head and neck squamous cell carcinoma and the additional therapeutic agent is an anti-EGFR antibody. In certain embodiments, the anti-EGFR antibody is cetuximab. In certain embodiments, the subject has HER2+ breast cancer and the additional therapeutic agent is an anti-HER2 antibody. In certain embodiments, the anti-HER2 antibody is trastuzumab. In certain embodiments, these methods further comprise administering a chemotherapeutic agent to the subject. In certain embodiments, the chemotherapeutic agent is administered systemically. In certain embodiments, the chemotherapeutic agent is gemcitabine. In certain embodiments, the anti-CTLA-4 antibody or pharmaceutical composition described herein is administered intratumorally and the subject has an advanced or metastatic solid tumor. In certain embodiments, the anti-CTLA-4 antibody or pharmaceutical composition described herein is administered intratumorally and the subject has head and neck cancer (e.g., relapsed/refractory head and neck squamous cell carcinoma). In certain embodiments, the anti-CTLA-4 antibody or pharmaceutical composition described herein is administered intratumorally and the subject has breast cancer (e.g., relapsed/refractory HER2+ breast cancer). In certain embodiments, the anti-CTLA-4 antibody or pharmaceutical composition described herein is delivered to a tumor draining lymph node. In certain embodiments, the anti-CTLA-4 antibody or pharmaceutical composition described herein is delivered via a localized administration (e.g., subcutaneous administration). In certain embodiments, the anti-CTLA-4 antibody or pharmaceutical composition described herein is delivered via a localized administration (e.g., subcutaneous administration) at 0.01 mg/kg, 0.03 mg/kg, 0.1 mg/kg, 0.3 mg/kg, 1 mg/kg, or 3 mg/kg. In certain embodiments, the anti-CTLA-4 antibody or pharmaceutical composition described herein is delivered via a localized administration (e.g., subcutaneous administration) at a dose that is up to 5-fold, 10-fold, 20-fold, 30-fold, 40-fold, 50-fold, 60-fold, 70-fold, 80-fold, 90-fold, 100-fold, or 200-fold lower than a dose given by systemic administration. In certain embodiments, the anti-CTLA-4 antibody or pharmaceutical composition described herein is delivered via a localized administration (e.g., subcutaneous administration) at a dose that is up to 10-fold lower than a dose given by

systemic administration. In certain embodiments, the anti-CTLA-4 antibody or pharmaceutical composition described herein is delivered via a localized administration (e.g., subcutaneous administration) at a dose that is up to 100-fold lower than a dose given by systemic administration. In certain embodiments, the anti-CTLA-4 antibody or pharmaceutical composition described herein is delivered via a localized administration (e.g., subcutaneous administration) and the method further comprises administering an additional therapeutic agent to the subject. In certain embodiments, the additional therapeutic agent is a vaccine. In certain embodiments, the vaccine comprises a heat shock protein peptide complex (HSPPC) comprising a heat shock protein complexed with an antigenic peptide. In one embodiment, the heat shock protein is gp96 protein and is complexed with a tumor-associated antigenic peptide, wherein the HSPPC is derived from a tumor obtained from a subject. In certain embodiments, the heat shock protein is selected from the group consisting of hsc70, hsp70, hsp90, hsp110, grp170, gp96, calreticulin, a mutant thereof, and combinations of two or more thereof. In certain embodiments, the heat shock protein is hsc70. In certain embodiments, the heat shock protein is hsp70. In certain embodiments, the antigenic peptide is synthetic. In certain embodiments, the subject has cancer. In certain embodiments, the subject has an infectious disease. In certain embodiments, these methods further comprise administering an additional therapeutic agent to the subject. In certain embodiments, the additional therapeutic agent is a chemotherapeutic or a checkpoint targeting agent. In certain embodiments, the checkpoint targeting agent is selected from the group consisting of an antagonist anti-PD-1 antibody, an antagonist anti-PD-L1 antibody, an antagonist anti-PD-L2 antibody, an antagonist anti-CTLA-4 antibody, an antagonist anti-TIM-3 antibody, an antagonist anti-LAG-3 antibody, an antagonist anti-CEACAM1 antibody, an agonist anti-GITR antibody, an agonist anti-OX40 antibody, and an agonist anti-CD137 antibody, an agonist anti-DR3 antibody, an agonist anti-TNFSF14 antibody, and an agonist anti-CD27 antibody. In certain embodiments, the additional therapeutic agent is radiotherapy. In certain embodiments, the additional therapeutic agent is an inhibitor of indoleamine-2,3-dioxygenase (IDO). Suitable IDO inhibitors include, without limitation, epacadostat, F001287, indoximod, and NLG919. In certain embodiments, the additional therapeutic agent is a vaccine. In certain embodiments, the vaccine comprises a heat shock protein peptide complex (HSPPC) comprising a heat shock protein complexed with an antigenic peptide. In one embodiment, the heat shock protein is gp96 protein and is complexed with a tumor-associated antigenic peptide, wherein the HSPPC is derived from a tumor obtained from a subject.

[0050] In another aspect, the instant disclosure provides a method of treating an infectious disease in a subject, the method comprising administering to the subject an effective amount of an anti-CTLA-4 antibody or pharmaceutical composition described herein. In another aspect, the instant disclosure provides a method of preventing an infectious disease in a subject, the method comprising administering to the subject an effective amount of an anti-CTLA-4 antibody or pharmaceutical composition described herein.

[0051] In one embodiment, the present invention relates to an antibody of the present invention, a polynucleotide of the invention, a vector of the invention, and/or a recombinant host cell of the invention, for use as a medicament.

[0052] In one embodiment, the present invention relates to an antibody of the present invention, a polynucleotide of the invention, a vector of the invention, and/or a recombinant host cell of the invention, for use as a diagnostic.

[0053] In one embodiment, the present invention relates to the use of an antibody of the present invention, a polynucleotide of the invention, a vector of the invention, and/or a recombinant host cell of the invention, for the *in vitro* detection of human CTLA-4 in a biological sample.

[0054] In one aspect, provided herein is a pharmaceutical composition comprising an anti-CTLA-4 antibody described herein and a pharmaceutically acceptable carrier or excipient, for use as a medicament.

[0055] In one aspect, provided herein is a pharmaceutical composition comprising an anti-CTLA-4 antibody described herein and a pharmaceutically acceptable carrier or excipient, for use as a diagnostic.

[0056] In one aspect, provided herein is a pharmaceutical composition comprising an anti-CTLA-4 antibody described herein, a polynucleotide of the invention, a vector of the invention, and/or a recombinant host cell of the invention, and a pharmaceutically acceptable carrier or excipient. In one aspect, the pharmaceutical composition is for use as a medicament and/or diagnostic.

[0057] In one aspect, the present invention relates to an antibody, polynucleotide, vector, recombinant host cell, and/or pharmaceutical composition of the present invention, for use in a method for increasing T-cell activation in response to an antigen.

[0058] In one aspect, the present invention relates to an antibody, polynucleotide, vector, recombinant host cell, and/or pharmaceutical composition of the present invention, for use in a method for increasing T-cell activation in response to an antigen in a subject.

[0059] In one aspect, the present invention relates to an antibody, polynucleotide, vector, recombinant host cell, and/or pharmaceutical composition of the present invention, for use in a method for increasing T-cell activation in response to an antigen in a subject comprising administering to the subject an effective amount of an antibody, polynucleotide, vector, recombinant host cell, and/or pharmaceutical composition of the invention.

[0060] In one aspect, the present invention relates to an antibody, polynucleotide, vector, recombinant host cell, and/or pharmaceutical composition of the present invention, for use in a method for the treatment of cancer.

[0061] In one aspect, the present invention relates to an antibody, polynucleotide, vector, recombinant host cell, and/or pharmaceutical composition of the present invention, for use in a method for the treatment of cancer in a subject.

[0062] In one aspect, the present invention relates to an antibody, polynucleotide, vector, recombinant host cell, and/or pharmaceutical composition of the present invention, for use in a method for the treatment of cancer in a subject comprising administering to the subject an effective amount of an antibody, polynucleotide, vector, recombinant host cell, and/or pharmaceutical composition of the invention.

[0063] In one aspect, the present invention relates to (a) an antibody, polynucleotide, vector, recombinant host cell, and/or pharmaceutical composition of the present invention and (b) an additional therapeutic agent, preferably an anti-PD-1 antibody, for use as a medicament.

[0064] In one aspect, the present invention relates to (a) an antibody, polynucleotide, vector, recombinant host cell, and/or pharmaceutical composition of the present invention and (b) an additional therapeutic agent, preferably an anti-PD-1 antibody, for use in a method for the treatment of cancer. In a preferred embodiment, the cancer is a solid tumor. In another preferred embodiment, the antibody, polynucleotide, vector, recombinant host cell, and/or pharmaceutical composition of the present invention is administered intratumorally to the subject, preferably administered intratumorally to the subject at 0.01 mg/kg, 0.03 mg/kg, 0.1 mg/kg, 0.3 mg/kg, 1 mg/kg, or 3 mg/kg, optionally at an interval of once every three weeks.

[0065] In one aspect, the present invention relates to a pharmaceutical composition, kit or kit-of-parts comprising (a) an antibody, polynucleotide, vector, recombinant host cell, and/or pharmaceutical composition of the present invention and (b) an additional therapeutic agent, preferably an anti-PD-1 antibody.

[0066] In one aspect, the present invention relates to (a) an antibody, polynucleotide, vector, recombinant host cell, and/or pharmaceutical composition of the present invention

and (b) an anti-EGFR antibody, and optionally (c) a chemotherapeutic agent, for use as a medicament.

[0067] In one aspect, the present invention relates to (a) an antibody, polynucleotide, vector, recombinant host cell, and/or pharmaceutical composition of the present invention and (b) an anti-EGFR antibody, and optionally (c) a chemotherapeutic agent, for use in a method for the treatment of cancer. In a preferred embodiment, the cancer is head and neck squamous cell carcinoma. In another preferred embodiment, the antibody, polynucleotide, vector, recombinant host cell, and/or pharmaceutical composition of the present invention is administered intratumorally to the subject, preferably administered intratumorally to the subject at 0.01 mg/kg, 0.03 mg/kg, 0.1 mg/kg, 0.3 mg/kg, 1 mg/kg, or 3 mg/kg, optionally at an interval of once every three weeks.

[0068] In one aspect, the present invention relates to a pharmaceutical composition, kit or kit-of-parts comprising (a) an antibody, polynucleotide, vector, recombinant host cell, and/or pharmaceutical composition of the present invention and (b) an anti-EGFR antibody, and optionally (c) a chemotherapeutic agent.

[0069] In one aspect, the present invention relates to (a) an antibody, polynucleotide, vector, recombinant host cell, and/or pharmaceutical composition of the present invention and (b) an anti-HER2 antibody, and optionally (c) a chemotherapeutic agent, for use as a medicament.

[0070] In one aspect, the present invention relates to (a) an antibody, polynucleotide, vector, recombinant host cell, and/or pharmaceutical composition of the present invention and (b) an anti-HER2 antibody, and optionally (c) a chemotherapeutic agent, for use in a method for the treatment of HER2+ breast cancer. In another preferred embodiment, the antibody, polynucleotide, vector, recombinant host cell, and/or pharmaceutical composition of the present invention is administered intratumorally to the subject, preferably administered intratumorally to the subject at 0.01 mg/kg, 0.03 mg/kg, 0.1 mg/kg, 0.3 mg/kg, 1 mg/kg, or 3 mg/kg, optionally at an interval of once every three weeks.

[0071] In one aspect, the present invention relates to a pharmaceutical composition, kit or kit-of-parts comprising (a) an antibody, polynucleotide, vector, recombinant host cell, and/or pharmaceutical composition of the present invention and (b) an anti-HER2 antibody, and optionally (c) a chemotherapeutic agent.

[0072] In one aspect, the present invention relates to an antibody, polynucleotide, vector, recombinant host cell, and/or pharmaceutical composition of the present invention, for use in a method for the treatment of cancer, wherein the antibody, polynucleotide, vector,

recombinant host cell, and/or pharmaceutical composition of the present invention is administered intratumorally to the subject, preferably administered intratumorally to the subject at 0.01 mg/kg, 0.03 mg/kg, 0.1 mg/kg, 0.3 mg/kg, 1 mg/kg, or 3 mg/kg, optionally at an interval of once every three weeks.

[0073] In one aspect, the present invention relates to an antibody, polynucleotide, vector, recombinant host cell, and/or pharmaceutical composition of the present invention, for use in a method for the treatment of cancer, wherein the antibody, polynucleotide, vector, recombinant host cell, and/or pharmaceutical composition of the present invention is administered subcutaneously or intravenously to the subject, preferably administered intravenously to the subject at 0.01 mg/kg, 0.03 mg/kg, 0.1 mg/kg, 0.3 mg/kg, 1 mg/kg, 3 mg/kg, 6 mg/kg, or 10 mg/kg, optionally at an interval of once every three weeks.

[0074] In one aspect, the present invention relates to (a) an antibody, polynucleotide, vector, recombinant host cell, and/or pharmaceutical composition of the present invention and (b) an additional therapeutic agent, for use as a medicament. In a preferred embodiment, the additional therapeutic agent is a chemotherapeutic agent or a checkpoint targeting agent or an inhibitor of indoleamine-2,3-dioxygenase (IDO) or a vaccine.

[0075] In one aspect, the present invention relates to (a) an antibody, polynucleotide, vector, recombinant host cell, and/or pharmaceutical composition of the present invention and (b) an additional therapeutic agent, for use in a method for the treatment of cancer. In a preferred embodiment, the additional therapeutic agent is a chemotherapeutic agent or a checkpoint targeting agent or an inhibitor of indoleamine-2,3-dioxygenase (IDO) or a vaccine.

[0076] In one aspect, the present invention relates to a pharmaceutical composition, kit or kit-of-parts comprising (a) an antibody, polynucleotide, vector, recombinant host cell, and/or pharmaceutical composition of the present invention and (b) an additional therapeutic agent. In a preferred embodiment, the additional therapeutic agent is a chemotherapeutic agent or a checkpoint targeting agent or an inhibitor of indoleamine-2,3-dioxygenase (IDO) or a vaccine.

[0077] In one aspect, the present invention relates to an antibody, polynucleotide, vector, recombinant host cell, and/or pharmaceutical composition of the present invention, for use in a method for the treatment of cancer, and/or for use in a method for increasing T-cell activation in response to an antigen, wherein the antibody, polynucleotide, vector, recombinant host cell, and/or pharmaceutical composition of the present invention is delivered to a tumor draining lymph node.

[0078] In one aspect, the present invention relates to the use of an antibody, polynucleotide, vector, recombinant host cell, and/or pharmaceutical composition of the present invention in a method for the treatment of cancer, and/or in a method for increasing T-cell activation in response to an antigen in a subject, wherein the antibody, polynucleotide, vector, recombinant host cell, and/or pharmaceutical composition of the present invention is delivered to a tumor draining lymph node.

[0079] In one aspect, the present invention relates to the use of an antibody, polynucleotide, vector, recombinant host cell, and/or pharmaceutical composition of the present invention, for preparing medicaments for immunotherapy, for example, for increasing T-cell activation in response to an antigen in a subject, treating cancer, or treating or preventing infectious diseases.

[0080] In one aspect, the present invention relates to the use of an antibody, polynucleotide, vector, recombinant host cell, and/or pharmaceutical composition of the present invention, for preparing medicaments for immunotherapy, for example, for increasing T-cell activation in response to an antigen in a subject, treating cancer, or treating or preventing infectious diseases, wherein the antibody, polynucleotide, vector, recombinant host cell, and/or pharmaceutical composition of the present invention is delivered to a tumor draining lymph node.

[0081] In one aspect, the present invention relates to the use of (a) an antibody, polynucleotide, vector, recombinant host cell, and/or pharmaceutical composition of the present invention and (b) an anti-HER2 antibody, and optionally (c) a chemotherapeutic agent, to prepare a medicament for immunotherapy, for example, for increasing T-cell activation in response to an antigen in a subject, treating cancer, or treating or preventing infectious diseases.

[0082] In one aspect, the present invention relates to the use of (a) an antibody, polynucleotide, vector, recombinant host cell, and/or pharmaceutical composition of the present invention and (b) an anti-HER2 antibody, and optionally (c) a chemotherapeutic agent, to prepare a medicament for immunotherapy, for example, for increasing T-cell activation in response to an antigen in a subject, treating cancer, or treating or preventing infectious diseases, wherein the antibody, polynucleotide, vector, recombinant host cell, and/or pharmaceutical composition of the present invention is delivered to a tumor draining lymph node.

5. BRIEF DESCRIPTION OF THE DRAWINGS

[0083] **Figures 1A**, **1B**, **1C**, **1D**, **1E**, **1F**, and **1G** are flow cytometry histograms showing the binding of anti-CTLA-4 antibodies or an IgG_1 isotype control antibody to Jurkat cells engineered to express human CTLA-4 on the cell surface. The anti-CTLA-4 antibodies tested are: AGEN1884.H1.1 (IgG_1), AGEN1884.H1.2 (IgG_1), AGEN1884.H1.3 (IgG_1), AGEN1884.H2.1 (IgG_1), AGEN1884.H2.2 (IgG_1), AGEN1884.H2.3 (IgG_1), and AGEN1884.H3 (IgG_1).

[0084] **Figure 2** is a graph showing IL-2 production of primary human PBMCs following incubation under sub-optimal stimulation with the Staphylococcal Enterotoxin A (SEA) superantigen in the absence or presence of the anti-CTLA-4 antibody AGEN1884.H3 (IgG_1) or an isotype control antibody (IgG_1). Replicates of eight were performed for each group and the mean values of the eight replicates are indicated with a black bar.

[0085] **Figure 3** is a graph showing results from an IL-2-luciferase reporter assay demonstrating that blockade of CTLA-4 leads to T cell activation. Fold response of luciferase expression, a surrogate marker for IL-2 gene activation, is plotted over a range of antibody concentrations for AGEN1884.H3 (IgG_1) or an isotype control antibody (IgG_1).

[0086] **Figure 4** is a graph showing results from a reporter assay where simultaneous engagement of AGEN1884.H3 (IgG_1) to target cells (*via* CTLA-4 binding) and effector cells (via FcγRIIIA binding) triggers expression of luciferase by the effector cell line. Luciferase activity is a surrogate marker for FcγRIIIA signaling. Fold response of RLU values is plotted against a range of antibody concentrations for AGEN1884.H3 (IgG_1) and an isotype control antibody (IgG_1).

[0087] **Figures 5A**, **5B**, **5C**, and **5D** are flow cytometry histograms showing CTLA-4-expressing Jurkat cells incubated with the anti-CTLA-4 antibody AGEN1884.H3 (IgG_1), AGEN1884.H3 (IgG_1 S239D/I332E), AGEN1884.H3 (IgG_1 S239D/A330L/I332E), or AGEN1884.H3 (IgG_1 L235V/F243L/R292P/Y300L/P396L), or an isotype control antibody. Antibody binding was detected using a fluorochrome-conjugated secondary antibody.

[0088] **Figures 6A** and **6B** are graphs showing blocking of binding between human CTLA-4 and its ligands, CD80 and CD86, respectively, by AGEN1884.H3 (IgG_1 S239D/A330L/I332E). Jurkat cells engineered to constitutively express human CTLA-4 were incubated with anti-CTLA-4 antibody AGEN1884.H3 (IgG_1-S239D/A330E/I332E), a reference anti-CTLA-4 antibody, or an isotype control antibody (IgG_1), and then stained with the indicated fluorescently labeled ligand. Ligand binding was then assessed by flow

cytometry.

[0089] **Figures 7A**, **7B**, and **7C** are graphs showing IL-2 production of primary human PBMCs cultured under sub-optimal stimulation with the SEA superantigen in the absence or presence of an isotype control antibody (IgG_1) or an anti-CTLA-4 antibody. Figures 7A and 7B are graphs showing IL-2 production stimulated by either a single dose or a dose titration of the isotype control antibody (IgG_1) or the anti-CTLA-4 antibodies AGEN1884.H3 (IgG_1), AGEN1884.H3 (IgG_1 S239D/I332E), AGEN1884.H3 (IgG_1 S239D/A330L/I332E), and AGEN1884.H3 (IgG_1 L235V/F243L/R292P/Y300L/P396L). In the study shown in Figure 7B, in addition to the isotype control antibody (IgG_1) or the anti-CTLA-4 antibody, the cells in each sample were also incubated with an IgG_4 S228P isotype control antibody. Figure 7C is a graph showing IL-2 production stimulated by a dose titration of the isotype control antibody (IgG_1) or the anti-CTLA-4 antibodies AGEN1884 (IgG_1), AGEN1884 (IgG_1 S239D/I332E), AGEN1884 (IgG_1 S239D/A330L/I332E), and afucosylated AGEN1884 (IgG_1).

Figure 8A is an immunoblot analysis for phosphorylated ZAP70 (Y493) in human PBMCs following stimulation with 50 ng/ml of SEA superantigen and 10 μg/ml of isotype control antibody (IgG_1) or the anti-CTLA-4 antibodies AGEN1884.H3 (IgG_1), AGEN1884.H3 (IgG_1 S239D/A330L/I332E), or AGEN1884.H3 (IgG_1 N297A). **Figure 8B** is a chart showing normalized densitometric analysis of the data shown in Figure 8A.

[0090] **Figures 9A**, **9B**, **9C**, and **9D** are graphs showing antitumor efficacy and intratumoral regulatory T cell (Treg) depletion induced by Fc variants of murine anti-CTLA-4 antibody 9D9. Figure 9A shows tumor growth in CT26 mice following single-dose treatment with murine anti-CTLA-4 antibody 9D9 (mIgG2a), an Fc-silent variant of anti-CTLA-4 antibody 9D9 (mIgG2a-N297A), an Fc variant of anti-CTLA-4 antibody 9D9 (mIgG2a-S239D/A330L/I332E), or an isotype control antibody (mIgG2a). The upper panel shows median tumor volume over time for each treatment group. The remaining panels show tumor volume over time for individual animals in each treatment group. Figure 9B shows the effect of anti-CTLA-4 antibody treatment on T cell populations from tumor infiltrates collected from mice treated with single doses of anti-CTLA-4 antibody 9D9 (mIgG2a), anti-CTLA-4 antibody 9D9 (mIgG2a-N297A), anti-CTLA-4 antibody 9D9 (mIgG2a-S239D/A330L/I332E), or isotype control antibody (mIgG2a). Tumor infiltrates were harvested and analyzed by flow cytometry at indicated time points after injection with antibody. Cell populations analyzed include: FoxP3+ Tregs (upper left panel), CD45+ leukocytes (upper right panel), and CD4+ non-Tregs (lower left panel). The lower right panel

shows the ratio of CD8+ T cells to Tregs observed in tumor infiltrates. Figure 9C shows FoxP3+ Treg populations over time in tumor-draining lymph nodes harvested from mice treated as described for Figure 9B. Figure 9D shows fold-change in splenic FoxP3+ Tregs at 72 hours after treatment as described for Figure 9B.

[0091] **Figure 10** is a series of graphs showing antitumor efficacy of murine anti-CTLA-4 antibodies when combined with tumor vaccine derived from a HPV+ tumor (viral antigens E6/E7). Shown are tumor volume over time for individual mice receiving no treatment, isotype control antibody (mIgG2a), anti-CTLA-4 antibody 9D9 (mIgG2a), or an Fc variant of anti-CTLA-4 antibody 9D9 (mIgG2a-S239D/A330L/I332E). Graphs in the top row show results for animals that were administered the indicated antibody treatment only. Graphs in the bottom row show results for animals that were administered the indicated antibody treatment in combination with tumor vaccine.

[0092] **Figures 11A** and **11B** are graphs showing gene expression and CpG methylation of human T cell populations. $CD4^+$ $CD25^{+/-}$ $FOXP3^-$ non-regulatory T cells (Teff) and $CD4^+$ $CD25^+$ $FOXP3^+$ regulatory T cells (Treg) were isolated from peripheral blood of healthy donors, expanded, and activated. Figure 11A shows FOXP3, intracellular CTLA-4, and membrane CTLA-4 levels in each activated T cell population, as determined by flow cytometry. Figure 11B shows the level of CpG methylation in CpG regions within the *FOXP3* (top panel) and *CTLA4* (bottom panel) loci in naïve T cells, activated effector T cells, and activated regulatory T cells, each from the same donor.

[0093] **Figures 12A** and **12B** are graphs showing time courses of antibody dependent cellular cytotoxicity (ADCC) of human CTLA-4+ target cells after incubation with anti-CTLA-4 antibody AGEN1884.H3 (IgG_1) **or Fc variants thereof. NK-92 cells (FcγRIIIA** V158-expressing) were co-cultured with CTLA-4+ target cells that were incubated with different Fc variants of anti-CTLA-4 antibodies or an IgG_1 isotype control (10 μg/ml). High content microscopy of caspase 3/7 activation was then used to quantify ADCC activity. Figure 12A shows ADCC activity in Jurkat cells engineered to express CTLA-4 on the cell surface, when incubated with AGEN1884.H3 (IgG_1), AGEN1884.H3 (IgG_1 N297A), AGEN1884.H3 (IgG_1 S239D/A330L/I332E), AGEN1884.H3 (IgG_1 S267E/L328F), afucosylated AGEN1884.H3 (IgG_1), or an isotype control antibody (IgG_1). Figure 12B shows ADCC activity in primary human activated effector T cells (left panel) or regulatory T cells (right panel) when incubated with these antibodies.

[0094] **Figures 13A**, **13B**, **13C**, and **13D** are graphs showing the effects of anti-CTLA-4 antibody variants on T cell function when administered alone or in combination with an anti-

PD-1 antibody. Human PBMCs were isolated from two donors and incubated under stimulatory culture conditions with anti-CTLA-4 antibody AGEN1884.H3 (IgG_1), an Fc variant anti-CTLA-4 antibody AGEN1884.H3 (IgG_1 S239D/A330L/I332E), or an isotype control antibody (IgG_1), in combination with a reference anti-PD-1 antibody or an isotype control antibody (IgG_4), as indicated. For each treatment condition listed, a dosage titration was used for the first-listed antibody, and a fixed concentration (5 μg/ml) was used for the second-listed antibody. This experiment was performed twice, for a total of two replicates per donor. The levels of IL-2 production induced by each antibody combination on PBMCs collected from the first donor are shown in Figure 13A (replicate 1) and Figure 13B (replicate 2). The levels of IL-2 production induced by each antibody combination on PBMCs collected from the second donor are shown in Figure 13C (replicate 1) and Figure 13D (replicate 2).

[0095] **Figures 14A**, **14B**, and **14C** are a series of sequence alignments. Figure 14A is a sequence alignment for human CTLA-4 (SEQ ID NO: 33), cynomolgus monkey CTLA-4 (SEQ ID NO: 40), mouse CTLA-4 (SEQ ID NO: 41), and rat CTLA-4 (SEQ ID NO: 42). Dots represent residues identical to corresponding human residues. An "*" (asterisk) indicates positions which have a single, fully conserved residue. A ":" (colon) indicates conservation between groups of strongly similar properties. A "." (period) indicates conservation between groups of weakly similar properties. Figures 14B and 14C are sequence alignments for human CTLA-4 (residues 1-144 and residues 145-223 of SEQ ID NO: 33, respectively), cynomolgus monkey CTLA-4 (residues 1-144 and residues 145-223 of SEQ ID NO: 40, respectively), human CD28 (residues 1-127 and residues 128-220 of SEQ ID NO: 43, respectively), human ICOS (residues 1-124 and residues 125-199 of SEQ ID NO: 44, respectively), human BTLA (residues 1-125 and residues 126-289 of SEQ ID NO: 45, respectively), and human PD-1 (residues 1-143 and residues 144-288 of SEQ ID NO: 46, respectively). The two regions showing strong decrease in deuterium uptake when human CTLA-4 was bound to AGEN1884-Fab are underlined in Figures 14A-14C: residues 80-82 (QVT, SEQ ID NO: 39) and residues 135-149 (YPPPYYLGIGNGTQI, SEQ ID NO: 37), numbered according to SEQ ID NO: 33.

6. DETAILED DESCRIPTION

[0096] The instant disclosure provides antibodies that specifically bind to CTLA-4 (*e.g.*, human CTLA-4) and antagonize CTLA-4 function, *e.g.*, CTLA-4-mediated immune

suppression. Also provided are pharmaceutical compositions comprising these antibodies, nucleic acids encoding these antibodies, expression vectors and host cells for making these antibodies, and methods of treating a subject using these antibodies. The antibodies described herein are particularly useful for increasing T cell activation in response to an antigen (*e.g.*, a tumor antigen or an infectious disease antigen), and hence for treating cancer in a subject or treating or preventing an infectious disease in a subject. All instances of "isolated antibodies" described herein are additionally contemplated as antibodies that may be, but need not be, isolated. All instances of "isolated polynucleotides" described herein are additionally contemplated as polynucleotides that may be, but need not be, isolated. All instances of "antibodies" described herein are additionally contemplated as antibodies that may be, but need not be, isolated. All instances of "polynucleotides" described herein are additionally contemplated as polynucleotides that may be, but need not be, isolated.

[0097] The skilled worker will appreciate that a glutamate (E) or glutamine (Q) amino acid residue at the N-terminus of a heavy chain variable region and/or a light chain variable region of any one of the antibodies described herein (e.g., an anti-CTLA4 antibody) can, under certain conditions, spontaneously convert to pyroglutamate by post-translational cyclization of the free amino group to form a lactam. Accordingly, in certain embodiments of each and every one of the methods, uses, pharmaceutical compositions, or kits described herein, the N-terminal amino acid residue of one or more heavy chain variable regions and/or light chain variable regions of the antibody has been converted to pyroglutamate (e.g., as a result of post-translational cyclization of the free amino group of the N-terminal E or Q residue).

6.1 Definitions

[0098] As used herein, the terms "about" and "approximately," when used to modify a numeric value or numeric range, indicate that deviations of 5% to 10% above (*e.g.*, up to 5% to 10% above) and 5% to 10% below (*e.g.*, up to 5% to 10% below) the value or range remain within the intended meaning of the recited value or range.

[0099] As used herein, the term "CTLA-4" refers to cytotoxic T-lymphocyte-associated protein 4. As used herein, the term "human CTLA-4" refers to a human CTLA-4 protein encoded by a wild type human CTLA-4 gene, *e.g.*, GenBank™ accession number NM_005214.4 or NM_001037631.2. An exemplary immature amino acid sequence of human CTLA-4 is provided as SEQ ID NO: 33.

[00100] As used herein, the terms "antibody" and "antibodies" include full length

antibodies, antigen-binding fragments of full length antibodies, and molecules comprising antibody CDRs, VH regions or VL regions. Examples of antibodies include monoclonal antibodies, recombinantly produced antibodies, monospecific antibodies, multispecific antibodies (including bispecific antibodies), human antibodies, humanized antibodies, chimeric antibodies, immunoglobulins, synthetic antibodies, tetrameric antibodies comprising two heavy chain and two light chain molecules, an antibody light chain monomer, an antibody heavy chain monomer, an antibody light chain dimer, an antibody heavy chain dimer, an antibody light chain- antibody heavy chain pair, intrabodies, heteroconjugate antibodies, antibody-drug conjugates, single domain antibodies, monovalent antibodies, single chain antibodies or single-chain Fvs (scFv), camelized antibodies, affybodies, Fab fragments, $F(ab')_2$ fragments, disulfide-linked Fvs (sdFv), anti-idiotypic (anti-Id) antibodies (including, *e.g.*, anti-anti-Id antibodies), and antigen-binding fragments of any of the above. In certain embodiments, antibodies described herein refer to polyclonal antibody populations. Antibodies can be of any type (*e.g.*, IgG, IgE, IgM, IgD, IgA or IgY), any class (*e.g.*, IgG_1, IgG_2, IgG_3, IgG_4, IgA_1 or IgA_2), or any subclass (*e.g.*, IgG_{2a} or IgG_{2b}) of immunoglobulin molecule. In certain embodiments, antibodies described herein are IgG antibodies, or a class (*e.g.*, human IgG_1 or IgG_4) or subclass thereof. In a specific embodiment, the antibody is a humanized monoclonal antibody. In another specific embodiment, the antibody is a human monoclonal antibody.

[00101] As used herein, the terms "VH region" and "VL region" refer to single antibody heavy and light chain variable regions, respectively, comprising FR (Framework Regions) 1, 2, 3 and 4 and CDR (Complementarity Determining Regions) 1, 2 and 3 (see Kabat *et al.*, (1991) Sequences of Proteins of Immunological Interest (NIH Publication No. 91-3242, Bethesda), which is herein incorporated by reference in its entirety).

[00102] As used herein, the term "CDR" or "complementarity determining region" means the noncontiguous antigen combining sites found within the variable region of both heavy and light chain polypeptides. These particular regions have been described by Kabat *et al.*, J. Biol. Chem. 252, 6609-6616 (1977) and Kabat *et al.*, Sequences of protein of immunological interest. (1991), by Chothia *et al.*, J. Mol. Biol. 196:901-917 (1987), and by MacCallum *et al.*, J. Mol. Biol. 262:732-745 (1996), all of which are herein incorporated by reference in their entireties, where the definitions include overlapping or subsets of amino acid residues when compared against each other. In certain embodiments, the term "CDR" is a CDR as defined by Kabat *et al.*, J. Biol. Chem. 252, 6609-6616 (1977) and Kabat *et al.*, Sequences of protein of immunological interest. (1991). In certain embodiments, the term "CDR" is a

CDR as defined by Chothia *et al.*, J. Mol. Biol. 196:901-917 (1987). In certain embodiments, the term "CDR" is a CDR as defined by MacCallum *et al.*, J. Mol. Biol. 262:732-745 (1996) and Martin A. "Protein Sequence and Structure Analysis of Antibody Variable Domains," in *Antibody Engineering*, Kontermann and Dübel, eds., Chapter 31, pp. 422-439, Springer-Verlag, Berlin (2001).

[00103] As used herein, the term "framework (FR) amino acid residues" refers to those amino acids in the framework region of an immunoglobulin chain. The term "framework region" or "FR region" as used herein, includes the amino acid residues that are part of the variable region, but are not part of the CDRs (*e.g.*, using the Kabat or Chothia definition of CDRs).

[00104] As used herein, the terms "variable region" and "variable domain" are used interchangeably and are common in the art. The variable region typically refers to a portion of an antibody, generally, a portion of a light or heavy chain, typically about the amino-terminal 110 to 125 amino acids in the mature heavy chain and about 90 to 115 amino acids in the mature light chain, which differ extensively in sequence among antibodies and are used in the binding and specificity of a particular antibody for its particular antigen. The variability in sequence is concentrated in those regions called complementarity determining regions (CDRs) while the more highly conserved regions in the variable domain are called framework regions (FR). Without wishing to be bound by any particular mechanism or theory, it is believed that the CDRs of the light and heavy chains are primarily responsible for the interaction and specificity of the antibody with antigen. In certain embodiments, the variable region is a human variable region. In certain embodiments, the variable region comprises rodent or murine CDRs and human framework regions (FRs). In particular embodiments, the variable region is a primate (*e.g.*, non-human primate) variable region. In certain embodiments, the variable region comprises rodent or murine CDRs and primate (*e.g.*, non-human primate) framework regions (FRs).

[00105] The terms "VL" and "VL domain" are used interchangeably to refer to the light chain variable region of an antibody.

[00106] The terms "VH" and "VH domain" are used interchangeably to refer to the heavy chain variable region of an antibody.

[00107] As used herein, the terms "constant region" and "constant domain" are interchangeable and are common in the art. The constant region is an antibody portion, *e.g.*, a carboxyl terminal portion of a light and/or heavy chain which is not directly involved in binding of an antibody to antigen but which can exhibit various effector functions, such as

interaction with an Fc receptor (*e.g.*, Fc gamma receptor). The constant region of an immunoglobulin molecule generally has a more conserved amino acid sequence relative to an immunoglobulin variable domain.

[00108] As used herein, the term "heavy chain" when used in reference to an antibody can refer to any distinct type, *e.g.*, **alpha (α), delta (δ), epsilon (ε), gamma (γ), and mu (μ), based** on the amino acid sequence of the constant domain, which give rise to IgA, IgD, IgE, IgG, and IgM classes of antibodies, respectively, including subclasses of IgG, *e.g.*, IgG_1, IgG_2, IgG_3, and IgG_4.

[00109] As used herein, the term "light chain" when used in reference to an antibody can refer to any distinct type, *e.g.*, **kappa (κ) or lambda (λ) based on the amino acid sequence of** the constant domains. Light chain amino acid sequences are well known in the art.

[00110] As used herein, the term "EU numbering system" refers to the EU numbering convention for the constant regions of an antibody, as described in Edelman, G.M. *et al.*, Proc. Natl. Acad. USA, 63, 78-85 (1969) and Kabat *et al.*, Sequences of Proteins of Immunological Interest, U.S. Dept. Health and Human Services, 5th edition, 1991, each of which is herein incorporated by reference in its entirety.

[00111] "Binding affinity" generally refers to the strength of the sum total of non-covalent interactions between a single binding site of a molecule (*e.g.*, an antibody) and its binding partner (*e.g.*, an antigen). Unless indicated otherwise, as used herein, "binding affinity" refers to intrinsic binding affinity which reflects a 1:1 interaction between members of a binding pair (*e.g.*, antibody and antigen). The affinity of a molecule X for its partner Y can generally be represented by the dissociation constant (K_D). Affinity can be measured and/or expressed in a number of ways known in the art, including, but not limited to, equilibrium dissociation constant (K_D), and equilibrium association constant (K_A). The K_D is calculated from the quotient of k_{off}/k_{on}, whereas K_A is calculated from the quotient of k_{on}/k_{off}. k_{on} refers to the association rate constant of, *e.g.*, an antibody to an antigen, and k_{off} refers to the dissociation rate constant of, *e.g.*, an antibody to an antigen. The k_{on} and k_{off} can be determined by techniques known to one of ordinary skill in the art, such as BIAcore® or KinExA. As used herein, a "lower affinity" refers to a larger K_D.

[00112] As used herein, the terms "specifically binds," "specifically recognizes," "immunospecifically binds," and "immunospecifically recognizes" are analogous terms in the context of antibodies and refer to molecules that bind to an antigen (*e.g.*, epitope or immune complex) as such binding is understood by one skilled in the art. For example, a molecule that specifically binds to an antigen can bind to other peptides or polypeptides, generally with

lower affinity as determined by, *e.g.*, immunoassays, BIAcore®, KinExA 3000 instrument (Sapidyne Instruments, Boise, ID), or other assays known in the art. In a specific embodiment, molecules that specifically bind to an antigen bind to the antigen with a K_A that is at least 2 logs (*i.e.*, factors of 10), 2.5 logs, 3 logs, 4 logs or greater than the K_A when the molecules bind non-specifically to another antigen.

[00113] In another specific embodiment, molecules that specifically bind to an antigen do not cross react with other proteins under similar binding conditions. In another specific embodiment, molecules that specifically bind to CTLA-4 do not cross react with other non-CTLA-4 proteins. In a specific embodiment, provided herein is an antibody that binds to CTLA-4 (*e.g.*, human CTLA-4) with higher affinity than to another unrelated antigen. In certain embodiments, provided herein is an antibody that binds to CTLA-4 (*e.g.*, human CTLA-4) with a 20%, 25%, 30%, 35%, 40%, 45%, 50%, 55%, 60%, 65%, 70%, 75%, 80%, 85%, 90%, 95% or higher affinity than to another, unrelated antigen as measured by, *e.g.*, a radioimmunoassay, surface plasmon resonance, or kinetic exclusion assay. In a specific embodiment, the extent of binding of an anti-CTLA-4 antibody described herein to an unrelated, non-CTLA-4 protein is less than 10%, 15%, or 20% of the binding of the antibody to CTLA-4 protein as measured by, *e.g.*, a radioimmunoassay.

[00114] As used herein, the term "afucosylation" or "afucosylated" in the context of an Fc refers to a substantial lack of a fucose covalently attached, directly or indirectly, to residue 297 of the human IgG_1 Fc region, numbered according to the EU numbering system, or the corresponding residue in non-IgG_1 or non-human IgG_1 immunoglobulins. Thus, in a composition comprising a plurality of afucosylated antibodies, at least 70% of the antibodies will not be fucosylated, directly or indirectly (*e.g.*, via intervening sugars) at residue 297 of the Fc region of the antibodies, and in some embodiments at least 80%, 85%, 90%, 95%, or 99% will not be fucosylated, directly or indirectly, at residue 297 of the Fc region.

[00115] As used herein, an "epitope" is a term in the art and refers to a localized region of an antigen to which an antibody can specifically bind. An epitope can be, for example, contiguous amino acids of a polypeptide (linear or contiguous epitope) or an epitope can, for example, come together from two or more non-contiguous regions of a polypeptide or polypeptides (conformational, non-linear, discontinuous, or non-contiguous epitope). In certain embodiments, the epitope to which an antibody binds can be determined by, *e.g.*, NMR spectroscopy, X-ray diffraction crystallography studies, ELISA assays, hydrogen/deuterium exchange coupled with mass spectrometry (*e.g.*, liquid chromatography electrospray mass spectrometry), array-based oligo-peptide scanning assays (*e.g.*,

constraining peptides using CLIPS (Chemical Linkage of Peptides onto Scaffolds) to map discontinuous or conformational epitopes), and/or mutagenesis mapping (*e.g.*, site-directed mutagenesis mapping). For X-ray crystallography, crystallization may be accomplished using any of the known methods in the art (*e.g.*, Giegé R *et al.*, (1994) Acta Crystallogr D Biol Crystallogr 50(Pt 4): 339-350; McPherson A (1990) Eur J Biochem 189: 1-23; Chayen NE (1997) Structure 5: 1269-1274; McPherson A (1976) J Biol Chem 251: 6300-6303, all of which are herein incorporated by reference in their entireties). Antibody:antigen crystals may be studied using well known X-ray diffraction techniques and may be refined using computer software such as X-PLOR (Yale University, 1992, distributed by Molecular Simulations, Inc.; *see*, *e.g.*, Meth Enzymol (1985) volumes 114 & 115, eds Wyckoff HW *et al.*,; U.S. 2004/0014194), and BUSTER (Bricogne G (1993) Acta Crystallogr D Biol Crystallogr 49(Pt 1): 37-60; Bricogne G (1997) Meth Enzymol 276A: 361-423, ed Carter CW; Roversi P *et al.*, (2000) Acta Crystallogr D Biol Crystallogr 56(Pt 10): 1316-1323), all of which are herein incorporated by reference in their entireties. Mutagenesis mapping studies may be accomplished using any method known to one of skill in the art. *See*, *e.g.*, Champe M *et al.*, (1995) J Biol Chem 270: 1388-1394 and Cunningham BC & Wells JA (1989) Science 244: 1081-1085, each of which is herein incorporated by reference in its entirety, for a description of mutagenesis techniques, including alanine scanning mutagenesis techniques. CLIPS (Chemical Linkage of Peptides onto Scaffolds) is a technology to present one or more peptides in a structurally constrained configuration to behave as functional mimics of complex protein domains. *See*, *e.g.*, U.S. Publication Nos. US 2008/0139407 A1 and US 2007/099240 A1, and US Patent No. 7,972,993, each of which is herein incorporated by reference in its entirety. In a specific embodiment, the epitope of an antibody is determined using alanine scanning mutagenesis studies. In a specific embodiment, the epitope of an antibody is determined using hydrogen/deuterium exchange coupled with mass spectrometry. In a specific embodiment, the epitope of an antibody is determined using CLIPS Epitope Mapping Technology from Pepscan Therapeutics.

[00116] As used herein, the term "an epitope located within a region of human CTLA-4" consisting of a particular amino acid sequence or a set of amino acid residues refers to an epitope comprising one or more of the amino acid residues of the specified region, wherein the specified region includes the first specified amino acid residue and the last specified amino acid residue of the region of human CTLA-4. In certain embodiments, the epitope comprises each one of the amino acid residues located within the specified region. In certain embodiments, one or more additional amino acid residues of human CTLA-4 outside the

specified region bind to an antibody together with an epitope located within the specified region.

[00117] As used herein, the terms "T cell receptor" and "TCR" are used interchangeably and refer to full length heterodimeric αβ or γδ TCRs, antigen-binding fragments of full length TCRs, and molecules comprising TCR CDRs or variable regions. Examples of TCRs include, but are not limited to, full length TCRs, antigen-binding fragments of full length TCRs, soluble TCRs lacking transmembrane and cytoplasmic regions, single-chain TCRs containing variable regions of TCRs attached by a flexible linker, TCR chains linked by an engineered disulfide bond, monospecific TCRs, multi-specific TCRs (including bispecific TCRs), TCR fusions, human TCRs, humanized TCRs, chimeric TCRs, recombinantly produced TCRs, and synthetic TCRs. The term encompasses wild-type TCRs and genetically engineered TCRs (*e.g.*, a chimeric TCR comprising a chimeric TCR chain which includes a first portion from a TCR of a first species and a second portion from a TCR of a second species).

[00118] As used herein, the terms "major histocompatibility complex" and "MHC" are used interchangeably and refer to an MHC class I molecule and/or an MHC class II molecule.

[00119] As used herein, the term "peptide-MHC complex" refers to an MHC molecule (MHC class I or MHC class II) with a peptide bound in the art-recognized peptide binding pocket of the MHC.

[00120] As used herein, the term "treat," "treating," and "treatment" refer to therapeutic or preventative measures described herein. The methods of "treatment" employ administration of an antibody to a subject having a disease or disorder, or predisposed to having such a disease or disorder, in order to prevent, cure, delay, reduce the severity of, or ameliorate one or more symptoms of the disease or disorder or recurring disease or disorder, or in order to prolong the survival of a subject beyond that expected in the absence of such treatment.

[00121] As used herein, the term "effective amount" in the context of the administration of a therapy to a subject refers to the amount of a therapy that achieves a desired prophylactic or therapeutic effect.

[00122] As used herein with respect to the response of a cancer to a therapy, the terms "refractory" and "resistant" have their art-recognized meaning and are used interchangeably.

[00123] As used herein, the term "subject" includes any human or non-human animal. In one embodiment, the subject is a human or non-human mammal. In one embodiment, the subject is a human.

[00124] The determination of "percent identity" between two sequences (*e.g.*, amino acid

sequences or nucleic acid sequences) can be accomplished using a mathematical algorithm. A specific, non-limiting example of a mathematical algorithm utilized for the comparison of two sequences is the algorithm of Karlin S & Altschul SF (1990) PNAS 87: 2264-2268, modified as in Karlin S & Altschul SF (1993) PNAS 90: 5873-5877, each of which is herein incorporated by reference in its entirety. Such an algorithm is incorporated into the NBLAST and XBLAST programs of Altschul SF *et al.*, (1990) J Mol Biol 215: 403, which is herein incorporated by reference in its entirety. BLAST nucleotide searches can be performed with the NBLAST nucleotide program parameters set, *e.g.*, for score=100, wordlength=12 to obtain nucleotide sequences homologous to a nucleic acid molecules described herein. BLAST protein searches can be performed with the XBLAST program parameters set, *e.g.*, to score 50, wordlength=3 to obtain amino acid sequences homologous to a protein molecule described herein. To obtain gapped alignments for comparison purposes, Gapped BLAST can be utilized as described in Altschul SF *et al.*, (1997) Nuc Acids Res 25: 3389-3402, which is herein incorporated by reference in its entirety. Alternatively, PSI BLAST can be used to perform an iterated search which detects distant relationships between molecules (*Id.*). When utilizing BLAST, Gapped BLAST, and PSI Blast programs, the default parameters of the respective programs (*e.g.*, of XBLAST and NBLAST) can be used (*see*, *e.g.*, National Center for Biotechnology Information (NCBI) on the worldwide web, ncbi.nlm.nih.gov). Another specific, non-limiting example of a mathematical algorithm utilized for the comparison of sequences is the algorithm of Myers and Miller, 1988, CABIOS 4:11-17, which is herein incorporated by reference in its entirety. Such an algorithm is incorporated in the ALIGN program (version 2.0) which is part of the GCG sequence alignment software package. When utilizing the ALIGN program for comparing amino acid sequences, a PAM120 weight residue table, a gap length penalty of 12, and a gap penalty of 4 can be used.

[00125] The percent identity between two sequences can be determined using techniques similar to those described above, with or without allowing gaps. In calculating percent identity, typically only exact matches are counted.

6.2 Anti-CTLA-4 Antibodies

[00126] In one aspect the instant disclosure provides antibodies that specifically bind to CTLA-4 (*e.g.*, human CTLA-4) and antagonize CTLA-4 function. The amino acid sequences of exemplary antibodies are set forth in Tables 1-4 herein.

Table 1. Amino acid sequences of exemplary anti-CTLA-4 antibodies.*

SEQ ID NO:	Description	Amino acid sequence
1	AGEN1884 VH	EVQLVESGGGLVKPGGSLRLSCAASGFTFSSYSM NWVRQAPGKGLEWVSSISSSSSYIYYADSVKGRF TISRDNAKNSLYLQMNSLRAEDTAVYYCARVGL MGPFDIWGQGTMVTVSS
2	AGEN1884_M102F VH	EVQLVESGGGLVKPGGSLRLSCAASGFTFSSYSM NWVRQAPGKGLEWVSSISSSSSYIYYADSVKGRF TISRDNAKNSLYLQMNSLRAEDTAVYYCARVGLF GPFDIWGQGTMVTVSS
3	AGEN1884_M113L VH	EVQLVESGGGLVKPGGSLRLSCAASGFTFSSYSM NWVRQAPGKGLEWVSSISSSSSYIYYADSVKGRF TISRDNAKNSLYLQMNSLRAEDTAVYYCARVGL MGPFDIWGQGTLVTVSS
4	AGEN1884_D62E VH	EVQLVESGGGLVKPGGSLRLSCAASGFTFSSYSM NWVRQAPGKGLEWVSSISSSSSYIYYAESVKGRF TISRDNAKNSLYLQMNSLRAEDTAVYYCARVGL MGPFDIWGQGTMVTVSS
5	AGEN1884_M102F_M11 3L VH	EVQLVESGGGLVKPGGSLRLSCAASGFTFSSYSM NWVRQAPGKGLEWVSSISSSSSYIYYADSVKGRF TISRDNAKNSLYLQMNSLRAEDTAVYYCARVGLF GPFDIWGQGTLVTVSS
6	AGEN1884_D62E_M102 F VH	EVQLVESGGGLVKPGGSLRLSCAASGFTFSSYSM NWVRQAPGKGLEWVSSISSSSSYIYYAESVKGRF TISRDNAKNSLYLQMNSLRAEDTAVYYCARVGLF GPFDIWGQGTMVTVSS
7	AGEN1884_D62E_M113 L VH	EVQLVESGGGLVKPGGSLRLSCAASGFTFSSYSM NWVRQAPGKGLEWVSSISSSSSYIYYAESVKGRF TISRDNAKNSLYLQMNSLRAEDTAVYYCARVGL MGPFDIWGQGTLVTVSS
8	AGEN1884_D62E_M102 F_M113L VH	EVQLVESGGGLVKPGGSLRLSCAASGFTFSSYSM NWVRQAPGKGLEWVSSISSSSSYIYYAESVKGRF TISRDNAKNSLYLQMNSLRAEDTAVYYCARVGLF GPFDIWGQGTLVTVSS
9	AGEN1884 VL	EIVLTQSPGTLSLSPGERATLSCRASQSVSRYLGW YQQKPGQAPRLLIYGASTRATGIPDRFSGSGSGTD FTLTITRLEPEDFAVYYCQQYGSSPWTFGQGTKVE IK
10	CDRH1	SYSMN
11	CDRH2	SISSSSSYIYYADSVKG
12	CDRH2	SISSSSSYIYYAESVKG
13	CDRH3	VGLMGPFDI
14	CDRH3	VGLFGPFDI
15	CDRL1	RASQSVSRYLG
16	CDRL2	GASTRAT
17	CDRL3	QQYGSSPWT
18	CDRH2 consensus sequence	SISSSSSYIYYAXSVKG, wherein: X is E or D

SEQ ID NO:	Description	Amino acid sequence
19	CDRH3 consensus sequence	VGLXGPFDI, wherein: X is F or M
20	VH consensus sequence	EVQLVESGGGLVKPGGSLRLSCAASGFTFSSYSM NWVRQAPGKGLEWVSSISSSSSYIYYAX$_1$SVKGRF TISRDNAKNSLYLQMNSLRAEDTAVYYCARVGL X$_2$GPFDIWGQGTX$_3$VTVSS, wherein: X$_1$ is E or D, X$_2$ is F or M, and X$_3$ is L or M.
23	AGEN1884.H3 (IgG$_1$) heavy chain	EVQLVESGGGLVKPGGSLRLSCAASGFTFSSYSM NWVRQAPGKGLEWVSSISSSSSYIYYAESVKGRF TISRDNAKNSLYLQMNSLRAEDTAVYYCARVGLF GPFDIWGQGTLVTVSSASTKGPSVFPLAPSSKSTS GGTAALGCLVKDYFPEPVTVSWNSGALTSGVHTF PAVLQSSGLYSLSSVVTVPSSSLGTQTYICNVNHK PSNTKVDKRVEPKSCDKTHTCPPCPAPELLGGPSV FLFPPKPKDTLMISRTPEVTCVVVDVSHEDPEVKF NWYVDGVEVHNAKTKPREEQYNSTYRVVSVLTV LHQDWLNGKEYKCKVSNKALPAPIEKTISKAKGQ PREPQVYTLPPSREEMTKNQVSLTCLVKGFYPSDI AVEWESNGQPENNYKTTPPVLDSDGSFFLYSKLT VDKSRWQQGNVFSCSVMHEALHNHYTQKSLSLS PG
24	AGEN1884.H3 (IgG$_1$ S239D/I332E) heavy chain	EVQLVESGGGLVKPGGSLRLSCAASGFTFSSYSM NWVRQAPGKGLEWVSSISSSSSYIYYAESVKGRF TISRDNAKNSLYLQMNSLRAEDTAVYYCARVGLF GPFDIWGQGTLVTVSSASTKGPSVFPLAPSSKSTS GGTAALGCLVKDYFPEPVTVSWNSGALTSGVHTF PAVLQSSGLYSLSSVVTVPSSSLGTQTYICNVNHK PSNTKVDKRVEPKSCDKTHTCPPCPAPELLGGPD VFLFPPKPKDTLMISRTPEVTCVVVDVSHEDPEVK FNWYVDGVEVHNAKTKPREEQYNSTYRVVSVLT VLHQDWLNGKEYKCKVSNKALPAPEEKTISKAK GQPREPQVYTLPPSREEMTKNQVSLTCLVKGFYP SDIAVEWESNGQPENNYKTTPPVLDSDGSFFLYSK LTVDKSRWQQGNVFSCSVMHEALHNHYTQKSLS LSPG

SEQ ID NO:	Description	Amino acid sequence
25	AGEN1884.H3 (IgG_1 S239D/A330L/I332E) heavy chain	EVQLVESGGGLVKPGGSLRLSCAASGFTFSSYSM NWVRQAPGKGLEWVSSISSSSSYIYYAESVKGRF TISRDNAKNSLYLQMNSLRAEDTAVYYCARVGLF GPFDIWGQGTLVTVSSASTKGPSVFPLAPSSKSTS GGTAALGCLVKDYFPEPVTVSWNSGALTSGVHTF PAVLQSSGLYSLSSVVTVPSSSLGTQTYICNVNHK PSNTKVDKRVEPKSCDKTHTCPPCPAPELLGGPD VFLFPPKPKDTLMISRTPEVTCVVVDVSHEDPEVK FNWYVDGVEVHNAKTKPREEQYNSTYRVVSVLT VLHQDWLNGKEYKCKVSNKALPLPEEKTISKAK GQPREPQVYTLPPSREEMTKNQVSLTCLVKGFYP SDIAVEWESNGQPENNYKTTPPVLDSDGSFFLYSK LTVDKSRWQQGNVFSCSVMHEALHNHYTQKSLS LSPG
26	AGEN1884.H3 (IgG_1 L235V/F243L/R292P/Y300L/P396L) heavy chain	EVQLVESGGGLVKPGGSLRLSCAASGFTFSSYSM NWVRQAPGKGLEWVSSISSSSSYIYYAESVKGRF TISRDNAKNSLYLQMNSLRAEDTAVYYCARVGLF GPFDIWGQGTLVTVSSASTKGPSVFPLAPSSKSTS GGTAALGCLVKDYFPEPVTVSWNSGALTSGVHTF PAVLQSSGLYSLSSVVTVPSSSLGTQTYICNVNHK PSNTKVDKRVEPKSCDKTHTCPPCPAPELVGGPS VFLLPPKPKDTLMISRTPEVTCVVVDVSHEDPEVK FNWYVDGVEVHNAKTKPPEEQYNSTLRVVSVLT VLHQDWLNGKEYKCKVSNKALPAPIEKTISKAKG QPREPQVYTLPPSREEMTKNQVSLTCLVKGFYPS DIAVEWESNGQPENNYKTTPLVLDSDGSFFLYSK LTVDKSRWQQGNVFSCSVMHEALHNHYTQKSLS LSPG
47	AGEN1884.H3 (IgG_1 N297A) heavy chain	EVQLVESGGGLVKPGGSLRLSCAASGFTFSSYSM NWVRQAPGKGLEWVSSISSSSSYIYYAESVKGRF TISRDNAKNSLYLQMNSLRAEDTAVYYCARVGLF GPFDIWGQGTLVTVSSASTKGPSVFPLAPSSKSTS GGTAALGCLVKDYFPEPVTVSWNSGALTSGVHTF PAVLQSSGLYSLSSVVTVPSSSLGTQTYICNVNHK PSNTKVDKRVEPKSCDKTHTCPPCPAPELLGGPSV FLFPPKPKDTLMISRTPEVTCVVVDVSHEDPEVKF NWYVDGVEVHNAKTKPREEQYASTYRVVSVLTV LHQDWLNGKEYKCKVSNKALPAPIEKTISKAKGQ PREPQVYTLPPSREEMTKNQVSLTCLVKGFYPSDI AVEWESNGQPENNYKTTPPVLDSDGSFFLYSKLT VDKSRWQQGNVFSCSVMHEALHNHYTQKSLSLS PG

SEQ ID NO:	Description	Amino acid sequence
48	AGEN1884.H3 (IgG_1 S267E/L328F) heavy chain	EVQLVESGGGLVKPGGSLRLSCAASGFTFSSYSM NWVRQAPGKGLEWVSSISSSSSYIYYAESVKGRF TISRDNAKNSLYLQMNSLRAEDTAVYYCARVGLF GPFDIWGQGTLVTVSSASTKGPSVFPLAPSSKSTS GGTAALGCLVKDYFPEPVTVSWNSGALTSGVHTF PAVLQSSGLYSLSSVVTVPSSSLGTQTYICNVNHK PSNTKVDKRVEPKSCDKTHTCPPCPAPELLGGPSV FLFPPKPKDTLMISRTPEVTCVVVDVEHEDPEVKF NWYVDGVEVHNAKTKPREEQYNSTYRVVSVLTV LHQDWLNGKEYKCKVSNKAFPAPIEKTISKAKGQ PREPQVYTLPPSREEMTKNQVSLTCLVKGFYPSDI AVEWESNGQPENNYKTTPPVLDSDGSFFLYSKLT VDKSRWQQGNVFSCSVMHEALHNHYTQKSLSLS PG
27	AGEN1884.H3 light chain	EIVLTQSPGTLSLSPGERATLSCRASQSVSRYLGW YQQKPGQAPRLLIYGASTRATGIPDRFSGSGSGTD FTLTITRLEPEDFAVYYCQQYGSSPWTFGQGTKVE IKRTVAAPSVFIFPPSDEQLKSGTASVVCLLNNFYP REAKVQWKVDNALQSGNSQESVTEQDSKDSTYS LSSTLTLSKADYEKHKVYACEVTHQGLSSPVTKS FNRGEC
28	IgG_1	ASTKGPSVFPLAPSSKSTSGGTAALGCLVKDYFPE PVTVSWNSGALTSGVHTFPAVLQSSGLYSLSSVV TVPSSSLGTQTYICNVNHKPSNTKVDKRVEPKSC DKTHTCPPCPAPELLGGPSVFLFPPKPKDTLMISRT PEVTCVVVDVSHEDPEVKFNWYVDGVEVHNAKT KPREEQYNSTYRVVSVLTVLHQDWLNGKEYKCK VSNKALPAPIEKTISKAKGQPREPQVYTLPPSREE MTKNQVSLTCLVKGFYPSDIAVEWESNGQPENNY KTTPPVLDSDGSFFLYSKLTVDKSRWQQGNVFSC SVMHEALHNHYTQKSLSLSPG
29	IgG_1 S239D/I332E	ASTKGPSVFPLAPSSKSTSGGTAALGCLVKDYFPE PVTVSWNSGALTSGVHTFPAVLQSSGLYSLSSVV TVPSSSLGTQTYICNVNHKPSNTKVDKRVEPKSC DKTHTCPPCPAPELLGGPDVFLFPPKPKDTLMISR TPEVTCVVVDVSHEDPEVKFNWYVDGVEVHNAK TKPREEQYNSTYRVVSVLTVLHQDWLNGKEYKC KVSNKALPAPEEKTISKAKGQPREPQVYTLPPSRE EMTKNQVSLTCLVKGFYPSDIAVEWESNGQPENN YKTTPPVLDSDGSFFLYSKLTVDKSRWQQGNVFS CSVMHEALHNHYTQKSLSLSPG

SEQ ID NO:	Description	Amino acid sequence
30	IgG_1 S239D/A330L/I332E	ASTKGPSVFPLAPSSKSTSGGTAALGCLVKDYFPE PVTVSWNSGALTSGVHTFPAVLQSSGLYSLSSVV TVPSSSLGTQTYICNVNHKPSNTKVDKRVEPKSC DKTHTCPPCPAPELLGGPDVFLFPPKPKDTLMISR TPEVTCVVVDVSHEDPEVKFNWYVDGVEVHNAK TKPREEQYNSTYRVVSVLTVLHQDWLNGKEYKC KVSNKALPLPEEKTISKAKGQPREPQVYTLPPSRE EMTKNQVSLTCLVKGFYPSDIAVEWESNGQPENN YKTTPPVLDSDGSFFLYSKLTVDKSRWQQGNVFS CSVMHEALHNHYTQKSLSLSPG
31	IgG_1 L235V/F243L/R292P/Y300L/P396L	ASTKGPSVFPLAPSSKSTSGGTAALGCLVKDYFPE PVTVSWNSGALTSGVHTFPAVLQSSGLYSLSSVV TVPSSSLGTQTYICNVNHKPSNTKVDKRVEPKSC DKTHTCPPCPAPELVGGPSVFLLPPKPKDTLMISR TPEVTCVVVDVSHEDPEVKFNWYVDGVEVHNAK TKPPEEQYNSTLRVVSVLTVLHQDWLNGKEYKC KVSNKALPAPIEKTISKAKGQPREPQVYTLPPSREE MTKNQVSLTCLVKGFYPSDIAVEWESNGQPENNY KTTPLVLDSDGSFFLYSKLTVDKSRWQQGNVFSC SVMHEALHNHYTQKSLSLSPG
32	Light chain constant region	RTVAAPSVFIFPPSDEQLKSGTASVVCLLNNFYPR EAKVQWKVDNALQSGNSQESVTEQDSKDSTYSL SSTLTLSKADYEKHKVYACEVTHQGLSSPVTKSF NRGEC

* CDRs are defined according to the Kabat numbering system.

Table 2. Heavy chain CDR amino acid sequences of exemplary anti-CTLA-4 antibodies.*

VH (SEQ ID NO:)	CDRH1 (SEQ ID NO:)	CDRH2 (SEQ ID NO:)	CDRH3 (SEQ ID NO:)
AGEN1884 VH (1)	SYSMN (10)	SISSSSSYIYYADSV KG (11)	VGLMGPFDI (13)
AGEN1884_M102F VH (2)	SYSMN (10)	SISSSSSYIYYADSV KG (11)	VGLFGPFDI (14)
AGEN1884_M113L VH (3)	SYSMN (10)	SISSSSSYIYYADSV KG (11)	VGLMGPFDI (13)
AGEN1884_D62E VH (4)	SYSMN (10)	SISSSSSYIYYAESV KG (12)	VGLMGPFDI (13)
AGEN1884_M102F_M113L VH (5)	SYSMN (10)	SISSSSSYIYYADSV KG (11)	VGLFGPFDI (14)
AGEN1884_D62E_M102F VH (6)	SYSMN (10)	SISSSSSYIYYAESV KG (12)	VGLFGPFDI (14)
AGEN1884_D62E_M113L VH (7)	SYSMN (10)	SISSSSSYIYYAESV KG (12)	VGLMGPFDI (13)
AGEN1884_D62E_M102F_M113L VH (8)	SYSMN (10)	SISSSSSYIYYAESV KG (12)	VGLFGPFDI (14)

*Defined according to the Kabat numbering system.

Table 3. Light chain CDR amino acid sequences of exemplary anti-CTLA-4 antibodies.*

VL (SEQ ID NO:)	CDRL1 (SEQ ID NO:)	CDRL2 (SEQ ID NO:)	CDRL3 (SEQ ID NO:)
AGEN1884 VL (9)	RASQSVSRYLG (15)	GASTRAT (16)	QQYGSSPWT (17)

*Defined according to the Kabat numbering system.

Table 4. Exemplary anti-CTLA-4 antibodies.

Antibody	Heavy chain variable region	SEQ ID NO:	Light chain variable region	SEQ ID NO:
AGEN1884	AGEN1884 VH	1	AGEN1884 VL	9
AGEN1884.H1.1	AGEN1884_M102F VH	2	AGEN1884 VL	9
AGEN1884.H1.2	AGEN1884_M113L VH	3	AGEN1884 VL	9
AGEN1884.H1.3	AGEN1884_D62E VH	4	AGEN1884 VL	9
AGEN1884.H2.1	AGEN1884_M102F_M113L VH	5	AGEN1884 VL	9
AGEN1884.H2.2	AGEN1884_D62E_M102F VH	6	AGEN1884 VL	9
AGEN1884.H2.3	AGEN1884_D62E_M113L VH	7	AGEN1884 VL	9
AGEN1884.H3	AGEN1884_D62E_M102F_M113L VH	8	AGEN1884 VL	9

Table 5. Closest germline genes.

SEQ ID NO:	Closest germline gene	Amino acid sequence
21	IGHV3-21*01	EVQLVESGGGLVKPGGSLRLSCAASGFTFSSYS MNWVRQAPGKGLEWVSSISSSSSYIYYADSVK GRFTISRDNAKNSLYLQMNSLRAEDTAVYYCA R
22	IGKV3-20*01	EIVLTQSPGTLSLSPGERATLSCRASQSVSSSYL AWYQQKPGQAPRLLIYGASSRATGIPDRFSGSG SGTDFTLTISRLEPEDFAVYYCQQYGSSP

Table 6. Exemplary sequences of CTLA-4 and family members.

SEQ ID NO:	Description	Amino acid Sequence
33	Human CTLA-4 immature protein (P16410)	MACLGFQRHKAQLNLATRTWPCTLLFFLLFIPVFC KAMHVAQPAVVLASSRGIASFVCEYASPGKATEV RVTVLRQADSQVTEVCAATYMMGNELTFLDDSI CTGTSSGNQVNLTIQGLRAMDTGLYICKVELMYP PPYYLGIGNGTQIYVIDPEPCPDSDFLLWILAAVSS GLFFYSFLLTAVSLSKMLKKRSPLTTGVYVKMPP TEPECEKQFQPYFIPIN
34	CTLA-4 epitope	YLGI
35	CTLA-4 epitope	YPPPYYLGI
36	CTLA-4 epitope	YLGIGNGTQI
37	CTLA-4 epitope	YPPPYYLGIGNGTQI

SEQ ID NO:	Description	Amino acid Sequence
38	CTLA-4 epitope	MYPPPYY
39	CTLA-4 epitope	QVT
40	MACFA CTLA-4 (G7PL88)	MACLGFQRHKARLNLATRTRPYTLLFSLLFIPVFS KAMHVAQPAVVLANSRGIASFVCEYASPGKATE VRVTVLRQADSQVTEVCAATYMMGNELTFLDDS ICTGTSSGNQVNLTIQGLRAMDTGLYICKVELMY PPPYYMGIGNGTQIYVIDPEPCPDSDFLLWILAAVS SGLFFYSFLLTAVSLSKMLKKRSPLTTGVYVKMP PTEPECEKQFQPYFIPIN
41	Mouse CTLA-4 (P09793)	MACLGLRRYKAQLQLPSRTWPFVALLTLLFIPVFS EAIQVTQPSVVLASSHGVASFPCEYSPSHNTDEVR VTVLRQTNDQMTEVCATTFTEKNTVGFLDYPFCS GTFNESRVNLTIQGLRAVDTGLYLCKVELMYPPP YFVGMGNGTQIYVIDPEPCPDSDFLLWILVAVSLG LFFYSFLVSAVSLSKMLKKRSPLTTGVYVKMPPT EPECEKQFQPYFIPIN
42	Rat CTLA-4 (Q62859)	MACLGLQRYKTHLQLPSRTWPFGVLLSLLFIPIFSE AIQVTQPSVVLASSHGVASFPCEYASSHNTDEVR VTVLRQTNDQVTEVCATTFTVKNTLGFLDDPFCS GTFNESRVNLTIQGLRAADTGLYFCKVELMYPPP YFVGMGNGTQIYVIDPEPCPDSDFLLWILAAVSSG LFFYSFLVTAVSLNRTLKKRSPLTTGVYVKMPPTE PECEKQFQPYFIPIN
43	Human CD28 (P10747)	MLRLLLALNLFPSIQVTGNKILVKQSPMLVAYDN AVNLSCKYSYNLFSREFRASLHKGLDSAVEVCVV YGNYSQQLQVYSKTGFNCDGKLGNESVTFYLQN LYVNQTDIYFCKIEVMYPPPYLDNEKSNGTIIHVK GKHLCPSPLFPGPSKPFWVLVVVGGVLACYSLLV TVAFIIFWVRSKRSRLLHSDYMNMTPRRPGPTRK HYQPYAPPRDFAAYRS
44	Human ICOS (Q9Y6W8)	MKSGLWYFFLFCLRIKVLTGEINGSANYEMFIFHN GGVQILCKYPDIVQQFKMQLLKGGQILCDLTKTK GSGNTVSIKSLKFCHSQLSNNSVSFFLYNLDHSHA NYYFCNLSIFDPPPFKVTLTGGYLHIYESQLCCQL KFWLPIGCAAFVVVCILGCILICWLTKKKYSSSVH DPNGEYMFMRAVNTAKKSRLTDVTL
45	Human BTLA (Q7Z6A9)	MKTLPAMLGTGKLFWVFFLIPYLDIWNIHGKESC DVQLYIKRQSEHSILAGDPFELECPVKYCANRPHV TWCKLNGTTCVKLEDRQTSWKEEKNISFFILHFEP VLPNDNGSYRCSANFQSNLIESHSTTLYVTDVKSA SERPSKDEMASRPWLLYRLLPLGGLPLLITTCFCL FCCLRRHQGKQNELSDTAGREINLVDAHLKSEQT EASTRQNSQVLLSETGIYDNDPDLCFRMQEGSEV YSNPCLEENKPGIVYASLNHSVIGPNSRLARNVKE APTEYASICVRS

SEQ ID NO:	Description	Amino acid Sequence
46	Human PD-1 (Q15116)	MQIPQAPWPVVWAVLQLGWRPGWFLDSPDRPW NPPTFSPALLVVTEGDNATFTCSFSNTSESFVLNW YRMSPSNQTDKLAAFPEDRSQPGQDCRFRVTQLP NGRDFHMSVVRARRNDSGTYLCGAISLAPKAQIK ESLRAELRVTERRAEVPTAHPSPSPRPAGQFQTLV VGVVGGLLGSLVLLVWVLAVICSRAARGTIGARR TGQPLKEDPSAVPVFSVDYGELDFQWREKTPEPP VPCVPEQTEYATIVFPSGMGTSSPARRGSADGPRS AQPLRPEDGHCSWPL

[00127] In certain embodiments, the instant disclosure provides an isolated antibody that specifically binds to CTLA-4 (*e.g.*, human CTLA-4), the antibody comprising a VH domain comprising one, two, or all three of the CDRs of a VH domain set forth in Table 1 herein. In certain embodiments, the antibody comprises the CDRH1 of one of VH domains set forth in Table 1. In certain embodiments, the antibody comprises the CDRH2 of one of the VH domains set forth in Table 1. In certain embodiments, the antibody comprises the CDRH3 of one of the VH domains set forth in Table 1.

[00128] In certain embodiments, the instant disclosure provides an isolated antibody that specifically binds to CTLA-4 (*e.g.*, human CTLA-4), the antibody comprising a VL domain comprising one, two, or all three of the CDRs of a VL domain disclosed in Table 1 herein. In certain embodiments, the antibody comprises the CDRL1 of one of VL domains set forth in Table 1. In certain embodiments, the antibody comprises the CDRL2 of one of the VL domains set forth in Table 1. In certain embodiments, the antibody comprises the CDRL3 of one of the VL domains set forth in Table 1.

[00129] In certain embodiments, the CDRs of an antibody can be determined according to Kabat *et al.*, J. Biol. Chem. 252, 6609-6616 (1977) and Kabat *et al.*, Sequences of protein of immunological interest (1991), each of which is herein incorporated by reference in its entirety.

[00130] In certain embodiments, the CDRs of an antibody can be determined according to the Chothia numbering scheme, which refers to the location of immunoglobulin structural loops (*see*, *e.g.*, Chothia C & Lesk AM, (1987), J Mol Biol 196: 901-917; Al-Lazikani B *et al.*, (1997) J Mol Biol 273: 927-948; Chothia C *et al.*, (1992) J Mol Biol 227: 799-817; Tramontano A *et al.*, (1990) J Mol Biol 215(1): 175-82; and U.S. Patent No. 7,709,226, all of which are herein incorporated by reference in their entireties). Typically, when using the Kabat numbering convention, the Chothia CDRH1 loop is present at heavy chain amino acids

26 to 32, 33, or 34, the Chothia CDRH2 loop is present at heavy chain amino acids 52 to 56, and the Chothia CDRH3 loop is present at heavy chain amino acids 95 to 102, while the Chothia CDRL1 loop is present at light chain amino acids 24 to 34, the Chothia CDRL2 loop is present at light chain amino acids 50 to 56, and the Chothia CDRL3 loop is present at light chain amino acids 89 to 97. The end of the Chothia CDRH1 loop when numbered using the Kabat numbering convention varies between H32 and H34 depending on the length of the loop (this is because the Kabat numbering scheme places the insertions at H35A and H35B; if neither 35A nor 35B is present, the loop ends at 32; if only 35A is present, the loop ends at 33; if both 35A and 35B are present, the loop ends at 34).

[00131] In certain embodiments, the instant disclosure provides an isolated antibody that specifically binds to CTLA-4 (*e.g.*, human CTLA-4), the antibody comprising the Chothia VH CDRs of a VH disclosed in Table 1 herein. In certain embodiments, the instant disclosure provides an isolated antibody that specifically binds to CTLA-4 (*e.g.*, human CTLA-4), the antibody comprising the Chothia VL CDRs of a VL disclosed in Table 1 herein. In certain embodiments, the instant disclosure provides an isolated antibody that specifically binds to CTLA-4 (*e.g.*, human CTLA-4), the antibody comprising the Chothia VH CDRs and Chothia VL CDRs of an antibody disclosed in Table 1 herein. In certain embodiments, antibodies that specifically bind to CTLA-4 (*e.g.*, human CTLA-4) comprise one or more CDRs, in which the Chothia and Kabat CDRs have the same amino acid sequence. In certain embodiments, the instant disclosure provides an isolated antibody that specifically binds to CTLA-4 (*e.g.*, human CTLA-4) and comprises combinations of Kabat CDRs and Chothia CDRs.

[00132] In certain embodiments, the CDRs of an antibody can be determined according to the IMGT numbering system as described in Lefranc M-P, (1999) The Immunologist 7: 132-136 and Lefranc M-P *et al.*, (1999) Nucleic Acids Res 27: 209-212, each of which is herein incorporated by reference in its entirety.

[00133] In certain embodiments, the instant disclosure provides antibodies that specifically bind to CTLA-4 (*e.g.*, human CTLA-4) and comprise CDRs of an antibody disclosed in Table 1 herein, as determined by the IMGT numbering system, for example, as described in Lefranc M-P (1999) *supra* and Lefranc M-P *et al.*, (1999) *supra.*

[00134] In certain embodiments, the CDRs of an antibody can be determined according to the AbM numbering scheme, which refers to AbM hypervariable regions, which represent a compromise between the Kabat CDRs and Chothia structural loops, and are used by Oxford Molecular's AbM antibody modeling software (Oxford Molecular Group, Inc.), herein

incorporated by reference in its entirety. In a particular embodiment, the instant disclosure provides antibodies that specifically bind to CTLA-4 (*e.g.*, human CTLA-4) and comprise CDRs of an antibody disclosed in Table 1 herein as determined by the AbM numbering scheme.

[00135] In certain embodiments, the instant disclosure provides an isolated antibody that specifically binds to CTLA-4 (*e.g.*, human CTLA-4), wherein the antibody comprises a heavy chain variable region comprising the CDRH1, CDRH2, and CDRH3 region amino acid sequences of a VH domain set forth in SEQ ID NO: 2, 4, 5, 6, 7, or 8, and a light chain variable region comprising the CDRL1, CDRL2, and CDRL3 region amino acid sequences of a VL domain set forth in SEQ ID NO: 9, wherein each CDR is defined in accordance with the MacCallum definition, the Kabat definition, the Chothia definition, the combination of the Kabat definition and the Chothia definition, the IMGT numbering system, or the AbM definition of CDR.

[00136] In certain embodiments, the instant disclosure provides an isolated antibody that specifically binds to CTLA-4 (*e.g.*, human CTLA-4), the antibody comprising:

(a) a CDRH1 comprises the amino acid sequence of SYSMN (SEQ ID NO: 10); and/or

(b) a CDRH2 comprises the amino acid sequence of SISSSSSYIYYAXSVKG (SEQ ID NO: 18), wherein X is E or D; and/or

(c) a CDRH3 comprises the amino acid sequence of VGLXGPFDI (SEQ ID NO: 19), wherein X is F or M; and/or

(d) CDRL1 comprises the amino acid sequence of RASQSVSRYLG (SEQ ID NO: 15); and/or

(e) CDRL2 comprises the amino acid sequence of GASTRAT (SEQ ID NO: 16); and/or

(f) CDRL3 comprises the amino acid sequence of QQYGSSPWT (SEQ ID NO: 17),

and wherein the CDRH1, CDRH2, and CDRH3 sequences of the antibody are not SEQ ID NOs: 10, 11, and 13, respectively.

[00137] In certain embodiments, CDRH2 comprises the amino acid sequence of SEQ ID NO: 11. In certain embodiments, CDRH2 comprises the amino acid sequence of SEQ ID NO: 12. In certain embodiments, CDRH3 comprises the amino acid sequence of SEQ ID NO: 13. In certain embodiments, CDRH3 comprises the amino acid sequence of SEQ ID NO: 14.

[00138] In certain embodiments, the instant disclosure provides an isolated antibody that specifically binds to CTLA-4 (*e.g.*, human CTLA-4), wherein the antibody comprises a VH domain comprising the CDRH1, CDRH2, and CDRH3 amino acid sequences set forth in

SEQ ID NOs: 10, 11, and 14; 10, 12, and 13; or 10, 12, and 14, respectively. In certain embodiments, the instant disclosure provides an isolated antibody that specifically binds to CTLA-4 (*e.g.*, human CTLA-4), wherein the antibody comprises a VH domain comprising the CDRH1, CDRH2, and CDRH3 amino acid sequences set forth in SEQ ID NOs: 10, 12, and 14, respectively.

[00139] In certain embodiments, the instant disclosure provides an isolated antibody that specifically binds to CTLA-4 (*e.g.*, human CTLA-4), wherein the antibody comprises a heavy chain variable region comprising CDRH1, CDRH2, and CDRH3 regions, and a light chain variable region comprising CDRL1, CDRL2, and CDRL3 regions, wherein the CDRH1, CDRH2, CDRH3, CDRL1, CDRL2, and CDRL3 regions comprise the amino acid sequences set forth in SEQ ID NOs: 10, 11, 14, 15, 16, and 17; 10, 12, 13, 15, 16, and 17; or 10, 12, 14, 15, 16, and 17, respectively. In certain embodiments, the instant disclosure provides an isolated antibody that specifically binds to CTLA-4 (*e.g.*, human CTLA-4), wherein the antibody comprises a heavy chain variable region comprising CDRH1, CDRH2, and CDRH3 regions, and a light chain variable region comprising CDRL1, CDRL2, and CDRL3 regions, wherein the CDRH1, CDRH2, CDRH3, CDRL1, CDRL2, and CDRL3 regions comprise the amino acid sequences set forth in SEQ ID NOs: 10, 12, 14, 15, 16, and 17, respectively.

[00140] In certain embodiments, the instant disclosure provides an isolated antibody that specifically binds to CTLA-4 (*e.g.*, human CTLA-4), comprising a heavy chain variable region comprising an amino acid sequence of SEQ ID NO: 20. In certain embodiments, the instant disclosure provides an isolated antibody that specifically binds to CTLA-4 (*e.g.*, human CTLA-4), comprising a heavy chain variable region comprising an amino acid sequence that is at least 75%, 80%, 85%, 90%, 95%, 99%, or 100% (*e.g.*, at least 86, 87, 88, 89, 90, 91, 92, 93, 94, 95, 96, 97, 98 or 99%) identical to the amino acid sequence set forth in SEQ ID NO: 2, 4, 5, 6, 7, or 8. In certain embodiments, the instant disclosure provides an isolated antibody that specifically binds to CTLA-4 (*e.g.*, human CTLA-4), comprising a heavy chain variable region comprising an amino acid sequence that is at least 75%, 80%, 85%, 90%, 95%, 99%, or 100% (*e.g.*, at least 86, 87, 88, 89, 90, 91, 92, 93, 94, 95, 96, 97, 98 or 99%) identical to the amino acid sequence set forth in SEQ ID NO: 3. In certain embodiments, the antibody comprises a heavy chain variable region having the amino acid sequence set forth in SEQ ID NO: 2, 4, 5, 6, 7, or 8. In certain embodiments, the antibody comprises a heavy chain variable region having the amino acid sequence set forth in SEQ ID NO: 2. In certain embodiments, the antibody comprises a heavy chain variable region having the amino acid sequence set forth in SEQ ID NO: 3. In certain embodiments, the antibody

comprises a heavy chain variable region having the amino acid sequence set forth in SEQ ID NO: 4. In certain embodiments, the antibody comprises a heavy chain variable region having the amino acid sequence set forth in SEQ ID NO: 5. In certain embodiments, the antibody comprises a heavy chain variable region having the amino acid sequence set forth in SEQ ID NO: 6. In certain embodiments, the antibody comprises a heavy chain variable region having the amino acid sequence set forth in SEQ ID NO: 7. In certain embodiments, the antibody comprises a heavy chain variable region having the amino acid sequence set forth in SEQ ID NO: 8.

[00141] In certain embodiments, the instant disclosure provides an isolated antibody that specifically binds to CTLA-4 (*e.g.*, human CTLA-4), comprising a light chain variable region comprising an amino acid sequence that is at least 75%, 80%, 85%, 90%, 95%, 99%, or 100% (*e.g.*, at least 86, 87, 88, 89, 90, 91, 92, 93, 94, 95, 96, 97, 98 or 99%) identical to the amino acid sequence set forth in SEQ ID NO: 9. In certain embodiments, the antibody comprises a light chain variable region having the amino acid sequence set forth in SEQ ID NO: 9.

[00142] In certain embodiments, the instant disclosure provides an isolated antibody that specifically binds to CTLA-4 (*e.g.*, human CTLA-4), comprising a heavy chain variable region comprising an amino acid sequence of SEQ ID NO: 20, and a light chain variable region comprising an amino acid sequence of SEQ ID NO: 9. In certain embodiments, the instant disclosure provides an isolated antibody that specifically binds to CTLA-4 (*e.g.*, human CTLA-4), comprising a heavy chain variable region comprising an amino acid sequence that is at least 75%, 80%, 85%, 90%, 95%, 99%, or 100% (*e.g.*, at least 86, 87, 88, 89, 90, 91, 92, 93, 94, 95, 96, 97, 98 or 99%) identical to the amino acid sequence set forth in SEQ ID NO: 2, 4, 5, 6, 7, or 8, and a light chain variable region comprising an amino acid sequence that is at least 75%, 80%, 85%, 90%, 95%, 99%, or 100% (*e.g.*, at least 86, 87, 88, 89, 90, 91, 92, 93, 94, 95, 96, 97, 98 or 99%) identical to the amino acid sequence set forth in SEQ ID NO: 9. In certain embodiments, the instant disclosure provides an isolated antibody that specifically binds to CTLA-4 (*e.g.*, human CTLA-4), comprising a heavy chain variable region comprising an amino acid sequence that is at least 75%, 80%, 85%, 90%, 95%, 99%, or 100% (*e.g.*, at least 86, 87, 88, 89, 90, 91, 92, 93, 94, 95, 96, 97, 98 or 99%) identical to the amino acid sequence set forth in SEQ ID NO: 3, and a light chain variable region comprising an amino acid sequence that is at least 75%, 80%, 85%, 90%, 95%, 99%, or 100% (*e.g.*, at least 86, 87, 88, 89, 90, 91, 92, 93, 94, 95, 96, 97, 98 or 99%) identical to the amino acid sequence set forth in SEQ ID NO: 9. In certain embodiments, the antibody

comprises a heavy chain variable region and light chain variable region having the amino acid sequences set forth in SEQ ID NO: 2 and 9; 4 and 9; 5 and 9; 6 and 9; 7 and 9; or 8 and 9, respectively. In certain embodiments, the antibody comprises a heavy chain variable region and light chain variable region having the amino acid sequences set forth in SEQ ID NO: 2 and 9, respectively. In certain embodiments, the antibody comprises a heavy chain variable region and light chain variable region having the amino acid sequences set forth in SEQ ID NO: 3 and 9, respectively. In certain embodiments, the antibody comprises a heavy chain variable region and light chain variable region having the amino acid sequences set forth in SEQ ID NO: 4 and 9, respectively. In certain embodiments, the antibody comprises a heavy chain variable region and light chain variable region having the amino acid sequences set forth in SEQ ID NO: 5 and 9, respectively. In certain embodiments, the antibody comprises a heavy chain variable region and light chain variable region having the amino acid sequences set forth in SEQ ID NO: 6 and 9, respectively. In certain embodiments, the antibody comprises a heavy chain variable region and light chain variable region having the amino acid sequences set forth in SEQ ID NO: 7 and 9, respectively. In certain embodiments, the antibody comprises a heavy chain variable region and light chain variable region having the amino acid sequences set forth in SEQ ID NO: 8 and 9, respectively.

[00143] In certain embodiments, the instant disclosure provides an isolated antibody that specifically binds to CTLA-4 (*e.g.*, human CTLA-4), comprising a heavy chain variable region having an amino acid sequence derived from a human IGHV3-21 germline sequence (*e.g.*, IGHV3-21*01, *e.g.*, having the amino acid sequence of SEQ ID NO: 21). One or more regions selected from framework 1, framework 2, framework 3, CDRH1, and CDRH2 (*e.g.*, two, three, four or five of these regions) can be derived from a human IGHV3-21 germline sequence (*e.g.*, IGHV3-21*01, *e.g.*, having the amino acid sequence of SEQ ID NO: 21). In one embodiment, framework 1, framework 2, framework 3, CDRH1, and CDRH2 are all derived from a human IGHV3-21 germline sequence (*e.g.*, IGHV3-21*01, *e.g.*, having the amino acid sequence of SEQ ID NO: 21).

[00144] In certain embodiments, the instant disclosure provides an isolated antibody that specifically binds to CTLA-4 (*e.g.*, human CTLA-4), comprising a light chain variable region having an amino acid sequence derived from a human germline sequence selected from the group consisting of IGKV3-20 (*e.g.*, IGKV3-20*01, *e.g.*, having the amino acid sequence of SEQ ID NO: 22). One or more regions selected from framework 1, framework 2, framework 3, CDRL1, and CDRL2 (*e.g.*, two, three, four or five of these regions) can be derived from a human germline sequence selected from the group consisting of IGKV3-20 (*e.g.*, IGKV3-

20*01, *e.g.*, having the amino acid sequence of SEQ ID NO: 22). In one embodiment, framework 1, framework 2, framework 3, CDRL1, and CDRL2 are all derived from a human germline sequence selected from the group consisting of IGKV3-20 (*e.g.*, IGKV3-20*01, *e.g.*, having the amino acid sequence of SEQ ID NO: 22).

[00145] In certain embodiments, the instant disclosure provides an isolated antibody that specifically binds to CTLA-4 (*e.g.*, human CTLA-4), comprising a heavy chain variable region having an amino acid sequence derived from a human IGHV3-21 germline sequence (*e.g.*, IGHV3-21*01, *e.g.*, having the amino acid sequence of SEQ ID NO: 21), and a light chain variable region having an amino acid sequence derived from a human germline sequence selected from the group consisting of IGKV3-20 (*e.g.*, IGKV3-20*01, *e.g.*, having the amino acid sequence of SEQ ID NO: 22).

[00146] In certain embodiments, the instant disclosure provides an isolated antibody that cross-competes for binding to CTLA-4 (*e.g.*, human CTLA-4) with an antibody comprising the heavy and light chain variable region amino acid sequences set forth in SEQ ID NOs: 2 and 9; 4 and 9; 5 and 9; 6 and 9; 7 and 9; or 8 and 9, respectively. In certain embodiments, the instant disclosure provides an isolated antibody that cross-competes for binding to CTLA-4 (*e.g.*, human CTLA-4) with an antibody comprising the heavy and light chain variable region amino acid sequences set forth in SEQ ID NOs: 3 and 9, respectively.

[00147] In certain embodiments, the instant disclosure provides an isolated antibody that binds to the same or an overlapping epitope of CTLA-4 (*e.g.*, an epitope of human CTLA-4) as an antibody described herein, *e.g.*, an antibody comprising the heavy and light chain variable region amino acid sequences set forth in SEQ ID NOs: 2 and 9; 4 and 9; 5 and 9; 6 and 9; 7 and 9; or 8 and 9, respectively. In certain embodiments, the instant disclosure provides an isolated antibody that binds to the same or an overlapping epitope of CTLA-4 (*e.g.*, an epitope of human CTLA-4) as an antibody described herein, *e.g.*, an antibody comprising the heavy and light chain variable region amino acid sequences set forth in SEQ ID NOs: 3 and 9, respectively. In certain embodiments, the epitope of an antibody can be determined by, *e.g.*, NMR spectroscopy, surface plasmon resonance (BIAcore®), X-ray diffraction crystallography studies, ELISA assays, hydrogen/deuterium exchange coupled with mass spectrometry (*e.g.*, liquid chromatography electrospray mass spectrometry), array-based oligo-peptide scanning assays, and/or mutagenesis mapping (*e.g.*, site-directed mutagenesis mapping). For X-ray crystallography, crystallization may be accomplished using any of the known methods in the art (*e.g.*, Giegé R *et al.*, (1994) Acta Crystallogr D Biol Crystallogr 50(Pt 4): 339-350; McPherson A (1990) Eur J Biochem 189: 1-23; Chayen

NE (1997) Structure 5: 1269-1274; McPherson A (1976) J Biol Chem 251: 6300-6303, all of which are herein incorporated by reference in their entireties). Antibody:antigen crystals may be studied using well known X-ray diffraction techniques and may be refined using computer software such as X-PLOR (Yale University, 1992, distributed by Molecular Simulations, Inc.; *see*, *e.g.*, Meth Enzymol (1985) volumes 114 & 115, eds Wyckoff HW *et al.*; U.S. Patent Application No. 2004/0014194), and BUSTER (Bricogne G (1993) Acta Crystallogr D Biol Crystallogr 49(Pt 1): 37-60; Bricogne G (1997) Meth Enzymol 276A: 361-423, ed Carter CW; Roversi P *et al.*, (2000) Acta Crystallogr D Biol Crystallogr 56(Pt 10): 1316-1323, all of which are herein incorporated by reference in their entireties). Mutagenesis mapping studies may be accomplished using any method known to one of skill in the art. *See*, *e.g.*, Champe M *et al.*, (1995) *supra* and Cunningham BC & Wells JA (1989) *supra* for a description of mutagenesis techniques, including alanine scanning mutagenesis techniques. In a specific embodiment, the epitope of an antibody is determined using alanine scanning mutagenesis studies. In addition, antibodies that recognize and bind to the same or overlapping epitopes of CTLA-4 (*e.g.*, human CTLA-4) can be identified using routine techniques such as an immunoassay, for example, by showing the ability of one antibody to block the binding of another antibody to a target antigen, *i.e.*, a competitive binding assay. Competition binding assays also can be used to determine whether two antibodies have similar binding specificity for an epitope. Competitive binding can be determined in an assay in which the immunoglobulin under test inhibits specific binding of a reference antibody to a common antigen, such as CTLA-4 (*e.g.*, human CTLA-4). Numerous types of competitive binding assays are known, for example: solid phase direct or indirect radioimmunoassay (RIA), solid phase direct or indirect enzyme immunoassay (EIA), sandwich competition assay (*see* Stahli C *et al.*, (1983) Methods Enzymol 9: 242-253); solid phase direct biotin-avidin EIA (*see* Kirkland TN *et al.*, (1986) J Immunol 137: 3614-9); solid phase direct labeled assay, solid phase direct labeled sandwich assay (*see* Harlow E & Lane D, (1988) Antibodies: A Laboratory Manual, Cold Spring Harbor Press); solid phase direct label RIA using I-125 label (*see* Morel GA *et al.*, (1988) Mol Immunol 25(1): 7-15); solid phase direct biotin-avidin EIA (*see* Cheung RC *et al.*, (1990) Virology 176: 546-52); and direct labeled RIA (*see* Moldenhauer G *et al.*, (1990) Scand J Immunol 32: 77-82), all of which are herein incorporated by reference in their entireties. Typically, such an assay involves the use of purified antigen (*e.g.*, CTLA-4 such as human CTLA-4) bound to a solid surface or cells bearing either of these, an unlabeled test immunoglobulin and a labeled reference immunoglobulin. Competitive inhibition can be measured by determining the amount of

label bound to the solid surface or cells in the presence of the test immunoglobulin. Usually the test immunoglobulin is present in excess. Usually, when a competing antibody is present in excess, it will inhibit specific binding of a reference antibody to a common antigen by at least 50-55%, 55-60%, 60-65%, 65-70%, 70-75% or more. A competition binding assay can be configured in a large number of different formats using either labeled antigen or labeled antibody. In a common version of this assay, the antigen is immobilized on a 96-well plate. The ability of unlabeled antibodies to block the binding of labeled antibodies to the antigen is then measured using radioactive or enzyme labels. For further details *see*, for example, Wagener C *et al.*, (1983) J Immunol 130: 2308-2315; Wagener C *et al.*, (1984) J Immunol Methods 68: 269-274; Kuroki M *et al.*, (1990) Cancer Res 50: 4872-4879; Kuroki M *et al.*, (1992) Immunol Invest 21: 523-538; Kuroki M *et al.*, (1992) Hybridoma 11: 391-407 and Antibodies: A Laboratory Manual, Ed Harlow E & Lane D editors *supra*, pp. 386-389, all of which are herein incorporated by reference in their entireties.

[00148] In certain embodiments, the instant disclosure provides an isolated antibody that specifically binds to CTLA-4 (*e.g.*, human CTLA-4), the antibody comprising a heavy chain comprising the amino acid sequence set forth in SEQ ID NO: 23, 24, 25, or 26. In certain embodiments, the antibody comprises a heavy chain comprising the amino acid sequence set forth in SEQ ID NO: 23. In certain embodiments, the antibody comprises a heavy chain comprising the amino acid sequence set forth in SEQ ID NO: 24. In certain embodiments, the antibody comprises a heavy chain comprising the amino acid sequence set forth in SEQ ID NO: 25. In certain embodiments, the antibody comprises a heavy chain comprising the amino acid sequence set forth in SEQ ID NO: 26.

[00149] In certain embodiments, the instant disclosure provides an isolated antibody that specifically binds to CTLA-4 (*e.g.*, human CTLA-4), the antibody comprising a light chain comprising the amino acid sequence set forth in SEQ ID NO: 27.

[00150] In certain embodiments, the instant disclosure provides an isolated antibody that specifically binds to CTLA-4 (*e.g.*, human CTLA-4), the antibody comprising a heavy chain comprising the amino acid sequence of SEQ ID NO: 23 and a light chain comprising the amino acid sequence of SEQ ID NO: 27. In certain embodiments, the instant disclosure provides an isolated antibody that specifically binds to CTLA-4 (*e.g.*, human CTLA-4), the antibody comprising a heavy chain comprising the amino acid sequence of SEQ ID NO: 24 and a light chain comprising the amino acid sequence of SEQ ID NO: 27. In certain embodiments, the instant disclosure provides an isolated antibody that specifically binds to CTLA-4 (*e.g.*, human CTLA-4), the antibody comprising a heavy chain comprising the

amino acid sequence of SEQ ID NO: 25 and a light chain comprising the amino acid sequence of SEQ ID NO: 27. In certain embodiments, the instant disclosure provides an isolated antibody that specifically binds to CTLA-4 (*e.g.*, human CTLA-4), the antibody comprising a heavy chain comprising the amino acid sequence of SEQ ID NO: 26 and a light chain comprising the amino acid sequence of SEQ ID NO: 27.

[00151] Any Ig constant region can be used in the antibodies described herein. In certain embodiments, the Ig region is a human IgG, IgE, IgM, IgD, IgA, or IgY immunoglobulin molecule, any class (*e.g.*, IgG_1, IgG_2, IgG_3, IgG_4, IgA_1, and IgA_2), or any subclass (*e.g.*, IgG_{2a} and IgG_{2b}) of immunoglobulin molecule.

[00152] In certain embodiments, the instant disclosure provides an isolated antibody that specifically binds to CTLA-4 (*e.g.*, human CTLA-4), the antibody comprising a heavy chain constant region comprising the amino acid sequence of SEQ ID NO: 28, 29, 30, or 31. In certain embodiments, the instant disclosure provides an isolated antibody that specifically binds to CTLA-4 (*e.g.*, human CTLA-4), the antibody comprising a light chain constant region comprising the amino acid sequence of SEQ ID NO: 32.

[00153] In certain embodiments, the instant disclosure provides an isolated antibody that specifically binds to CTLA-4 (*e.g.*, human CTLA-4), the antibody comprising a heavy chain constant region, *e.g.*, an IgG_1 constant region, or fragment thereof comprising a mutation selected from the group consisting of: S239D, I332E, and a combination thereof, numbered according to the EU numbering system. In certain embodiments, the antibody comprises an IgG_1 heavy chain constant region comprising S239D and I332E mutations, numbered according to the EU numbering system. In certain embodiments, the antibody comprises a heavy chain constant region comprising the amino acid sequence of SEQ ID NO: 29.

[00154] In certain embodiments, the instant disclosure provides an isolated antibody that specifically binds to CTLA-4 (*e.g.*, human CTLA-4), the antibody comprising a heavy chain constant region, *e.g.*, an IgG_1 constant region, or fragment thereof comprising a mutation selected from the group consisting of: S239D, A330L, I332E, and combinations thereof, numbered according to the EU numbering system. In certain embodiments, the antibody comprises an IgG_1 heavy chain constant region comprising S239D, A330L, and I332E mutations, numbered according to the EU numbering system. In certain embodiments, the antibody comprises a heavy chain constant region comprising the amino acid sequence of SEQ ID NO: 30.

[00155] In certain embodiments, the instant disclosure provides an isolated antibody that specifically binds to CTLA-4 (*e.g.*, human CTLA-4), the antibody comprising a heavy chain

constant region, *e.g.*, an IgG_1 constant region, or fragment thereof comprising a mutation selected from the group consisting of: L235V, F243L, R292P, Y300L, P396L, and combinations thereof, numbered according to the EU numbering system. In certain embodiments, the antibody comprises an IgG_1 heavy chain constant region comprising L235V, F243L, R292P, Y300L, and P396L mutations, numbered according to the EU numbering system. In certain embodiments, the antibody comprises a heavy chain constant region comprising the amino acid sequence of SEQ ID NO: 31.

[00156] In certain embodiments, the IgG regions of the antibodies described herein have **an increased affinity for FcγRIIIA,** *e.g.*, as compared with an antibody with a wild-type Fc region, *e.g.*, an IgG_1 **Fc. Sequence alterations that result in increased affinity for FcγRIIIA** are known in the art, for example, in Kellner *et al., Methods 65: 105-113* (2014), Lazar *et al., Proc Natl Acad Sci 103: 4005-4010* (2006), Shields *et al., J Biol Chem. 276(9):6591-6604* (2001), each of which is herein incorporated by reference in its entirety. In certain embodiments, the antibody comprises a heavy chain constant region, *e.g.*, an IgG_1 constant region, or fragment thereof comprising a mutation selected from the group consisting of: G236A, S239D, F243L, T256A, K290A, R292P, S298A, Y300L, V305I, A330L, I332E, E333A, K334A, A339T, and P396L, and combinations thereof, numbered according to the EU numbering system. In certain embodiments, the antibody comprises a heavy chain constant region, *e.g.*, an IgG1 constant region, or fragment thereof comprising S239D, numbered according to the EU numbering system. In certain embodiments, the antibody comprises a heavy chain constant region, *e.g.*, an IgG1 constant region, or fragment thereof comprising T256A, numbered according to the EU numbering system. In certain embodiments, the antibody comprises a heavy chain constant region, *e.g.*, an IgG1 constant region, or fragment thereof comprising K290A, numbered according to the EU numbering system. In certain embodiments, the antibody comprises a heavy chain constant region, *e.g.*, an IgG1 constant region, or fragment thereof comprising S298A, numbered according to the EU numbering system. In certain embodiments, the antibody comprises a heavy chain constant region, *e.g.*, an IgG1 constant region, or fragment thereof comprising I332E, numbered according to the EU numbering system. In certain embodiments, the antibody comprises a heavy chain constant region, *e.g.*, an IgG1 constant region, or fragment thereof comprising E333A, numbered according to the EU numbering system. In certain embodiments, the antibody comprises a heavy chain constant region, *e.g.*, an IgG1 constant region, or fragment thereof comprising K334A, numbered according to the EU numbering system. In certain embodiments, the antibody comprises a heavy chain constant region, *e.g.*,

an IgG1 constant region, or fragment thereof comprising A339T, numbered according to the EU numbering system. In certain embodiments, the antibody comprises a heavy chain constant region, *e.g.*, an IgG1 constant region, or fragment thereof comprising S239D and I332E, numbered according to the EU numbering system. In certain embodiments, the antibody comprises a heavy chain constant region, *e.g.*, an IgG1 constant region, or fragment thereof comprising S239D, A330L, and I332E, numbered according to the EU numbering system. In certain embodiments, the antibody comprises a heavy chain constant region, *e.g.*, an IgG1 constant region, or fragment thereof comprising S298A, E333A, and K334A, numbered according to the EU numbering system. In certain embodiments, the antibody comprises a heavy chain constant region, *e.g.*, an IgG1 constant region, or fragment thereof comprising G236A, S239D, and I332E, numbered according to the EU numbering system. In certain embodiments, the antibody comprises a heavy chain constant region, *e.g.*, an IgG1 constant region, or fragment thereof comprising F243L, R292P, Y300L, V305I, and P396L, numbered according to the EU numbering system.

[00157] In certain embodiments, the antibodies described herein exhibit antibody-dependent cellular cytotoxicity (ADCC) activity. In certain embodiments, the antibodies described herein initiate natural killer cell mediated cell depletion. In certain embodiments, the antibodies described herein are used for treating tumor infiltrated with natural killer cells. In certain embodiments, the antibodies described herein exhibit antibody-dependent cellular phagocytosis (ADCP) activity. In certain embodiments, the antibodies described herein initiate macrophage mediated cell depletion. In certain embodiments, the antibodies described herein are used for treating tumor infiltrated with macrophages. In certain embodiments, the antibodies described herein selectively deplete intratumoral regulatory T cells.

[00158] In certain embodiments, an antibody described herein is an activatable antibody that in an activated state binds human CTLA-4 protein. In certain embodiments, the activatable antibody comprises a masking moiety that inhibits the binding of the antibody in an uncleaved state to human CTLA-4 protein, and at least one cleavable moiety coupled to the antibody, e.g., wherein the cleavable moiety is a polypeptide that functions as a substrate for a protease that is enriched in the tumor microenvironment. Exemplary activatable antibodies are described, e.g., in U.S. Patent Nos. 8,513,390 and 8,518,404, and U.S. Patent Application Publication Nos. US 2014/0255313, US 2014/0010810, US 2014/0023664, which are incorporated herein by reference. In certain embodiments, the activatable antibody comprises a human IgG heavy chain constant region that is a variant of a wild type

human IgG heavy chain constant region, wherein the variant human IgG heavy chain constant **region binds to human FcγRIIIA with higher affinity than the wild type human IgG heavy chain constant region binds to human FcγRIIIA.**

[00159] In certain embodiments, one, two, or more mutations (*e.g.*, amino acid substitutions) are introduced into the Fc region of an antibody described herein (*e.g.*, CH2 domain (residues 231-340 of human IgG_1) and/or CH3 domain (residues 341-447 of human IgG_1) and/or the hinge region, numbered according to the EU numbering system, to alter one or more functional properties of the antibody, such as serum half-life, complement fixation, Fc receptor binding and/or antigen-dependent cellular cytotoxicity.

[00160] In certain embodiments, one, two, or more mutations (*e.g.*, amino acid substitutions) are introduced into the hinge region of the Fc region (CH1 domain) such that the number of cysteine residues in the hinge region are altered (*e.g.*, increased or decreased) as described in, *e.g.*, U.S. Patent No. 5,677,425, herein incorporated by reference in its entirety. The number of cysteine residues in the hinge region of the CH1 domain may be altered to, *e.g.*, facilitate assembly of the light and heavy chains, or to alter (*e.g.*, increase or decrease) the stability of the antibody.

[00161] In a specific embodiment, one, two, or more amino acid mutations (*e.g.*, substitutions, insertions or deletions) are introduced into an IgG constant domain, or FcRn-binding fragment thereof (preferably an Fc or hinge-Fc domain fragment) to alter (*e.g.*, decrease or increase) half-life of the antibody *in vivo*. *See, e.g.,* International Publication Nos. WO 02/060919; WO 98/23289; and WO 97/34631; and U.S. Patent Nos. 5,869,046, 6,121,022, 6,277,375 and 6,165,745, all of which are herein incorporated by reference in their entireties, for examples of mutations that will alter (*e.g.*, decrease or increase) the half-life of an antibody *in vivo*. In some embodiments, one, two or more amino acid mutations (*e.g.*, substitutions, insertions, or deletions) are introduced into an IgG constant domain, or FcRn-binding fragment thereof (preferably an Fc or hinge-Fc domain fragment) to decrease the half-life of the antibody *in vivo*. In other embodiments, one, two or more amino acid mutations (*e.g.*, substitutions, insertions or deletions) are introduced into an IgG constant domain, or FcRn-binding fragment thereof (preferably an Fc or hinge-Fc domain fragment) to increase the half-life of the antibody *in vivo*. In a specific embodiment, the antibodies may have one or more amino acid mutations (*e.g.*, substitutions) in the second constant (CH2) domain (residues 231-340 of human IgG_1) and/or the third constant (CH3) domain (residues 341-447 of human IgG_1), numbered according to the EU numbering system. In a specific embodiment, the constant region of the IgG_1 of an antibody described herein comprises a

methionine (M) to tyrosine (Y) substitution in position 252, a serine (S) to threonine (T) substitution in position 254, and a threonine (T) to glutamic acid (E) substitution in position 256, numbered according to the EU numbering system. *See* U.S. Patent No. 7,658,921, which is herein incorporated by reference in its entirety. This type of mutant IgG, referred to as "YTE mutant" has been shown to display fourfold increased half-life as compared to wild-type versions of the same antibody (*see* Dall'Acqua WF *et al.*, (2006) J Biol Chem 281: 23514-24, which is herein incorporated by reference in its entirety). In certain embodiments, an antibody comprises an IgG constant domain comprising one, two, three or more amino acid substitutions of amino acid residues at positions 251-257, 285-290, 308-314, 385-389, and 428-436, numbered according to the EU numbering system.

[00162] In some embodiments, one, two, or more mutations (*e.g.*, amino acid substitutions) are introduced into the Fc region of an antibody described herein (*e.g.*, CH2 domain (residues 231-340 of human IgG_1) and/or CH3 domain (residues 341-447 of human IgG_1) and/or the hinge region, numbered according to the EU numbering system, to increase or decrease the affinity of the antibody for an Fc receptor (*e.g.*, an activated Fc receptor) on the surface of an effector cell. Mutations in the Fc region of an antibody that decrease or increase the affinity of an antibody for an Fc receptor and techniques for introducing such mutations into the Fc receptor or fragment thereof are known to one of skill in the art. Examples of mutations in the Fc receptor of an antibody that can be made to alter the affinity of the antibody for an Fc receptor are described in, *e.g.*, Smith P *et al.*, (2012) PNAS 109: 6181-6186, U.S. Patent No. 6,737,056, and International Publication Nos. WO 02/060919; WO 98/23289; and WO 97/34631, all of which are herein incorporated by reference in their entireties.

[00163] In a further embodiment, one, two, or more amino acid substitutions are introduced into an IgG constant domain Fc region to alter the effector function(s) of the antibody. For example, one or more amino acids selected from amino acid residues 234, 235, 236, 237, 297, 318, 320 and 322, numbered according to the EU numbering system, can be replaced with a different amino acid residue such that the antibody has an altered affinity for an effector ligand but retains the antigen-binding ability of the parent antibody. The effector ligand to which affinity is altered can be, for example, an Fc receptor or the C1 component of complement. This approach is described in further detail in U.S. Patent Nos. 5,624,821 and 5,648,260, each of which is herein incorporated by reference in its entirety. In some embodiments, the deletion or inactivation (through point mutations or other means) of a constant region domain may reduce Fc receptor binding of the circulating antibody thereby

increasing tumor localization. *See*, *e.g.*, U.S. Patent Nos. 5,585,097 and 8,591,886, each of which is herein incorporated by reference in its entirety, for a description of mutations that delete or inactivate the constant domain and thereby increase tumor localization. In certain embodiments, one or more amino acid substitutions may be introduced into the Fc region of an antibody described herein to remove potential glycosylation sites on Fc region, which may reduce Fc receptor binding (*see*, *e.g.*, Shields RL *et al.*, (2001) J Biol Chem 276: 6591-604, which is herein incorporated by reference in its entirety). In various embodiments, one or more of the following mutations in the constant region of an antibody described herein may be made: an N297A substitution; an N297Q substitution; a L235A substitution and a L237A substitution; a L234A substitution and a L235A substitution; a E233P substitution; a L234V substitution; a L235A substitution; a C236 deletion; a P238A substitution; a D265A substitution; a A327Q substitution; or a P329A substitution, numbered according to the EU numbering system. In certain embodiments, a mutation selected from the group consisting of D265A, P329A, and a combination thereof, numbered according to the EU numbering system, may be made in the constant region of an antibody described herein.

[00164] In a specific embodiment, an antibody described herein comprises the constant domain of an IgG_1 with an N297Q or N297A amino acid substitution, numbered according to the EU numbering system. In one embodiment, an antibody described herein comprises the constant domain of an IgG_1 with a mutation selected from the group consisting of D265A, P329A, and a combination thereof, numbered according to the EU numbering system. In another embodiment, an antibody described herein comprises the constant domain of an IgG_1 with a mutation selected from the group consisting of L234A, L235A, and a combination thereof, numbered according to the EU numbering system. In certain embodiments, amino acid residues in the constant region of an antibody described herein in the positions corresponding to positions L234, L235, and D265 in a human IgG_1 heavy chain, numbered according to the EU numbering system, are not L, L, and D, respectively. This approach is described in detail in International Publication No. WO 14/108483, which is herein incorporated by reference in its entirety. In a particular embodiment, the amino acids corresponding to positions L234, L235, and D265 in a human IgG_1 heavy chain are F, E, and A; or A, A, and A, respectively, numbered according to the EU numbering system.

[00165] In certain embodiments, one or more amino acids selected from amino acid residues 329, 331, and 322 in the constant region of an antibody described herein, numbered according to the EU numbering system, can be replaced with a different amino acid residue such that the antibody has altered C1q binding and/or reduced or abolished complement

dependent cytotoxicity (CDC). This approach is described in further detail in U.S. Patent No. 6,194,551 (Idusogie *et al.*), which is herein incorporated by reference in its entirety. In some embodiments, one or more amino acid residues within amino acid positions 231 to 238 in the N-terminal region of the CH2 domain of an antibody described herein are altered to thereby alter the ability of the antibody to fix complement, numbered according to the EU numbering system. This approach is described further in International Publication No. WO 94/29351, which is herein incorporated by reference in its entirety. In certain embodiments, the Fc region of an antibody described herein is modified to increase the ability of the antibody to mediate antibody dependent cellular cytotoxicity (ADCC) and/or to increase the affinity of the antibody for an Fcγ receptor by mutating one or more amino acids (*e.g.*, introducing amino acid substitutions) at the following positions: 238, 239, 248, 249, 252, 254, 255, 256, 258, 265, 267, 268, 269, 270, 272, 276, 278, 280, 283, 285, 286, 289, 290, 292, 293, 294, 295, 296, 298, 301, 303, 305, 307, 309, 312, 315, 320, 322, 324, 326, 327, 328, 329, 330, 331, 333, 334, 335, 337, 338, 340, 360, 373, 376, 378, 382, 388, 389, 398, 414, 416, 419, 430, 434, 435, 437, 438, or 439, numbered according to the EU numbering system. This approach is described further in International Publication No. WO 00/42072, which is herein incorporated by reference in its entirety.

[00166] In certain embodiments, an antibody described herein comprises the constant region of an IgG_4 antibody and the serine at amino acid residue 228 of the heavy chain, numbered according to the EU numbering system, is substituted for proline.

[00167] In certain embodiments, any of the constant region mutations or modifications described herein can be introduced into one or both heavy chain constant regions of an antibody described herein having two heavy chain constant regions.

[00168] In certain embodiments, the instant disclosure provides an isolated antibody that specifically binds to CTLA-4 (*e.g.*, human CTLA-4) and functions as an antagonist.

[00169] In certain embodiments, the instant disclosure provides an isolated antibody that specifically binds to CTLA-4 (*e.g.*, human CTLA-4) and decreases CTLA-4 (*e.g.*, human CTLA-4) activity by at least 5%, 10%, 15%, 20%, 25%, 30%, 35%, 40%, 45%, 50%, 55%, 60%, 65%, 70%, 75%, 80%, 85%, 90%, 95%, 98%, or 99% as assessed by methods described herein and/or known to one of skill in the art, relative to CTLA-4 (*e.g.*, human CTLA-4) activity without any antibody or with an unrelated antibody (*e.g.*, an antibody that does not specifically bind to CTLA-4 (*e.g.*, human CTLA-4)). In certain embodiments, the instant disclosure provides an isolated antibody that specifically binds to CTLA-4 (*e.g.*, human

CTLA-4) and decreases CTLA-4 (*e.g.*, human CTLA-4) activity by at least about 1.2 fold, 1.3 fold, 1.4 fold, 1.5 fold, 2 fold, 2.5 fold, 3 fold, 3.5 fold, 4 fold, 4.5 fold, 5 fold, 6 fold, 7 fold, 8 fold, 9 fold, 10 fold, 15 fold, 20 fold, 30 fold, 40 fold, 50 fold, 60 fold, 70 fold, 80 fold, 90 fold, or 100 fold as assessed by methods described herein and/or known to one of skill in the art, relative to CTLA-4 (*e.g.*, human CTLA-4) activity without any antibody or with an unrelated antibody (*e.g.*, an antibody that does not specifically bind to CTLA-4 (*e.g.*, human CTLA-4)). Non-limiting examples of CTLA-4 (*e.g.*, human CTLA-4) activity can include CTLA-4 (*e.g.*, human CTLA-4) signaling, CTLA-4 (*e.g.*, human CTLA-4) binding to CTLA-4 (*e.g.*, human CTLA-4) ligand (*e.g.*, CD80 or CD86), and inhibition of cytokine production (*e.g.*, **IL-2, IFN-γ, or TNF-α). In certain embodiments, the instant disclosure** provides an isolated antibody that specifically binds to CTLA-4 (*e.g.*, human CTLA-4) and deactivates, reduces, or inhibits a CTLA-4 (*e.g.*, human CTLA-4) activity. In specific embodiments, a decrease in a CTLA-4 (*e.g.*, human CTLA-4) activity is assessed as described in the Examples, *infra*.

[00170] In specific embodiments, the instant disclosure provides an isolated antibody that specifically binds to CTLA-4 (*e.g.*, human CTLA-4) and reduces CTLA-4 (*e.g.*, human CTLA-4) binding to its ligand (*e.g.*, CD80 or CD86) by at least about 5%, 10%, 15%, 20%, 25%, 30%, 35%, 40%, 45%, 50%, 55%, 60%, 65%, 70%, 75%, 80%, 85%, 90%, 95%, 98%, or 99%, as assessed by methods known to one of skill in the art, relative to CTLA-4 (*e.g.*, human CTLA-4) binding to its ligand (*e.g.*, CD80 or CD86) without any antibody or with an unrelated antibody (*e.g.*, an antibody that does not specifically bind to CTLA-4 (*e.g.*, human CTLA-4)). In specific embodiments, the instant disclosure provides an isolated antibody that specifically binds to CTLA-4 (*e.g.*, human CTLA-4) and reduces CTLA-4 (*e.g.*, human CTLA-4) binding to its ligand (*e.g.*, CD80 or CD86) by at least about 1.2 fold, 1.3 fold, 1.4 fold, 1.5 fold, 2 fold, 2.5 fold, 3 fold, 3.5 fold, 4 fold, 4.5 fold, 5 fold, 6 fold, 7 fold, 8 fold, 9 fold, 10 fold, 15 fold, 20 fold, 30 fold, 40 fold, 50 fold, 60 fold, 70 fold, 80 fold, 90 fold, or 100 fold, as assessed by methods known to one of skill in the art, relative to CTLA-4 (*e.g.*, human CTLA-4) binding to its ligand (*e.g.*, CD80 or CD86) without any antibody or with an unrelated antibody (*e.g.*, an antibody that does not specifically bind to CTLA-4 (*e.g.*, human CTLA-4)).

[00171] In specific embodiments, the instant disclosure provides an isolated antibody that specifically binds to CTLA-4 (*e.g.*, human CTLA-4) and increases cytokine production (*e.g.*, **IL-2, IFN-γ, or TNF-α) by at least about 5%, 10%, 15%, 20%, 25%, 30%, 35%, 40%, 45%,** 50%, 55%, 60%, 65%, 70%, 75%, 80%, 85%, 90%, 95%, 98%, or 99%, as assessed by

methods described herein (*see* the Examples, *infra*) or known to one of skill in the art, relative to cytokine production without any antibody or with an unrelated antibody (*e.g.*, an antibody that does not specifically bind to CTLA-4 (*e.g.*, human CTLA-4)). In specific embodiments, the instant disclosure provides an isolated antibody that specifically binds to CTLA-4 (*e.g.*, human CTLA-4) and increases cytokine production (*e.g.*, **IL-2, IFN-γ, or TNF-α) by at least about 1.2 fold, 1.3 fold, 1.4 fold, 1.5 fold, 2 fold, 2.5 fold, 3 fold, 3.5 fold,** 4 fold, 4.5 fold, 5 fold, 6 fold, 7 fold, 8 fold, 9 fold, 10 fold, 15 fold, 20 fold, 30 fold, 40 fold, 50 fold, 60 fold, 70 fold, 80 fold, 90 fold, or 100 fold, as assessed by methods described herein (*see* the Examples, *infra*) or known to one of skill in the art, relative to cytokine production without any antibody or with an unrelated antibody (*e.g.*, an antibody that does not specifically bind to CTLA-4 (*e.g.*, human CTLA-4)).

[00172] In certain embodiments, human peripheral blood mononuclear cells (PBMCs) stimulated with Staphylococcus Enterotoxin A (SEA) in the presence of an antibody described herein, which specifically binds to CTLA-4 (*e.g.*, human CTLA-4), have increased IL-2 production by at least about 1.2 fold, 1.3 fold, 1.4 fold, 1.5 fold, 2 fold, 2.5 fold, 3 fold, 3.5 fold, 4 fold, 4.5 fold, 5 fold, 6 fold, 7 fold, 8 fold, 9 fold, 10 fold, 15 fold, 20 fold, 30 fold, 40 fold, 50 fold, 60 fold, 70 fold, 80 fold, 90 fold, or 100 fold relative to PBMCs only stimulated with SEA without any antibody or with an unrelated antibody (*e.g.*, an antibody that does not specifically bind to CTLA-4 (*e.g.*, human CTLA-4)), as assessed by methods described herein (*see* the Examples, *infra*) or known to one of skill in the art.

6.3 Pharmaceutical Compositions

[00173] Provided herein are compositions comprising an anti-CTLA-4 antibody described herein having the desired degree of purity in a physiologically acceptable carrier, excipient or stabilizer (Remington's Pharmaceutical Sciences (1990) Mack Publishing Co., Easton, PA). Acceptable carriers, excipients, or stabilizers are nontoxic to recipients at the dosages and concentrations employed, and include buffers such as phosphate, citrate, and other organic acids; antioxidants including ascorbic acid and methionine; preservatives (such as octadecyldimethylbenzyl ammonium chloride; hexamethonium chloride; benzalkonium chloride, benzethonium chloride; phenol, butyl or benzyl alcohol; alkyl parabens such as methyl or propyl paraben; catechol; resorcinol; cyclohexanol; 3-pentanol; and m-cresol); low molecular weight (less than about 10 residues) polypeptides; proteins, such as serum albumin, gelatin, or immunoglobulins; hydrophilic polymers such as polyvinylpyrrolidone; amino acids such as glycine, glutamine, asparagine, histidine, arginine, or lysine; monosaccharides,

disaccharides, and other carbohydrates including glucose, mannose, or dextrins; chelating agents such as EDTA; sugars such as sucrose, mannitol, trehalose or sorbitol; salt-forming counter-ions such as sodium; metal complexes (*e.g.,* Zn-protein complexes); and/or non-ionic surfactants such as TWEEN™, PLURONICS™ or polyethylene glycol (PEG).

[00174] In a specific embodiment, pharmaceutical compositions comprise an anti-CTLA-4 antibody described herein, and optionally one or more additional prophylactic or therapeutic agents, in a pharmaceutically acceptable carrier. In a specific embodiment, pharmaceutical compositions comprise an effective amount of an antibody or antigen-binding fragment thereof described herein, and optionally one or more additional prophylactic or therapeutic agents, in a pharmaceutically acceptable carrier. In some embodiments, the antibody is the only active ingredient included in the pharmaceutical composition. Pharmaceutical compositions described herein can be useful in inhibiting, CTLA-4 activity and treating a condition, such as cancer or an infectious disease.

[00175] In one aspect, provided herein is a pharmaceutical composition comprising an anti-CTLA-4 antibody of the invention and a pharmaceutically acceptable carrier or excipient, for use as a medicament.

[00176] In one aspect, provided herein is a pharmaceutical composition comprising an anti-CTLA-4 antibody of the invention and a pharmaceutically acceptable carrier or excipient, for use in a method for the treatment of cancer.

[00177] Pharmaceutically acceptable carriers used in parenteral preparations include aqueous vehicles, nonaqueous vehicles, antimicrobial agents, isotonic agents, buffers, antioxidants, local anesthetics, suspending and dispersing agents, emulsifying agents, sequestering or chelating agents and other pharmaceutically acceptable substances. Examples of aqueous vehicles include Sodium Chloride Injection, Ringers Injection, Isotonic Dextrose Injection, Sterile Water Injection, Dextrose and Lactated Ringers Injection. Nonaqueous parenteral vehicles include fixed oils of vegetable origin, cottonseed oil, corn oil, sesame oil and peanut oil. Antimicrobial agents in bacteriostatic or fungistatic concentrations can be added to parenteral preparations packaged in multiple-dose containers which include phenols or cresols, mercurials, benzyl alcohol, chlorobutanol, methyl and propyl p-hydroxybenzoic acid esters, thimerosal, benzalkonium chloride and benzethonium chloride. Isotonic agents include sodium chloride and dextrose. Buffers include phosphate and citrate. Antioxidants include sodium bisulfate. Local anesthetics include procaine hydrochloride. Suspending and dispersing agents include sodium carboxymethylcelluose, hydroxypropyl methylcellulose and polyvinylpyrrolidone. Emulsifying agents include

Polysorbate 80 (TWEEN® 80). A sequestering or chelating agent of metal ions includes EDTA. Pharmaceutical carriers also include ethyl alcohol, polyethylene glycol and propylene glycol for water miscible vehicles; and sodium hydroxide, hydrochloric acid, citric acid or lactic acid for pH adjustment.

[00178] A pharmaceutical composition may be formulated for any route of administration to a subject. Specific examples of routes of administration include intranasal, oral, pulmonary, transdermal, intradermal, and parenteral. Parenteral administration, characterized by either subcutaneous, intramuscular or intravenous injection, is also contemplated herein. Injectables can be prepared in conventional forms, either as liquid solutions or suspensions, solid forms suitable for solution or suspension in liquid prior to injection, or as emulsions. The injectables, solutions and emulsions also contain one or more excipients. Suitable excipients are, for example, water, saline, dextrose, glycerol or ethanol. In addition, if desired, the pharmaceutical compositions to be administered can also contain minor amounts of non-toxic auxiliary substances such as wetting or emulsifying agents, pH buffering agents, stabilizers, solubility enhancers, and other such agents, such as for example, sodium acetate, sorbitan monolaurate, triethanolamine oleate and cyclodextrins.

[00179] Preparations for parenteral administration of an antibody include sterile solutions ready for injection, sterile dry soluble products, such as lyophilized powders, ready to be combined with a solvent just prior to use, including hypodermic tablets, sterile suspensions ready for injection, sterile dry insoluble products ready to be combined with a vehicle just prior to use and sterile emulsions. The solutions may be either aqueous or nonaqueous.

[00180] If administered intravenously, suitable carriers include physiological saline or phosphate buffered saline (PBS), and solutions containing thickening and solubilizing agents, such as glucose, polyethylene glycol, and polypropylene glycol and mixtures thereof.

[00181] Topical mixtures comprising an antibody are prepared as described for the local and systemic administration. The resulting mixture can be a solution, suspension, emulsions or the like and can be formulated as creams, gels, ointments, emulsions, solutions, elixirs, lotions, suspensions, tinctures, pastes, foams, aerosols, irrigations, sprays, suppositories, bandages, dermal patches or any other formulations suitable for topical administration.

[00182] An anti-CTLA-4 antibody described herein can be formulated as an aerosol for topical application, such as by inhalation (see, *e.g.*, U.S. Patent Nos. 4,044,126, 4,414,209 and 4,364,923, which describe aerosols for delivery of a steroid useful for treatment of inflammatory diseases, particularly asthma and are herein incorporated by reference in their entireties). These formulations for administration to the respiratory tract can be in the form

of an aerosol or solution for a nebulizer, or as a microfine powder for insufflations, alone or in combination with an inert carrier such as lactose. In such a case, the particles of the formulation will, in one embodiment, have diameters of less than 50 microns, in one embodiment less than 10 microns.

[00183] An anti-CTLA-4 antibody described herein can be formulated for local or topical application, such as for topical application to the skin and mucous membranes, such as in the eye, in the form of gels, creams, and lotions and for application to the eye or for intracisternal or intraspinal application. Topical administration is contemplated for transdermal delivery and also for administration to the eyes or mucosa, or for inhalation therapies. Nasal solutions of the antibody alone or in combination with other pharmaceutically acceptable excipients can also be administered.

[00184] Transdermal patches, including iontophoretic and electrophoretic devices, are well known to those of skill in the art, and can be used to administer an antibody. For example, such patches are disclosed in U.S. Patent Nos. 6,267,983, 6,261,595, 6,256,533, 6,167,301, 6,024,975, 6,010715, 5,985,317, 5,983,134, 5,948,433, and 5,860,957, all of which are herein incorporated by reference in their entireties.

[00185] In certain embodiments, a pharmaceutical composition comprising an antibody or antigen-binding fragment thereof described herein is a lyophilized powder, which can be reconstituted for administration as solutions, emulsions and other mixtures. It may also be reconstituted and formulated as solids or gels. The lyophilized powder is prepared by dissolving an antibody or antigen-binding fragment thereof described herein, or a pharmaceutically acceptable derivative thereof, in a suitable solvent. In some embodiments, the lyophilized powder is sterile. The solvent may contain an excipient which improves the stability or other pharmacological component of the powder or reconstituted solution, prepared from the powder. Excipients that may be used include, but are not limited to, dextrose, sorbitol, fructose, corn syrup, xylitol, glycerin, glucose, sucrose or other suitable agent. The solvent may also contain a buffer, such as citrate, sodium or potassium phosphate or other such buffer known to those of skill in the art at, in one embodiment, about neutral pH. Subsequent sterile filtration of the solution followed by lyophilization under standard conditions known to those of skill in the art provides the desired formulation. In one embodiment, the resulting solution will be apportioned into vials for lyophilization. Each vial will contain a single dosage or multiple dosages of the compound. The lyophilized powder can be stored under appropriate conditions, such as at about 4°C to room temperature. Reconstitution of this lyophilized powder with water for injection provides a formulation for

use in parenteral administration. For reconstitution, the lyophilized powder is added to sterile water or other suitable carrier. The precise amount depends upon the selected compound. Such amount can be empirically determined.

[00186] The anti-CTLA-4 antibodies described herein and other compositions provided herein can also be formulated to be targeted to a particular tissue, receptor, or other area of the body of the subject to be treated. Many such targeting methods are well known to those of skill in the art. All such targeting methods are contemplated herein for use in the instant compositions. For non-limiting examples of targeting methods, see, e.g., U.S. Patent Nos. 6,316,652, 6,274,552, 6,271,359, 6,253,872, 6,139,865, 6,131,570, 6,120,751, 6,071,495, 6,060,082, 6,048,736, 6,039,975, 6,004,534, 5,985,307, 5,972,366, 5,900,252, 5,840,674, 5,759,542 and 5,709,874, all of which are herein incorporated by reference in their entireties. In a specific embodiment, an antibody or antigen-binding fragment thereof described herein is targeted to a tumor.

[00187] The compositions to be used for *in vivo* administration can be sterile. This is readily accomplished by filtration through, *e.g.,* sterile filtration membranes.

6.4 Methods of Use and Uses

[00188] In another aspect, the instant disclosure provides a method of treating a subject using the anti-CTLA-4 antibodies described herein. Any disease or disorder in a subject that would benefit from inhibition of CTLA-4 function can be treated using the anti-CTLA-4 antibodies described herein. The anti-CTLA-4 antibodies described herein are particularly useful for inhibiting immune system tolerance to tumors, and accordingly can be used as an immunotherapy for subjects with cancer. For example, in certain embodiments, the instant disclosure provides a method of increasing T-cell activation in response to an antigen in a subject, the method comprising administering to the subject an effective amount of an anti-CTLA-4 antibody or pharmaceutical composition thereof, as described herein. In certain embodiments, the instant disclosure provides a method of treating cancer in a subject, the method comprising administering to the subject an effective amount of the antibody or pharmaceutical composition, as described herein.

[0001] Cancers that can be treated with the antibodies, therapeutic combinations, or pharmaceutical compositions described herein include, without limitation, solid cancer (e.g., relapsed or refractory solid cancer, and advanced or metastatic solid cancer), carcinoma, sarcoma, melanoma (e.g., stage III or stage IV melanoma), small cell lung cancer, non-small cell lung cancer, urothelial cancer, ovarian cancer, prostate cancer (e.g., metastatic hormone-

refractory prostate cancer and progressive metastatic prostate cancer), pancreatic cancer, breast cancer (e.g., $HER2^+$ breast cancer (e.g., relapsed/refractory $HER2^+$ breast cancer)), head and neck cancer (e.g., relapsed/refractory head and neck squamous cell carcinoma (HNSCC)), glioma, malignant glioma, glioblastoma multiforme, brain metastasis, merkel cancer, gastric cancer, gastroesophageal cancer, renal cell carcinoma, uveal melanoma, colon cancer, cervical cancer, lymphoma (e.g., relapsed or refractory lymphoma), non-Hodgkin's lymphoma, Hodgkin's lymphoma, leukemia, and multiple myeloma. In certain embodiments, the cancer is treated with intratumoral administration of an antibody, therapeutic combination, or pharmaceutical composition described herein. Cancers that can be treated with intratumoral administration of the antibodies, therapeutic combinations, or pharmaceutical compositions described herein include, without limitation, solid tumors (e.g., advanced or metastatic solid tumors), head and neck cancer (e.g., relapsed/refractory head and neck squamous cell carcinoma (HNSCC)), and breast cancer (e.g., $HER2^+$ breast cancer (e.g., relapsed/refractory $HER2^+$ breast cancer)).

[0002] In certain embodiments, the cancer treated in accordance with the methods described herein is a solid tumor. In certain embodiments, the cancer treated in accordance with the methods described herein is a metastatic or locally advanced cancer (e.g., a metastatic or locally advanced solid tumor). In certain embodiments, the cancer is treated in accordance with a method described herein as a first cancer therapy after diagnosis of the metastatic or locally advanced tumor (e.g., within 1, 2, 3, 4, 5, or 6 days; 1, 2, 3, 4, 6, 8, or 12 weeks; or, 1, 2, 3, 4, 6, 8, or 12 months after diagnosis). In certain embodiments, the cancer is treated in accordance with a method described herein as the first cancer therapy after diagnosis of tumor progression (e.g., within 1, 2, 3, 4, 5, or 6 days; 1, 2, 3, 4, 6, 8, or 12 weeks; or, 1, 2, 3, 4, 6, 8, or 12 months after diagnosis of tumor progression) that has occurred despite previous treatment of the tumor with a different cancer therapy, optionally wherein the method described herein is provided as the second cancer therapy administered. In certain embodiments, the cancer is treated in accordance with a method described herein as the first cancer therapy after diagnosis of toxicity of a different cancer therapy (e.g., within 1, 2, 3, 4, 5, or 6 days; 1, 2, 3, 4, 6, 8, or 12 weeks; or, 1, 2, 3, 4, 6, 8, or 12 months after diagnosis of toxicity of the different cancer therapy), optionally wherein the method described herein is provided as the second cancer therapy administered. In certain embodiments, the cancer treated in accordance with the methods described herein is a metastatic or locally advanced cancer (e.g., solid tumor) for which no standard therapy is available. In other embodiments, the cancer treated in accordance with the methods

described herein is a metastatic or locally advanced cancer (e.g., solid tumor) for which a standard therapy has failed (*i.e.*, the cancer has progressed after the standard therapy). In certain embodiments, a therapy fails if the cancer is refractory to the therapy. In certain embodiments, a therapy fails if the cancer relapses after responding, fully or partially, to the therapy. In certain embodiments, metastatic or locally advanced cancer (e.g., solid tumor) has been confirmed histologically or cytologically.

[0003] In certain embodiments, the cancer is a solid tumor. In certain embodiments, the cancer (e.g., solid tumor) expresses PD-L1. In certain embodiments, the percentage of tumor cells in a sample of the cancer (e.g., solid tumor) that exhibit detectable expression (e.g., partial or complete expression) of PD-L1 is at least 1% (e.g., at least 2%, 3%, 4%, 5%, 10%, 15%, 20%, 25%, 30%, 35%, 40%, 45%, 50%, 60%, 70%, 80%, or 90%). In certain embodiments, the percentage of tumor cells in a sample of the cancer (e.g., solid tumor) that exhibit detectable membrane expression (e.g., partial or complete membrane expression) of PD-L1 is at least 1% (e.g., at least 2%, 3%, 4%, 5%, 10%, 15%, 20%, 25%, 30%, 35%, 40%, 45%, 50%, 60%, 70%, 80%, or 90%). In certain embodiments, the percentage of tumor cells in a sample of the cancer (e.g., solid tumor) that exhibit detectable membrane expression (e.g., partial or complete membrane expression) of PD-L1 is at least 1%. In certain embodiments, the percentage of tumor cells in a sample of the cancer (e.g., solid tumor) that exhibit detectable membrane expression (e.g., partial or complete membrane expression) of PD-L1 is at least 5%. In certain embodiments, the percentage of tumor cells in a sample of the cancer (e.g., solid tumor) that exhibit detectable membrane expression (e.g., partial or complete membrane expression) of PD-L1 is at least 25%. In certain embodiments, the percentage of tumor cells in a sample of the cancer (e.g., solid tumor) that exhibit detectable membrane expression (e.g., partial or complete membrane expression) of PD-L1 is at least 50%.

[0004] In certain embodiments, the metastatic or locally advanced cancer (e.g., solid tumor) expresses PD-L1. In certain embodiments, the percentage of tumor cells in a sample of the metastatic or locally advanced cancer (e.g., solid tumor) that exhibit detectable expression (e.g., partial or complete expression) of PD-L1 is at least 1% (e.g., at least 2%, 3%, 4%, 5%, 10%, 15%, 20%, 25%, 30%, 35%, 40%, 45%, 50%, 60%, 70%, 80%, or 90%). In certain embodiments, the percentage of tumor cells in a sample of the metastatic or locally advanced cancer (e.g., solid tumor) that exhibit detectable membrane expression (e.g., partial or complete membrane expression) of PD-L1 is at least 1% (e.g., at least 2%, 3%, 4%, 5%, 10%, 15%, 20%, 25%, 30%, 35%, 40%, 45%, 50%, 60%, 70%, 80%, or 90%). In certain

embodiments, the percentage of tumor cells in a sample of the metastatic or locally advanced cancer (e.g., solid tumor) that exhibit detectable membrane expression (e.g., partial or complete membrane expression) of PD-L1 is at least 1%. In certain embodiments, the percentage of tumor cells in a sample of the metastatic or locally advanced cancer (e.g., solid tumor) that exhibit detectable membrane expression (e.g., partial or complete membrane expression) of PD-L1 is at least 5%. In certain embodiments, the percentage of tumor cells in a sample of the metastatic or locally advanced cancer (e.g., solid tumor) that exhibit detectable membrane expression (e.g., partial or complete membrane expression) of PD-L1 is at least 25%. In certain embodiments, the percentage of tumor cells in a sample of the metastatic or locally advanced cancer (e.g., solid tumor) that exhibit detectable membrane expression (e.g., partial or complete membrane expression) of PD-L1 is at least 50%.

[0005] For each and every one of the methods described herein that requires a certain percentage of cells in a sample exhibit detectable expression (e.g., membrane expression, partial or complete membrane expression) of PD-L1, the expression of PD-L1 can be detected by any method well known in the art, including but not limited to immunohistochemistry. Exemplary immunohistochemistry assays for measuring PD-L1 expression in tumor cells are provided in Hirsch et al. (2017, J. Thoracic Oncol. 12(2): 208-222), Rimm et al. (2017, JAMA Oncol. 3(8): 1051-1058), and Diggs and Hsueh (2017, Biomarker Res. 5:12), which are incorporated by reference herein in their entirety.

[0006] In certain embodiments, the cancer treated in accordance with a method described herein is a metastatic or locally advanced non-small cell lung cancer (NSCLC). In certain embodiments, the cancer treated in accordance with a method described herein is a metastatic non-small cell lung cancer (NSCLC). In certain embodiments, the cancer treated in accordance with a method described herein is a Stage IV, metastatic or locally advanced NSCLC. In certain embodiments, the cancer treated in accordance with a method described herein is a Stage IV, metastatic NSCLC. In certain embodiments, the percentage of tumor cells in a sample of the metastatic or locally advanced NSCLC that exhibit detectable expression (e.g., partial or complete expression) of PD-L1 is at least 1%, 2%, 3%, 4%, 5%, 10%, 15%, 20%, 25%, 30%, 35%, 40%, 45%, 50%, 60%, 70%, 80%, or 90%. In certain embodiments, the percentage of tumor cells in a sample of the metastatic or locally advanced NSCLC that exhibit detectable membrane expression (e.g., partial or complete membrane expression) of PD-L1 is at least 1%, 2%, 3%, 4%, 5%, 10%, 15%, 20%, 25%, 30%, 35%, 40%, 45%, 50%, 60%, 70%, 80%, or 90%. In certain embodiments, the percentage of tumor cells in a sample of the metastatic or locally advanced NSCLC that exhibit detectable

membrane expression (e.g., partial or complete membrane expression) of PD-L1 is at least 1%. In certain embodiments, the percentage of tumor cells in a sample of the metastatic or locally advanced NSCLC that exhibit detectable membrane expression (e.g., partial or complete membrane expression) of PD-L1 is at least 5%. In certain embodiments, the percentage of tumor cells in a sample of the metastatic or locally advanced NSCLC that exhibit detectable membrane expression (e.g., partial or complete membrane expression) of PD-L1 is at least 25%. In certain embodiments, the percentage of tumor cells in a sample of the metastatic or locally advanced NSCLC that exhibit detectable membrane expression (e.g., partial or complete membrane expression) of PD-L1 is at least 50%. In certain embodiments, the metastatic or locally advanced NSCLC has no EGFR or ALK genomic tumor aberrations. In certain embodiments, the metastatic or locally advanced NSCLC has no EGFR sensitizing mutation (e.g., mutation that is amenable to treatment with a tyrosine kinase inhibitor including erlotinib, gefitinib, or afatanib) or ALK translocation. In certain embodiments, the subject having the metastatic or locally advanced NSCLC has received no prior systemic chemotherapy treatment for metastatic or locally advanced NSCLC. In certain embodiments, the metastatic or locally advanced NSCLC is treated in accordance with a method described herein as a first cancer therapy after diagnosis (e.g., within 1, 2, 3, 4, 5, or 6 days; 1, 2, 3, 4, 6, 8, or 12 weeks; or, 1, 2, 3, 4, 6, 8, or 12 months after diagnosis) of the metastatic or locally advanced NSCLC. In certain embodiments, the method comprises treating a subject having NSCLC (e.g., Stage IV, metastatic, or locally advanced NSCLC) using an anti-CTLA-4 antibody described herein, e.g., AGEN1884.H3 (IgG_1 S239D/A330L/I332E), or pharmaceutical composition comprising such anti-CTLA-4 antibody, wherein the percentage of tumor cells in a sample of the NSCLC that exhibit detectable membrane expression (e.g., partial or complete membrane expression) of PD-L1 is at least 1%, 2%, 3%, 4%, 5%, 10%, 15%, 20%, 25%, 30%, 35%, 40%, 45%, 50%, 60%, 70%, 80%, or 90%, and wherein the method is provided as a first cancer therapy after diagnosis of the cervical cancer (e.g., within 1, 2, 3, 4, 5, or 6 days; 1, 2, 3, 4, 6, 8, or 12 weeks; or, 1, 2, 3, 4, 6, 8, or 12 months after diagnosis).

[0007] In certain embodiments, the cancer treated in accordance with the methods described herein is a cervical cancer. In certain embodiments, the cancer treated in accordance with the methods described herein is a metastatic or locally advanced, unresectable squamous cell carcinoma, adenosquamous carcinoma, or adenocarcinoma of the cervix. In certain embodiments, the cancer treated in accordance with the methods described herein is an unresectable or metastatic cervical cancer. In certain embodiments, the cervical

cancer has progressed after a standard therapy (e.g., has relapsed after the standard therapy, or is refractory to the standard therapy). In certain embodiments, the standard therapy comprises a platinum-containing chemotherapy. In certain embodiments, the platinum-containing chemotherapy is selected from the group consisting of cisplatin, carboplatin, oxaliplatin, nedaplatin, satraplatin, picoplatin, triplatin, phenanthriplatin, iproplatin, lobapatin, heptaplatin, lipoplatin, and a combination thereof. In certain embodiments, the standard therapy further comprises a second chemotherapy. In certain embodiments, the second chemotherapy is selected from the group consisting of a nucleotide analog (e.g., gemcitabine), a folate antimetabolite (e.g., pemetrexed), and a taxane (e.g., paclitaxel). In certain embodiments, the standard therapy is any platinum-based doublet chemotherapy (PT-DC) (also known as platinum-containing doublet) known in the art. In certain embodiments, the PT-DC comprises cisplatin and gemcitabine, cisplatin and pemetrexed, cisplatin and paclitaxel, carboplatin and paclitaxel, or cisplatin and topotecan. The standard therapy (e.g., one comprising a PT-DC) can optionally further comprise one or more additional therapies, such as bevacizumab. In certain embodiments, the standard therapy comprises paclitaxel and topotecan. In certain embodiments, the cervical cancer is HPV positive. In certain embodiments, the cervical cancer is associated with microsatellite instability. In certain embodiments, the cancer treated in accordance with the methods described herein is a metastatic or locally advanced, unresectable squamous cell carcinoma, adenosquamous carcinoma, or adenocarcinoma of the cervix that has relapsed after a platinum-containing doublet administered for treatment of advanced (recurrent, unresectable, or metastatic) disease. In certain embodiments, the cancer of the cervix is treated in accordance with a method described herein as a first cancer therapy after diagnosis of the cervical cancer (e.g., within 1, 2, 3, 4, 5, or 6 days; 1, 2, 3, 4, 6, 8, or 12 weeks; or, 1, 2, 3, 4, 6, 8, or 12 months after diagnosis). In certain embodiments, the cancer of the cervix is treated in accordance with a method described herein as the first cancer therapy after diagnosis of tumor progression (e.g., within 1, 2, 3, 4, 5, or 6 days; 1, 2, 3, 4, 6, 8, or 12 weeks; or, 1, 2, 3, 4, 6, 8, or 12 months after diagnosis of tumor progression) that has occurred despite previous treatment of the cancer of the cervix with a different cancer therapy, optionally wherein the method described herein is provided as the second cancer therapy administered. In certain embodiments, the cancer of the cervix is treated in accordance with a method described herein as the first cancer therapy after diagnosis of toxicity of a different cancer therapy (e.g., within 1, 2, 3, 4, 5, or 6 days; 1, 2, 3, 4, 6, 8, or 12 weeks; or, 1, 2, 3, 4, 6, 8, or 12 months after diagnosis of toxicity of the different cancer therapy), optionally wherein the method

described herein is provided as the second cancer therapy administered. In certain embodiments, the method comprises treating a subject having cervical cancer (e.g., a metastatic or locally advanced, unresectable squamous cell carcinoma, adenosquamous carcinoma, or adenocarcinoma of the cervix) using an anti-CTLA-4 antibody described herein, e.g., AGEN1884.H3 (IgG_1 S239D/A330L/I332E), or pharmaceutical composition comprising such anti-CTLA-4 antibody, wherein the method is provided as a first cancer therapy after diagnosis of the cervical cancer (e.g., within 1, 2, 3, 4, 5, or 6 days; 1, 2, 3, 4, 6, 8, or 12 weeks; or, 1, 2, 3, 4, 6, 8, or 12 months after diagnosis). In certain embodiments, the method comprises treating a subject having cervical cancer (e.g., a metastatic or locally advanced, unresectable squamous cell carcinoma, adenosquamous carcinoma, or adenocarcinoma of the cervix) using an anti-CTLA-4 antibody described herein, e.g., AGEN1884.H3 (IgG_1 S239D/A330L/I332E), or pharmaceutical composition comprising such anti-CTLA-4 antibody, wherein the method is provided after diagnosis of tumor progression (e.g., within 1, 2, 3, 4, 5, or 6 days; 1, 2, 3, 4, 6, 8, or 12 weeks; or, 1, 2, 3, 4, 6, 8, or 12 months after diagnosis of tumor progression) that has occurred despite previous treatment of the cervical cancer with a different cancer therapy, or provided after diagnosis of toxicity of a different cancer therapy (e.g., within 1, 2, 3, 4, 5, or 6 days; 1, 2, 3, 4, 6, 8, or 12 weeks; or, 1, 2, 3, 4, 6, 8, or 12 months after diagnosis of toxicity of the different cancer therapy), and wherein the method described herein is provided as the second cancer therapy administered.

[0008] In certain embodiments, the cancer treated in accordance with the methods described herein is a cutaneous squamous-cell carcinoma (cSCC). In certain embodiments, the cancer treated in accordance with the methods described herein is a Stage IV cutaneous squamous-cell carcinoma (cSCC). In certain embodiments, the cSCC (e.g., Stage IV cSCC) is not curable with radiation therapy. In certain embodiments, the Stage IV cSCC is diagnosed histologically or cytologically according to the eighth edition of the American Joint Committee on Cancer staging manual (AJCC-8). In certain embodiments, the cSCC (e.g., Stage IV cSCC) is treated in accordance with a method described herein as a first cancer therapy after diagnosis of the cSCC (e.g., Stage IV cSCC) (e.g., within 1, 2, 3, 4, 5, or 6 days; 1, 2, 3, 4, 6, 8, or 12 weeks; or, 1, 2, 3, 4, 6, 8, or 12 months after diagnosis).. In certain embodiments, the cSCC (e.g., Stage IV cSCC) is treated in accordance with a method described herein as the first cancer therapy after diagnosis of tumor progression (e.g., within 1, 2, 3, 4, 5, or 6 days; 1, 2, 3, 4, 6, 8, or 12 weeks; or, 1, 2, 3, 4, 6, 8, or 12 months after diagnosis of tumor progression) that has occurred despite previous treatment of the cSCC

(e.g., Stage IV cSCC) with a different cancer therapy, optionally wherein the method described herein is provided as the second cancer therapy administered. In certain embodiments, the cSCC (e.g., Stage IV cSCC) is treated in according with a method described herein as the first cancer therapy after diagnosis of toxicity of a different cancer therapy (e.g., within 1, 2, 3, 4, 5, or 6 days; 1, 2, 3, 4, 6, 8, or 12 weeks; or, 1, 2, 3, 4, 6, 8, or 12 months after diagnosis of toxicity of the different cancer therapy), optionally wherein the method described herein is provided as the second cancer therapy administered.

[0009] In certain embodiments, the cancer treated in accordance with the methods described herein is B cell lymphoma (*e.g.*, B cell chronic lymphocytic leukemia, B cell non-Hodgkin lymphoma, cutaneous B cell lymphoma, diffuse large B cell lymphoma), basal cell carcinoma, bladder cancer, blastoma, brain metastasis, breast cancer, Burkitt lymphoma, carcinoma (*e.g.*, adenocarcinoma (*e.g.*, of the gastroesophageal junction)), cervical cancer, colon cancer, colorectal cancer (colon cancer and rectal cancer), endometrial carcinoma, esophageal cancer, Ewing sarcoma, follicular lymphoma, gastric cancer, gastroesophageal junction carcinoma, gastrointestinal cancer, glioblastoma (*e.g.*, glioblastoma multiforme, e.g., newly diagnosed or recurrent), glioma, head and neck cancer (*e.g.*, head and neck squamous cell carcinoma), hepatic metastasis, Hodgkin's and non-Hodgkin's lymphoma, kidney cancer (*e.g.*, renal cell carcinoma and Wilms' tumors), laryngeal cancer, leukemia (*e.g.*, chronic myelocytic leukemia, hairy cell leukemia), liver cancer (e.g., hepatic carcinoma and hepatoma), lung cancer (*e.g.*, non-small cell lung cancer and small-cell lung cancer), lymphblastic lymphoma, lymphoma, mantle cell lymphoma, metastatic brain tumor, metastatic cancer, myeloma (*e.g.*, multiple myeloma), neuroblastoma, ocular melanoma, oropharyngeal cancer, osteosarcoma, ovarian cancer, pancreatic cancer (*e.g.*, pancreatis ductal adenocarcinoma), prostate cancer (*e.g.*, hormone refractory (*e.g.*, castration resistant), metastatic, metastatic hormone refractory (*e.g.*, castration resistant, androgen independent)), renal cell carcinoma (*e.g.*, metastatic), salivary gland carcinoma, sarcoma (e.g., rhabdomyosarcoma), skin cancer (*e.g.*, melanoma (*e.g.*, metastatic melanoma)), soft tissue sarcoma, solid tumor, squamous cell carcinoma, synovia sarcoma, testicular cancer, thyroid cancer, transitional cell cancer (urothelial cell cancer), uveal melanoma (*e.g.*, metastatic), verrucous carcinoma, vulval cancer, and Waldenstrom macroglobulinemia.

[0010] In certain embodiments, the cancer treated in accordance with the methods described herein is human sarcoma or carcinoma, *e.g.*, fibrosarcoma, myxosarcoma, liposarcoma, chondrosarcoma, osteogenic sarcoma, chordoma, angiosarcoma, endotheliosarcoma, lymphangiosarcoma, lymphangioendotheliosarcoma, synovioma,

mesothelioma, Ewing's tumor, leiomyosarcoma, rhabdomyosarcoma, colon carcinoma, pancreatic cancer, breast cancer, ovarian cancer, prostate cancer, squamous cell carcinoma, basal cell carcinoma, adenocarcinoma, sweat gland carcinoma, sebaceous gland carcinoma, papillary carcinoma, papillary adenocarcinomas, cystadenocarcinoma, medullary carcinoma, bronchogenic carcinoma, renal cell carcinoma (e.g., metastatic), hepatoma, bile duct carcinoma, choriocarcinoma, seminoma, embryonal carcinoma, Wilms' tumor, cervical cancer, testicular tumor, lung carcinoma, small cell lung carcinoma, bladder carcinoma, epithelial carcinoma, glioma, glioblastoma multiforme, astrocytoma, medulloblastoma, craniopharyngioma, ependymoma, pinealoma, hemangioblastoma, acoustic neuroma, oligodendroglioma, meningioma, melanoma, neuroblastoma, or retinoblastoma.

[0011] In certain embodiments, the cancer treated in accordance with the methods described herein is angiosarcoma.

[0012] In certain embodiments, the cancer treated in accordance with the methods described herein is an acute lymphocytic leukemia or acute myelocytic leukemia (*e.g.*, myeloblastic, promyelocytic, myelomonocytic, monocytic and erythroleukemia); chronic leukemia (chronic myelocytic (granulocytic) leukemia or chronic lymphocytic leukemia); Hodgkin's disease; non-Hodgkin's disease; acute myeloid leukemia; B-cell lymphoma; T-cell lymphoma; anaplastic large cell lymphoma; intraocular lymphoma; follicular lymphoma; small intestine lymphoma; or orsplenic marginal zone lymphoma.

[0013] In certain embodiments, the cancer treated in accordance with the methods described herein is multiple myeloma, Waldenstrom's macroglobulinemia, heavy chain disease, gastrointestinal stromal tumors, head and/or neck cancer (*e.g.*, squamous cell carcinoma of the hypopharynx, squamous cell carcinoma of the larynx, cell carcinoma of the oropharynx, or verrucous carcinoma of the larynx), endometrial stromal sarcoma, mast cell sarcoma, adult soft tissue sarcoma, uterine sarcoma, merkel cell carcinoma, urothelial carcinoma, melanoma with brain metastases, uveal melanoma, uveal melanoma with liver metastases, non-small cell lung cancer, rectal cancer, or myelodysplastic syndrome. In some embodiments, the cancer treated in accordance with the methods is metastatic.

[0014] In certain embodiments, the cancer treated in accordance with the methods described herein is prostate cancer, breast cancer, lung cancer, colorectal cancer, melanoma, bronchial cancer, bladder cancer, brain or central nervous system cancer, peripheral nervous system cancer, uterine or endometrial cancer, cancer of the oral cavity or pharynx, non-Hodgkin’s lymphoma, thyroid cancer, kidney cancer, biliary tract cancer, small bowel or appendix cancer, salivary gland cancer, thyroid gland cancer, adrenal gland cancer, squamous

cell cancer, mesothelioma, osteocarcinoma, thyoma/thymic carcinoma, glioblastoma, myelodysplastic syndrome, soft tissue sarcoma, DIPG, adenocarcinoma, osteosarcoma, chondrosarcoma, leukemia, or pancreatic cancer. In some embodiments, the cancer treated in accordance with the methods described herein includes a carcinoma (*e.g.*, an adenocarcinoma), lymphoma, blastoma, melanoma, sarcoma, or leukemia.

[0015] In certain embodiments, the cancer treated in accordance with the methods described herein is squamous cell cancer, small-cell lung cancer, non-small cell lung cancer, gastrointestinal cancer, Hodgkin's lymphoma, non-Hodgkin's lymphoma, pancreatic cancer, glioblastoma, glioma, cervical cancer, ovarian cancer, liver cancer (*e.g.*, hepatic carcinoma and hepatoma), bladder cancer, breast cancer, inflammatory breast cancer, Merkel cell carcinoma, colon cancer, colorectal cancer, stomach cancer, urinary bladder cancer, endometrial carcinoma, myeloma (*e.g.*, multiple myeloma), salivary gland, carcinoma, kidney cancer (*e.g.*, renal cell carcinoma and Wilms' tumors), basal cell carcinoma, melanoma, prostate cancer, vulval cancer, thyroid cancer, testicular cancer, esophageal cancer, serous adenocarcinoma or various types of head and neck cancer. In certain embodiments, the cancer treated in accordance with the methods described herein includes desmoplastic melanoma, inflammatory breast cancer, thymoma, rectal cancer, anal cancer, or surgically treatable or non-surgically treatable brain stem glioma. In a specific embodiment, the cancer is a solid tumor. In another specific embodiment, the cancer is glioblastoma multiforme. In some embodiments, the glioblastoma multiforme is recurrent. In some embodiments, the glioblastoma multiforme is newly diagnosed. In some embodiments, the glioblastoma multiforme is in a subject having non-methylated MGMT promoters. In some embodiments, the glioblastoma multiforme is refractory to Bevacizumab therapy. In some embodiments, the glioblastoma multiforme is in a subject that has not received Bevacizumab therapy.

[0016] In certain embodiments, the cancer treated in accordance with the methods described herein is metastatic melanoma (e.g., resistant metastatic melanoma), metastatic ovarian cancer, or metastatic renal cell carcinoma. In certain embodiments, the cancer treated in accordance with the methods described herein is melanoma that is resistant to Ipilimumab. In some embodiments, the cancer treated in accordance with the methods described herein is melanoma that is resistant to Nivolumab or Pembrolizumab. In some embodiments, the cancer treated in accordance with the methods described herein is melanoma that is resistant to Ipilimumab and Nivolumab or Pembrolizumab.

[00189] In certain embodiments, the cancer treated in accordance with the methods described herein is breast cancer (*e.g.*, herceptin resistant breast cancer and trastuzumab-DM1

(T-DM1) resistant breast cancer), prostate cancer, glioblastoma multiforme, colorectal cancer, sarcoma, bladder cancer, cervical cancer, HPV-associated cancers, cancers of the vagina, cancers of the vulva, cancers of the penis, cancer of the anus, cancer of the rectum, cancer of the oropharynx, multiple myeloma, renal cell carcinoma, ovarian cancer, hepatocellular cancer, endometrial cancer, pancreatic cancer, lymphoma, and leukemia (*e.g.*, elderly leukemia, acute myeloid leukemia (AML), and elderly AML).

[00190] In certain embodiments, the cancer treated in accordance with the methods described herein is metastatic malignant melanoma (e.g., cutaneous or intraocular malignant melanoma), renal cancer (*e.g.*, clear cell carcinoma), prostate cancer (*e.g.*, hormone refractory prostate adenocarcinoma), breast cancer, colon cancer, lung cancer (*e.g.*, non-small cell lung cancer), bone cancer, pancreatic cancer, skin cancer, cancer of the head or neck, uterine cancer, ovarian cancer, rectal cancer, cancer of the anal region, stomach cancer, testicular cancer, uterine cancer, carcinoma of the fallopian tubes, carcinoma of the endometrium, carcinoma of the cervix, carcinoma of the vagina, carcinoma of the vulva, cancer of the esophagus, cancer of the small intestine, cancer of the endocrine system, cancer of the thyroid gland, cancer of the parathyroid gland, cancer of the adrenal gland, sarcoma of soft tissue, cancer of the urethra, cancer of the penis, chronic or acute leukemias including acute myeloid leukemia, chronic myeloid leukemia, acute lymphoblastic leukemia, chronic lymphocytic leukemia, solid tumors of childhood, lymphocytic lymphoma, cancer of the bladder, cancer of the kidney or ureter, carcinoma of the renal pelvis, neoplasm of the central nervous system (CNS), primary CNS lymphoma, tumor angiogenesis, spinal axis tumor, brain stem glioma, glioma, pituitary adenoma, Kaposi's sarcoma, epidermoid cancer, squamous cell cancer, T-cell lymphoma, environmentally induced cancers including those induced by asbestos, esophageal cancer, liver cancer, refractory or recurrent malignancies, metastatic cancers, cancers that express PD-L1, and combinations of said cancers.

[00191] In certain embodiments, the subject has previously received an immunotherapy. In certain embodiments, the subject has not previously received any immunotherapy. In certain embodiments, the cancer is an advanced or metastatic cancer.

[00192] In certain embodiments, the instant disclosure provides a method of preventing or treating an infectious disease in a subject, the method comprising administering to the subject an effective amount of an anti-CTLA-4 antibody or pharmaceutical composition thereof, as described herein. In one embodiment, provided herein are methods for preventing and/or treating an infection (*e.g.*, a viral infection, a bacterial infection, a fungal infection, a protozoal infection, or a parasitic infection). The infection prevented and/or treated in

accordance with the methods can be caused by an infectious agent identified herein. In a specific embodiment, an anti-CTLA-4 antibody described herein or a composition thereof is the only active agent administered to a subject. In some embodiments, an anti-CTLA-4 antibody described herein or a composition thereof is used in combination with anti-infective interventions (e.g., antivirals, antibacterials, antifungals, or anti-helminthics) for the treatment of infectious diseases.

[00193] Infectious diseases that can be treated and/or prevented by anti-CTLA-4 antibodies or pharmaceutical compositions described herein are caused by infectious agents including but not limited to bacteria, parasites, fungi, protozae, and viruses. In a specific embodiment, the infectious disease treated and/or prevented by anti-CTLA-4 antibodies or pharmaceutical compositions described herein is caused by a virus. Viral diseases or viral infections that can be prevented and/or treated in accordance with the methods described herein include, but are not limited to, those caused by hepatitis type A, hepatitis type B, hepatitis type C, influenza (*e.g.*, influenza A or influenza B), varicella, adenovirus, herpes simplex type I (HSV-I), herpes simplex type II (HSV-II), rinderpest, rhinovirus, echovirus, rotavirus, respiratory syncytial virus, papilloma virus, papova virus, cytomegalovirus, echinovirus, arbovirus, huntavirus, coxsackie virus, mumps virus, measles virus, rubella virus, polio virus, small pox, Epstein Barr virus, human immunodeficiency virus type I (HIV-I), human immunodeficiency virus type II (HIV-II), and agents of viral diseases such as viral meningitis, encephalitis, dengue or small pox.

[00194] Bacterial infections that can be prevented and/or treated include infections caused by Escherichia coli, Klebsiella pneumoniae, Staphylococcus aureus, Enterococcus faecalis, Proteus vulgaris, Staphylococcus viridans, and Pseudomonas aeruginosa. Bacterial diseases caused by bacteria (e.g., Escherichia coli, Klebsiella pneumoniae, Staphylococcus aureus, Enterococcus faecalis, Proteus vulgaris, Staphylococcus viridans, and Pseudomonas aeruginosa) that can be prevented and/or treated in accordance with the methods described herein include, but are not limited to, Mycobacteria rickettsia, Mycoplasma, Neisseria, S. pneumonia, Borrelia burgdorferi (Lyme disease), Bacillus antracis (anthrax), tetanus, Streptococcus, Staphylococcus, mycobacterium, pertissus, cholera, plague, diptheria, chlamydia, S. aureus and legionella.

[00195] Protozoal diseases or protozoal infections caused by protozoa that can be prevented and/or treated in accordance with the methods described herein include, but are not limited to, leishmania, coccidiosis, trypanosoma schistosoma or malaria. Parasitic diseases or parasitic infections caused by parasites that can be prevented and/or treated in accordance

with the methods described herein include, but are not limited to, chlamydia and rickettsia.

[00196] Fungal diseases or fungal infections that can be prevented and/or treated in accordance with the methods described herein include, but are not limited to, those caused by *Candida* infections, zygomycosis, *Candida* mastitis, progressive disseminated trichosporonosis with latent trichosporonemia, disseminated candidiasis, pulmonary paracoccidioidomycosis, pulmonary aspergillosis, *Pneumocystis carinii* pneumonia, cryptococcal meningitis, *coccidioidal* meningoencephalitis and cerebrospinal vasculitis, *Aspergillus niger* infection, *Fusarium keratitis*, paranasal sinus mycoses, *Aspergillus fumigatus* endocarditis, tibial dyschondroplasia, *Candida glabrata* vaginitis, oropharyngeal candidiasis, X-linked chronic granulomatous disease, tinea pedis, cutaneous candidiasis, mycotic placentitis, disseminated trichosporonosis, allergic bronchopulmonary aspergillosis, mycotic keratitis, *Cryptococcus neoformans* infection, fungal peritonitis, *Curvularia geniculata* infection, staphylococcal endophthalmitis, sporotrichosis, and dermatophytosis.

[00197] In certain embodiments, the infectious disease is acute. In certain embodiments, the infectious disease is chronic. In certain embodiments, the infectious disease is caused by flavivirus, e.g., West Nile virus, *Saint Louis encephalitis* virus, Powassan virus, tick-borne encephalitis virus, dengue virus, zika virus, Kyasanur Forest disease virus, yellow fever virus, and chikungunya virus. In certain embodiments, the infectious disease is caused by Ebola virus. In certain embodiments, the infectious disease is caused by influenza virus. In certain embodiments, the infectious disease is caused by Human Immunodeficiency Virus (HIV), Hepatitis B virus (HBV) or Hepatitis C virus (HCV). In certain embodiments, the anti-CTLA-4 antibody or pharmaceutical composition thereof, as described herein, promotes viral control. In certain embodiments, the anti-CTLA-4 antibody or pharmaceutical composition thereof, as described herein, eliminates viral reservoirs.

[00198] The present invention relates in one aspect to an anti-CTLA-4 antibody of the invention and/or a pharmaceutical composition of the invention comprising an anti-CTLA-4 antibody of the invention and a pharmaceutically acceptable carrier or excipient, for use as a medicament.

[00199] The present invention relates, in one aspect, to an anti-CTLA-4 antibody of the invention, and/or its use in combination with pharmaceutically acceptable carriers or excipients, for preparing pharmaceutical compositions or medicaments for immunotherapy (e.g., an immunotherapy for increasing T-cell activation in response to an antigen in a subject, treating cancer, or treating or preventing infectious diseases).

[00200] The present invention relates in one aspect to an anti-CTLA-4 antibody of the

invention and/or a pharmaceutical composition of the invention comprising an anti-CTLA-4 antibody of the invention and a pharmaceutically acceptable carrier or excipient, for use in a method for the treatment of cancer.

[00201] The present invention relates in one aspect to an anti-CTLA-4 antibody of the invention and/or a pharmaceutical composition of the invention comprising an anti-CTLA-4 antibody of the invention and a pharmaceutically acceptable carrier or excipient, for use in a method for inhibiting immune system tolerance to tumors and/or for immunotherapy for subjects with cancer.

[00202] The present invention relates in one aspect to an anti-CTLA-4 antibody of the invention and/or a pharmaceutical composition of the invention comprising an anti-CTLA-4 antibody of the invention and a pharmaceutically acceptable carrier or excipient, for use in a method for the treatment of an infectious disease.

[00203] In certain embodiments, the anti-CTLA-4 antibody or pharmaceutical composition described herein is administered as a monotherapy.

[00204] In certain embodiments, these methods further comprise administering an additional therapeutic agent to the subject. In certain embodiments, the additional therapeutic agent is a chemotherapeutic or a checkpoint targeting agent. In certain embodiments, the checkpoint targeting agent is selected from the group consisting of an antagonist anti-PD-1 antibody, an antagonist anti-PD-L1 antibody, an antagonist anti-PD-L2 antibody, an antagonist anti-CTLA-4 antibody, an antagonist anti-TIM-3 antibody, an antagonist anti-LAG-3 antibody, an antagonist anti-CEACAM1 antibody, an agonist anti-GITR antibody, an agonist anti-OX40 antibody, an agonist anti-CD137 antibody, an agonist anti-DR3 antibody, an agonist anti-TNFSF14 antibody, and an agonist anti-CD27 antibody. In certain embodiments, the checkpoint targeting agent is an antagonist anti-PD-1 antibody. In certain embodiments, the checkpoint targeting agent is an antagonist anti-PD-L1 antibody. In certain embodiments, the checkpoint targeting agent is an antagonist anti-LAG-3 antibody. In certain embodiments, the additional therapeutic agent is an agonist to a tumor necrosis factor receptor superfamily member or a tumor necrosis factor superfamily member.

[00205] In certain embodiments, the present invention relates to (a) an anti-CTLA-4 antibody of the invention and/or a pharmaceutical composition of the invention comprising an anti-CTLA-4 antibody of the invention and a pharmaceutically acceptable carrier or excipient and (b) an additional therapeutic agent, for use as a medicament. In a preferred embodiment, the additional therapeutic agent is a chemotherapeutic or a checkpoint targeting agent.

[00206] In certain embodiments, the present invention relates to (a) an anti-CTLA-4 antibody of the invention and/or a pharmaceutical composition of the invention comprising an anti-CTLA-4 antibody of the invention and a pharmaceutically acceptable carrier or excipient and (b) an additional therapeutic agent, for use in a method for the treatment of cancer.

[00207] In certain embodiments, the present invention relates to (a) an anti-CTLA-4 antibody of the invention and/or a pharmaceutical composition of the invention comprising an anti-CTLA-4 antibody of the invention and a pharmaceutically acceptable carrier or excipient and (b) an additional therapeutic agent, for use in a method for the treatment of an infectious disease.

[00208] In certain embodiments, an anti-CTLA-4 antibody described herein is administered to a subject in combination with a compound that targets an immunomodulatory enzyme(s) such as IDO (indoleamine-(2,3)-dioxygenase) and/or TDO (tryptophan 2,3-dioxygenase). In certain embodiments, such compound is selected from the group consisting of epacadostat (Incyte Corp; see, e.g., WO 2010/005958 which is incorporated by reference herein in its entirety), F001287 (Flexus Biosciences), indoximod (NewLink Genetics), and NLG919 (NewLink Genetics). In one embodiment, the compound is epacadostat. In another embodiment, the compound is F001287. In another embodiment, the compound is indoximod. In another embodiment, the compound is NLG919.

[00209] In certain embodiments, the present invention relates to (a) an anti-CTLA-4 antibody of the invention and/or a pharmaceutical composition of the invention comprising an anti-CTLA-4 antibody of the invention and a pharmaceutically acceptable carrier or excipient and (b) a compound that targets an immunomodulatory enzyme, for use as a medicament. In a preferred embodiment, the compound targets IDO and/or TDO.

[00210] In certain embodiments, the present invention relates to (a) an anti-CTLA-4 antibody of the invention and/or a pharmaceutical composition of the invention comprising an anti-CTLA-4 antibody of the invention and a pharmaceutically acceptable carrier or excipient and (b) a compound that targets an immunomodulatory enzyme, for use in a method for the treatment of cancer. In a preferred embodiment, the compound targets IDO and/or TDO.

[00211] In certain embodiments, an anti-CTLA-4 antibody described herein is administered to a subject in combination with a vaccine. In certain embodiments, the vaccine is a heat shock protein based tumor vaccine or a heat shock protein based pathogen vaccine. In a specific embodiment, an anti-CTLA-4 antibody described herein is administered to a

subject in combination with a heat shock protein based tumor-vaccine. Heat shock proteins (HSPs) are a family of highly conserved proteins found ubiquitously across all species. Their expression can be powerfully induced to much higher levels as a result of heat shock or other forms of stress, including exposure to toxins, oxidative stress or glucose deprivation. Five families have been classified according to molecular weight: HSP-110, -90, -70, -60 and -28. HSPs deliver immunogenic peptides through the cross-presentation pathway in antigen presenting cells (APCs) such as macrophages and dendritic cells (DCs), leading to T cell activation. HSPs function as chaperone carriers of tumor-associated antigenic peptides forming complexes able to induce tumor-specific immunity. Upon release from dying tumor cells, the HSP-antigen complexes are taken up by antigen-presenting cells (APCs) wherein the antigens are processed into peptides that bind MHC class I and class II molecules leading to the activation of anti-tumor CD8+ and CD4+ T cells. The immunity elicited by HSP complexes derived from tumor preparations is specifically directed against the unique antigenic peptide repertoire expressed by the cancer of each subject.

[00212] A heat shock protein peptide complex (HSPPC) is a protein peptide complex consisting of a heat shock protein non-covalently complexed with antigenic peptides. HSPPCs elicit both innate and adaptive immune responses. In a specific embodiment, the antigenic peptide(s) displays antigenicity for the cancer being treated. HSPPCs are efficiently seized by APCs via membrane receptors (mainly CD91) or by binding to Toll-like receptors. HSPPC internalization results in functional maturation of the APCs with chemokine and cytokine production leading to activation of natural killer cells (NK), monocytes and Th1 and Th-2-mediated immune responses. In certain embodiments, HSPPCs used in methods described herein comprise one or more heat shock proteins from the hsp60, hsp70, or hsp90 family of stress proteins complexed with antigenic peptides. In certain embodiments, HSPPCs comprise hsc70, hsp70, hsp90, hsp110, grp170, gp96, calreticulin, or combinations of two or more thereof.

[00213] In a specific embodiment, an anti-CTLA-4 antibody described herein is administered to a subject in combination with a heat shock protein peptide complex (HSPPC), *e.g.*, heat shock protein peptide complex-96 (HSPPC-96), to treat cancer. HSPPC-96 comprises a 96 kDa heat shock protein (Hsp), gp96, complexed to antigenic peptides. HSPPC-96 is a cancer immunotherapy manufactured from a subject's tumor and contains the cancer's antigenic "fingerprint." In certain embodiments, this fingerprint contains unique antigens that are present only in that particular subject's specific cancer cells and injection of the vaccine is intended to stimulate the subject's immune system to recognize and attack any

cells with the specific cancer fingerprint.

[00214] In certain embodiments, the HSPPC, *e.g.*, HSPPC-96, is produced from the tumor tissue of a subject. In a specific embodiment, the HSPPC (*e.g.*, HSPPC-96) is produced from a tumor of the type of cancer or metastasis thereof being treated. In another specific embodiment, the HSPPC (*e.g.*, HSPPC-96) is autologous to the subject being treated. In certain embodiments, the tumor tissue is non-necrotic tumor tissue. In certain embodiments, at least 1 gram (*e.g.*, at least 1, at least 2, at least 3, at least 4, at least 5, at least 6, at least 7, at least 8, at least 9, or at least 10 grams) of non-necrotic tumor tissue is used to produce a vaccine regimen. In certain embodiments, after surgical resection, non-necrotic tumor tissue is frozen prior to use in vaccine preparation. In some embodiments, the HSPPC, *e.g.*, HSPPC-96, is isolated from the tumor tissue by purification techniques, filtered and prepared for an injectable vaccine. In certain embodiments, a subject is administered 6-12 doses of the HSPPC, *e.g.*, HSPCC-96. In such embodiments, the HSPPC, *e.g.*, HSPPC-96, doses may be administered weekly for the first 4 doses and then biweekly for the 2-8 additional doses.

[00215] Further examples of HSPPCs that may be used in accordance with the methods described herein are disclosed in the following patents and patent applications, which are incorporated herein by reference herein in their entireties, U.S. Patent Nos. 6,391,306, 6,383,492, 6,403,095, 6,410,026, 6,436,404, 6,447,780, 6,447,781 and 6,610,659, all of which are herein incorporated by reference in their entireties.

[00216] In certain embodiments, the present invention relates to (a) an anti-CTLA-4 antibody of the invention and/or a pharmaceutical composition of the invention comprising an anti-CTLA-4 antibody of the invention and a pharmaceutically acceptable carrier or excipient and (b) a vaccine, for use as a medicament. In a preferred embodiment, the vaccine is a heat shock protein based tumor vaccine or a heat shock protein based pathogen vaccine. In a preferred embodiment, the vaccine is a heat shock protein based viral vaccine.

[00217] In certain embodiments, the present invention relates to (a) an anti-CTLA-4 antibody of the invention and/or a pharmaceutical composition of the invention comprising an anti-CTLA-4 antibody of the invention and a pharmaceutically acceptable carrier or excipient and (b) a vaccine, for use in a method for the treatment of cancer. In a preferred embodiment, the vaccine is a heat shock protein based tumor vaccine.

[00218] The anti-CTLA-4 antibody and the additional therapeutic agent (e.g., chemotherapeutic, checkpoint targeting agent, IDO inhibitor, and/or vaccine) can be administered separately, sequentially or concurrently as separate dosage forms. In one embodiment, an anti-CTLA-4 antibody is administered parenterally, and an IDO inhibitor is

administered orally.

[00219] In certain embodiments, an anti-CTLA-4 antibody described herein is administered to a subject intratumorally. In certain embodiments, an anti-CTLA-4 antibody described herein is administered to a subject intratumorally in combination with an additional therapeutic agent. In certain embodiments, the additional therapeutic agent is administered systemically. In certain embodiments, the subject has solid tumors. In certain embodiments, the subject has head and neck squamous cell carcinoma (HNSCC). In certain embodiments, the subject has $HER2^+$ breast cancer. In certain embodiments, the additional therapeutic agent that is administered systemically is an anti-PD-1 antibody (e.g., pembrolizumab or nivolumab). In certain embodiments, the additional therapeutic agent that is administered systemically is an anti-EGFR antibody (e.g., cetuximab). In certain embodiments, the additional therapeutic agent that is administered systemically is an anti-HER2 antibody (e.g., trastuzumab). In certain embodiments, the additional therapeutic agent that is administered systemically is a chemotherapeutic agent (e.g., gemcitabine). In certain embodiments, the subject has solid tumors and the additional therapeutic agent that is administered systemically is an anti-PD-1 antibody (e.g., pembrolizumab or nivolumab). In certain embodiments, the anti-PD-1 antibody is pembrolizumab administered at 200 mg every three weeks. In certain embodiments, the subject has head and neck squamous cell carcinoma (HNSCC) and the additional therapeutic agent that is administered systemically is an anti-EGFR antibody (e.g., cetuximab). In certain embodiments, the subject has $HER2^+$ breast cancer and the additional therapeutic agent that is administered systemically is an anti-HER2 antibody (e.g., trastuzumab). In certain embodiments, the subject further received a chemotherapeutic agent (e.g., gemcitabine). In one aspect, the present invention relates to an anti-CTLA-4 antibody and/or pharmaceutical composition of the present invention, and optionally an additional therapeutic agent, for use in a method for the treatment of cancer, wherein the anti-CTLA-4 antibody and/or pharmaceutical composition of the present invention is administered intratumorally to the subject. In one preferred embodiment, an additional therapeutic agent is administered to the subject, more preferably, an additional therapeutic agent is administered systemically to the subject.

[00220] In certain embodiments, an anti-PD-1 antibody is used in methods described herein. In certain embodiments, the anti-PD-1 antibody is Nivolumab, also known as BMS-936558 or MDX1106, developed by Bristol-Myers Squibb. In certain embodiments, the anti-PD-1 antibody is Pembrolizumab, also known as Lambrolizumab or MK-3475, developed by Merck & Co. In certain embodiments, the anti-PD-1 antibody is Pidilizumab, also known as

CT-011, developed by CureTech. In certain embodiments, the anti-PD-1 antibody is MEDI0680, also known as AMP-514, developed by Medimmune. In certain embodiments, the anti-PD-1 antibody is PDR001 developed by Novartis Pharmaceuticals. In certain embodiments, the anti-PD-1 antibody is REGN2810 developed by Regeneron Pharmaceuticals. In certain embodiments, the anti-PD-1 antibody is PF-06801591 developed by Pfizer. In certain embodiments, the anti-PD-1 antibody is BGB-A317 developed by BeiGene. In certain embodiments, the anti-PD-1 antibody is TSR-042 developed by AnaptysBio and Tesaro. In certain embodiments, the anti-PD-1 antibody is SHR-1210 developed by Hengrui.

[00221] Further non-limiting examples of anti-PD-1 antibodies that may be used in treatment methods described herein are disclosed in the following patents and patent applications, which are incorporated herein by reference in their entireties for all purposes: U.S. Patent No. 6,808,710; U.S. Patent No. 7,332,582; U.S. Patent No. 7,488,802; U.S. Patent No. 8,008,449; U.S. Patent No. 8,114,845; U.S. Patent No. 8,168,757; U.S. Patent No. 8,354,509; U.S. Patent No. 8,686,119; U.S. Patent No. 8,735,553; U.S. Patent No. 8,747,847; U.S. Patent No. 8,779,105; U.S. Patent No. 8,927,697; U.S. Patent No. 8,993,731; U.S. Patent No. 9,102,727; U.S. Patent No. 9,205,148; U.S. Publication No. US 2013/0202623 A1; U.S. Publication No. US 2013/0291136 A1; U.S. Publication No. US 2014/0044738 A1; U.S. Publication No. US 2014/0356363 A1; U.S. Publication No. US 2016/0075783 A1; and PCT Publication No. WO 2013/033091 A1; PCT Publication No. WO 2015/036394 A1; PCT Publication No. WO 2014/179664 A2; PCT Publication No. WO 2014/209804 A1; PCT Publication No. WO 2014/206107 A1; PCT Publication No. WO 2015/058573 A1; PCT Publication No. WO 2015/085847 A1; PCT Publication No. WO 2015/200119 A1; PCT Publication No. WO 2016/015685 A1; and PCT Publication No. WO 2016/020856 A1.

[00222] In certain embodiments, an anti-PD-L1 antibody is used in methods described herein. In certain embodiments, the anti-PD-L1 antibody is atezolizumab developed by Genentech. In certain embodiments, the anti-PD-L1 antibody is durvalumab developed by AstraZeneca, Celgene and Medimmune. In certain embodiments, the anti-PD-L1 antibody is avelumab, also known as MSB0010718C, developed by Merck Serono and Pfizer. In certain embodiments, the anti-PD-L1 antibody is MDX-1105 developed by Bristol-Myers Squibb. In certain embodiments, the anti-PD-L1 antibody is AMP-224 developed by Amplimmune and GSK.

[00223] Non-limiting examples of anti-PD-L1 antibodies that may be used in treatment methods described herein are disclosed in the following patents and patent applications,

which are incorporated herein by reference in their entireties for all purposes: US Patent No. 7,943,743; US Patent No. 8,168,179; US Patent No. 8,217,149; U.S. Patent No. 8,552,154; U.S. Patent No. 8,779,108; U.S. Patent No. 8,981,063; U.S. Patent No. 9,175,082; U.S. Publication No. US 2010/0203056 A1; U.S. Publication No. US 2003/0232323 A1; U.S. Publication No. US 2013/0323249 A1; U.S. Publication No. US 2014/0341917 A1; U.S. Publication No. US 2014/0044738 A1; U.S. Publication No. US 2015/0203580 A1; U.S. Publication No. US 2015/0225483 A1; U.S. Publication No. US 2015/0346208 A1; U.S. Publication No. US 2015/0355184 A1; and PCT Publication No. WO 2014/100079 A1; PCT Publication No. WO 2014/022758 A1; PCT Publication No. WO 2014/055897 A2; PCT Publication No. WO 2015/061668 A1; PCT Publication No. WO 2015/109124 A1; PCT Publication No. WO 2015/195163 A1; PCT Publication No. WO 2016/000619 A1; and PCT Publication No. WO 2016/030350 A1.

[00224] In certain embodiments, an anti-LAG-3 antibody is used in methods described herein. In certain embodiments, the anti-LAG-3 antibody is BMS-986016 developed by Bristol-Myers Squibb. In certain embodiments, the anti-LAG-3 antibody is LAG525 developed by Novartis. In certain embodiments, the anti-LAG-3 antibody is GSK2831781 developed by GSK.

[00225] Non-limiting examples of anti-LAG-3 antibodies that may be used in treatment methods described herein are disclosed in the following patents and patent applications, which are incorporated herein by reference in their entireties for all purposes: US Patent No. 9,244,059; U.S. Publication No. US 2011/0150892 A1; U.S. Publication No. US 2014/0093511 A1; U.S. Publication No. US 2014/0286935 A1; U.S. Publication No. US 2015/0259420 A1; and PCT Publication No. WO 2015/042246 A1; PCT Publication No. WO 2015/116539 A1; PCT Publication No. WO 2015/200119 A1; and PCT Publication No. WO 2016/028672 A1.

[00226] In certain embodiments, an anti-EGFR antibody is used in methods described herein. In certain embodiments, the anti-EGFR antibody is cetuximab developed by Bristol-Myers Squibb and ImClone, panitumumab developed by Abgenix and Amgen, nimotuzumab developed by CMI Cuba and YM BioSciences, necitumumab developed by ImClone, zalutumumab developed by Genmab, matuzumab developed by Takeda, Sym004 developed by Merck Serono and Symphogen, imgatuzumab developed by Glycart and Roche, duligotumab developed by Genentech and Roche, depatuxizumab developed by Abbott, depatuxizumab mafodotin developed by Abbvie, MM-151 developed by Adimab and Merrimack, GC1118 developed by Green Cross, AMG 595 developed by Amgen and

ImmunoGen, CetuGEX developed by Glycotope, laprituximab emtansine developed by ImmunoGen, JNJ-61186372 developed by Genmab and Janssen Biotech, SCT200 developed by Sinocelltech, LY3164530 developed by Lilly, HLX07 developed by Shanghai Henlius, or SYN004 developed by Synermore.

[00227] In certain embodiments, an anti-HER2 antibody is used in methods described herein. In certain embodiments, the anti-HER2 antibody is trastuzumab developed by Genentech and Roche, trastuzumab emtansine developed by Genentech and Roche, pertuzumab developed by Genentech, ertumaxomab developed by Fresenius, margetuximab developed by MacroGenics, MM-111 developed by Merrimack, CT-P06 developed by Celltrion, PF-05280014 developed by Pfizer, MM-302 developed by Merrimack, SB3 developed by Merck & Co, CMAB302 developed by Shanghai CP Guojian, TrasGEX developed by Glycotope, ARX788 developed by Ambrx and Zhejiang Medicine, SYD985 developed by Synthon, FS102 developed by Bristol-Myers Squibb and f-star, BCD-022 developed by Biocad, ABP 980 developed by Amgen, DS-8201a developed by Daiichi Sankyo, HLX02 developed by Shanghai Henlius, or CANMAb developed by Biocon and Mylan.

[00228] An antibody or pharmaceutical composition described herein may be delivered to a subject by a variety of routes. These include, but are not limited to, parenteral, intranasal, intratracheal, oral, intradermal, topical, intramuscular, intraperitoneal, transdermal, intravenous, intratumoral, conjunctival and subcutaneous routes. Pulmonary administration can also be employed, *e.g.*, by use of an inhaler or nebulizer, and formulation with an aerosolizing agent for use as a spray. In certain embodiments, the antibody or pharmaceutical composition described herein is delivered subcutaneously or intravenously. In certain embodiments, the antibody or pharmaceutical composition described herein is delivered intratumorally. In certain embodiments, the anti-CTLA-4 antibody or pharmaceutical composition described herein is delivered to a tumor draining lymph node. In certain embodiments, the antibody or pharmaceutical composition described herein is delivered via a localized administration (e.g., subcutaneous administration). In certain embodiments, the anti-CTLA-4 antibody or pharmaceutical composition described herein is delivered systemically. In certain embodiments, the anti-CTLA-4 antibody or pharmaceutical composition described herein is delivered locally.

[00229] In one aspect, the present invention relates to an anti-CTLA-4 antibody and/or pharmaceutical composition of the present invention, and optionally an additional therapeutic agent, for use in a method for the treatment of cancer, wherein the anti-CTLA-4 antibody

and/or pharmaceutical composition of the present invention is delivered intratumorally to the subject, is delivered to a tumor draining lymph node of a subject, or is delivered via a localized administration (e.g., subcutaneous administration) to a subject.

[00230] The amount of an antibody or composition which will be effective in the treatment and/or prevention of a condition will depend on the nature of the disease, and can be determined by standard clinical techniques.

[00231] The precise dose to be employed in a composition will also depend on the route of administration, and the seriousness of the infection or disease caused by it, and should be decided according to the judgment of the practitioner and each subject's circumstances. For example, effective doses may also vary depending upon means of administration, target site, physiological state of the patient (including age, body weight and health), whether the patient is human or an animal, other medications administered, or whether treatment is prophylactic or therapeutic. Usually, the patient is a human but non-human mammals including transgenic mammals can also be treated. Treatment dosages are optimally titrated to optimize safety and efficacy.

[00232] In certain embodiments, an anti-CTLA-4 antibody or pharmaceutical composition described herein is administered to a subject (e.g., via intravenous injection) at 0.01 mg/kg, 0.03 mg/kg, 0.1 mg/kg, 0.3 mg/kg, 1 mg/kg, 3 mg/kg, 6 mg/kg, 10 mg/kg, about 0.1 mg/kg, about 0.3 mg/kg, about 1 mg/kg, about 3 mg/kg, about 6 mg/kg, or about 10 mg/kg. In certain embodiments, an anti-CTLA-4 antibody or pharmaceutical composition described herein is administered to a subject (e.g., via intravenous injection) every week, every two weeks, every three weeks, every four weeks, every six weeks, every eight weeks, every twelve weeks, every month, every two months, every three months, every four months, every five months, every six months, every eight months, and every year, e.g., at the doses described above. In certain embodiments, an anti-CTLA-4 antibody or pharmaceutical composition described herein is administered to a subject (e.g., via intravenous injection) every three weeks at the doses described above.

[00233] In one aspect, the present invention relates to an anti-CTLA-4 antibody and/or pharmaceutical composition of the present invention, and optionally an additional therapeutic agent, for use in a method for the treatment of cancer, wherein the anti-CTLA-4 antibody and/or pharmaceutical composition of the present invention is administered to a subject at 0.01 mg/kg, 0.03 mg/kg, 0.1 mg/kg, 0.3 mg/kg, 1 mg/kg, 3 mg/kg, 6 mg/kg, 10 mg/kg, about 0.1 mg/kg, about 0.3 mg/kg, about 1 mg/kg, about 3 mg/kg, about 6 mg/kg, or about 10 mg/kg, more preferably every two weeks, every three weeks, every four weeks, every six

weeks, or every twelve weeks.

[00234] In certain embodiments, an anti-CTLA-4 antibody or pharmaceutical composition described herein is administered to a subject (e.g., via intravenous injection) at 0.1 mg/kg every two weeks, every three weeks, every four weeks, every six weeks, or every twelve weeks. In certain embodiments, an anti-CTLA-4 antibody or pharmaceutical composition described herein is administered to a subject (e.g., via intravenous injection) at 0.3 mg/kg every two weeks, every three weeks, every four weeks, every six weeks, or every twelve weeks. In certain embodiments, an anti-CTLA-4 antibody or pharmaceutical composition described herein is administered to a subject (e.g., via intravenous injection) at 1 mg/kg every two weeks, every three weeks, every four weeks, every six weeks, or every twelve weeks. In certain embodiments, an anti-CTLA-4 antibody or pharmaceutical composition described herein is administered to a subject (e.g., via intravenous injection) at 3 mg/kg every two weeks, every three weeks, every four weeks, every six weeks, or every twelve weeks. In certain embodiments, an anti-CTLA-4 antibody or pharmaceutical composition described herein is administered to a subject (e.g., via intravenous injection) at 6 mg/kg every two weeks, every three weeks, every four weeks, every six weeks, or every twelve weeks. In certain embodiments, an anti-CTLA-4 antibody or pharmaceutical composition described herein is administered to a subject (e.g., via intravenous injection) at 10 mg/kg every two weeks, every three weeks, every four weeks, every six weeks, or every twelve weeks.

[00235] In certain embodiments, an anti-CTLA-4 antibody or pharmaceutical composition described herein is administered to a subject (e.g., via intravenous injection) at 0.1 mg/kg or about 0.1 mg/kg every three weeks. In certain embodiments, an anti-CTLA-4 antibody or pharmaceutical composition described herein is administered to a subject (e.g., via intravenous injection) at 0.3 mg/kg or about 0.3 mg/kg every three weeks. In certain embodiments, an anti-CTLA-4 antibody or pharmaceutical composition described herein is administered to a subject (e.g., via intravenous injection) at 1 mg/kg or about 1 mg/kg every three weeks. In certain embodiments, an anti-CTLA-4 antibody or pharmaceutical composition described herein is administered to a subject (e.g., via intravenous injection) at 3 mg/kg or about 3 mg/kg every three weeks. In certain embodiments, an anti-CTLA-4 antibody or pharmaceutical composition described herein is administered to a subject (e.g., via intravenous injection) at 6 mg/kg or about 6 mg/kg every three weeks. In certain embodiments, an anti-CTLA-4 antibody or pharmaceutical composition described herein is administered to a subject (e.g., via intravenous injection) at 10 mg/kg or about 10 mg/kg every three weeks.

[00236] In certain embodiments, an anti-CTLA-4 antibody or pharmaceutical composition described herein is administered to a subject via intratumoral injection at 0.01 mg/kg, 0.03 mg/kg, 0.1 mg/kg, 0.3 mg/kg, 1 mg/kg, 3 mg/kg, about 0.01 mg/kg, about 0.03 mg/kg, about 0.1 mg/kg, about 0.3 mg/kg, about 1 mg/kg, or about 3 mg/kg. In certain embodiments, an anti-CTLA-4 antibody or pharmaceutical composition described herein is administered to a subject via intratumoral injection every week, every two weeks, every three weeks, every four weeks, every six weeks, every eight weeks, every twelve weeks, every month, every two months, every three months, every four months, every five months, every six months, every eight months, and every year, e.g., at the doses described above. In certain embodiments, an anti-CTLA-4 antibody or pharmaceutical composition described herein is administered to a subject via intratumoral injection every three weeks at the doses described above.

[00237] In certain embodiments, an anti-CTLA-4 antibody or pharmaceutical composition described herein is administered to a subject via intratumoral injection at 0.01 mg/kg or about 0.01 mg/kg every three weeks. In certain embodiments, an anti-CTLA-4 antibody or pharmaceutical composition described herein is administered to a subject via intratumoral injection at 0.03 mg/kg or about 0.03 mg/kg every three weeks. In certain embodiments, an anti-CTLA-4 antibody or pharmaceutical composition described herein is administered to a subject via intratumoral injection at 0.1 mg/kg or about 0.1 mg/kg every three weeks. In certain embodiments, an anti-CTLA-4 antibody or pharmaceutical composition described herein is administered to a subject via intratumoral injection at 0.3 mg/kg or about 0.3 mg/kg every three weeks. In certain embodiments, an anti-CTLA-4 antibody or pharmaceutical composition described herein is administered to a subject via intratumoral injection at 1 mg/kg or about 1 mg/kg every three weeks. In certain embodiments, an anti-CTLA-4 antibody or pharmaceutical composition described herein is administered to a subject via intratumoral injection at 3 mg/kg or about 3 mg/kg every three weeks.

[00238] In certain embodiments, an anti-CTLA-4 antibody or pharmaceutical composition described herein is administered to a subject via a localized administration (e.g., subcutaneous administration) at 0.01 mg/kg, 0.03 mg/kg, 0.1 mg/kg, 0.3 mg/kg, 1 mg/kg, 3 mg/kg, about 0.01 mg/kg, about 0.03 mg/kg, about 0.1 mg/kg, about 0.3 mg/kg, about 1 mg/kg, or about 3 mg/kg. In certain embodiments, an anti-CTLA-4 antibody or pharmaceutical composition described herein is administered to a subject via a localized administration (e.g., subcutaneous administration) every week, every two weeks, every three weeks, every four weeks, every six weeks, every eight weeks, every twelve weeks, every month, every two months, every three months, every four months, every five months, every

six months, every eight months, and every year, e.g., at the doses described above.

[00239] In certain embodiments, the therapeutic combination is administered to a subject for at least 3, 6, 9, 12, 18, or 24 months. In certain embodiments, the therapeutic combination is administered to a subject for up to 3, 6, 9, 12, 18, or 24 months.

[00240] In certain embodiments, the instant disclosure provides a method of treating a subject having angiosarcoma, the method comprising administering to the subject (e.g., intravenously, intratumorally, or subcutaneously) an effective amount of an anti-CTLA-4 antibody or pharmaceutical composition described herein. In certain embodiments, the instant disclosure provides a method of treating a subject having angiosarcoma, the method comprising administering to the subject intravenously an antibody that specifically binds to human CTLA-4 at 0.01 mg/kg, 0.03 mg/kg, 0.1 mg/kg, 0.3 mg/kg, 1 mg/kg, 3 mg/kg, about 0.01 mg/kg, about 0.03 mg/kg, about 0.1 mg/kg, about 0.3 mg/kg, about 1 mg/kg, or about 3 mg/kg, optionally every one, two or three weeks. In certain embodiments, the instant disclosure provides a method of treating a subject having angiosarcoma, the method comprising administering to the subject intravenously an antibody that specifically binds to human CTLA-4 at 0.1 mg/kg once every three weeks. In certain embodiments, the antibody comprises a heavy chain variable region comprising the amino acid sequence of SEQ ID NO: 8 and a light chain variable region comprising the amino acid sequence of SEQ ID NO: 9. In certain embodiments, the antibody comprises a heavy chain comprising the amino acid sequence of SEQ ID NO: 23; and a light chain comprising the amino acid sequence of SEQ ID NO: 27. In certain embodiments, the antibody comprises a heavy chain comprising the amino acid sequence of SEQ ID NO: 24; and a light chain comprising the amino acid sequence of SEQ ID NO: 27. In certain embodiments, the antibody comprises a heavy chain comprising the amino acid sequence of SEQ ID NO: 25; and a light chain comprising the amino acid sequence of SEQ ID NO: 27. In certain embodiments, the antibody comprises a heavy chain comprising the amino acid sequence of SEQ ID NO: 26; and a light chain comprising the amino acid sequence of SEQ ID NO: 27.

[00241] An anti-CTLA-4 antibody described herein can also be used to assay CTLA-4 protein levels in a biological sample using classical immunohistological methods known to those of skill in the art, including immunoassays, such as the enzyme linked immunosorbent assay (ELISA), immunoprecipitation, or Western blotting. Suitable antibody assay labels are known in the art and include enzyme labels, such as, glucose oxidase; radioisotopes, such as iodine (^{125}I, ^{121}I), carbon (^{14}C), sulfur (^{35}S), tritium (^{3}H), indium (^{121}In), and technetium (^{99}Tc); luminescent labels, such as luminol; and fluorescent

labels, such as fluorescein and rhodamine, and biotin. Such labels can be used to label an antibody or an antigen-binding fragment thereof described herein. Alternatively, a second antibody that recognizes an anti-CTLA-4 antibody or antigen-binding fragment thereof described herein can be labeled and used in combination with an anti-CTLA-4 antibody or antigen-binding fragment thereof to detect CTLA-4 protein levels. In one embodiment, the present invention relates to the use of an anti-CTLA-4 antibody of the invention, for assaying and/or detecting CTLA-4 protein levels in a biological sample *in vitro*.

[00242] Assaying for the expression level of CTLA-4 protein is intended to include qualitatively or quantitatively measuring or estimating the level of a CTLA-4 protein in a first biological sample either directly (*e.g.*, by determining or estimating absolute protein level) or relatively (*e.g.*, by comparing to the disease associated protein level in a second biological sample). CTLA-4 polypeptide expression level in the first biological sample can be measured or estimated and compared to a standard CTLA-4 protein level, the standard being taken from a second biological sample obtained from an individual not having the disorder or being determined by averaging levels from a population of individuals not having the disorder. As will be appreciated in the art, once the "standard" CTLA-4 polypeptide level is known, it can be used repeatedly as a standard for comparison.

[00243] As used herein, the term "biological sample" refers to any biological sample obtained from a subject, cell line, tissue, or other source of cells potentially expressing CTLA-4. Methods for obtaining tissue biopsies and body fluids from animals (*e.g.*, humans) are well known in the art. Biological samples include peripheral mononuclear blood cells.

[00244] An anti-CTLA-4 antibody or antigen-binding fragment thereof described herein can be used for prognostic, diagnostic, monitoring and screening applications, including *in vitro* and *in vivo* applications well known and standard to the skilled artisan and based on the present description. Prognostic, diagnostic, monitoring and screening assays and kits for *in vitro* assessment and evaluation of immune system status and/or immune response may be utilized to predict, diagnose and monitor to evaluate patient samples including those known to have or suspected of having an immune system-dysfunction or with regard to an anticipated or desired immune system response, antigen response or vaccine response. The assessment and evaluation of immune system status and/or immune response is also useful in determining the suitability of a patient for a clinical trial of a drug or for the administration of a particular chemotherapeutic agent or an antibody or antigen-binding fragment thereof,

including combinations thereof, versus a different agent or antibody or antigen-binding fragment thereof. This type of prognostic and diagnostic monitoring and assessment is already in practice utilizing antibodies against the HER2 protein in breast cancer (HercepTest™, Dako) where the assay is also used to evaluate patients for antibody therapy using Herceptin®. *In vivo* applications include directed cell therapy and immune system modulation and radio imaging of immune responses.

[00245] In one aspect, the present invention relates to an anti-CTLA-4 antibody and/or pharmaceutical composition of the present invention for use as a diagnostic.

[00246] In one aspect, the present invention relates to an anti-CTLA-4 antibody and/or pharmaceutical composition of the present invention for use in a method for the prediction, diagnosis and/or monitoring of an immune system-dysfunction and/or cancer.

[00247] In one embodiment, the present invention relates to the use of an anti-CTLA-4 antibody of the invention, for predicting, diagnosing and/or monitoring an immune system-dysfunction and/or cancer in a subject by assaying and/or detecting CTLA-4 protein levels in a biological sample of the subject of *in vitro*.

[00248] In one embodiment, an anti-CTLA-4 antibody or antigen-binding fragment thereof can be used in immunohistochemistry of biopsy samples. In another embodiment, an anti-CTLA-4 antibody or antigen-binding fragment thereof can be used to detect levels of CTLA-4, or levels of cells which contain CTLA-4 on their membrane surface, which levels can then be linked to certain disease symptoms. Anti-CTLA-4 antibodies or antigen-binding fragments thereof described herein may carry a detectable or functional label. When fluorescence labels are used, currently available microscopy and fluorescence-activated cell sorter analysis (FACS) or combination of both methods procedures known in the art may be utilized to identify and to quantitate the specific binding members. Anti-CTLA-4 antibodies or antigen-binding fragments thereof described herein may carry a fluorescence label. Exemplary fluorescence labels include, for example, reactive and conjugated probes e.g. Aminocoumarin, Fluorescein and Texas red, Alexa Fluor dyes, Cy dyes and DyLight dyes. An anti-CTLA-4 antibody or antigen-binding fragment thereof may carry a radioactive label, such as the isotopes ^{3}H, ^{14}C, ^{32}P, ^{35}S, ^{36}Cl, ^{51}Cr, ^{57}Co, ^{58}Co, ^{59}Fe, ^{67}Cu, ^{90}Y, ^{99}Tc, ^{111}In, ^{117}Lu, ^{121}I, ^{124}I, ^{125}I, ^{131}I, ^{198}Au, ^{211}At, ^{213}Bi, ^{225}Ac and ^{186}Re. When radioactive labels are used, currently available counting procedures known in the art may be utilized to identify and quantitate the specific binding of anti-CTLA-4 antibody or antigen-binding fragment thereof to CTLA-4 (*e.g.*, human CTLA-4). In the instance where the label is an enzyme, detection may be accomplished by any of the presently utilized colorimetric, spectrophotometric,

fluorospectrophotometric, amperometric or gasometric techniques as known in the art. This can be achieved by contacting a sample or a control sample with an anti-CTLA-4 antibody or antigen-binding fragment thereof under conditions that allow for the formation of a complex between the antibody or antigen-binding fragment thereof and CTLA-4. Any complexes formed between the antibody or antigen-binding fragment thereof and CTLA-4 are detected and compared in the sample and the control. In light of the specific binding of the antibodies described herein for CTLA-4, the antibodies or antigen-binding fragments thereof can be used to specifically detect CTLA-4 expression on the surface of cells. The antibodies or antigen-binding fragments thereof described herein can also be used to purify CTLA-4 via immunoaffinity purification. Also included herein is an assay system which may be prepared in the form of a test kit for the quantitative analysis of the extent of the presence of, for instance, CTLA-4 or CTLA-4/CTLA-4 ligand complexes. The system or test kit may comprise a labeled component, *e.g.*, a labeled antibody, and one or more additional immunochemical reagents.

[00249] In one embodiment, the present invention relates to an *in vitro* method for assaying and/or detecting CTLA-4 protein levels in a biological sample comprising (1) contacting a sample and optionally a control sample with an anti-CTLA-4 antibody or antigen-binding fragment thereof of the invention under conditions that allow for the formation of a complex between the antibody or antigen-binding fragment thereof and CTLA-4, and (2) detecting and comparing the complexes formed in the sample and optionally the control.

6.5 Polynucleotides, Vectors and Methods of Producing Anti-CTLA-4 Antibodies

[00250] In another aspect, provided herein are polynucleotides comprising a nucleotide sequence encoding an antibody described herein or a fragment thereof (*e.g.*, a light chain variable region and/or heavy chain variable region) that specifically binds to a CTLA-4 (*e.g.*, human CTLA-4) antigen, and vectors, *e.g.*, vectors comprising such polynucleotides for recombinant expression in host cells (*e.g.*, *E. coli* and mammalian cells). Provided herein are polynucleotides comprising nucleotide sequences encoding any of the antibodies provided herein, as well as vectors comprising such polynucleotide sequences, *e.g.*, expression vectors for their efficient expression in host cells, *e.g.*, mammalian cells.

[00251] As used herein, an "isolated" polynucleotide or nucleic acid molecule is one which is separated from other nucleic acid molecules which are present in the natural source (*e.g.*, in a mouse or a human) of the nucleic acid molecule. Moreover, an "isolated" nucleic

acid molecule, such as a cDNA molecule, can be substantially free of other cellular material, or culture medium when produced by recombinant techniques, or substantially free of chemical precursors or other chemicals when chemically synthesized. For example, the language "substantially free" includes preparations of polynucleotide or nucleic acid molecule having less than about 15%, 10%, 5%, 2%, 1%, 0.5%, or 0.1% (in particular less than about 10%) of other material, *e.g.*, cellular material, culture medium, other nucleic acid molecules, chemical precursors and/or other chemicals. In a specific embodiment, a nucleic acid molecule(s) encoding an antibody described herein is isolated or purified.

[00252] In particular aspects, provided herein are polynucleotides comprising nucleotide sequences encoding antibodies or antigen-binding fragments thereof, which specifically bind to a CTLA-4 polypeptide (*e.g.*, human CTLA-4) and comprises an amino acid sequence as described herein, as well as antibodies which compete with such antibodies for binding to a CTLA-4 polypeptide (*e.g.*, in a dose-dependent manner), or which binds to the same epitope as that of such antibodies.

[00253] In certain aspects, provided herein are polynucleotides comprising a nucleotide sequence encoding the light chain or heavy chain of an antibody described herein. The polynucleotides can comprise nucleotide sequences encoding a light chain comprising the VL FRs and CDRs of antibodies described herein (see, *e.g.*, Table 1).

[00254] Also provided herein are polynucleotides encoding an anti-CTLA-4 antibody that are optimized, *e.g.*, by codon/RNA optimization, replacement with heterologous signal sequences, and elimination of mRNA instability elements. Methods to generate optimized nucleic acids encoding an anti-CTLA-4 antibody or a fragment thereof (*e.g.*, light chain, heavy chain, VH domain, or VL domain) for recombinant expression by introducing codon changes and/or eliminating inhibitory regions in the mRNA can be carried out by adapting the optimization methods described in, *e.g.*, U.S. Patent Nos. 5,965,726; 6,174,666; 6,291,664; 6,414,132; and 6,794,498, accordingly, all of which are herein incorporated by reference in their entireties. For example, potential splice sites and instability elements (*e.g.*, A/T or A/U rich elements) within the RNA can be mutated without altering the amino acids encoded by the nucleic acid sequences to increase stability of the RNA for recombinant expression. The alterations utilize the degeneracy of the genetic code, *e.g.*, using an alternative codon for an identical amino acid. In some embodiments, it can be desirable to alter one or more codons to encode a conservative mutation, *e.g.*, a similar amino acid with similar chemical structure and properties and/or function as the original amino acid. Such methods can increase expression of an anti-CTLA-4 antibody or fragment thereof by at least

1 fold, 2 fold, 3 fold, 4 fold, 5 fold, 10 fold, 20 fold, 30 fold, 40 fold, 50 fold, 60 fold, 70 fold, 80 fold, 90 fold, or 100 fold or more relative to the expression of an anti-CTLA-4 antibody encoded by polynucleotides that have not been optimized.

[00255] In certain embodiments, an optimized polynucleotide sequence encoding an anti-CTLA-4 antibody described herein or a fragment thereof (*e.g.*, VL domain and/or VH domain) can hybridize to an antisense (*e.g.*, complementary) polynucleotide of an unoptimized polynucleotide sequence encoding an anti-CTLA-4 antibody described herein or a fragment thereof (*e.g.*, VL domain and/or VH domain). In specific embodiments, an optimized nucleotide sequence encoding an anti-CTLA-4 antibody described herein or a fragment hybridizes under high stringency conditions to antisense polynucleotide of an unoptimized polynucleotide sequence encoding an anti-CTLA-4 antibody described herein or a fragment thereof. In a specific embodiment, an optimized nucleotide sequence encoding an anti-CTLA-4 antibody described herein or a fragment thereof hybridizes under high stringency, intermediate or lower stringency hybridization conditions to an antisense polynucleotide of an unoptimized nucleotide sequence encoding an anti-CTLA-4 antibody described herein or a fragment thereof. Information regarding hybridization conditions has been described, see, *e.g.*, U.S. Patent Application Publication No. US 2005/0048549 (*e.g.*, paragraphs 72-73), which is incorporated herein by reference.

[00256] The polynucleotides can be obtained, and the nucleotide sequence of the polynucleotides determined, by any method known in the art. Nucleotide sequences encoding antibodies described herein, *e.g.*, antibodies described in Table 1, and modified versions of these antibodies can be determined using methods well known in the art, *i.e.*, nucleotide codons known to encode particular amino acids are assembled in such a way to generate a nucleic acid that encodes the antibody. Such a polynucleotide encoding the antibody can be assembled from chemically synthesized oligonucleotides (*e.g.*, as described in Kutmeier G *et al.*, (1994), BioTechniques 17: 242-6, incorporated by reference in its entirety), which, briefly, involves the synthesis of overlapping oligonucleotides containing portions of the sequence encoding the antibody, annealing and ligating of those oligonucleotides, and then amplification of the ligated oligonucleotides by PCR.

[00257] Alternatively, a polynucleotide encoding an antibody described herein can be generated from nucleic acid from a suitable source (*e.g.*, a hybridoma) using methods well known in the art (*e.g.*, PCR and other molecular cloning methods). For example, PCR amplification using synthetic primers hybridizable to the 3' and 5' ends of a known sequence can be performed using genomic DNA obtained from hybridoma cells producing the antibody

of interest. Such PCR amplification methods can be used to obtain nucleic acids comprising the sequence encoding the light chain and/or heavy chain of an antibody. Such PCR amplification methods can be used to obtain nucleic acids comprising the sequence encoding the variable light chain region and/or the variable heavy chain region of an antibody. The amplified nucleic acids can be cloned into vectors for expression in host cells and for further cloning, for example, to generate chimeric and humanized antibodies.

[00258] If a clone containing a nucleic acid encoding a particular antibody is not available, but the sequence of the antibody molecule is known, a nucleic acid encoding the immunoglobulin can be chemically synthesized or obtained from a suitable source (*e.g.*, an antibody cDNA library or a cDNA library generated from, or nucleic acid, preferably poly A+ RNA, isolated from, any tissue or cells expressing the antibody, such as hybridoma cells selected to express an antibody described herein) by PCR amplification using synthetic primers hybridizable to the 3' and 5' ends of the sequence or by cloning using an oligonucleotide probe specific for the particular gene sequence to identify, *e.g.*, a cDNA clone from a cDNA library that encodes the antibody. Amplified nucleic acids generated by PCR can then be cloned into replicable cloning vectors using any method well known in the art.

[00259] DNA encoding anti-CTLA-4 antibodies described herein can be readily isolated and sequenced using conventional procedures (*e.g.*, by using oligonucleotide probes that are capable of binding specifically to genes encoding the heavy and light chains of the anti-CTLA-4 antibodies). Hybridoma cells can serve as a source of such DNA. Once isolated, the DNA can be placed into expression vectors, which are then transfected into host cells such as *E. coli* cells, simian COS cells, Chinese hamster ovary (CHO) cells (*e.g.*, CHO cells from the CHO GS System™ (Lonza)), or myeloma cells that do not otherwise produce immunoglobulin protein, to obtain the synthesis of anti-CTLA-4 antibodies in the recombinant host cells.

[00260] To generate whole antibodies, PCR primers including VH or VL nucleotide sequences, a restriction site, and a flanking sequence to protect the restriction site can be used to amplify the VH or VL sequences in scFv clones. Utilizing cloning techniques known to those of skill in the art, the PCR amplified VH domains can be cloned into vectors expressing a heavy chain constant region, *e.g.*, the human gamma 4 constant region, and the PCR amplified VL domains can be cloned into vectors expressing a light chain constant region, *e.g.*, human kappa or lambda constant regions. In certain embodiments, the vectors for **expressing the VH or VL domains comprise an EF-1α promoter, a secretion signal, a cloning**

site for the variable region, constant domains, and a selection marker such as neomycin. The VH and VL domains can also be cloned into one vector expressing the necessary constant regions. The heavy chain conversion vectors and light chain conversion vectors are then co-transfected into cell lines to generate stable or transient cell lines that express full-length antibodies, *e.g.*, IgG, using techniques known to those of skill in the art.

[00261] The DNA also can be modified, for example, by substituting the coding sequence for human heavy and light chain constant domains in place of the murine sequences, or by covalently joining to the immunoglobulin coding sequence all or part of the coding sequence for a non-immunoglobulin polypeptide.

[00262] Also provided are polynucleotides that hybridize under high stringency, intermediate or lower stringency hybridization conditions to polynucleotides that encode an antibody described herein. In specific embodiments, polynucleotides described herein hybridize under high stringency, intermediate or lower stringency hybridization conditions to polynucleotides encoding a VH domain and/or VL domain provided herein.

[00263] Hybridization conditions have been described in the art and are known to one of skill in the art. For example, hybridization under stringent conditions can involve hybridization to filter-bound DNA in 6x sodium chloride/sodium citrate (SSC) at about 45° C followed by one or more washes in 0.2xSSC/0.1% SDS at about 50-65° C; hybridization under highly stringent conditions can involve hybridization to filter-bound nucleic acid in 6xSSC at about 45° C followed by one or more washes in 0.1xSSC/0.2% SDS at about 68° C. Hybridization under other stringent hybridization conditions are known to those of skill in the art and have been described, see, for example, Ausubel FM *et al.,* eds., (1989) Current Protocols in Molecular Biology, Vol. I, Green Publishing Associates, Inc. and John Wiley & Sons, Inc., New York at pages 6.3.1-6.3.6 and 2.10.3, incorporated by reference in its entirety.

[00264] In certain aspects, provided herein are cells (*e.g.*, host cells) expressing (*e.g.*, recombinantly) antibodies described herein (or an antigen-binding fragment thereof) which specifically bind to CTLA-4 (*e.g.*, human CTLA-4) and related polynucleotides and expression vectors. Provided herein are vectors (*e.g.*, expression vectors) comprising polynucleotides comprising nucleotide sequences encoding anti-CTLA-4 antibodies or a fragment for recombinant expression in host cells, preferably in mammalian cells. Also provided herein are host cells comprising such vectors for recombinantly expressing anti-CTLA-4 antibodies described herein (*e.g.*, human or humanized antibody). In a particular aspect, provided herein are methods for producing an antibody described herein, comprising

expressing such antibody from a host cell.

[00265] Recombinant expression of an antibody described herein (*e.g.*, a full-length antibody, heavy and/or light chain of an antibody, or a single chain antibody described herein) that specifically binds to CTLA-4 (*e.g.*, human CTLA-4) involves construction of an expression vector containing a polynucleotide that encodes the antibody. Once a polynucleotide encoding an antibody molecule, heavy and/or light chain of an antibody, or a fragment thereof (*e.g.*, heavy and/or light chain variable regions) described herein has been obtained, the vector for the production of the antibody molecule can be produced by recombinant DNA technology using techniques well known in the art. Thus, methods for preparing a protein by expressing a polynucleotide containing an antibody or antibody fragment (*e.g.*, light chain or heavy chain) encoding nucleotide sequence are described herein. Methods which are well known to those skilled in the art can be used to construct expression vectors containing antibody or antibody fragment (*e.g.*, light chain or heavy chain) coding sequences and appropriate transcriptional and translational control signals. These methods include, for example, *in vitro* recombinant DNA techniques, synthetic techniques, and *in vivo* genetic recombination. Also provided are replicable vectors comprising a nucleotide sequence encoding an antibody molecule described herein, a heavy or light chain of an antibody, a heavy or light chain variable region of an antibody or a fragment thereof, or a heavy or light chain CDR, operably linked to a promoter. Such vectors can, for example, include the nucleotide sequence encoding the constant region of the antibody molecule (see, *e.g.,* International Publication Nos. WO 86/05807 and WO 89/01036; and U.S. Patent No. 5,122,464, which are herein incorporated by reference in their entireties) and variable regions of the antibody can be cloned into such a vector for expression of the entire heavy, the entire light chain, or both the entire heavy and light chains.

[00266] An expression vector can be transferred to a cell (*e.g.*, host cell) by conventional techniques and the resulting cells can then be cultured by conventional techniques to produce an antibody described herein or a fragment thereof. Thus, provided herein are host cells containing a polynucleotide encoding an antibody described herein or fragments thereof, or a heavy or light chain thereof, or fragment thereof, or a single chain antibody described herein, operably linked to a promoter for expression of such sequences in the host cell. In certain embodiments, for the expression of double-chained antibodies, vectors encoding both the heavy and light chains, individually, can be co-expressed in the host cell for expression of the entire immunoglobulin molecule, as detailed below. In certain embodiments, a host cell contains a vector comprising a polynucleotide encoding both the heavy chain and light chain

of an antibody described herein, or a fragment thereof. In specific embodiments, a host cell contains two different vectors, a first vector comprising a polynucleotide encoding a heavy chain or a heavy chain variable region of an antibody described herein, or a fragment thereof, and a second vector comprising a polynucleotide encoding a light chain or a light chain variable region of an antibody described herein, or a fragment thereof. In other embodiments, a first host cell comprises a first vector comprising a polynucleotide encoding a heavy chain or a heavy chain variable region of an antibody described herein, or a fragment thereof, and a second host cell comprises a second vector comprising a polynucleotide encoding a light chain or a light chain variable region of an antibody described herein. In specific embodiments, a heavy chain/heavy chain variable region expressed by a first cell associated with a light chain/light chain variable region of a second cell to form an anti-CTLA-4 antibody described herein or an antigen-binding fragment thereof. In certain embodiments, provided herein is a population of host cells comprising such first host cell and such second host cell.

[00267] In a particular embodiment, provided herein is a population of vectors comprising a first vector comprising a polynucleotide encoding a light chain/light chain variable region of an anti-CTLA-4 antibody described herein, and a second vector comprising a polynucleotide encoding a heavy chain/heavy chain variable region of an anti-CTLA-4 antibody described herein.

[00268] A variety of host-expression vector systems can be utilized to express antibody molecules described herein (see, *e.g.,* U.S. Patent No. 5,807,715). Such host-expression systems represent vehicles by which the coding sequences of interest can be produced and subsequently purified, but also represent cells which can, when transformed or transfected with the appropriate nucleotide coding sequences, express an antibody molecule described herein *in situ.* These include but are not limited to microorganisms such as bacteria (*e.g., E. coli* and *B. subtilis*) transformed with recombinant bacteriophage DNA, plasmid DNA or cosmid DNA expression vectors containing antibody coding sequences; yeast (*e.g., Saccharomyces Pichia*) transformed with recombinant yeast expression vectors containing antibody coding sequences; insect cell systems infected with recombinant virus expression vectors (*e.g.,* baculovirus) containing antibody coding sequences; plant cell systems (*e.g.,* green algae such as *Chlamydomonas reinhardtii*) infected with recombinant virus expression vectors (*e.g.,* cauliflower mosaic virus, CaMV; tobacco mosaic virus, TMV) or transformed with recombinant plasmid expression vectors (*e.g.,* Ti plasmid) containing antibody coding sequences; or mammalian cell systems (*e.g.,* COS (*e.g.*, COS1 or COS), CHO, BHK, MDCK,

HEK 293, NS0, PER.C6, VERO, CRL7O3O, HsS78Bst, HeLa, and NIH 3T3, HEK-293T, HepG2, SP210, R1.1, B-W, L-M, BSC1, BSC40, YB/20 and BMT10 cells) harboring recombinant expression constructs containing promoters derived from the genome of mammalian cells (*e.g.,* metallothionein promoter) or from mammalian viruses (*e.g.,* the adenovirus late promoter; the vaccinia virus 7.5K promoter). In a specific embodiment, cells for expressing antibodies described herein or an antigen-binding fragment thereof are CHO cells, for example CHO cells from the CHO GS System™ (Lonza). In a particular embodiment, cells for expressing antibodies described herein are human cells, *e.g.*, human cell lines. In a specific embodiment, a mammalian expression vector is pOptiVEC™ or pcDNA3.3. In a particular embodiment, bacterial cells such as *Escherichia coli*, or eukaryotic cells (*e.g.*, mammalian cells), especially for the expression of whole recombinant antibody molecule, are used for the expression of a recombinant antibody molecule. For example, mammalian cells such as Chinese hamster ovary (CHO) cells, in conjunction with a vector such as the major intermediate early gene promoter element from human cytomegalovirus is an effective expression system for antibodies (Foecking MK & Hofstetter H (1986) Gene 45: 101-5; and Cockett MI *et al.,* (1990) Biotechnology 8(7): 662-7, which are herein incorporated by reference in their entireties). In certain embodiments, antibodies described herein are produced by CHO cells or NS0 cells. In a specific embodiment, the expression of nucleotide sequences encoding antibodies described herein which specifically bind CTLA-4 (*e.g.*, human CTLA-4) is regulated by a constitutive promoter, inducible promoter or tissue specific promoter.

[00269] In bacterial systems, a number of expression vectors can be advantageously selected depending upon the use intended for the antibody molecule being expressed. For example, when a large quantity of such an antibody is to be produced, for the generation of pharmaceutical compositions of an antibody molecule, vectors which direct the expression of high levels of fusion protein products that are readily purified can be desirable. Such vectors include, but are not limited to, the *E. coli* expression vector pUR278 (Ruether U & Mueller-Hill B (1983) EMBO J 2: 1791-1794, herein incorporated by reference in its entirety), in which the antibody coding sequence can be ligated individually into the vector in frame with the lac Z coding region so that a fusion protein is produced; pIN vectors (Inouye S & Inouye M (1985) Nuc Acids Res 13: 3101-3109; Van Heeke G & Schuster SM (1989) J Biol Chem 24: 5503-5509, which are herein incorporated by reference in their entireties); and the like. For example, pGEX vectors can also be used to express foreign polypeptides as fusion proteins with glutathione 5-transferase (GST). In general, such fusion proteins are soluble

and can easily be purified from lysed cells by adsorption and binding to matrix glutathione agarose beads followed by elution in the presence of free glutathione. The pGEX vectors are designed to include thrombin or factor Xa protease cleavage sites so that the cloned target gene product can be released from the GST moiety.

[00270] In an insect system, *Autographa californica* nuclear polyhedrosis virus (AcNPV), for example, can be used as a vector to express foreign genes. The virus grows in *Spodoptera frugiperda* cells. The antibody coding sequence can be cloned individually into non-essential regions (for example the polyhedrin gene) of the virus and placed under control of an AcNPV promoter (for example the polyhedrin promoter).

[00271] In mammalian host cells, a number of viral-based expression systems can be utilized. In cases where an adenovirus is used as an expression vector, the antibody coding sequence of interest can be ligated to an adenovirus transcription/translation control complex, *e.g.,* the late promoter and tripartite leader sequence. This chimeric gene can then be inserted in the adenovirus genome by *in vitro* or *in vivo* recombination. Insertion in a non-essential region of the viral genome (*e.g.,* region El or E3) will result in a recombinant virus that is viable and capable of expressing the antibody molecule in infected hosts (*e.g.,* see Logan J & Shenk T (1984) PNAS 81(12): 3655-9, herein incorporated by reference in its entirety). Specific initiation signals can also be required for efficient translation of inserted antibody coding sequences. These signals include the ATG initiation codon and adjacent sequences. Furthermore, the initiation codon must be in phase with the reading frame of the desired coding sequence to ensure translation of the entire insert. These exogenous translational control signals and initiation codons can be of a variety of origins, both natural and synthetic. The efficiency of expression can be enhanced by the inclusion of appropriate transcription enhancer elements, transcription terminators, *etc.* (see, *e.g.*, Bitter G *et al.,* (1987) Methods Enzymol. 153: 516-544, herein incorporated by reference in its entirety).

[00272] In addition, a host cell strain can be chosen which modulates the expression of the inserted sequences, or modifies and processes the gene product in the specific fashion desired. Such modifications (*e.g.,* glycosylation) and processing (*e.g.,* cleavage) of protein products can be important for the function of the protein. Different host cells have characteristic and specific mechanisms for the post-translational processing and modification of proteins and gene products. Appropriate cell lines or host systems can be chosen to ensure the correct modification and processing of the foreign protein expressed. To this end, eukaryotic host cells which possess the cellular machinery for proper processing of the primary transcript, glycosylation, and phosphorylation of the gene product can be used. Such

mammalian host cells include but are not limited to CHO, VERO, BHK, Hela, MDCK, HEK 293, NIH 3T3, W138, BT483, Hs578T, HTB2, BT2O and T47D, NS0 (a murine myeloma cell line that does not endogenously produce any immunoglobulin chains), CRL7O3O, COS (*e.g.*, COS1 or COS), PER.C6, VERO, HsS78Bst, HEK-293T, HepG2, SP210, R1.1, B-W, L-M, BSC1, BSC40, YB/20, BMT10 and HsS78Bst cells. In certain embodiments, anti-CTLA-4 antibodies described herein are produced in mammalian cells, such as CHO cells.

[00273] In a specific embodiment, the antibodies described herein or antigen-binding fragments thereof have reduced fucose content or no fucose content. Such antibodies can be produced using techniques known one skilled in the art. For example, the antibodies can be expressed in cells deficient or lacking the ability of to fucosylate. In a specific example, cell lines with a knockout of both alleles of α1,6-fucosyltransferase can be used to produce antibodies or antigen-binding fragments thereof with reduced fucose content. The Potelligent® system (Lonza) is an example of such a system that can be used to produce antibodies or antigen-binding fragments thereof with reduced fucose content.

[00274] For long-term, high-yield production of recombinant proteins, stable expression cells can be generated. For example, cell lines which stably express an anti-CTLA-4 antibody described herein or an antigen-binding fragment thereof can be engineered. In specific embodiments, a cell provided herein stably expresses a light chain/light chain variable region and a heavy chain/heavy chain variable region which associate to form an antibody described herein or an antigen-binding fragment thereof.

[00275] In certain aspects, rather than using expression vectors which contain viral origins of replication, host cells can be transformed with DNA controlled by appropriate expression control elements (*e.g.,* promoter, enhancer, sequences, transcription terminators, polyadenylation sites, *etc.*), and a selectable marker. Following the introduction of the foreign DNA/polynucleotide, engineered cells can be allowed to grow for 1-2 days in an enriched media, and then are switched to a selective media. The selectable marker in the recombinant plasmid confers resistance to the selection and allows cells to stably integrate the plasmid into their chromosomes and grow to form foci which in turn can be cloned and expanded into cell lines. This method can advantageously be used to engineer cell lines which express an anti-CTLA-4 antibody described herein or a fragment thereof. Such engineered cell lines can be particularly useful in screening and evaluation of compositions that interact directly or indirectly with the antibody molecule.

[00276] A number of selection systems can be used, including but not limited to the herpes simplex virus thymidine kinase (Wigler M *et al.,* (1977) Cell 11(1): 223-32),

hypoxanthineguanine phosphoribosyltransferase (Szybalska EH & Szybalski W (1962) PNAS 48(12): 2026-2034, herein incorporated by reference in its entirety) and adenine phosphoribosyltransferase (Lowy I *et al.,* (1980) Cell 22(3): 817-23, herein incorporated by reference in its entirety) genes in tk-, hgprt- or aprt-cells, respectively. Also, antimetabolite resistance can be used as the basis of selection for the following genes: *dhfr*, which confers resistance to methotrexate (Wigler M *et al.,* (1980) PNAS 77(6): 3567-70; O'Hare K *et al.,* (1981) PNAS 78: 1527-31); *gpt*, which confers resistance to mycophenolic acid (Mulligan RC & Berg P (1981) PNAS 78(4): 2072-6); neo, which confers resistance to the aminoglycoside G-418 (Wu GY & Wu CH (1991) Biotherapy 3: 87-95; Tolstoshev P (1993) Ann Rev Pharmacol Toxicol 32: 573-596; Mulligan RC (1993) Science 260: 926-932; and Morgan RA & Anderson WF (1993) Ann Rev Biochem 62: 191-217; Nabel GJ & Felgner PL (1993) Trends Biotechnol 11(5): 211-5); and *hygro*, which confers resistance to hygromycin (Santerre RF *et al.,* (1984) Gene 30(1-3): 147-56), all of which are herein incorporated by reference in their entireties. Methods commonly known in the art of recombinant DNA technology can be routinely applied to select the desired recombinant clone and such methods are described, for example, in Ausubel FM *et al.,* (eds.), Current Protocols in Molecular Biology, John Wiley & Sons, NY (1993); Kriegler M, Gene Transfer and Expression, A Laboratory Manual, Stockton Press, NY (1990); and in Chapters 12 and 13, Dracopoli NC *et al.,* (eds.), Current Protocols in Human Genetics, John Wiley & Sons, NY (1994); Colbère-Garapin F *et al.,* (1981) J Mol Biol 150: 1-14, which are incorporated by reference herein in their entireties.

[00277] The expression levels of an antibody molecule can be increased by vector amplification (for a review, see Bebbington CR & Hentschel CCG, The use of vectors based on gene amplification for the expression of cloned genes in mammalian cells in DNA cloning, Vol. 3 (Academic Press, New York, 1987), herein incorporated by reference in its entirety). When a marker in the vector system expressing antibody is amplifiable, increase in the level of inhibitor present in culture of host cell will increase the number of copies of the marker gene. Since the amplified region is associated with the antibody gene, production of the antibody will also increase (Crouse GF *et al.,* (1983) Mol Cell Biol 3: 257-66, herein incorporated by reference in its entirety).

[00278] The host cell can be co-transfected with two or more expression vectors described herein, the first vector encoding a heavy chain derived polypeptide and the second vector encoding a light chain derived polypeptide. The two vectors can contain identical selectable markers which enable equal expression of heavy and light chain polypeptides. The host cells

can be co-transfected with different amounts of the two or more expression vectors. For example, host cells can be transfected with any one of the following ratios of a first expression vector and a second expression vector: 1:1, 1:2, 1:3, 1:4, 1:5, 1:6, 1:7, 1:8, 1:9, 1:10, 1:12, 1:15, 1:20, 1:25, 1:30, 1:35, 1:40, 1:45, or 1:50.

[00279] Alternatively, a single vector can be used which encodes, and is capable of expressing, both heavy and light chain polypeptides. In such situations, the light chain should be placed before the heavy chain to avoid an excess of toxic free heavy chain (Proudfoot NJ (1986) Nature 322: 562-565; and Köhler G (1980) PNAS 77: 2197-2199, which are herein incorporated by reference in their entireties). The coding sequences for the heavy and light chains can comprise cDNA or genomic DNA. The expression vector can be monocistronic or multicistronic. A multicistronic nucleic acid construct can encode 2, 3, 4, 5, 6, 7, 8, 9, 10 or more, or in the range of 2-5, 5-10 or 10-20 genes/nucleotide sequences. For example, a bicistronic nucleic acid construct can comprise in the following order a promoter, a first gene (*e.g.*, heavy chain of an antibody described herein), and a second gene and (*e.g.*, light chain of an antibody described herein). In such an expression vector, the transcription of both genes can be driven by the promoter, whereas the translation of the mRNA from the first gene can be by a cap-dependent scanning mechanism and the translation of the mRNA from the second gene can be by a cap-independent mechanism, *e.g.*, by an IRES.

[00280] Once an antibody molecule described herein has been produced by recombinant expression, it can be purified by any method known in the art for purification of an immunoglobulin molecule, for example, by chromatography (*e.g.,* ion exchange, affinity, particularly by affinity for the specific antigen after Protein A, and sizing column chromatography), centrifugation, differential solubility, or by any other standard technique for the purification of proteins. Further, the antibodies described herein can be fused to heterologous polypeptide sequences described herein or otherwise known in the art to facilitate purification.

[00281] In specific embodiments, an antibody or an antigen-binding fragment thereof described herein is isolated or purified. Generally, an isolated antibody is one that is substantially free of other antibodies with different antigenic specificities than the isolated antibody. For example, in a particular embodiment, a preparation of an antibody described herein is substantially free of cellular material and/or chemical precursors. The language "substantially free of cellular material" includes preparations of an antibody in which the antibody is separated from cellular components of the cells from which it is isolated or recombinantly produced. Thus, an antibody that is substantially free of cellular material

includes preparations of antibody having less than about 30%, 20%, 10%, 5%, 2%, 1%, 0.5%, or 0.1% (by dry weight) of heterologous protein (also referred to herein as a "contaminating protein") and/or variants of an antibody, for example, different post-translational modified forms of an antibody or other different versions of an antibody (*e.g.*, antibody fragments). When the antibody is recombinantly produced, it is also generally substantially free of culture medium, *i.e.,* culture medium represents less than about 20%, 10%, 2%, 1%, 0.5%, or 0.1% of the volume of the protein preparation. When the antibody is produced by chemical synthesis, it is generally substantially free of chemical precursors or other chemicals, *i.e.,* it is separated from chemical precursors or other chemicals which are involved in the synthesis of the protein. Accordingly, such preparations of the antibody have less than about 30%, 20%, 10%, or 5% (by dry weight) of chemical precursors or compounds other than the antibody of interest. In a specific embodiment, antibodies described herein are isolated or purified.

[00282] Antibodies or fragments thereof that specifically bind to CTLA-4 (*e.g.*, human CTLA-4) can be produced by any method known in the art for the synthesis of antibodies, for example, by chemical synthesis or by recombinant expression techniques. The methods described herein employs, unless otherwise indicated, conventional techniques in molecular biology, microbiology, genetic analysis, recombinant DNA, organic chemistry, biochemistry, PCR, oligonucleotide synthesis and modification, nucleic acid hybridization, and related fields within the skill of the art. These techniques are described, for example, in the references cited herein and are fully explained in the literature. See, *e.g.,* Maniatis T *et al.,* (1982) Molecular Cloning: A Laboratory Manual, Cold Spring Harbor Laboratory Press; Sambrook J *et al.,* (1989), Molecular Cloning: A Laboratory Manual, Second Edition, Cold Spring Harbor Laboratory Press; Sambrook J *et al.,* (2001) Molecular Cloning: A Laboratory Manual, Cold Spring Harbor Laboratory Press, Cold Spring Harbor, NY; Ausubel FM *et al.,* Current Protocols in Molecular Biology, John Wiley & Sons (1987 and annual updates); Current Protocols in Immunology, John Wiley & Sons (1987 and annual updates) Gait (ed.) (1984) Oligonucleotide Synthesis: A Practical Approach, IRL Press; Eckstein (ed.) (1991) Oligonucleotides and Analogues: A Practical Approach, IRL Press; Birren B *et al.,* (eds.) (1999) Genome Analysis: A Laboratory Manual, Cold Spring Harbor Laboratory Press, all of which are herein incorporated by reference in their entireties.

[00283] In a specific embodiment, an antibody described herein is an antibody (*e.g.*, recombinant antibody) prepared, expressed, created or isolated by any means that involves creation, *e.g.*, via synthesis, genetic engineering of DNA sequences. In certain embodiments, such antibody comprises sequences (*e.g.*, DNA sequences or amino acid sequences) that do

not naturally exist within the antibody germline repertoire of an animal or mammal (*e.g.*, human) *in vivo*.

[00284] In one aspect, provided herein is a method of making an antibody or an antigen-binding fragment thereof which specifically binds to CTLA-4 (*e.g.*, human CTLA-4) comprising culturing a cell or host cell described herein. In a certain aspect, provided herein is a method of making an antibody or an antigen-binding fragment thereof which specifically binds to CTLA-4 (*e.g.*, human CTLA-4) comprising expressing (*e.g.*, recombinantly expressing) the antibody or antigen-binding fragment thereof using a cell or host cell described herein (*e.g.*, a cell or a host cell comprising polynucleotides encoding an antibody described herein). In a particular embodiment, the cell is an isolated cell. In a particular embodiment, the exogenous polynucleotides have been introduced into the cell. In a particular embodiment, the method further comprises the step of purifying the antibody or antigen-binding fragment thereof obtained from the cell or host cell. Preferably, the method is performed *in vitro*.

[00285] Methods for producing polyclonal antibodies are known in the art (see, for example, Chapter 11 in: Short Protocols in Molecular Biology, (2002) 5th Ed., Ausubel FM *et al.*, eds., John Wiley and Sons, New York, herein incorporated by reference in its entirety).

[00286] Monoclonal antibodies can be prepared using a wide variety of techniques known in the art including the use of hybridoma, recombinant, and phage display technologies, or a combination thereof. For example, monoclonal antibodies can be produced using hybridoma techniques including those known in the art and taught, for example, in Harlow E & Lane D, Antibodies: A Laboratory Manual, (Cold Spring Harbor Laboratory Press, 2nd ed. 1988); Hammerling GJ *et al.*, in: Monoclonal Antibodies and T-Cell Hybridomas 563 681 (Elsevier, N.Y., 1981), which are herein incorporated by reference in their entireties. The term "monoclonal antibody" as used herein is not limited to antibodies produced through hybridoma technology. For example, monoclonal antibodies can be produced recombinantly from host cells exogenously expressing an antibody described herein or a fragment thereof, for example, light chain and/or heavy chain of such antibody.

[00287] In specific embodiments, a "monoclonal antibody," as used herein, is an antibody produced by a single cell (*e.g.*, hybridoma or host cell producing a recombinant antibody), wherein the antibody specifically binds to CTLA-4 (*e.g.*, human CTLA-4) as determined, *e.g.*, by ELISA or other antigen-binding or competitive binding assay known in the art or in the examples provided herein. In particular embodiments, a monoclonal antibody can be a chimeric antibody or a humanized antibody. In certain embodiments, a monoclonal antibody

is a monovalent antibody or multivalent (*e.g.*, bivalent) antibody. In particular embodiments, a monoclonal antibody is a monospecific or multispecific antibody (*e.g.*, bispecific antibody). Monoclonal antibodies described herein can, for example, be made by the hybridoma method as described in Kohler G & Milstein C (1975) Nature 256: 495, herein incorporated by reference in its entirety, or can, *e.g.*, be isolated from phage libraries using the techniques as described herein, for example. Other methods for the preparation of clonal cell lines and of monoclonal antibodies expressed thereby are well known in the art (see, for example, Chapter 11 in: Short Protocols in Molecular Biology, (2002) 5th Ed., Ausubel FM *et al., supra*).

[00288] Methods for producing and screening for specific antibodies using hybridoma technology are routine and well known in the art. For example, in the hybridoma method, a mouse or other appropriate host animal, such as a sheep, goat, rabbit, rat, hamster or macaque monkey, is immunized to elicit lymphocytes that produce or are capable of producing antibodies that will specifically bind to the protein (*e.g.*, CTLA-4 (*e.g.*, human CTLA-4)) used for immunization. Alternatively, lymphocytes may be immunized *in vitro*. Lymphocytes then are fused with myeloma cells using a suitable fusing agent, such as polyethylene glycol, to form a hybridoma cell (Goding JW (Ed), Monoclonal Antibodies: Principles and Practice, pp. 59-103 (Academic Press, 1986), herein incorporated by reference in its entirety). Additionally, a RIMMS (repetitive immunization multiple sites) technique can be used to immunize an animal (Kilpatrick KE *et al.,* (1997) Hybridoma 16:381-9, herein incorporated by reference in its entirety).

[00289] In some embodiments, mice (or other animals, such as rats, monkeys, donkeys, pigs, sheep, hamster, or dogs) can be immunized with an antigen (*e.g.*, CTLA-4 (*e.g.*, human CTLA-4)) and once an immune response is detected, *e.g.*, antibodies specific for the antigen are detected in the mouse serum, the mouse spleen is harvested and splenocytes isolated. The splenocytes are then fused by well-known techniques to any suitable myeloma cells, for example cells from cell line SP20 available from the American Type Culture Collection (ATCC®) (Manassas, VA), to form hybridomas. Hybridomas are selected and cloned by limited dilution. In certain embodiments, lymph nodes of the immunized mice are harvested and fused with NS0 myeloma cells.

[00290] The hybridoma cells thus prepared are seeded and grown in a suitable culture medium that preferably contains one or more substances that inhibit the growth or survival of the unfused, parental myeloma cells. For example, if the parental myeloma cells lack the enzyme hypoxanthine guanine phosphoribosyl transferase (HGPRT or HPRT), the culture medium for the hybridomas typically will include hypoxanthine, aminopterin, and thymidine

(HAT medium), which substances prevent the growth of HGPRT-deficient cells.

[00291] Specific embodiments employ myeloma cells that fuse efficiently, support stable high-level production of antibody by the selected antibody-producing cells, and are sensitive to a medium such as HAT medium. Among these myeloma cell lines are murine myeloma lines, such as NS0 cell line or those derived from MOPC-21 and MPC-11 mouse tumors available from the Salk Institute Cell Distribution Center, San Diego, CA, USA, and SP-2 or X63-Ag8.653 cells available from the American Type Culture Collection, Rockville, MD, USA. Human myeloma and mouse-human heteromyeloma cell lines also have been described for the production of human monoclonal antibodies (Kozbor D (1984) J Immunol 133: 3001-5; Brodeur *et al.*, Monoclonal Antibody Production Techniques and Applications, pp. 51-63 (Marcel Dekker, Inc., New York, 1987), which are herein incorporated by reference in their entireties).

[00292] Culture medium in which hybridoma cells are growing is assayed for production of monoclonal antibodies directed against CTLA-4 (*e.g.*, human CTLA-4). The binding specificity of monoclonal antibodies produced by hybridoma cells is determined by methods known in the art, for example, immunoprecipitation or by an *in vitro* binding assay, such as radioimmunoassay (RIA) or enzyme-linked immunoabsorbent assay (ELISA).

[00293] After hybridoma cells are identified that produce antibodies of the desired specificity, affinity, and/or activity, the clones may be subcloned by limiting dilution procedures and grown by standard methods (Goding JW (Ed), Monoclonal Antibodies: Principles and Practice, *supra*). Suitable culture media for this purpose include, for example, D-MEM or RPMI 1640 medium. In addition, the hybridoma cells may be grown *in vivo* as ascites tumors in an animal.

[00294] The monoclonal antibodies secreted by the subclones are suitably separated from the culture medium, ascites fluid, or serum by conventional immunoglobulin purification procedures such as, for example, protein A-Sepharose, hydroxylapatite chromatography, gel electrophoresis, dialysis, or affinity chromatography.

[00295] Antibodies described herein include antibody fragments which recognize specific CTLA-4 (*e.g.*, human CTLA-4) and can be generated by any technique known to those of skill in the art. For example, Fab and $F(ab')_2$ fragments described herein can be produced by proteolytic cleavage of immunoglobulin molecules, using enzymes such as papain (to produce Fab fragments) or pepsin (to produce $F(ab')_2$ fragments). A Fab fragment corresponds to one of the two identical arms of an antibody molecule and contains the complete light chain paired with the VH and CH1 domains of the heavy chain. A $F(ab')_2$

fragment contains the two antigen-binding arms of an antibody molecule linked by disulfide bonds in the hinge region.

[00296] Further, the antibodies described herein or antigen-binding fragments thereof can also be generated using various phage display methods known in the art. In phage display methods, functional antibody domains are displayed on the surface of phage particles which carry the polynucleotide sequences encoding them. In particular, DNA sequences encoding VH and VL domains are amplified from animal cDNA libraries (*e.g.,* human or murine cDNA libraries of affected tissues). The DNA encoding the VH and VL domains are recombined together with a scFv linker by PCR and cloned into a phagemid vector. The vector is electroporated in *E. coli* and the *E. coli* is infected with helper phage. Phage used in these methods are typically filamentous phage including fd and M13, and the VH and VL domains are usually recombinantly fused to either the phage gene III or gene VIII. Phage expressing an antigen binding domain that binds to a particular antigen can be selected or identified with antigen, *e.g.,* using labeled antigen or antigen bound or captured to a solid surface or bead. Examples of phage display methods that can be used to make the antibodies described herein include those disclosed in Brinkman U *et al.,* (1995) J Immunol Methods 182: 41-50; Ames RS *et al.,* (1995) J Immunol Methods 184: 177-186; Kettleborough CA *et al.,* (1994) Eur J Immunol 24: 952-958; Persic L *et al.,* (1997) Gene 187: 9-18; Burton DR & Barbas CF (1994) Advan Immunol 57: 191-280; PCT Application No. PCT/GB91/001134; International Publication Nos. WO 90/02809, WO 91/10737, WO 92/01047, WO 92/18619, WO 93/1 1236, WO 95/15982, WO 95/20401, and WO 97/13844; and U.S. Patent Nos. 5,698,426, 5,223,409, 5,403,484, 5,580,717, 5,427,908, 5,750,753, 5,821,047, 5,571,698, 5,427,908, 5,516,637, 5,780,225, 5,658,727, 5,733,743 and 5,969,108, all of which are herein incorporated by reference in their entireties.

[00297] As described in the above references, after phage selection, the antibody coding regions from the phage can be isolated and used to generate whole antibodies, including human antibodies, or any other desired antigen binding fragment, and expressed in any desired host, including mammalian cells, insect cells, plant cells, yeast, and bacteria, *e.g.,* as described below. Techniques to recombinantly produce antibody fragments such as Fab, Fab' and $F(ab')_2$ fragments can also be employed using methods known in the art such as those disclosed in PCT publication No. WO 92/22324; Mullinax RL *et al.,* (1992) BioTechniques 12(6): 864-9; Sawai H *et al.,* (1995) Am J Reprod Immunol 34: 26-34; and Better M *et al.,* (1988) Science 240: 1041-1043, all of which are herein incorporated by reference in their entireties.

[00298] In certain embodiments, to generate whole antibodies, PCR primers including VH or VL nucleotide sequences, a restriction site, and a flanking sequence to protect the restriction site can be used to amplify the VH or VL sequences from a template, *e.g.*, scFv clones. Utilizing cloning techniques known to those of skill in the art, the PCR amplified VH domains can be cloned into vectors expressing a VH constant region, and the PCR amplified VL domains can be cloned into vectors expressing a VL constant region, *e.g.*, human kappa or lambda constant regions. The VH and VL domains can also be cloned into one vector expressing the necessary constant regions. The heavy chain conversion vectors and light chain conversion vectors are then co-transfected into cell lines to generate stable or transient cell lines that express full-length antibodies, *e.g.*, IgG, using techniques known to those of skill in the art.

[00299] A chimeric antibody is a molecule in which different portions of the antibody are derived from different immunoglobulin molecules. For example, a chimeric antibody can contain a variable region of a mouse or rat monoclonal antibody fused to a constant region of a human antibody. Methods for producing chimeric antibodies are known in the art. See, *e.g.*, Morrison SL (1985) Science 229: 1202-7; Oi VT & Morrison SL (1986) BioTechniques 4: 214-221; Gillies SD *et al.*, (1989) J Immunol Methods 125: 191-202; and U.S. Patent Nos. 5,807,715, 4,816,567, 4,816,397, and 6,331,415, all of which are herein incorporated by reference in their entireties.

[00300] A humanized antibody is capable of binding to a predetermined antigen and which comprises a framework region having substantially the amino acid sequence of a human immunoglobulin and CDRs having substantially the amino acid sequence of a non-human immunoglobulin (*e.g.*, a murine immunoglobulin). In particular embodiments, a humanized antibody also comprises at least a portion of an immunoglobulin constant region (Fc), typically that of a human immunoglobulin. The antibody also can include the CH1, hinge, CH2, CH3, and CH4 regions of the heavy chain. A humanized antibody can be selected from any class of immunoglobulins, including IgM, IgG, IgD, IgA and IgE, and any isotype, including IgG_1, IgG_2, IgG_3 and IgG_4. Humanized antibodies can be produced using a variety of techniques known in the art, including but not limited to, CDR-grafting (European Patent No. EP 239400; International Publication No. WO 91/09967; and U.S. Patent Nos. 5,225,539, 5,530,101, and 5,585,089), veneering or resurfacing (European Patent Nos. EP 592106 and EP 519596; Padlan EA (1991) Mol Immunol 28(4/5): 489-498; Studnicka GM *et al.*, (1994) Prot Engineering 7(6): 805-814; and Roguska MA *et al.*, (1994) PNAS 91: 969-973), chain shuffling (U.S. Patent No. 5,565,332), and techniques disclosed in, *e.g.*, U.S. Pat. No.

6,407,213, U.S. Pat. No. 5,766,886, International Publication No. WO 93/17105; Tan P *et al.,* (2002) J Immunol 169: 1119-25; Caldas C *et al.,* (2000) Protein Eng. 13(5): 353-60; Morea V *et al.,* (2000) Methods 20(3): 267-79; Baca M *et al.,* (1997) J Biol Chem 272(16): 10678-84; Roguska MA *et al.,* (1996) Protein Eng 9(10): 895 904; Couto JR *et al.,* (1995) Cancer Res. 55 (23 Supp): 5973s-5977s; Couto JR *et al.,* (1995) Cancer Res 55(8): 1717-22; Sandhu JS (1994) Gene 150(2): 409-10 and Pedersen JT *et al.,* (1994) J Mol Biol 235(3): 959-73, all of which are herein incorporated by reference in their entireties. See also U.S. Application Publication No. US 2005/0042664 A1 (Feb. 24, 2005), which is incorporated by reference herein in its entirety.

[00301] Methods for making multispecific (*e.g.*, bispecific antibodies) have been described, see, for example, U.S. Patent Nos. 7,951,917; 7,183,076; 8,227,577; 5,837,242; 5,989,830; 5,869,620; 6,132,992 and 8,586,713, all of which are herein incorporated by reference in their entireties.

[00302] Single domain antibodies, for example, antibodies lacking the light chains, can be produced by methods well known in the art. See Riechmann L & Muyldermans S (1999) J Immunol 231: 25-38; Nuttall SD *et al.,* (2000) Curr Pharm Biotechnol 1(3): 253-263; Muyldermans S, (2001) J Biotechnol 74(4): 277-302; U.S. Patent No. 6,005,079; and International Publication Nos. WO 94/04678, WO 94/25591 and WO 01/44301, all of which are herein incorporated by reference in their entireties.

[00303] Further, antibodies that specifically bind to a CTLA-4 antigen can, in turn, be utilized to generate anti-idiotype antibodies that "mimic" an antigen using techniques well known to those skilled in the art. (See, *e.g.,* Greenspan NS & Bona CA (1989) FASEB J 7(5): 437-444; and Nissinoff A (1991) J Immunol 147(8): 2429-2438, which are herein incorporated by reference in their entireties).

[00304] In particular embodiments, an antibody described herein, which binds to the same epitope of CTLA-4 (*e.g.*, human CTLA-4) as an anti-CTLA-4 antibody described herein, is a human antibody or an antigen-binding fragment thereof. In particular embodiments, an antibody described herein, which competitively blocks (*e.g.*, in a dose-dependent manner) any one of the antibodies described herein, from binding to CTLA-4 (*e.g.*, human CTLA-4), is a human antibody or an antigen-binding fragment thereof. Human antibodies can be produced using any method known in the art. For example, transgenic mice which are incapable of expressing functional endogenous immunoglobulins, but which can express human immunoglobulin genes, can be used. In particular, the human heavy and light chain immunoglobulin gene complexes can be introduced randomly or by homologous

recombination into mouse embryonic stem cells. Alternatively, the human variable region, constant region, and diversity region can be introduced into mouse embryonic stem cells in addition to the human heavy and light chain genes. The mouse heavy and light chain immunoglobulin genes can be rendered non-functional separately or simultaneously with the introduction of human immunoglobulin loci by homologous recombination. In particular, homozygous deletion of the J_H region prevents endogenous antibody production. The modified embryonic stem cells are expanded and microinjected into blastocysts to produce chimeric mice. The chimeric mice are then bred to produce homozygous offspring which express human antibodies. The transgenic mice are immunized in the normal fashion with a selected antigen, *e.g.,* all or a portion of an antigen (*e.g.*, CTLA-4). Monoclonal antibodies directed against the antigen can be obtained from the immunized, transgenic mice using conventional hybridoma technology. The human immunoglobulin transgenes harbored by the transgenic mice rearrange during B cell differentiation, and subsequently undergo class switching and somatic mutation. Thus, using such a technique, it is possible to produce therapeutically useful IgG, IgA, IgM and IgE antibodies. For an overview of this technology for producing human antibodies, see Lonberg N & Huszar D (1995) Int Rev Immunol 13:65-93, which is herein incorporated by reference in its entirety. For a detailed discussion of this technology for producing human antibodies and human monoclonal antibodies and protocols for producing such antibodies, see, *e.g.,* International Publication Nos. WO 98/24893, WO 96/34096 and WO 96/33735; and U.S. Patent Nos. 5,413,923, 5,625,126, 5,633,425, 5,569,825, 5,661,016, 5,545,806, 5,814,318 and 5,939,598. Examples of mice capable of producing human antibodies include the Xenomouse™ (Abgenix, Inc.; U.S. Patent Nos. 6,075,181 and 6,150,184), the HuAb-Mouse™ (Mederex, Inc./Gen Pharm; U.S. Patent Nos. 5,545,806 and 5,569, 825), the Trans Chromo Mouse™ (Kirin) and the KM Mouse™ (Medarex/Kirin), all of which are herein incorporated by reference in their entireties.

[00305] Human antibodies which specifically bind to CTLA-4 (*e.g.*, human CTLA-4) can be made by a variety of methods known in the art including phage display methods described above using antibody libraries derived from human immunoglobulin sequences. *See* also U.S. Patent Nos. 4,444,887, 4,716,111, and 5,885,793; and International Publication Nos. WO 98/46645, WO 98/50433, WO 98/24893, WO 98/16654, WO 96/34096, WO 96/33735, and WO 91/10741, all of which are herein incorporated by reference in their entireties.

[00306] In some embodiments, human antibodies can be produced using mouse–human hybridomas. For example, human peripheral blood lymphocytes transformed with Epstein-Barr virus (EBV) can be fused with mouse myeloma cells to produce mouse–human

hybridomas secreting human monoclonal antibodies, and these mouse–human hybridomas can be screened to determine ones which secrete human monoclonal antibodies that specifically bind to a target antigen (*e.g.*, CTLA-4 (*e.g.*, human CTLA-4)). Such methods are known and are described in the art, see, *e.g.*, Shinmoto H *et al.*, (2004) Cytotechnology 46: 19-23; Naganawa Y *et al.*, (2005) Human Antibodies 14: 27-31, which are herein incorporated by reference in their entireties.

6.6 Kits

[00307] Also provided, are kits comprising one or more antibodies described herein, or pharmaceutical composition or conjugates thereof. In a specific embodiment, provided herein is a pharmaceutical pack or kit comprising one or more containers filled with one or more of the ingredients of the pharmaceutical compositions described herein, such as one or more antibodies provided herein or an antigen-binding fragment thereof. In some embodiments, the kits contain a pharmaceutical composition described herein and any prophylactic or therapeutic agent, such as those described herein. In certain embodiments, the kits may contain a T-cell mitogen, such as, *e.g.*, phytohaemagglutinin (PHA) and/or phorbol myristate acetate (PMA), or a TCR complex stimulating antibody, such as an anti-CD3 antibody and anti-CD28 antibody. Optionally associated with such container(s) can be a notice in the form prescribed by a governmental agency regulating the manufacture, use or sale of pharmaceuticals or biological products, which notice reflects approval by the agency of manufacture, use or sale for human administration.

[00308] Also provided, are kits that can be used in the above methods. In one embodiment, a kit comprises an antibody described herein, preferably a purified antibody, in one or more containers. In a specific embodiment, kits described herein contain a substantially isolated CTLA-4 antigen (*e.g.*, human CTLA-4) as a control. In another specific embodiment, the kits described herein further comprise a control antibody which does not react with a CTLA-4 antigen. In another specific embodiment, kits described herein contain one or more elements for detecting the binding of an antibody to a CTLA-4 antigen (*e.g.*, the antibody can be conjugated to a detectable substrate such as a fluorescent compound, an enzymatic substrate, a radioactive compound or a luminescent compound, or a second antibody which recognizes the first antibody can be conjugated to a detectable substrate). In specific embodiments, a kit provided herein can include a recombinantly produced or chemically synthesized CTLA-4 antigen. The CTLA-4 antigen provided in the kit can also be attached to a solid support. In a more specific embodiment, the detecting

means of the above described kit includes a solid support to which a CTLA-4 antigen is attached. Such a kit can also include a non-attached reporter-labeled anti-human antibody or anti-mouse/rat antibody. In this embodiment, binding of the antibody to the CTLA-4 antigen can be detected by binding of the said reporter-labeled antibody.

[00309] In one embodiment, the present invention relates to the use of a kit of the present invention for *in vitro* assaying and/or detection of human CTLA-4 in a biological sample.

7. EXAMPLES

[00310] The examples in this Section (*i.e.*, Section 6) are offered by way of illustration, and not by way of limitation.

7.1 Example 1: Characterization of novel anti-CTLA-4 antibodies

[00311] This example describes the characterization of antibodies that bind to human CTLA-4. In particular, this example describes the characterization of antibodies that specifically bind to human CTLA-4 and inhibit the function of CTLA-4. The sequence information of the variable regions of these antibodies is provided in Table 4. All the antibodies were expressed as IgG_1 antibodies and analyzed in the assays described below.

7.1.1 Binding of anti-CTLA-4 antibodies to CTLA-4-expressing cells

[00312] Jurkat cells engineered to constitutively express human CTLA-4 (Promega) were used to analyze the binding of anti-CTLA-4 antibodies. Briefly, the cells were stained at $5x10^5$ cells/well using 2 µg/ml of antibody in a 96-well plate for 30 minutes at 4°C. The cells were washed twice and incubated for 20 minutes at 4°C with an anti-human IgG secondary antibody (Thermo Scientific, Cat #31529). The cells were washed and suspended in 50 µl of 2% paraformaldehyde (Alfa Aesar, Cat #43368) prepared in PBS. Data were collected with BD FACS Canto II.

[00313] As shown in Figures 1A-1G, all the anti-CTLA-4 antibodies tested bound to CTLA-4-expressing cells.

7.1.2 Effect of anti-CTLA-4 antibody on human PBMCs following Staphylococcal Enterotoxin A (SEA) stimulation

[00314] The functional activities of the anti-CTLA-4 antibody AGEN1884.H3 (IgG_1) on primary human PBMCs were assessed following Staphylococcal Enterotoxin A (SEA) stimulation. Briefly, cryopreserved PBMCs were stimulated with 100 ng/ml of the SEA superantigen (Toxin Technologies, Cat# at101red) in the absence or presence of 10 µg/ml of an anti-CTLA-4 antibody or an isotype control antibody (IgG_1) for 5 days at 37°C and 5% CO_2. IL-2 concentrations in the culture supernatant were analyzed by AlphaLISA (Perkin

Elmer, Cat# AL221F).

[00315] The anti-CTLA-4 antibody AGEN1884.H3 (IgG_1) increased IL-2 production in human PBMCs stimulated with the SEA superantigen (Figure 2).

7.1.3 Effect of anti-CTLA-4 antibody on IL-2-luciferase reporter cell line

[0017] Next, the functional activities of the anti-CTLA-4 antibody AGEN1884.H3 (IgG_1) were further analyzed using an IL-2-luciferase reporter assay. Briefly, a human T cell line (Jurkat) that endogenously expressed CD3 and CD28 was engineered to constitutively express cell surface CTLA-4 and a luciferase reporter gene driven by an IL-2 promoter. The Jurkat reporter cell line was co-cultured with an antigen presenting cell line (Raji) that endogenously expressed CD80 and CD86 and was engineered to express a proprietary T cell activator (Promega). T cell receptor (TCR) triggering (signal 1) was achieved by the T cell activator; and costimulatory signaling (signal 2) was provided in trans by CD80 and CD86 expressed on Raji cells. TCR signaling in the Jurkat T cell line triggered IL-2 expression, leading to luciferase production, a surrogate marker for T cell activation. Co-culture of these two cell lines resulted in engagement of the inhibitory co-receptor CTLA-4 (expressed on Jurkat cells) with its natural ligands CD80 and CD86 (expressed on Raji cells) inhibiting T cell activation, demonstrated by a lack of luciferase expression. This inhibition was relieved upon addition of increasing concentrations of anti-CTLA-4 blocking antibodies. Luciferase expression was quantified using Bio-Glo™ reagent and the resulting data were used to determine fold response values (fold increase with AGEN1884.H3 (IgG_1) compared with an isotype control antibody (IgG_1)).

[0018] As shown in Figure 3, the anti-CTLA-4 antibody AGEN1884.H3 (IgG_1) dose-dependently released CTLA-4 mediated inhibition of T cells in this IL-2-luciferase reporter assay.

7.1.4 Effect of anti-CTLA-4 antibody on Fc gamma receptor IIIA reporter cell line

[00316] The ability of anti-CTLA-4 antibody to co-engage CTLA-4 and signal *via* activating Fc gamma receptors was evaluated using a reporter cell line expressing Fc gamma receptor IIIA (FcγRIIIA) (Promega). Briefly, Jurkat cells were engineered to constitutively express human CTLA-4 on the cell surface. These target cells were co-cultured with an effector cell line (Jurkat) engineered to express FcγRIIIA (the V158 variant) upstream of an NFAT response element (RE) driving expression of firefly luciferase. A titrated dose of AGEN1884.H3 (IgG_1) or an isotype control antibody (IgG_1) was added to the co-culture and incubated at 37°C overnight. Simultaneous engagement of AGEN1884.H3 by the target cell

line (binding to CTLA-4 by the Fab region) and effector cell line (binding to FcγRIIIA by the Fc region) triggers NFAT RE reporter gene activation and luciferase expression. The next day, Bio-Glo reagent (Promega) was added to the co-culture, luminescence was measured by EnVison Multimode Plate Reader (Perkin Elmer), and relative light units (RLU) were recorded to calculate fold response values (fold increase with AGEN1884.H3 (IgG_1) compared with an isotype control antibody (IgG_1)).

[00317] When bound to target cells expressing human CTLA-4 on the cell surface, the IgG_1 **antibody AGEN1884.H3 activated FcγRIIIA signaling in the effector cells (Figure 4).**

7.2 Example 2: Characterization of anti-CTLA-4 antibodies with different Fc regions

[00318] This example analyzes the impact of Fc/Fc receptor interaction on the functional activity of anti-CTLA-4 antibodies. AGEN1884.H3 was expressed as antibodies in which the IgG_1 Fc region comprises the S239D/I332E, S239D/A330L/I332E, or L235V/F243L/R292P/Y300L/P396L mutations, numbered according to the EU numbering system, and tested in functional assays described below. The antibody AGEN1884.H3 (IgG_1 S239D/I332E) comprises a heavy chain comprising the amino acid sequence of SEQ ID NO: 24 and a light chain comprising the amino acid sequence of SEQ ID NO: 27. The antibody AGEN1884.H3 (IgG_1 S239D/A330L/I332E) comprises a heavy chain comprising the amino acid sequence of SEQ ID NO: 25 and a light chain comprising the amino acid sequence of SEQ ID NO: 27. The antibody AGEN1884.H3 (IgG_1 L235V/F243L/R292P/Y300L/P396L) comprises a heavy chain comprising the amino acid sequence of SEQ ID NO: 26 and a light chain comprising the amino acid sequence of SEQ ID NO: 27. For comparison, AGEN1884 was also expressed as a wild type IgG_1 antibody, an IgG_1 antibody comprising S239D/I332E or S239D/A330L/I332E mutations, numbered according to the EU numbering system, or an afucosylated IgG_1 antibody, and tested in some functional assays.

7.2.1 Binding of anti-CTLA-4 antibodies to CTLA-4-expressing cells

[00319] The binding of anit-CTLA-4 antibodies AGEN1884.H3 (IgG_1 S239D/I332E), AGEN1884.H3 (IgG_1 S239D/A330L/I332E), and AGEN1884.H3 (IgG_1 L235V/F243L/R292P/Y300L/P396L) to CTLA-4-expressing cells was characterized similarly as described above. Briefly, Jurkat cells engineered to express human CTLA-4 (Promega) were stained first with 5 μg/ml of an anti-CTLA-4 antibody or an isotype control antibody and then with an anti-human IgG secondary antibody (Thermo Scientific, Cat #31529). The cells were analyzed using BD FACS Canto II.

[00320] As shown in Figures 5A-5D, AGEN1884.H3 antibodies with different Fc regions all bound to cells expressing human CTLA-4.

7.2.2 Effect of anti-CTLA-4 antibody on ligand binding to human CTLA-4

[00321] In this example, the ability of an Fc variant anti-CTLA-4 antibody AGEN1884.H3 (IgG_1-S239D/A330E/I332E) to block binding between human CTLA-4 and its ligands, CD80 and CD86, was tested.

[00322] Briefly, recombinant CD80-Fc and CD86-Fc proteins were conjugated to the fluorochrome Alexa Fluor 647 (Invitrogen, A20186). Jurkat cells were transduced with tr*CTLA4* **(truncated intracellular domain) under the control of the EF1α promoter, as** described in Nakaseko et al. (J Exp Med. 1999 Sep 20; 190(6): 765–774), thus producing a cell line that constitutively expressed human CTLA-4 on the cell surface. CTLA-4-expressing cells were incubated with a dose titration of anti-CTLA-4 antibody AGEN1884.H3 (IgG_1-S239D/A330E/I332E), a reference anti-CTLA-4 antibody, or an isotype control antibody (IgG_1). The cells were then stained with fluorescently labelled CD80-Fc or CD86-Fc protein. Following staining, fluorescence was analyzed using the LSRFortessa flow cytometer (BD Biosciences). FACS plots were analyzed using a combination of FACS DIVA and WEHI Weasel software. Values were plotted using Graphpad Prism software.

[00323] As shown in Figure 6A, AGEN1884.H3 (IgG_1-S239D/A330E/I332E) and the reference anti-CTLA-4 antibody each blocked binding between human CTLA-4 and CD80 in a dose-dependent manner, whereas isotype control antibody (IgG_1) had no effect. As shown in Figure 6B, AGEN1884.H3 (IgG_1-S239D/A330E/I332E) and the reference anti-CTLA-4 antibody each also blocked binding between human CTLA-4 and CD86 in a dose-dependent manner, whereas isotype control antibody (IgG_1) had no effect. These data show that AGEN1884.H3 (IgG_1-S239D/A330E/I332E) functions as a ligand-blocking antibody for CTLA-4.

7.2.3 Effect of anti-CTLA-4 antibodies on human PBMCs following Staphylococcal Enterotoxin A (SEA) stimulation

[00324] In this example, the impact of Fc regions on the functional activity of anti-CTLA-4 antibodies was analyzed using the SEA stimulation assay described above. In brief, human PBMCs were cultured in vitro with 100 ng/ml of the SEA peptide (Toxin Technologies, Cat# at101red) in the absence or presence of anti-CTLA-4 antibodies with different Fc regions or an isotype control antibody. After five days, concentrations of IL-2 in the culture supernatant, a marker of T cell activation, were measured using AlphaLISA (Perkin Elmer,

Cat# AL221F).

[00325] As shown in Figure 7A, the three AGEN1884.H3 antibodies containing mutations in the IgG_1 **Fc regions, all of which enhanced binding to FcγRIIIA, stimulated more IL-2** secretion than AGEN1884.H3 with a wild type IgG_1 Fc region.

[00326] In similar studies, AGEN1884.H3 or AGEN1884 antibodies with different Fc regions were tested in the SEA stimulation assay. Introducing S239D/I332E, S239D/A330L/I332E, or L235V/F243L/R292P/Y300L/P396L substitutions in the IgG_1 Fc region significantly enhanced the functional activity of AGEN1884.H3 (Figure 7B). Similarly, AGEN1884 (IgG_1 S239D/I332E), AGEN1884 (IgG_1 S239D/A330L/I332E), and afucosylated AGEN1884 (IgG_1) enhanced IL-2 production at substantially lower concentrations compared to AGEN1884 with a wild type IgG_1 Fc region (Figure 7C).

7.2.4 Effect of anti-CTLA-4 antibodies on ZAP70 phosphorylation

[00327] In this example, the impact of Fc regions on the functional activity of anti-CTLA-4 antibodies in the T cell-antigen presenting cell (APC) synapse was analyzed using an assay that measures extent of phosphorylation of the protein tyrosine kinase ZAP70, which is recruited to the TCR following TCR engagement, where it becomes phosphorylated and facilitates downstream signaling events.

[00328] Briefly, human PBMCs were incubated with a suboptimal concentration of SEA peptide and 10 μg/mL of isotype control antibody (IgG_1) or the anti-CTLA-4 antibodies AGEN1884.H3 (IgG_1), AGEN1884.H3 (IgG_1 S239D/A330L/I332E), or AGEN1884.H3 (IgG_1 N297A). Cells were then incubated at 37°C for 0 (pre) 1, 5, 10, 30, or 60 minutes. At the end of the incubation, cells were lysed with cold 1×RIPA buffer supplemented with a phosphatase/protease inhibitor cocktail (Cell Signaling Technologies). Following supernatant clarification, protein concentration was quantified using bicinchoninic acid (BCA) analysis (Pierce Biotechnology). Cell lysates (20 μg/lane) were prepared in Bolt LDS sample buffer diluted and heated for 10 minutes at 70°C before being loaded onto a 4-12% Bolt Bis Tris gels (Novex). Proteins were separated in 1x Bolt MOPS-buffer (ThermoFisher) and then blotted onto a PVDF membrane. Following blockade with 5% bovine serum albumin (BSA, 1 hour), samples were incubated with primary anti-human rabbit phospho-ZAP70 (Tyr493)/Syk (Tyr526) antibody (Cell Signaling Technologies) in blocking buffer overnight at 4°C. Membranes were probed with goat anti-rabbit secondary HRP-conjugate and visualized with SignalFire ECL reagent (Cell Signaling Technology). Images were captured using the Chemidoc imaging system (BioRad). As a control, total ZAP70 protein was evaluated

following membrane stripping with Restore™ PLUS Western Blot Stripping Buffer. Densitometric analysis of phospho-ZAP70 normalized to that of total ZAP70 was performed using Image J (Wayne Rasband; National Institute of Mental Health, Bethesda, MD, USA) and expressed as the fold change relative to the isotype control treated samples that was incubated for 1 minute.

[00329] As shown in Figures 8A-8B, in the isotype control antibody sample, ZAP70 phosphorylation was transiently increased within ten minutes after stimulation and rapidly diminished with no detectable levels after 15 minutes. In contrast, the addition of anti-CTLA-4 antibodies AGEN1884.H3 (IgG_1) or AGEN1884.H3 (IgG_1 S239D/A330L/I332E) extended detectable ZAP70 activation to 30 minutes, with the most pronounced activity and relative abundance observed with AGEN1884.H3 (IgG_1 S239D/A330L/I332E).

7.2.5 Effect of murine anti-CTLA-4 antibodies on tumor growth and intratumoral regulatory T cell depletion in a mouse model

[00330] In this example, the impact of Fc regions on the antitumor and intratumoral regulatory T cell (Treg) depletion activities of anti-CTLA-4 antibodies was analyzed using a mouse model for colon cancer (CT26 tumor-bearing mice).

[00331] Briefly, $5x10^4$ CT26 tumor cells were suspended in 100 ml PBS and injected subcutaneously into 6-8 week old female BALB/cJ mice (Jackson Laboratories). Following engraftment to a tumor volume of 50-80mm^3, mice were treated with a single 100 μg dose of murine anti-CTLA-4 antibody 9D9 (mIgG2a), an Fc-silent variant of anti-CTLA-4 antibody 9D9 (mIgG2a-N297A), an Fc variant of anti-CTLA-4 antibody 9D9 (mIgG2a-S239D/A330L/I332E), or an isotype control antibody (mIgG2a). Amino acid sequences for the murine antibodies tested are shown in Table 7.

Table 7. Amino acid sequences of murine anti-CTLA-4 antibodies

Description	Sequence	SEQ ID NO
Murine anti-CTLA-4 antibody (mIgG2a) heavy chain	EAKLQESGPVLVKPGASVKMSCKASGYTFTDY YMNWVKQSHGKSLEWIGVINPYNGDTSYNQKF KGKATLTVDKSSSTAYMELNSLTSEDSAVYYC ARYYGSWFAYWGQGTLVTVSSAKTTAPSVYPL APVCGDTTGSSVTLGCLVKGYFPEPVTLTWNSG SLSSGVHTFPAVLQSDLYTLSSSVTVTSSTWPSQ SITCNVAHPASSTKVDKKIEPRGPTIKPCPPCKCP APNLLGGPSVFIFPPKIKDVLMISLSPIVTCVVVD VSEDDPDVQISWFVNNVEVHTAQTQTHREDYN STLRVVSALPIQHQDWMSGKEFKCKVNNKDLP APIERTISKPKGSVRAPQVYVLPPPEEEMTKKQV TLTCMVTDFMPEDIYVEWTNNGKTELNYKNTE PVLDSDGSYFMYSKLRVEKKNWVERNSYSCSV	49

	VHEGLHNHHTTKSFSRTPG	
Murine anti-CTLA-4 antibody (mIgG2a-S239D/A330L/I332E) heavy chain	EAKLQESGPVLVKPGASVKMSCKASGYTFTDY YMNWVKQSHGKSLEWIGVINPYNGDTSYNQKF KGKATLTVDKSSSTAYMELNSLTSEDSAVYYC ARYYGSWFAYWGQGTLVTVSSAKTTAPSVYPL APVCGDTTGSSVTLGCLVKGYFPEPVTLTWNSG SLSSGVHTFPAVLQSDLYTLSSSVTVTSSTWPSQ SITCNVAHPASSTKVDKKIEPRGPTIKPCPPCKCP APNLLGGPDVFIFPPKIKDVLMISLSPIVTCVVVD VSEDDPDVQISWFVNNVEVHTAQTQTHREDYN STLRVVSALPIQHQDWMSGKEFKCKVNNKDLP LPEERTISKPKGSVRAPQVYVLPPPEEEMTKKQ VTLTCMVTDFMPEDIYVEWTNNGKTELNYKNT EPVLDSDGSYFMYSKLRVEKKNWVERNSYSCS VVHEGLHNHHTTKSFSRTPG	50
Murine anti-CTLA-4 antibody (mIgG2a-N297A) heavy chain	EAKLQESGPVLVKPGASVKMSCKASGYTFTDY YMNWVKQSHGKSLEWIGVINPYNGDTSYNQKF KGKATLTVDKSSSTAYMELNSLTSEDSAVYYC ARYYGSWFAYWGQGTLVTVSSAKTTAPSVYPL APVCGDTTGSSVTLGCLVKGYFPEPVTLTWNSG SLSSGVHTFPAVLQSDLYTLSSSVTVTSSTWPSQ SITCNVAHPASSTKVDKKIEPRGPTIKPCPPCKCP APNLLGGPSVFIFPPKIKDVLMISLSPIVTCVVVD VSEDDPDVQISWFVNNVEVHTAQTQTHREDYA STLRVVSALPIQHQDWMSGKEFKCKVNNKDLP APIERTISKPKGSVRAPQVYVLPPPEEEMTKKQV TLTCMVTDFMPEDIYVEWTNNGKTELNYKNTE PVLDSDGSYFMYSKLRVEKKNWVERNSYSCSV VHEGLHNHHTTKSFSRTPG	51
Murine anti-CTLA-4 antibody (mIgG2a) light chain	DIVMTQTTLSLPVSLGDQASISCRSSQSIVHSNG NTYLEWYLQKPGQSPKLLIYKVSNRFSGVPDRF SGSGSGTDFTLKISRVEAEDLGVYYCFQGSHVP YTFGGGTKLEIKRADAAPTVSIFPPSSEQLTSGG ASVVCFLNNFYPKDINVKWKIDGSERQNGVLNS WTDQDSKDSTYSMSSTLTLTKDEYERHNSYTC EATHKTSTSPIVKSFNRNEC	52

[00332] In a first experiment, mice treated with antibodies were then measured biweekly for tumor growth. As shown in Figure 9A, an Fc variant of anti-CTLA-4 antibody 9D9 (shown as mIgG2a-S239D/A330L/I332E) induced complete regression in all CT26 tumor-bearing mice (eight out of eight mice tested). In contrast, other variants of antibody 9D9 failed to elicit the same efficacy: antibody 9D9 (mIgG2a) itself induced complete regressions in three out of nine mice tested, and the Fc-silent variant of antibody 9D9 (mIgG2a-N297A) failed to induce regression in any of the nine mice tested.

[00333] In a second experiment, CT26 tumor-bearing mice were treated as described

above and then sacrificed at 0, 24, 72, or 240 hours post-treatment for collection of tumor tissue, tumor draining lymph nodes, and spleens. Collected tissues were evaluated for $FoxP3^+$ Treg expansion by flow cytometry. Single cell suspensions were obtained by mechanical dissociation followed by filtration (70 μM cell strainer). To reduce non-specific binding, the cells were incubated with an FcγR blocking antibody (Biolegend) in FACS buffer (PBS, 2mM EDTA, 0.5% BSA, pH 7.2) for 15 minutes at ambient temperature. Samples were then washed twice in FACS buffer and stained for a lineage panel of CD3, CD4, CD8, and CD25, as well as a fixable live/dead marker, for 30 minutes at 4°C. For Treg delineation, samples were then washed twice, fixed, permeabilized, and then incubated with an anti-FoxP3 antibody (FJK-16s) for 30 minutes at 4°C. Samples were analyzed using the LSRFortessa flow cytometer (BD Biosciences). FACS plots were analyzed using a combination of FACS DIVA and WEHI Weasel software. As shown in Figure 9B, the anti-CTLA-4 antibody 9D9 (mIgG2a) and the Fc variant anti-CTLA-4 antibody 9D9 (mIgG2a-S239D/A330L/I332E) each reduced the quantities of intratumoral FoxP3+ Tregs compared to the isotype control antibody, with the Fc variant anti-CTLA-4 antibody 9D9 (mIgG2a-S239D/A330L/I332E) decreasing the quantity of intratumoral FoxP3+ Tregs most significantly. The Fc-silent variant of anti-CTLA-4 antibody 9D9 (mIgG2a-N297A) did not substantially reduce the quantity of intratumoral FoxP3+ Tregs relative to the isotype control antibody. None of the treatment groups showed substantial changes in the quantities of intratumoral CD45+ leukocytes or CD4+ non-Tregs. The Fc variant anti-CTLA-4 antibody 9D9 (mIgG2a-S239D/A330L/I332E) induced the largest increase in intratumoral CD8/Treg ratio over time, followed by antibody 9D9 (mIgG2a), and then by the Fc-silent variant antibody 9D9 (mIgG2a-N297A) and the isotype control antibody (mIgG2a).

[00334] As shown in Figure 9C, the anti-CTLA-4 antibody 9D9 (mIgG2a), the Fc variant anti-CTLA-4 antibody 9D9 (mIgG2a-S239D/A330L/I332E) and the Fc-silent variant of anti-CTLA-4 antibody 9D9 (mIgG2a-N297A) had no substantial effect on the quantities of tumor draining lymph node (TDLN) FoxP3+ Tregs compared to the isotype control antibody. Similarly, as shown in Figure 9D, the anti-CTLA-4 antibody 9D9 (mIgG2a), the Fc variant anti-CTLA-4 antibody 9D9 (mIgG2a-S239D/A330L/I332E) and Fc-silent variant of anti-CTLA-4 antibody 9D9 (mIgG2a-N297A) had no substantial effect on the quantities of splenic FoxP3+ Tregs compared to the isotype control antibody.

7.2.6 Effect of murine anti-CTLA-4 antibodies in combination with a tumor vaccine on tumor growth

[00335] In this example, the effect on tumor growth of a combination of murine anti-

CTLA-4 antibodies and an HPV tumor vaccine was tested in the HPV+ TC-1 syngeneic tumor mouse model.

[00336] The TC-1 cell line was developed by co-transformation of primary lung epithelial cells (C57BL/6) with c-Ha-ras and HPV-16 (E6/E7) oncogenes, as described in Lin et al. (1996, Cancer Res. 56(1): 21-26). For tumor implantation, $2x10^5$ TC-1 cells were injected subcutaneously into 6-8 week old female C57BL/6 mice (Jackson Laboratories). At each of days day 5, 10 and 15 post-tumor implantation, mice were administered 100 μg of anti-CTLA-4 antibody 9D9 (mIgG2a), an Fc variant anti-CTLA-4 antibody 9D9 (mIgG2a-S239D/A330L/I332E), or an isotype control antibody (mIgG2a), in combination with a dose of HPV vaccine (HPV^+ tumor, viral antigens E6/E7) or no additional treatment. Each dose of HPV vaccine contained 30 μg of HSP protein (0.4 nM) complexed with HPV pool peptide (1.2 nM) and was supplemented with 10 μg QS-21 Stimulon® adjuvant. After treatment, mice were assessed biweekly for tumor growth and were sacrificed when tumors reached 2000 mm^3 or upon ulceration.

[00337] As shown in Figure 10, the antitumor efficacy of anti-CTLA-4 antibody 9D9 (mIgG2a) and the Fc variant anti-CTLA-4 antibody 9D9 (mIgG2a-S239D/A330L/I332E) each showed improvement when administered in combination with HPV tumor vaccine. This effect was greater for the Fc variant anti-CTLA-4 antibody (mIgG2a-S239D/A330L/I332E). In particular, the Fc variant anti-CTLA-4 antibody 9D9 (mIgG2a-S239D/A330L/I332E) induced a noticeable additional decrease in TC-1 tumor growth when combined with the HPV tumor vaccine, relative to when the antibody was administered as a single agent. This additional decrease in tumor growth was greater than that observed for the combinations of antibody 9D9 (mIgG2a) or the isotype control antibody (mIgG2a) with the HPV tumor vaccine.

7.2.7 Characterization of expanded and activated T cell populations

[00338] In this example, expanded and activated T cell populations were characterized for gene expression and CpG methylation. In brief, natural $CD4^+$ $CD25^+$ $FOXP3^+$ regulatory T cells or $CD4^+$ $CD25^{+/-}$ $FOXP3^-$ non-regulatory T cells were isolated from peripheral blood of a healthy human donor, expanded, and activated. The T cells were then characterized for expression of FOXP3 and CTLA-4 by flow cytometry, and assessed for lineage stability by examining DNA CpG methylation at CpG regions within the *FOXP3* and *CTLA4* loci. As known in the art, hypomethylation at these CpG sites can be used to accurately delineate effector versus regulatory T cell lineages (Waight et al., 2015, J. Immunol. 194(3): 878-882).

[00339] PBMCs were isolated via Ficoll gradient from healthy donor buffy coats (Research Blood Components, LLC) and were then enriched for effector T cells (Teffs) or natural regulatory T cells (Tregs) using magnetic bead isolation (MACS, Miltenyi). The enriched Teffs or Tregs were activated with CD3-CD28 microbeads (1:1 bead:cell ratio; Invitrogen) and with recombinant human IL-2 for seven days in RPMI media supplemented with 10% heat-inactivated FBS at 37°C and 5% CO_2. Following stimulation, the cells were evaluated for FOXP3 and CTLA-4 expression via flow cytometry. To reduce non-specific binding, the cells were pre-incubated with an FcγR blocking antibody (Biolegend) in FACS buffer (PBS, 2mM EDTA, 0.5% BSA, pH 7.2) for 15 minutes at ambient temperature. Samples were then washed twice in FACS buffer and stained with a lineage panel of CD3, CD4, CD8, CD25, as well as a fixable cell death marker, for 30 minutes at 4°C. To assess membrane CTLA-4 expression, CTLA-4 staining was conducted at 37°C. For intracellular FOXP3 and CTLA-4 staining, samples were washed twice, fixed, permeabilized, and incubated with an anti-FOXP3 antibody (PCH101) and anti-CTLA-4 antibody (BNI3), respectively, for 30 minutes at 4°C. Samples were then washed twice and analyzed using the LSRFortessa flow cytometer (BD Biosciences). FACS plots were analyzed using a combination of FACS DIVA and WEHI Weasel software. For CpG methylation analysis, total DNA was isolated from approximately $1x10^5$ naïve $CD4^+$ T cells, activated Teffs, or activated Tregs and subjected to pyrosequencing.

[00340] As shown in Figure 11A, a high level of FOXP3 expression was detected on activated Tregs, as well as high levels of both intracellular and membrane CTLA-4 expression. In contrast, activated Teffs showed reduced levels of FOXP3, intracellular CTLA-4, and membrane CTLA-4 relative to activated Tregs. In particular, substantially less membrane CTLA-4 expression was observed for activated Teffs compared to activated Tregs. Figure 11B further shows that activated Tregs also exhibited hypomethylated *FOXP3* and *CTLA4* CpG regions compared to naïve and activated Teffs from the same donor.

7.2.8 Effect of anti-CTLA-4 antibodies on antibody dependent cellular cytotoxicity of CTLA-4-expressing human T cells

[00341] In this example, the effect of anti-CTLA-4 antibody AGEN1884.H3 (IgG_1) or Fc variants thereof on antibody dependent cellular cytotoxicity (ADCC) of human CTLA-4-expressing T cells was assessed using high content microscopy of caspase 3/7 activation to quantify ADCC activity.

[00342] Briefly, CTLA-4-expressing target cells were co-cultured with NK-92 cells expressing FcγRIIIA, following opsonization with 10 μg/ml of anti-CTLA-4 antibody or Fc

variants thereof, as described below. In a first experiment, Jurkat cells engineered to constitutively express cell-surface human CTLA-4 were used as target cells. CTLA-4-expressing Jurkat cells were generated by transducing the Jurkat cell line with tr*CTLA4* **(intracellular domain removed) under the control of the EF1α promoter, as described in** Nakaseko et al. (1999, J. Exp. Med. 190(6): 765-774). In a second experiment, primary human activated effector and regulatory T cells were used as target cells. CTLA-4-**expressing target cells and FcγRIIIA-158V-expressing NK-92 cells were differentially** stained using red and blue live-cell tracers (Thermo Fisher) and co-cultured at a 1:1 cell ratio ($1.5x10^3$ cells/well in 384-well plates). Samples were treated with 10 μg/ml of AGEN1884.H3 (IgG_1), AGEN1884.H3 (IgG_1 N297A), AGEN1884.H3 (IgG_1 S239D/A330L/I332E), AGEN1884.H3 (IgG_1 S267E/L328F), afucosylated AGEN1884.H3 (IgG_1), or an isotype control antibody (IgG_1). Samples were then evaluated for the induction of apoptosis over time by live confocal imaging of caspase 3/7 substrate, which fluoresces following cleavage by activated caspase. Sample images were acquired every 20 minutes for six hours. Percentage ADCC activity is measured as the number of apoptotic cells relative to the total cell count under each condition.

[00343] As shown in Figure 12A, the Fc variant AGEN1884.H3 (IgG_1 S239D/A330L/I332E) antibody, the afucosylated AGEN1884.H3 antibody, and the AGEN1884.H3 (IgG_1) antibody each induced substantially greater ADCC activity in Jurkat cells engineered to express cell-surface CTLA-4 relative to the AGEN1884.H3 (IgG_1 N297A) variant, the AGEN1884.H3 (IgG_1 S267E/L328F) variant, and isotype control antibody (IgG_1). The AGEN1884.H3 (IgG_1 S239D/A330L/I332E) Fc variant antibody and the afucosylated AGEN1884.H3 antibody induced greater increases in ADCC activity compared to the AGEN1884.H3 (IgG_1) antibody. As shown in Figure 12B, the AGEN1884.H3 (IgG_1 S239D/A330L/I332E) Fc variant antibody induced the highest levels of ADCC in both primary human activated effector T cells (left panel) and primary human activated regulatory T cells (right panel), followed by afucosylated AGEN1884.H3 antibody. The AGEN1884.H3 (IgG_1) antibody also induced slightly higher levels of ADCC compared to controls. The remaining antibodies tested induced little to no ADCC activity in either effector or regulatory T cells. Notably, the AGEN1884.H3 (IgG_1 S239D/A330L/I332E) Fc variant antibody and the afucosylated AGEN1884.H3 antibody each induced substantially greater ADCC in regulatory T cells compared to effector T cells.

7.2.9 Effect of anti-CTLA-4 antibodies in combination with an anti-PD-1 antibody on T cell functionality

[00344] In this example, the effect of anti-CTLA-4 antibodies in combination with an anti-PD-1 antibody on primary human T cell function was examined.

[00345] Briefly, PBMCs were isolated via Ficoll gradient from healthy donor buffy coats (Research Blood Components, LLC) of two human donors. This experiment was performed twice on PBMCs collected from each donor, for a total of two replicates per donor. For each replicate, isolated PBMCs were incubated for four days under stimulatory culture conditions with a dosage titration of anti-CTLA-4 antibody AGEN1884.H3 (IgG_1), an Fc variant anti-CTLA-4 antibody AGEN1884.H3 (IgG_1 S239D/A330L/I332E), or an isotype control antibody (IgG_1), in combination with a fixed dosage (5 μg/ml) of a reference anti-PD-1 antagonist antibody or an isotype control antibody (IgG_4). Stimulatory culture conditions were defined as cells suspended in RPMI media, supplemented with 100 ng/ml SEA superantigen (Sigma-Aldrich), 10% heat-inactivated FBS at 37°C, and 5% CO_2. Following incubation, cell-free supernatants were assayed for IL-2 production using an AlphaLISA immunoassay (Perkin-Elmer). Data was collected using the EnVision® Multilabel Plate Reader (Perkin-Elmer), and the concentration of IL-2 was determined using an IL-2 standard curve. Values were interpolated and plotted using Graphpad Prism software.

[00346] As shown in Figures 13A-13D, the anti-CTLA-4 antibody AGEN1884.H3 (IgG_1) and the Fc variant anti-CTLA-4 antibody AGEN1884.H3 (IgG_1 S239D/A330L/I332E) each induced increased IL-2 production relative to isotype controls or reference anti-PD-1 antibody alone. IL-2 production was further enhanced when AGEN1884.H3 or AGEN1884.H3 (IgG_1 S239D/A330L/I332E) was combined with reference anti-PD-1 antibody. Whether administered with isotype control antibody or in combination with anti-PD-1 reference antibody, the Fc variant anti-CTLA-4 antibody AGEN1884.H3 (IgG_1 S239D/A330L/I332E) induced a greater increase in IL-2 production compared to AGEN1884.H3 (IgG_1). This effect was consistent in replicates for the first donor (Figures 13A and 13B) and the second donor (Figures 13C and 13D).

7.3 Example 3: Epitope mapping of anti-CTLA-4 antibody

[00347] The interaction of the Fab fragment of AGEN1884 (AGEN1884-Fab) with the extracellular domain of human CTLA-4 was studied by hydrogen-deuterium exchange (HDX) mass spectrometry. CTLA-4 extracellular domain alone or in combination with AGEN1884-Fab, in phosphate buffered saline solution at pH 7.4, was diluted with a ten-fold

volume of deuterium oxide labeling buffer and incubated for varying periods of time (0, 60, 300, 1800, and 7200 seconds) at room temperature. Exchange of deuterium for hydrogen was quenched by adding one volume of 4 M guanidine hydrochloride, 0.85 M TCEP (tris(2-carboxyethyl)phosphine) buffer and the final pH was 2.5. Samples were then subjected to on-column pepsin/protease type XIII digestion and LC-MS analysis. Mass spectra were recorded in MS only mode. For the calculation of deuterium incorporation, the mass spectra for a given peptide were combined across the extracted ion chromatogram peak and the weighted average m/z was calculated. The mass increase from the mass of the native peptide (0 minute) to the weighted averaged mass corresponds to the level of deuterium incorporation. The deuterium buildup curves over exchange time for all the peptides were plotted for further analysis and were compared with HDExaminer software.

[00348] Most of the CTLA-4 peptides displayed identical or similar deuterium levels with and without the anti-human CTLA-4 Fab present. Several peptide segments, however, were found to have significantly decreased deuterium incorporation upon Fab binding. All the residues in this paragraph are numbered according to SEQ ID NO: 33. Two regions, residues 80-82 (QVT, SEQ ID NO: 39) and residues 135-149 (YPPPYYLGIGNGTQI, SEQ ID NO: 37), experienced strong deuterium protection when human CTLA-4 was bound to Fab. The strongest decrease in deuterium uptake was observed at residues 140-141 (YL) which thus appeared to be a main feature of the epitope of AGEN1884 on CTLA-4. Inspection of the sequences of human and cynomolgus monkey CTLA-4, both of which AGEN1884 binds strongly (data not shown), reveals almost complete sequence identity in the two regions described above, except for a methionine substitution for leucine at position 141 (Figure 14A). In contrast, AGEN1884 does not bind to any significant extent to either mouse or rat CTLA-4 (data not shown) which differ from human CTLA-4 at residues 140-143 (YLGI, SEQ ID NO: 34) at three out of four positions (Figure 14A). Further selectivity data show that AGEN1884 binds with high specificity to human and cynomolgus monkey CTLA-4 and not to other related CD28 family members including CD28, ICOS, BTLA, and PD-1 (data not shown). Sequence comparison among these related proteins shows that the non-CTLA-4 proteins all differ at residues 140-143 (YLGI, SEQ ID NO: 34) (Figure 14B), further supporting the importance of this epitope to the binding of AGEN1884.

* * *

[00349] The invention is not to be limited in scope by the specific embodiments described herein. Indeed, various modifications of the invention in addition to those described will become apparent to those skilled in the art from the foregoing description and accompanying

figures. Such modifications are intended to fall within the scope of the appended claims.

[00350] All references (*e.g.*, publications or patents or patent applications) cited herein are incorporated herein by reference in their entireties and for all purposes to the same extent as if each individual reference (*e.g.*, publication or patent or patent application) was specifically and individually indicated to be incorporated by reference in its entirety for all purposes.

[00351] Other embodiments are within the following claims.

WHAT IS CLAIMED:

1. An isolated antibody that specifically binds to human CTLA-4, the antibody comprising a heavy chain variable region comprising complementarity determining regions CDRH1, CDRH2, and CDRH3 and a light chain variable region comprising complementarity determining regions CDRL1, CDRL2, and CDRL3, wherein:

(a) CDRH1 comprises the amino acid sequence of SYSMN (SEQ ID NO: 10);

(b) CDRH2 comprises the amino acid sequence of SISSSSSYIYYAXSVKG (SEQ ID NO: 18), wherein X is E or D;

(c) CDRH3 comprises the amino acid sequence of VGLXGPFDI (SEQ ID NO: 19), wherein X is F or M;

(d) CDRL1 comprises the amino acid sequence of RASQSVSRYLG (SEQ ID NO: 15);

(e) CDRL2 comprises the amino acid sequence of GASTRAT (SEQ ID NO: 16); and

(f) CDRL3 comprises the amino acid sequence of QQYGSSPWT (SEQ ID NO: 17),

and wherein the CDRH1, CDRH2, and CDRH3 sequences of the antibody are not SEQ ID NOs: 10, 11, and 13, respectively.

2. The isolated antibody of claim 1, wherein CDRH2 comprises the amino acid sequence of SEQ ID NO: 12.

3. The isolated antibody of claim 1 or 2, wherein CDRH3 comprises the amino acid sequence of SEQ ID NO: 14.

4. An isolated antibody that specifically binds to human CTLA-4, the antibody comprising a heavy chain variable region comprising complementarity determining regions CDRH1, CDRH2, and CDRH3 and a light chain variable region comprising complementarity determining regions CDRL1, CDRL2, and CDRL3, wherein CDRH1, CDRH2, CDRH3, CDRL1, CDRL2, and CDRL3 comprise the amino acid sequences set forth in SEQ ID NOs: 10, 12, 14, 15, 16, and 17, respectively.

5. The isolated antibody of claim 1, wherein the antibody comprises a heavy chain variable region comprising the amino acid sequence of SEQ ID NO: 20.

6. The isolated antibody of claim 1, wherein the antibody comprises a heavy chain variable region comprising an amino acid sequence which is at least 75%, 80%, 85%, 90%, 95%, 99%, or 100% identical to an amino acid sequence selected from the group consisting of SEQ ID NOs: 2 and 4-8.

7. The isolated antibody of claim 6, wherein the heavy chain variable region comprises an amino acid sequence selected from the group consisting of SEQ ID NOs: 2 and 4-8.

8. The isolated antibody of claim 7, wherein the heavy chain variable region comprises the amino acid sequence of SEQ ID NO: 8.
9. The isolated antibody of any one of the preceding claims, wherein the antibody comprises a light chain variable region comprising an amino acid sequence which is at least 75%, 80%, 85%, 90%, 95%, 99%, or 100% identical to the amino acid sequence of SEQ ID NO: 9.
10. The isolated antibody of claim 9, wherein the antibody comprises a light chain variable region comprising the amino acid sequence of SEQ ID NO: 9.
11. An isolated antibody that specifically binds to human CTLA-4, the antibody comprising a heavy chain variable region comprising an amino acid sequence selected from the group consisting of SEQ ID NOs: 2-8.
12. The isolated antibody of claim 11, wherein the heavy chain variable region comprises the amino acid sequence of SEQ ID NO: 8.
13. The isolated antibody of claim 12, wherein the antibody comprises a heavy chain comprising an amino acid sequence selected from the group consisting of SEQ ID NOs: 23-26.
14. An isolated antibody that specifically binds to human CTLA-4, the antibody comprising a heavy chain variable region and a light chain variable region, wherein the heavy chain variable region and the light chain variable region comprise the amino acid sequences set forth in SEQ ID NOs: 2 and 9; 3 and 9; 4 and 9; 5 and 9; 6 and 9; 7 and 9; or 8 and 9, respectively.
15. The isolated antibody of claim 14, wherein the heavy chain variable region and the light chain variable region comprise the amino acid sequences set forth in SEQ ID NOs: 8 and 9, respectively.
16. An isolated antibody that specifically binds to human CTLA-4, the antibody comprising a heavy chain variable region having an amino acid sequence derived from a human IGHV3-21 germline sequence, wherein the heavy chain variable region comprises the amino acid sequence set forth in SEQ ID NO: 14.
17. The isolated antibody of claim 16, wherein the antibody comprises a light chain variable region having an amino acid sequence derived from a human IGKV3-20 germline sequence.
18. An isolated antibody that specifically binds to human CTLA-4, the antibody comprising a heavy chain comprising the amino acid sequence of SEQ ID NO: 23, and a light chain comprising the amino acid sequence of SEQ ID NO: 27.

19. An isolated antibody that specifically binds to human CTLA-4, the antibody comprising a heavy chain comprising the amino acid sequence of SEQ ID NO: 24, and a light chain comprising the amino acid sequence of SEQ ID NO: 27.
20. An isolated antibody that specifically binds to human CTLA-4, the antibody comprising a heavy chain comprising the amino acid sequence of SEQ ID NO: 25, and a light chain comprising the amino acid sequence of SEQ ID NO: 27.
21. An isolated antibody that specifically binds to human CTLA-4, the antibody comprising a heavy chain comprising the amino acid sequence of SEQ ID NO: 26, and a light chain comprising the amino acid sequence of SEQ ID NO: 27.
22. The isolated antibody of any one of claims 1-12 or 14-17, wherein the antibody comprises a heavy chain constant region selected from the group consisting of human IgG_1, IgG_2, IgG_3, IgG_4, IgA_1, and IgA_2.
23. The isolated antibody of any one of claims 1-12 or 14-17, wherein the antibody comprises an IgG_1 heavy chain constant region.
24. The isolated antibody of claim 23, wherein the antibody comprises a heavy chain constant region comprising the amino acid sequence of SEQ ID NO: 28.
25. The isolated antibody of claim 23, wherein the amino acid sequence of the IgG_1 heavy chain constant region comprises S239D/I332E mutations, numbered according to the EU numbering system.
26. The isolated antibody of claim 25, wherein the antibody comprises a heavy chain constant region comprising the amino acid sequence of SEQ ID NO: 29.
27. The isolated antibody of claim 23, wherein the amino acid sequence of the IgG_1 heavy chain constant region comprises S239D/A330L/I332E mutations, numbered according to the EU numbering system.
28. The isolated antibody of claim 27, wherein the antibody comprises a heavy chain constant region comprising the amino acid sequence of SEQ ID NO: 30.
29. The isolated antibody of claim 23, wherein the amino acid sequence of the IgG_1 heavy chain constant region comprises L235V/F243L/R292P/Y300L/P396L mutations, numbered according to the EU numbering system.
30. The isolated antibody of claim 29, wherein the antibody comprises a heavy chain constant region comprising the amino acid sequence of SEQ ID NO: 31.
31. The isolated antibody of claim 23, wherein the IgG_1 heavy chain constant region is afucosylated IgG_1.

32. The isolated antibody of any one of claims 1-12 or 14-17, wherein the antibody comprises a human IgG heavy chain constant region that is a variant of a wild type human IgG heavy chain constant region, wherein the variant human IgG heavy chain constant region **binds to FcγRIIIA with a higher affinity than the wild type human IgG heavy chain constant region binds to FcγRIIIA.**

33. The isolated antibody of claim 32, wherein the variant human IgG heavy chain constant region is a variant human IgG_1 heavy chain constant region.

34. The isolated antibody of any one of claims 1-17 or 22-33, wherein the antibody comprises **a light chain constant region selected from the group consisting of human Igκ and Igλ.**

35. **The isolated antibody of claim 34, wherein the antibody comprises an Igκ light chain** constant region.

36. The isolated antibody of claim 35, wherein the antibody comprises a light chain constant region comprising the amino acid sequence of SEQ ID NO: 32.

37. The isolated antibody of any one of the preceding claims, wherein the antibody binds to the same epitope of human CTLA-4 as an antibody comprising a heavy chain variable region comprising the amino acid sequence of SEQ ID NO: 8 and a light chain variable region comprising the amino acid sequence of SEQ ID NO: 9.

38. The isolated antibody of any one of the preceding claims, wherein the antibody binds to an epitope located within a region of human CTLA-4 consisting of an amino acid sequence selected from the group consisting of SEQ ID NOs: 34-39.

39. The isolated antibody of any one of the preceding claims, wherein the antibody is a human antibody.

40. The isolated antibody of any one of the preceding claims, wherein the antibody is a bispecific antibody.

41. The isolated antibody of any one of the preceding claims, wherein the antibody is antagonistic to human CTLA-4.

42. The isolated antibody of any one of the preceding claims, wherein the antibody deactivates, reduces, or inhibits an activity of human CTLA-4.

43. The isolated antibody of claim 42, wherein the antibody inhibits binding of human CTLA-4 to human CD80 or human CD86.

44. The isolated antibody of claim 42, wherein the antibody induces IL-2 production by peripheral blood mononuclear cells (PBMCs) stimulated with staphylococcal enterotoxin A (SEA).

45. The isolated antibody of any one of the preceding claims conjugated to a cytotoxic agent, cytostatic agent, toxin, radionuclide, or detectable label.
46. The isolated antibody of any one of the preceding claims, wherein the N-terminal amino acid residue of the heavy chain variable region and/or the light chain variable region of the antibody has been converted to pyroglutamate.
47. A pharmaceutical composition comprising the antibody of any one of the preceding claims and a pharmaceutically acceptable carrier or excipient.
48. An isolated polynucleotide encoding a heavy and/or light chain of the antibody of any one of claims 1-45.
49. A vector comprising the polynucleotide of claim 48.
50. A recombinant host cell comprising the polynucleotide of claim 48 or the vector of claim 49.
51. A method of producing an antibody that specifically binds to human CTLA-4, the method comprising culturing the host cell of claim 50 so that the polynucleotide is expressed and the antibody is produced.
52. A method of increasing T cell activation in response to an antigen in a subject, the method comprising administering to the subject an effective amount of the antibody or pharmaceutical composition of any one of claims 1-47.
53. A method of treating cancer in a subject, the method comprising administering to the subject an effective amount of the antibody or pharmaceutical composition of any one of claims 1-47.
54. The method of claim 53, wherein the cancer is a metastatic or locally advanced tumor.
55. The method of claim 53 or 54, wherein the cancer is a solid tumor.
56. The method of any one of claims 53-55, wherein the cancer is a metastatic or locally advanced, unresectable squamous cell carcinoma, adenosquamous carcinoma, or adenocarcinoma of the cervix.
57. The method of any one of claims 53-56, wherein no standard therapy is available for the cancer.
58. The method of any one of claims 53-56, wherein the cancer is refractory to a standard therapy.
59. The method of any one of claims 53-56, wherein the cancer has relapsed after a standard therapy.
60. The method of claim 58 or 59, wherein the standard therapy comprises a platinum-containing chemotherapy.

61. The method of claim 60, wherein the standard therapy comprises a platinum-containing doublet.
62. The method of any one of claims 53-61, wherein the cancer is HPV positive.
63. The method of any one of claims 53-55, wherein the cancer is a non-small cell lung cancer (NSCLC).
64. The method of claim 63, wherein the cancer is a metastatic NSCLC.
65. The method of claim 63 or 64, wherein the NSCLC has no EGFR or ALK genomic tumor aberrations.
66. The method of any one of claims 63-65, wherein the NSCLC has no EGFR sensitizing mutation or ALK translocation.
67. The method of any one of claims 63-66, wherein the subject has received no prior systemic chemotherapy treatment for the NSCLC.
68. The method of any one of claims 53-55, wherein the cancer is a cutaneous squamous-cell carcinoma (cSCC).
69. The method of claim 68, wherein the cancer is a Stage IV cSCC.
70. The method of claim 69, wherein the cSCC is diagnosed histologically or cytologically according to the eighth edition of the American Joint Committee on Cancer staging manual.
71. The method of any one of claims 68-70, wherein the cSCC is not curable with radiation therapy.
72. The method of any one of claims 53-71, wherein the percentage of tumor cells in a sample of the cancer that exhibit detectable membrane expression of PD-L1 is at least 1%, 2%, 3%, 4%, 5%, 10%, 15%, 20%, 25%, 30%, 35%, 40%, 45%, 50%, 60%, 70%, 80%, or 90%.
73. The method of any one of claims 53-72, wherein the antibody is administered as a first cancer therapy after diagnosis of the cancer.
74. The method of any one of claims 53-73, wherein the antibody is administered as the first cancer therapy after:

(a) diagnosis of tumor progression that has occurred despite previous treatment of the cancer with a different cancer therapy; or

(b) diagnosis of toxicity of a different cancer therapy,

optionally wherein the antibody is the second cancer therapy administered to the subject.
75. The method of any one of claims 52-75, wherein the antibody or pharmaceutical composition is administered intravenously.

76. The method of claim 75, wherein the antibody or pharmaceutical composition is administered intravenously at 0.01 mg/kg, 0.03 mg/kg, 0.1 mg/kg, 0.3 mg/kg, 1 mg/kg, 3 mg/kg, 6 mg/kg, or 10 mg/kg, optionally at an interval of once every two weeks.
77. The method of claim 75, wherein the antibody or pharmaceutical composition is administered intravenously at 0.01 mg/kg, 0.03 mg/kg, 0.1 mg/kg, 0.3 mg/kg, 1 mg/kg, 3 mg/kg, 6 mg/kg, or 10 mg/kg, optionally at an interval of once every three weeks.
78. The method of claim 75, wherein the antibody or pharmaceutical composition is administered intravenously at 0.01 mg/kg, 0.03 mg/kg, 0.1 mg/kg, 0.3 mg/kg, 1 mg/kg, 3 mg/kg, 6 mg/kg, or 10 mg/kg, optionally at an interval of once every four weeks.
79. The method of claim 75, wherein the antibody or pharmaceutical composition is administered intravenously at 0.01 mg/kg, 0.03 mg/kg, 0.1 mg/kg, 0.3 mg/kg, 1 mg/kg, 3 mg/kg, 6 mg/kg, or 10 mg/kg, optionally at an interval of once every six weeks.
80. The method of claim 75, wherein the antibody or pharmaceutical composition is administered intravenously at 0.01 mg/kg, 0.03 mg/kg, 0.1 mg/kg, 0.3 mg/kg, 1 mg/kg, 3 mg/kg, 6 mg/kg, or 10 mg/kg, optionally at an interval of once every twelve weeks.
81. The method of any one of claims 52-75, wherein the antibody or pharmaceutical composition is administered subcutaneously.
82. The method of any one of claims 53-75, wherein the antibody or pharmaceutical composition is administered intratumorally.
83. The method of claim 82, wherein the antibody or pharmaceutical composition is administered intratumorally at 0.03 mg/kg, 0.1 mg/kg, or 0.3 mg/kg, optionally at an interval of once every three weeks.
84. The method of any one of claims 52-75, wherein the antibody or pharmaceutical composition is delivered to a tumor draining lymph node.
85. The method of any one of claims 81-84, wherein the antibody is the antibody of any one of claims 25-33.
86. The method of any one of claims 52-85, wherein the antibody is administered as a monotherapy.
87. The method of any one of claims 52-85, further comprising administering an additional therapeutic agent to the subject.
88. The method of claim 87, wherein the additional therapeutic agent is administered systemically.
89. The method of claim 87 or 88, wherein the additional therapeutic agent is an anti-PD-1 antibody, optionally wherein the anti-PD-1 antibody is pembrolizumab or nivolumab.

90. The method of claim 89, wherein the additional therapeutic agent is pembrolizumab administered at 200 mg every three weeks.

91. The method of claim 87 or 88, wherein the subject has head and neck squamous cell carcinoma and wherein the additional therapeutic agent is an anti-EGFR antibody, optionally wherein the anti-EGFR antibody is cetuximab,

optionally wherein the method further comprises administering a chemotherapeutic agent to the subject, optionally wherein the chemotherapeutic agent is administered systemically, optionally wherein the chemotherapeutic agent is gemcitabine.

92. The method of claim 87 or 88, wherein the subject has HER2+ breast cancer and wherein the additional therapeutic agent is an anti-HER2 antibody, optionally wherein the anti-HER2 antibody is trastuzumab,

optionally wherein the method further comprises administering a chemotherapeutic agent to the subject, optionally wherein the chemotherapeutic agent is administered systemically, optionally wherein the chemotherapeutic agent is gemcitabine.

93. The method of claim 87 or 88, wherein the additional therapeutic agent is a chemotherapeutic or a checkpoint targeting agent.

94. The method of claim 93, wherein the checkpoint targeting agent is selected from the group consisting of an antagonist anti-PD-1 antibody, an antagonist anti-PD-L1 antibody, an antagonist anti-PD-L2 antibody, an antagonist anti-CTLA-4 antibody, an antagonist anti-TIM-3 antibody, an antagonist anti-LAG-3 antibody, an antagonist anti-CEACAM1 antibody, an agonist anti-GITR antibody, an agonist anti-OX40 antibody, and an agonist anti-CD137 antibody.

95. The method of claim 87 or 88, wherein the additional therapeutic agent is an inhibitor of indoleamine-2,3-dioxygenase (IDO).

96. The method of claim 95, wherein the inhibitor is selected from the group consisting of epacadostat, F001287, indoximod, and NLG919.

97. The method of claim 87 or 88, wherein the additional therapeutic agent is a vaccine.

98. The method of claim 97, wherein the vaccine comprises a heat shock protein peptide complex (HSPPC) comprising a heat shock protein complexed with an antigenic peptide.

99. The method of claim 98, wherein the heat shock protein is hsc70 and is complexed with a tumor-associated antigenic peptide.

100. The method of claim 98, wherein the heat shock protein is gp96 protein and is complexed with a tumor-associated antigenic peptide, wherein the HSPPC is derived from a tumor obtained from a subject.

101. A method of treating an infectious disease in a subject, the method comprising administering to the subject an effective amount of the antibody or pharmaceutical composition of any one of claims 1-47.