

CLAIMS

1. An isolated antibody that specifically binds to human OX40, comprising:
 - (a) a heavy chain variable region comprising
a heavy chain complementarity determining region 1 (CDR1) comprising
the amino acid sequence of GSAMH (SEQ ID NO: 4),
a heavy chain CDR2 comprising the amino acid sequence of
RIRSKANSYATAYAASVKG (SEQ ID NO: 5), and
a heavy chain CDR3 comprising the amino acid sequence of
GIYDSSGYDY (SEQ ID NO: 6); and
 - (b) a light chain variable region comprising
a light chain CDR1 comprising the amino acid sequence of
RSSQSLLSNGYNYLD (SEQ ID NO: 1),
a light chain CDR2 comprising the amino acid sequence of LGSNRAS
(SEQ ID NO: 2), and
a light chain CDR3 comprising the amino acid sequence of MQALQTPLT
(SEQ ID NO: 3).
2. The antibody of claim 1, wherein
 - (a) the heavy chain variable region comprises the amino acid sequence of SEQ
ID NO: 16; and/or
 - (b) the light chain variable region comprises the amino acid sequence of SEQ
ID NO: 15.
3. The antibody of claim 1 or claim 2, wherein
 - (a) the antibody comprises a heavy chain sequence comprising an amino acid
sequence selected from the group consisting of SEQ ID NOs: 60-63; and/or
 - (b) the antibody comprises a light chain sequence comprising the amino acid
sequence of SEQ ID NO: 20.

4. An isolated antibody that specifically binds to human OX40, wherein the antibody comprises a heavy chain variable region and a light chain variable region, wherein the heavy chain variable region comprises the amino acid sequence of SEQ ID NO: 16; and wherein the light chain variable region comprises the amino acid sequence of SEQ ID NO: 15.

5. The antibody of claim 4, wherein the antibody comprises:

- (a) (i) a heavy chain comprising the amino acid sequence of SEQ ID NO: 60; and (ii) a light chain comprising the amino acid sequence of SEQ ID NO: 20;
- (b) (i) a heavy chain comprising the amino acid sequence of SEQ ID NO: 61; and (ii) a light chain comprising the amino acid sequence of SEQ ID NO: 20; or
- (c) (i) a heavy chain comprising the amino acid sequence of SEQ ID NO: 62 or 63; and (ii) a light chain comprising the amino acid sequence of SEQ ID NO: 20.

6. The antibody of any one of claims 1, 2, or 4, further comprising a heavy chain constant region; wherein the heavy chain constant region is selected from the group consisting of human immunoglobulins IgG₁, IgG₂, IgG₃, IgG₄, IgA₁, and IgA₂.

7. The antibody of claim 6, wherein:

- (a) the IgG₁ is non-fucosylated IgG₁;
- (b) the amino acid sequence of the IgG₁ comprises a N297A mutation or a mutation selected from the group consisting of D265A, P329A, and a combination thereof;
- (c) the amino acid sequence of the IgG₁ comprises a N297Q mutation;
- (d) the amino acid sequence of the IgG₄ comprises a S228P mutation;
- (e) the amino acid sequence of the IgG₂ comprises a C127S mutation;

- (f) the heavy chain constant region comprises an amino acid sequence selected from the group consisting of SEQ ID NOs: 64-71; or
 - (g) the amino acid sequence of the IgG₁ comprises a mutation selected from the group consisting of N297A, N297Q, D265A, and a combination thereof or a mutation selected from the group consisting of D265A, P329A, and a combination thereof.
8. The antibody of any one of claims 1, 2, 4, 6, or 7, further comprising a light chain constant region, wherein the light chain constant region is selected from the group consisting of a human kappa light chain constant region or a human lambda light chain constant region.
9. The antibody of any one of claims 1-8, wherein the antibody is a human antibody.
10. The antibody of any one of claims 1-9, wherein:
- (a) the antibody is agonistic;
 - (b) the antibody activates, enhances, or induces an activity of human OX40;
 - (c) the antibody induces production of TNF α , TNF β , IFN γ , GM-CSF, IL-2, IL-4, IL-10, IL-13, or a combination thereof by anti-CD3-stimulated peripheral blood mononuclear cells (PBMCs);
 - (d) the antibody induces production of TNF α , TNF β , IFN γ , GM-CSF, IL-2, IL-4, IL-10, IL-13, or a combination thereof by anti-CD3-stimulated peripheral blood mononuclear cells (PBMCs), wherein the production of TNF α , TNF β , IFN γ , GM-CSF, IL-2, IL-10, or IL-13 is a substantially increasing function of the concentration of the antibody;
 - (e) the antibody induces production of TNF α , TNF β , IFN γ , GM-CSF, IL-2, IL-4, IL-10, IL-13, or a combination thereof by anti-CD3-stimulated peripheral blood mononuclear cells (PBMCs), wherein the production of TNF α , TNF β , IFN γ , GM-CSF, IL-2, IL-10, or IL-13 shows a sigmoidal dose response curve;

- (f) the antibody induces IL-2 production by SEA-stimulated PBMCs and suppresses IL-10 production by SEA-stimulated PBMCs;
 - (g) the antibody induces IL-2 production by SEA-stimulated PBMCs, wherein the IL-2 production is a substantially increasing function of the concentration of the antibody;
 - (h) the antibody induces IL-2 production by SEA-stimulated PBMCs, wherein the IL-2 production shows a sigmoidal dose response curve;
 - (i) the antibody relieves suppression of T effector cells by T regulatory cells; and/or
 - (j) the antibody induces IL-2 production by a co-culture of T effector cells and T regulatory cells and suppresses IL-10 production by a co-culture of T effector cells and T regulatory cells.
11. The antibody of any one of claims 1-10, wherein:
- (a) the antibody induces CD4⁺ T cell proliferation;
 - (b) the antibody induces CD4⁺ T cell proliferation, wherein the CD4⁺ T cell proliferation is a substantially increasing function of the concentration of the antibody; or
 - (c) the antibody induces CD4⁺ T cell proliferation, wherein the CD4⁺ T cell proliferation shows a sigmoidal dose response curve.
12. The antibody of any one of claims 1-11, further comprising a detectable label.
13. An isolated antibody that specifically binds to human OX40, comprising:
- (a) a heavy chain variable region sequence and a light chain variable region sequence of any one of claims 1-9, wherein the antibody is selected from the group consisting of a Fab, Fab', F(ab')₂, and scFv fragment; or
 - (b) one heavy chain and one light chain, wherein the heavy chain and light chain comprise the heavy chain variable region and the light chain variable region, respectively, of any one of claims 1-9.

14. The antibody of claim 13, further comprising a human immunoglobulin IgG₁ heavy chain constant region, wherein the amino acid sequence of the IgG₁ heavy chain constant region comprises a mutation selected from the group consisting of N297A, N297Q, D265A, and a combination thereof or a mutation selected from the group consisting of D265A, P329A, and a combination thereof.

15. An isolated antibody that specifically binds to human OX40, wherein the antibody comprises (i) a first antigen-binding domain that specifically binds to human OX40; and (ii) a second antigen-binding domain that does not specifically bind to OX40, wherein the antigen-binding domain that specifically binds to human OX40 comprises:

- (a) a first heavy chain variable domain (VH) comprising a VH complementarity determining region (CDR) 1 comprising the amino acid sequence of GSAMH (SEQ ID NO: 4); a VH-CDR2 comprising the amino acid sequence of RIRSKANSYATAYAASVKG (SEQ ID NO: 5); and a VH-CDR3 comprising the amino acid sequence of GIYDSSGYDY (SEQ ID NO: 6); and
- (b) a first light chain variable domain (VL) comprising a VL-CDR1 comprising the amino acid sequence of RSSQSLHLSNGYNYLD (SEQ ID NO: 1); a VL-CDR2 comprising the amino acid sequence of LGSNRAS (SEQ ID NO: 2); and a VL-CDR3 comprising the amino acid sequence of MQALQTPLT (SEQ ID NO: 3).

16. The antibody of claim 15, wherein:

- (a) the antigen-binding domain that specifically binds to human OX40 specifically binds to the same epitope of human OX40 as an antibody comprising a VH comprising the amino acid sequence of SEQ ID NO: 16 and a VL comprising the amino acid sequence of SEQ ID NO: 15;
- (b) the antigen-binding domain that specifically binds to human OX40 exhibits, as compared to binding to a human OX40 sequence of SEQ ID NO: 55,

reduced or absent binding to a protein identical to SEQ ID NO: 55 except for the presence of an amino acid mutation selected from the group consisting of: N60A, R62A, R80A, L88A, P93A, P99A, P115A, and a combination thereof;

- (c) the antigen-binding domain that specifically binds to human OX40 comprises a VH and a VL, wherein the VH comprises the amino acid sequence of SEQ ID NO: 16; or
- (d) the antigen-binding domain that specifically binds to human OX40 comprises a VH and a VL, wherein the VL comprises the amino acid sequence of SEQ ID NO: 15.

17. The antibody of claim 15 or claim 16, wherein:

- (a) the second antigen-binding domain specifically binds to a non-human antigen;
- (b) the second antigen-binding domain specifically binds to a viral antigen;
- (c) the second antigen-binding domain specifically binds to an HIV antigen; or
- (d) the second antigen-binding domain specifically binds to chicken albumin or hen egg lysozyme.

18. An isolated antibody that specifically binds to OX40, wherein the antibody comprises:

- (a) an antigen-binding domain that specifically binds to human OX40, comprising a first heavy chain and a light chain; and
 - (b) a second heavy chain or a fragment thereof,
- wherein, the antigen-binding domain that specifically binds to human OX40

comprises:

- (i) a first heavy chain variable domain (VH) comprising a VH complementarity determining region (CDR) 1 comprising the amino acid sequence of GSAMH (SEQ ID NO: 4); a VH-CDR2 comprising the amino acid sequence of RIRSKANSYATAYAASVKG (SEQ ID NO: 5); and a

VH-CDR3 comprising the amino acid sequence of GIYDSSGYDY (SEQ ID NO: 6); and

a first light chain variable domain (VL) comprising a VL-CDR1 comprising the amino acid sequence of RSSQSLLHSNGYNYLD (SEQ ID NO: 1); a VL-CDR2 comprising the amino acid sequence of LGSNRAS (SEQ ID NO: 2); and a VL-CDR3 comprising the amino acid sequence of MQALQTPLT (SEQ ID NO: 3); or

(ii) the antigen-binding domain that specifically binds to human OX40 specifically binds to the same epitope of human OX40 as an antibody comprising a VH comprising the amino acid sequence of SEQ ID NO: 16 and a VL comprising the amino acid sequence of SEQ ID NO: 15.

19. The antibody of any one of claims 15-18, wherein:

- (a) the antigen-binding domain that specifically binds to human OX40 comprises a VH comprising an amino acid sequence that is at least 75%, 80%, 85%, 90%, 95%, or 99% identical to the amino acid sequence of SEQ ID NO: 16;
- (b) the antigen-binding domain that specifically binds to human OX40 comprises a VH comprising the amino acid sequence of SEQ ID NO: 16;
- (c) the antigen-binding domain that specifically binds to human OX40 comprises a VH comprising an amino acid sequence derived from a human IGHV3-73 germline sequence;
- (d) the antigen-binding domain that specifically binds to human OX40 comprises a VL comprising an amino acid sequence that is at least 75%, 80%, 85%, 90%, 95%, or 99% identical to the amino acid sequence of SEQ ID NO: 15;
- (e) the antigen-binding domain that specifically binds to human OX40 comprises a VL-CDR3 comprising the amino acid sequence of SEQ ID NO: 3;

- (f) the antigen-binding domain that specifically binds to human OX40 comprises a VL comprising the amino acid sequence of SEQ ID NO: 15;
- (g) the antigen-binding domain that specifically binds to human OX40 comprises a light chain comprising the amino acid sequence of SEQ ID NO: 20;
- (h) the antigen-binding domain that specifically binds to human OX40 comprises a light chain comprising the amino acid sequence of SEQ ID NO: 50;
- (i) the antigen-binding domain that specifically binds to human OX40 comprises a VL comprising an amino acid sequence derived from a human IGKV2-28 germline sequence;
- (j) the antigen-binding domain that specifically binds to human OX40 comprises the VH and VL sequences set forth in SEQ ID NOS: 16 and 15, respectively;
- (k) the antigen-binding domain that specifically binds to human OX40 comprises a heavy chain comprising the amino acid sequence of SEQ ID NO: 60;
- (l) the first antigen-binding domain and the second antigen-binding domain comprise an identical mutation selected from the group consisting of N297A, N297Q, D265A, and a combination thereof or an identical mutation selected from the group consisting of D265A, P329A, and a combination thereof; and/or
- (m) the antigen-binding domain that specifically binds to human OX40 and the second heavy chain or fragment thereof comprise an identical mutation selected from the group consisting of N297A, N297Q, D265A, and a combination thereof or an identical mutation selected from the group consisting of D265A, P329A, and a combination thereof.

20. An isolated nucleic acid molecule encoding (i) the heavy chain variable region or the heavy chain of the antibody of any one of claims 1-19 and/or (ii) the light chain variable region or the light chain of the antibody of any one of claims 1-19.
21. The isolated nucleic acid molecule of claim 20, wherein (i) the nucleic acid molecule encodes a heavy chain variable region comprising the amino acid sequence of SEQ ID NO: 16; and/or (ii) the nucleic acid molecule encodes a light chain variable region comprising the amino acid sequence of SEQ ID NO: 15.
22. An isolated vector comprising the nucleic acid molecule of claim 20 or claim 21.
23. A host cell comprising:
- (a) the nucleic acid molecule of claim 20 or claim 21;
 - (b) the vector of claim 22; or
 - (c) a first vector comprising the nucleic acid of claim 20(i) or claim 21(i) and a second vector comprising the nucleic acid of claim 20(ii) or claim 21(ii).
24. The host cell of claim 23, wherein the host cell is selected from the group consisting of E. coli, Pseudomonas, Bacillus, Streptomyces, yeast, CHO, YB/20, NSO, PER-C6, HEK-293T, NIH-3T3, HeLa, BHK, Hep 02, SP2/0, R1.1, B-W, L-M, COS 1, COS 7, BSC1, BSC40, BMT10 cell, plant cell, insect cell, and human cell in tissue culture.
25. A method of producing an antibody that binds to human OX40 comprising culturing the host cell of claim 23 or claim 24 so that the nucleic acid molecule is expressed and the antibody is produced.
26. An isolated antibody that specifically binds to human OX40 and is encoded by the isolated nucleic acid molecule of claim 20 or claim 21.
27. A pharmaceutical composition comprising:

- (a) (i) the antibody of any one of claims 1-19 or claim 26;
(ii) the nucleic acid molecule of claim 20 or claim 21;
(iii) the vector of claim 22; or
(iv) the host cell of claim 23 or claim 24; and
- (b) a pharmaceutically acceptable excipient.

28. An effective amount of the antibody of any one of claims 1-19 or claim 26, the nucleic acid molecule of claim 20 or claim 21, the vector of claim 22, the host cell of claim 23 or claim 24, or the pharmaceutical composition of claim 27 for use in therapy by i) modulating an immune response in a subject or ii) modulating an immune response in a subject, wherein modulating the immune response comprises enhancing or inducing the immune response of the subject.

29. An effective amount of the antibody of any one of claims 1-14, 19 or claim 26, the nucleic acid molecule of claim 20 or 21, the vector of claim 22, the host cell of claim 23 or claim 24, or the pharmaceutical composition of claim 27 for use in therapy by enhancing the expansion of T cells and T cell effector function in a subject.

30. Use of an effective amount of the antibody of any one of claims 1-14, 19 or claim 26, the nucleic acid molecule of claim 20 or claim 21, the vector of claim 22, the host cell of claim 23 or claim 24, or the pharmaceutical composition of claim 27 in the manufacture of a medicament for the treatment of cancer in a subject.

31. The use of claim 30, wherein the cancer is selected from the group consisting of melanoma, renal cancer, prostate cancer, colon cancer, and lung cancer.

32. Use of an effective amount of the antibody of any one of claims 1, 2, 4, 6-9, 12-19, or claim 26, or the pharmaceutical composition of claim 27 in the manufacture of a medicament for the treatment of an autoimmune or inflammatory disease or disorder in a subject.

33. The use of claim 32, wherein the disease or disorder is selected from the group consisting of: transplant rejection, vasculitis, asthma, rheumatoid arthritis, dermatitis, inflammatory bowel disease, uveitis, and lupus.
34. An effective amount of the antibody of any one of claims 1-14, 19 or claim 26, the nucleic acid molecule of claim 20 or claim 21, the vector of claim 22, the host cell of claim 23 or claim 24, or the pharmaceutical composition of claim 27 for use in the treatment of an infectious disease in a subject.
35. The use of any one of claims 30-34, wherein the subject is human.
36. A method for detecting OX40 in a sample comprising contacting the sample with the antibody of any one of claims 1-19 or claim 26.