

CLAIMS

1. An isolated antibody that specifically binds to human CTLA-4 protein, 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 SYX₁MX₂ (SEQ ID NO: 22), wherein: X₁ is S or A; and X₂ is N or S;
 - (b) CDRH2 comprises the amino acid sequence of SSSSSSYIYYADSVKG (SEQ ID NO: 2);
 - (c) CDRH3 comprises the amino acid sequence of VGLMGPFXI (SEQ ID NO: 23), wherein X is D or N;
 - (d) CDRL1 comprises the amino acid sequence of RASQSVX₁X₂YLX₃ (SEQ ID NO: 24), wherein: X₁ is S or G; X₂ is R, S, or T; and X₃ is G or A;
 - (e) CDRL2 comprises the amino acid sequence of X₁X₂SX₃RAT (SEQ ID NO: 25), wherein: X₁ is G or A; X₂ is A or T; and X₃ is T, S, R, or N; and
 - (f) CDRL3 comprises the amino acid sequence of QQYGX₁SPX₂T (SEQ ID NO: 26), wherein: X₁ is S or T; and X₂ is W or F.
2. The isolated antibody of claim 1, wherein:
 - (a) CDRH1 comprises an amino acid sequence selected from the group consisting of SEQ ID NOs: 1 and 27;
 - (b) CDRH3 comprises an amino acid sequence selected from the group consisting of SEQ ID NOs: 3 and 28;
 - (c) CDRL1 comprises an amino acid sequence selected from the group consisting of SEQ ID NOs: 4, 29, and 30;
 - (d) CDRL2 comprises an amino acid sequence selected from the group consisting of SEQ ID NOs: 5 and 31-35; and/or
 - (e) CDRL3 comprises an amino acid sequence selected from the group consisting of SEQ ID NOs: 6, 36, and 37.
3. The isolated antibody of claim 1 or 2, wherein CDRH1, CDRH2 and CDRH3, respectively, comprise the CDRH1, CDRH2 and CDRH3 amino acid sequences set forth in SEQ ID NOs: 1, 2, and 3; 27, 2, and 3; or, 27, 2, and 28.
4. The isolated antibody of any one of claims 1 to 3, wherein CDRL1, CDRL2 and CDRL3, respectively, comprise the CDRL1, CDRL2 and CDRL3 amino acid sequences set forth in SEQ ID NOs: 4, 5, and 6; 29, 32, and 36; 29, 33, and 37; 30, 31, and 6; 29, 34, and 6; or, 29, 35, and 37.

5. The isolated antibody of any one of claims 1 to 4, wherein CDRH1, CDRH2, CDRH3, CDRL1, CDRL2, and CDRL3, respectively, comprise the amino acid sequences set forth in SEQ ID NOs: 1, 2, 3, 4, 5, and 6; 1, 2, 3, 29, 32, and 36; 1, 2, 3, 29, 33, and 37; 27, 2, 3, 4, 5, and 6; 27, 2, 3, 29, 33, and 37; 1, 2, 3, 30, 31, and 6; 1, 2, 3, 29, 34, and 6; 1, 2, 3, 29, 35, and 37; 27, 2, 28, 4, 5, and 6; 27, 2, 28, 29, 32, and 36; 27, 2, 28, 29, 33, and 37; or, 27, 2, 28, 29, 35, and 37.
6. The isolated antibody of any one of claims 1 to 5, wherein CDRH1, CDRH2, CDRH3, CDRL1, CDRL2, and CDRL3 comprise the amino acid sequences set forth in SEQ ID NOs: 1, 2, 3, 4, 5, and 6, respectively.
7. The isolated antibody of any one of claims 1 to 6, wherein the heavy chain variable region comprises the amino acid sequence of SEQ ID NO: 72.
8. The isolated antibody of any one of claims 1 to 6, wherein the heavy chain variable region comprises an amino acid sequence which is at least 75%, 80%, 85%, 90%, 95%, or 100% identical to an amino acid sequence selected from the group consisting of SEQ ID NOs: 7 and 38-42.
9. The isolated antibody of any one of claims 1 to 8, wherein the heavy chain variable region comprises an amino acid sequence derived from a human IGHV3-21 germline sequence.
10. The isolated antibody of claim 1, wherein the antibody comprises a heavy chain comprising the amino acid sequence of SEQ ID NO: 12, 14, 93, or 94.
11. The isolated antibody of any one of claims 1 to 10, wherein the light chain variable region comprises an amino acid sequence derived from a human IGKV3-20 germline sequence or a human IGKV3-11 germline sequence.
12. The isolated antibody of any one of claims 1 to 11, wherein the light chain variable region comprises the amino acid sequence of SEQ ID NO: 73.
13. The isolated antibody of any one of claims 1 to 11, wherein the light chain variable region comprises an amino acid sequence which is at least 75%, 80%, 85%, 90%, 95%, or 100% identical to an amino acid sequence selected from the group consisting of SEQ ID NOs: 8 and 43-47.
14. The isolated antibody of claim 1, wherein the heavy chain variable region and the light chain variable region, respectively, comprise the amino acid sequences set forth in SEQ ID NOs: 7 and

44; 7 and 45; 38 and 8; 38 and 45; 39 and 43; 39 and 45; 39 and 46; 39 and 47; 40 and 43; 40 and 8; 40 and 44; 40 and 45; 41 and 8; 41 and 44; 41 and 45; 41 and 47; 42 and 43; or, 42 and 45.

15. The isolated antibody of claim 1, wherein the amino acid sequences of the heavy chain variable region and the light chain variable region, respectively, consist of the amino acid sequences set forth in SEQ ID NOs: 7 and 44; 7 and 45; 38 and 8; 38 and 45; 39 and 43; 39 and 45; 39 and 46; 39 and 47; 40 and 43; 40 and 8; 40 and 44; 40 and 45; 41 and 8; 41 and 44; 41 and 45; 41 and 47; 42 and 43; or, 42 and 45.
16. The isolated antibody of claim 1, comprising a heavy chain and a light chain, wherein the heavy chain and the light chain, respectively, comprise the amino acid sequences set forth in SEQ ID NOs: 12 and 15; 93 and 15; 14 and 13; 14 and 15; 94 and 13; or 94 and 15.
17. The isolated antibody of claim 1, comprising a heavy chain and a light chain, wherein the amino acid sequences of the heavy chain and the light chain, respectively, consist of the amino acid sequences set forth in SEQ ID NOs: 12 and 15; 93 and 15; 14 and 13; 14 and 15; 94 and 13; or 94 and 15.
18. An isolated antibody that specifically binds to human CTLA-4 protein, comprising: a heavy chain variable region comprising the CDRH1, CDRH2 and CDRH3 amino acid sequences of SEQ ID NOs: 1, 2, and 3, respectively; and a light chain variable region comprising the amino acid sequence of SEQ ID NO: 8.
19. An isolated antibody that specifically binds to human CTLA-4 protein, comprising: a heavy chain variable region comprising the amino acid sequence of SEQ ID NO: 7; and a light chain variable region comprising the CDRL1, CDRL2 and CDRL3 amino acid sequences of SEQ ID NOs: 4, 5, and 6, respectively.
20. An isolated antibody that specifically binds to human CTLA-4 protein, comprising: a heavy chain variable region comprising the amino acid sequence of SEQ ID NO: 7; and a light chain variable region comprising the amino acid sequence of SEQ ID NO: 8.
21. The isolated antibody of claim 20, wherein the amino acid sequence of the heavy chain variable region consists of the amino acid sequence of SEQ ID NO: 7, and the amino acid sequence of the light chain variable region consists of the amino acid sequence of SEQ ID NO: 8.

22. An isolated antibody that specifically binds to human CTLA-4 protein, comprising: a heavy chain and a light chain, wherein the heavy chain comprises the amino acid sequence of SEQ ID NO: 93; and the light chain comprises the amino acid sequence of SEQ ID NO: 13.
23. The isolated antibody of any one of claims 1, 19, 20, or 22, wherein the heavy chain comprises the amino acid sequence of SEQ ID NO: 12.
24. The isolated antibody of claim 23, wherein the amino acid sequence of the heavy chain consists of the amino acid sequence of SEQ ID NO: 12.
25. The isolated antibody of claim 22, wherein the amino acid sequence of the heavy chain consists of the amino acid sequence of SEQ ID NO: 93, and the amino acid sequence of the light chain consists of the amino acid sequence of SEQ ID NO: 13.
26. The isolated antibody of claim 22, wherein the heavy chain comprises the amino acid sequence of SEQ ID NO: 12, and the amino acid sequence of the light chain consists of the amino acid sequence of SEQ ID NO: 13.
27. The isolated antibody of claim 26, wherein the amino acid sequence of the heavy chain consists of the amino acid sequence of SEQ ID NO: 12, and the amino acid sequence of the light chain consists of the amino acid sequence of SEQ ID NO: 13.
28. The isolated antibody of claim 21, wherein the antibody comprises a heavy chain and a light chain, wherein the heavy chain comprises the amino acid sequence of SEQ ID NO: 97; and the light chain comprises the amino acid sequence of SEQ ID NO: 13.
29. An isolated antibody that specifically binds to human CTLA-4 protein, comprising a heavy chain variable region comprising an amino acid sequence selected from the group consisting of SEQ ID NOs: 7 and 38-42.
30. The isolated antibody of claim 1 or 29, wherein the heavy chain variable region comprises the amino acid sequence of SEQ ID NO: 7.
31. The isolated antibody of claim 19 or 30, wherein the amino acid sequence of the heavy chain variable region consists of the amino acid sequence of SEQ ID NO: 7.
32. An isolated antibody that specifically binds to human CTLA-4 protein, comprising a heavy chain comprising the amino acid sequence of SEQ ID NO: 12, 14, 93, or 94.

33. The isolated antibody of any one of claims 1, 19, 20, or 32, wherein the heavy chain comprises the amino acid sequence of SEQ ID NO: 93.
34. The isolated antibody of claim 22 or 33, wherein the amino acid sequence of the heavy chain consists of the amino acid sequence of SEQ ID NO: 93.
35. An isolated antibody that specifically binds to human CTLA-4 protein, comprising a light chain variable region comprising an amino acid sequence selected from the group consisting of SEQ ID NOs: 8 and 43-47.
36. The isolated antibody of claim 1 or 35, wherein the light chain variable region comprises the amino acid sequence of SEQ ID NO: 8.
37. The isolated antibody of claim 18 or 36, wherein the amino acid sequence of the light chain variable region consists of the amino acid sequence of SEQ ID NO: 8.
38. An isolated antibody that specifically binds to human CTLA-4 protein, comprising a light chain comprising the amino acid sequence of SEQ ID NO: 13 or 15.
39. The isolated antibody of any one of claims 18, 21, or 38, wherein the light chain comprises the amino acid sequence of SEQ ID NO: 13.
40. The isolated antibody of claim 22 or 39, wherein amino acid sequence of the light chain consists of the amino acid sequence of SEQ ID NO: 13.
41. The isolated antibody of claim 1, wherein the antibody comprises a heavy chain constant region selected from the group consisting of human IgG₁, IgG₂, IgG₃, IgG₄, IgA₁, and IgA₂; and/or a light chain constant region selected from the group consisting of human IgGκ and IgGλ.
42. The isolated antibody of claim 1, 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 a human Fc gamma receptor with higher affinity than the corresponding wild type human IgG heavy chain constant region binds to the human Fc gamma receptor, optionally wherein the variant human IgG heavy chain constant region is a variant human IgG₁, a variant human IgG₂, or a variant human IgG₄ heavy chain constant region.

43. The isolated antibody of claim 42, wherein the human Fc gamma receptor is FcγRIIB, FcγRIIIA, FcγRIIA, or FcγRI.
44. The isolated antibody of any one of claims 41 to 43, wherein the human Fc gamma receptor is FcγRIIIA or FcγRIIB and the variant human IgG heavy chain constant region comprises:
- (a) one or more of the following amino acid mutations, according to the EU numbering system: G236D, P238D, S239D, S267E, L328F, and L328E; or
 - (b) a set of amino acid mutations selected from the group consisting of: S267E and L328F; P238D and L328E; P238D and one or more substitutions selected from the group consisting of E233D, G237D, H268D, P271G, and A330R; P238D, E233D, G237D, H268D, P271G, and A330R; G236D and S267E; S239D and S267E; V262E, S267E, and L328F; and V264E, S267E, and L328F, according to the EU numbering system.
45. The isolated antibody of any one of claims 41 to 43, wherein the human Fc gamma receptor is FcγRIIB, and the variant human IgG heavy chain constant region further comprises one or more amino acid mutations that reduce the affinity of the IgG for human FcγRIIIA, human FcγRIIA, or human FcγRI.
46. The isolated antibody of any one of claims 41 to 43, wherein the human Fc gamma receptor is FcγRIIIA, FcγRIIA, or FcγRI and the variant human IgG heavy chain constant region comprises:
- (a) one or more of the following amino acid mutations, according to the EU numbering system: G236A, S239D, F243L, T256A, K290A, R292P, S298A, Y300L, V305I, A330L, I332E, E333A, K334A, A339T, and P396L; or
 - (b) a set of amino acid mutations selected from the group consisting of: S239D; T256A; K290A; S298A; I332E; E333A; K334A; A339T; S239D and I332E; S239D, A330L, and I332E; S298A, E333A, and K334A; G236A, S239D, and I332E; and F243L, R292P, Y300L, V305I, and P396L, according to the EU numbering system.
47. The isolated antibody of any one of claims 1 to 46, wherein the antibody comprises an afucosylated Fc region.
48. An isolated antibody that specifically binds to human CTLA-4 protein, comprising a heavy chain and a light chain, wherein the light chain comprises the amino acid sequence set forth in SEQ ID NO: 8, and the heavy chain comprises a variant human IgG₁ heavy chain constant region that binds to human FcγRIIIA with higher affinity than the corresponding wild type human IgG₁ heavy chain constant region binds to human FcγRIIIA.

49. The isolated antibody of claim 48, wherein the variant human IgG₁ heavy chain constant region comprises the mutations S239D, A330L, and I332E, according to the EU numbering system.
50. An isolated antibody that specifically binds to human CTLA-4 protein, comprising a heavy chain and a light chain, wherein the light chain comprises the amino acid sequence set forth in SEQ ID NO: 13, and the heavy chain comprises a variant human IgG₁ heavy chain constant region that binds to human FcγRIIIA with higher affinity than the corresponding wild type human IgG₁ heavy chain constant region binds to human FcγRIIIA.
51. The isolated antibody of claim 50, wherein the variant human IgG₁ heavy chain constant region comprises the mutations S239D, A330L, and I332E, according to the EU numbering system.
52. The isolated antibody of any one of claims 1 to 51, wherein the antibody is a human antibody.
53. The isolated antibody of any one of claims 1 to 52, wherein the antibody is antagonistic to CTLA-4.
54. The isolated antibody of any one of claims 1 to 53, wherein the antibody inhibits binding of the human CTLA-4 protein to human CD80 or to human CD86.
55. The isolated antibody of any one of claims 1 to 54 conjugated to a cytotoxic agent, cytostatic agent, toxin, radionuclide, or detectable label.
56. A pharmaceutical composition comprising the antibody of any one of claims 1 to 55 and a pharmaceutically acceptable carrier or excipient.
57. An isolated polynucleotide encoding a heavy chain variable region and/or a light chain variable region of the antibody of any one of claims 1 to 55.
58. A vector comprising the polynucleotide of claim 57.
59. A recombinant host cell comprising the polynucleotide of claim 57 or the vector of claim 58.
60. A method of producing an antibody that binds to human CTLA-4, the method comprising culturing the host cell of claim 59 so that the polynucleotide is expressed and the antibody is produced.

61. Use of the pharmaceutical composition of claim 56 in the manufacture of a medicament for increasing T-cell activation in response to an antigen in a subject, wherein the medicament is administered to the subject in an effective amount.
62. Use of the pharmaceutical composition of claim 56 in the manufacture of a medicament for the treatment of cancer in a subject, wherein the medicament is administered to the subject in an effective amount.
63. The use of claim 62, wherein the cancer is selected from the group consisting of acoustic neuroma, acute lymphocytic leukemia, acute myelocytic leukemia, adenocarcinoma, adenocarcinoma of the gastroesophageal junction, adrenal gland cancer, adult soft tissue sarcoma, anal cancer, anaplastic large cell lymphoma, angiosarcoma, astrocytoma, basal cell carcinoma, B-cell lymphoma, bile duct carcinoma, biliary tract cancer, bladder cancer, blastoma, brain metastasis, brain or central nervous system cancer, breast cancer, HER2+ breast cancer, relapsed refractory HER2+ breast cancer, bronchial cancer, bronchogenic carcinoma, Burkitt lymphoma, cancer of the oral cavity or pharynx, cervical cancer, chondrosarcoma, chordoma, choriocarcinoma, chronic leukemia, chronic myelocytic leukemia, chronic lymphocytic leukemia, colon cancer, craniopharyngioma, cystadenocarcinoma, desmoplastic melanoma, DIPG, embryonal carcinoma, endometrial carcinoma, endometrial stromal sarcoma, endotheliosarcoma, ependymoma, epithelial carcinoma, esophageal cancer, Ewing sarcoma, fibrosarcoma, follicular lymphoma, gastric cancer, gastroesophageal cancer, gastroesophageal junction carcinoma, gastrointestinal cancer, gastrointestinal stromal tumors, glioblastoma multiforme, glioblastoma multiforme that is refractory to bevacizumab therapy, hairy cell leukemia, head and neck cancer, head and neck squamous cell carcinoma, relapsed/refractory head and neck squamous cell carcinoma, heavy chain disease, hemangioblastoma, hepatic metastasis, hepatoma, hepatic carcinoma, Hodgkin's lymphoma, inflammatory breast cancer, intraocular lymphoma, kidney cancer, laryngeal cancer, squamous cell carcinoma of the hypopharynx, squamous cell carcinoma of the larynx, carcinoma of the oropharynx, verrucous carcinoma of the larynx, leiomyosarcoma, leukemia, liposarcoma, liver cancer, lung cancer, lymphangioendotheliosarcoma, lymphangiosarcoma, lymphoblastic lymphoma, lymphoma, mantle cell lymphoma, mast cell sarcoma, medullary carcinoma, medulloblastoma, melanoma, stage III melanoma, stage IV melanoma, melanoma with brain metastases, melanoma that is refractory to nivolumab or pembrolizumab, meningioma, Merkel cell carcinoma, mesothelioma, metastatic brain tumor, metastatic cancer, metastatic hormone refractory cancer, multiple myeloma, myelodysplastic syndrome, myeloma, myxosarcoma, neuroblastoma, non-Hodgkin's lymphoma, non-small cell lung cancer, ocular melanoma, oligodendroglioma, omyelodysplastic syndrome, surgically treatable or non-surgically treatable brain stem glioma, oropharyngeal cancer, orsplenic marginal zone lymphoma, osteocarcinoma, osteogenic sarcoma, ovarian cancer, metastatic ovarian cancer, pancreatic cancer, pancreatic ductal adenocarcinoma,

papillary adenocarcinomas, peripheral nervous system cancer, pinealoma, prostate cancer, hormone refractory prostate cancer, metastatic hormone refractory prostate cancer, progressive metastatic prostate cancer, rectal cancer, renal cell carcinoma, metastatic renal cell carcinoma, retinoblastoma, rhabdomyosarcoma, salivary gland cancer, sarcoma, sebaceous gland carcinoma, seminoma, serous adenocarcinoma, skin cancer, small bowel or appendix cancer, small cell lung cancer, small intestine lymphoma, soft tissue sarcoma, solid cancer, squamous cell cancer, stomach cancer, sweat gland carcinoma, synovia sarcoma, synovioma, T-cell lymphoma, testicular cancer, thymoma, thymic carcinoma, thyroid cancer, transitional cell cancer, urothelial cancer, uterine sarcoma, uveal melanoma, uveal melanoma with liver metastases, verrucous carcinoma, vulval cancer, Waldenstrom macroglobulinemia, and Wilms' tumor.

64. The use of any one of claims 61 to 63, wherein the antibody or pharmaceutical composition is administered subcutaneously, intratumorally, or intravenously.
65. The use of any one of claims 61 to 64, wherein the antibody or pharmaceutical composition is administered at 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.
66. The use of any one of claims 61 to 65, further comprising administering an additional therapeutic agent to the subject.
67. The use of claim 66, wherein the additional therapeutic agent is a chemotherapeutic or a checkpoint targeting agent.
68. The use of claim 67, 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, and an agonist anti-OX40 antibody.
69. The use of claim 66, wherein the additional therapeutic agent is an inhibitor of indoleamine-2,3-dioxygenase (IDO).
70. The use of claim 69, wherein the inhibitor is selected from the group consisting of epacadostat, F001287, indoximod, and NLG919.
71. The use of claim 66, wherein the additional therapeutic agent is a vaccine.

72. The use of claim 71, wherein the vaccine comprises a heat shock protein peptide complex (HSPPC) comprising a heat shock protein complexed with an antigenic peptide.
73. The use of claim 72, wherein the heat shock protein is hsc70 and the antigenic peptide is synthetic.