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(54) Title: ANTI-OX40 ANTIBODIES AND METHODS OF USE THEREOF

(57) Abstract: The present disclosure provides antibodies that specifically bind to human OX40 receptor (OX40) and compositions comprising such antibodies. In a specific aspect, the antibodies specifically bind to human OX40 and modulate OX40 activity, *e.g.*, reduce, deactivate, or inhibit OX40 activity. The present disclosure also provides methods for autoimmune or inflammatory diseases or disorders, by administering an antibody that specifically binds to human OX40 and modulates OX40 activity, *e.g.*, reduces, deactivates, or inhibits OX40 activity.

ANTI-OX40 ANTIBODIES AND METHODS OF USE THEREOF

1. RELATED APPLICATIONS

[0001] The instant application claims priority to U.S. Provisional Application No. 62/262,371, filed on December 2, 2015, the disclosure of which is herein incorporated by reference in its entirety.

2. SEQUENCE LISTING

[0002] The instant application contains a sequence listing which has been submitted electronically in ASCII format and is hereby incorporated by reference in its entirety (said ASCII copy, created on December 1, 2016, is named 3617_015PC01_SeqListing.txt and is 170,077 bytes in size).

3. FIELD

[0003] The present disclosure relates to antibodies that specifically bind to human OX40 receptor ("OX40"), compositions comprising such antibodies, and methods of producing and using those antibodies.

4. BACKGROUND

[0004] The OX40 receptor is an important stimulatory receptor that modulates T cell, Natural Killer T (NKT) cell, and NK cell function (also known as OX40, CD134, TNFRSF4, TXGP1L, ACT35, and ACT-4) (Sugamura K *et al.*, (2004) Nat Rev Immunol 4: 420-431). OX40 is a member of the tumor necrosis factor receptor superfamily (TNFRSF), and signaling via OX40 can modulate important immune functions.

[0005] OX40 can be upregulated by antigen-specific T cells following T cell receptor (TCR) stimulation by professional antigen presenting cells (APCs) displaying MHC class I or II molecules loaded with a cognate peptide (Sugamura K *et al.*, (2004) Nat Rev Immunol 4: 420-431). Upon maturation APCs such as dendritic cells (DCs) upregulate stimulatory B7 family members (*e.g.*, CD80 and CD86), as well as accessory co-stimulatory molecules including OX40 ligand (OX40L), which help to sculpt the kinetics and magnitude of the T cell immune response,

as well as effective memory cell differentiation. Notably, other cell types can also express constitutive and/or inducible levels of OX40L such as B cells, vascular endothelial cells, mast cells, and in some instances activated T cells (Soroosh P *et al.*, (2006) J Immunol 176: 5975-5987). OX40:OX40L co-engagement is believed to drive the higher order clustering of receptor trimers and subsequent signal transduction (Compaan DM *et al.*, (2006) Structure 14: 1321-1330).

[0006] OX40 and OX40L interactions have been associated with immune responses in inflammatory and autoimmune diseases and disorders, including mouse models of asthma/atopy, encephalomyelitis, rheumatoid arthritis, colitis/inflammatory bowel disease, graft-versus-host disease (*e.g.*, transplant rejection), diabetes in non-obese diabetic mice, and atherosclerosis (Croft M *et al.*, (2009) Immunol Rev 229(1): 173-191, and references cited therein). Reduced symptomatology associated with the diseases and disorders has been reported in OX40- and OX40L-deficient mice, in mice receiving anti-OX40 liposomes loaded with a cytostatic drug, and in mice in which OX40 and OX40L interactions were blocked with an anti-OX40L blocking antibody or a recombinant OX40 fused to the Fc portion of human immunoglobulin (Croft M *et al.*; Boot EPJ *et al.*, (2005) Arthritis Res Ther 7: R604-615; Weinberg AD *et al.*, (1999) J Immunol 162: 1818-1826). Treatment with a blocking anti-OX40L antibody was also shown to inhibit Th2 inflammation in a rhesus monkey model of asthma (Croft M *et al.*; Seshasayee D *et al.*, (2007) J Clin Invest 117: 3868-3878). Additionally, polymorphisms in OX40L have been associated with lupus (Croft M *et al.*).

[0007] Given the role of human OX40 in modulating immune responses, provided herein are antibodies that specifically bind to OX40 and the use of these antibodies to modulate OX40 activity.

5. SUMMARY

[0008] In one aspect, provided herein are antagonist antibodies that specifically bind to OX40 (*e.g.*, human OX40).

[0009] In one aspect, an isolated antibody that specifically binds to human OX40, comprises: (A) a first antigen-binding domain that specifically binds to human OX40; comprising: (i) a first heavy chain comprising (a) a first heavy chain constant region comprising a F405L or a K409R mutation, numbered according to the EU numbering system; and (b) 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:47); a VH-CDR2 comprising the amino acid sequence of RIRSKANSYATAYAASVKG (SEQ ID NO:48); and a VH-CDR3 comprising the amino acid sequence of GIYDSSGYDY (SEQ ID NO:49); and ii) a first light chain comprising (a) a first light chain constant region; and (b) a first light chain variable domain (VL) comprising a VL–CDR1 comprising the amino acid sequence of RSSQSLLHSNGYNYLD (SEQ ID NO:50); a VL-CDR2 comprising the amino acid sequence of LGSNRAS (SEQ ID NO:51); and a VL-CDR3 comprising the amino acid sequence of MQGSKWPLT (SEQ ID NO:52) or MQALQTPLT (SEQ ID NO:53); and (B) a second antigen-binding domain that does not bind to an antigen expressed by a human immune cell; comprising: (i) a second heavy chain comprising (a) a second heavy chain constant region comprising a F405L or a K409R mutation, numbered according to the EU numbering system; and (b) a second heavy chain variable domain; and (ii) a second light chain comprising (a) a second light chain constant region; and (b) a second light chain variable domain, wherein the first heavy chain constant region and the second heavy chain constant region contain different mutations.

[0010] In one aspect, an isolated antibody that specifically binds to human OX40, comprises:(A) a first antigen-binding domain that specifically binds to the same epitope of human OX40 as an antibody comprising a VH comprising the amino acid sequence of SEQ ID NO:54 and a VL comprising the amino acid sequence of SEQ ID NO:55 or 56, wherein the first antigen-binding domain comprises a first heavy chain constant region comprising a F405L or a K409R mutation, numbered according to the EU numbering system; and (B) a second antigen-binding domain that does not specifically bind to an antigen expressed by a human immune cell, comprising a second heavy chain constant region comprising a F405L or a K409R mutation, numbered according to the EU numbering system; wherein the first heavy chain constant region and the second heavy chain constant region contain different mutations.

[0011] In one aspect, an isolated antibody that specifically binds to human OX40, wherein the antibody comprises: (A) a first antigen-binding domain that specifically binds to human OX40 and exhibits, as compared to binding to a human OX40 sequence of SEQ ID NO:72, reduced or absent binding to a protein identical to SEQ ID NO:72 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, numbered according to SEQ ID NO:72, wherein the first antigen-binding domain comprises a first heavy chain constant region comprising a F405L

or a K409R mutation, numbered according to the EU numbering system; and (B) a second antigen-binding domain that does not specifically bind to an antigen expressed by a human immune cell, comprising a second heavy chain constant region comprising a F405L or a K409R mutation, numbered according to the EU numbering system; wherein the first heavy chain constant region and the second heavy chain constant region contain different mutations.

[0012] In one aspect, an isolated antibody that specifically binds to human OX40, comprises:(A) a first antigen-binding domain that specifically binds to human OX40 comprising a VH and a VL, wherein the VH comprises the amino acid sequence of SEQ ID NO:54, wherein the first antigen-binding domain comprises a first heavy chain constant region comprising a F405L or a K409R mutation, numbered according to the EU numbering system; and (B) a second antigen-binding domain that does not specifically bind to an antigen expressed by a human immune cell, comprising a second heavy chain constant region comprising a F405L or a K409R mutation, numbered according to the EU numbering system; wherein the first heavy chain constant region and the second heavy chain constant region contain different mutations.

[0013] In one aspect, an isolated antibody that specifically binds to human OX40, comprises:(A) a first antigen-binding domain that specifically binds to human OX40 comprising a VH and a VL, wherein the VL comprises the amino acid sequence of SEQ ID NO:55 or SEQ ID NO:56, wherein the first antigen-binding domain comprises a first heavy chain constant region comprising a F405L or a K409R mutation, numbered according to the EU numbering system; and (B) a second antigen-binding domain that does not specifically bind to an antigen expressed by a human immune cell, comprising a second heavy chain constant region comprising a F405L or a K409R mutation, numbered according to the EU numbering system; wherein the first heavy chain constant region and the second heavy chain constant region contain different mutations.

[0014] In one aspect, the second antigen-binding domain specifically binds to a non-human antigen. In one aspect, the second antigen-binding domain specifically binds to a viral antigen. In one aspect, the viral antigen is a HIV antigen. In one aspect, the second antigen-binding domain specifically binds to chicken albumin or hen egg lysozyme.

[0015] In one aspect, an isolated antibody that specifically binds to human OX40, comprises:(A) an antigen-binding domain that specifically binds to human OX40; comprising: (i) a first heavy chain comprising (a) a first heavy chain constant region comprising a F405L or a

K409R mutation, numbered according to the EU numbering system; and (b) 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:47); a VH-CDR2 comprising the amino acid sequence of RIRSKANSYATAYAASVKG (SEQ ID NO:48); and a VH-CDR3 comprising the amino acid sequence of GIYDSSGYDY (SEQ ID NO:49); and (ii) a light chain comprising (a) a light chain constant region; and (b) a light chain variable domain (VL) comprising a VL–CDR1 comprising the amino acid sequence of RSSQSLLHSNGYNYLD (SEQ ID NO:50); a VL-CDR2 comprising the amino acid sequence of LGSNRAS (SEQ ID NO:51); and a VL-CDR3 comprising the amino acid sequence of MQGSKWPLT (SEQ ID NO:52) or MQALQTPLT (SEQ ID NO:53); and (B) a second heavy chain comprising a second heavy chain constant region comprising a F405L or a K409R mutation, or a fragment thereof comprising the F405L or K409R mutation, numbered according to the EU numbering system; wherein the first heavy chain constant region and the second heavy chain constant region contain different mutations.

[0016] In one aspect, the second heavy chain further comprises a second heavy chain variable domain and wherein the isolated antibody that specifically binds to human OX40 further comprises a second light chain comprising a second light chain constant region and a second light chain variable region.

[0017] In one aspect, an isolated antibody that specifically binds to human OX40 comprises: (A) an antigen-binding domain that specifically binds to the same epitope of human OX40 as an antibody comprising a VH comprising the amino acid sequence of SEQ ID NO:54 and a VL comprising the amino acid sequence of SEQ ID NO:55 or 56, wherein the antigen-binding domain comprises a first heavy chain constant region comprising a F405L or a K409R mutation, numbered according to the EU numbering system; and (B) a heavy chain comprising a second heavy chain constant region comprising a F405L or a K409R mutation, or a fragment thereof comprising the F405L or K409R mutation, numbered according to the EU numbering system; wherein the first heavy chain constant region and the second heavy chain constant region contain different mutations.

[0018] In one aspect, an isolated antibody that specifically binds to human OX40, comprises: (A) an antigen-binding domain that specifically binds to human OX40 and exhibits, as compared to binding to a human OX40 sequence of SEQ ID NO:72, reduced or absent binding to a protein

identical to SEQ ID NO:72 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, numbered according to SEQ ID NO:72, wherein the antigen-binding domain comprises a first heavy chain constant region comprising a F405L or a K409R mutation, numbered according to the EU numbering system; and (B) a heavy chain comprising a second IgG_1 heavy chain constant region comprising a F405L or a K409R mutation, or a fragment thereof comprising the F405L or K409R mutation, numbered according to the EU numbering system; wherein the first heavy chain constant region and the second heavy chain constant region contain different mutations.

[0019] In one aspect, an isolated antibody that specifically binds to human OX40 comprises:(A) an antigen-binding domain that specifically binds to human OX40 comprising a VH and a VL, wherein the VH comprises the amino acid sequence of SEQ ID NO:54, wherein the antigen-binding domain comprises a first heavy chain constant region comprising a F405L or a K409R mutation, numbered according to the EU numbering system; and (B) a heavy chain comprising a second heavy chain constant region comprising a F405L or a K409R mutation, or a fragment thereof comprising the F405L or K409R mutation, numbered according to the EU numbering system; wherein the first heavy chain constant region and the second heavy chain constant region contain different mutations.

[0020] In one aspect, an isolated antibody that specifically binds to human OX40, wherein the antibody comprises: (A) an antigen-binding domain that specifically binds to human OX40 comprising a VH and a VL, wherein the VL comprises the amino acid sequence of SEQ ID NO:55 or SEQ ID NO:56, wherein the antigen-binding domain comprises a first heavy chain constant region comprising a F405L or a K409R mutation, numbered according to the EU numbering system; and (B) a heavy chain comprising a second heavy chain constant region comprising a F405L or a K409R mutation, or a fragment thereof comprising the F405L or K409R mutation, numbered according to the EU numbering system; wherein the first heavy chain constant region and the second heavy chain constant region contain different mutations.

[0021] In one aspect, the heavy chain further comprises a second heavy chain variable domain and wherein the isolated antibody that specifically binds to human OX40 further comprises a second light chain comprising a second light chain constant region and a second light chain variable region.

[0022] In one aspect, the antibody is a bispecific antibody.

[0023] In one aspect, the fragment of the heavy chain comprising a second heavy chain constant region is an Fc fragment.

[0024] In one aspect, the heavy chain comprising a second heavy chain constant region or fragment thereof is from an antigen-binding domain that specifically binds to a non-human antigen. In one aspect, the heavy chain comprising a second heavy chain constant region or fragment thereof is from an antigen-binding domain that specifically binds to a viral antigen. In one aspect, the viral antigen is a HIV antigen. In one aspect, the heavy chain comprising a second heavy chain constant region or fragment thereof is from an antigen-binding domain that specifically binds to chicken albumin or hen egg lysozyme.

[0025] In one aspect, 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:54. In one aspect, the antigen-binding domain that specifically binds to human OX40 comprises a VH comprising the amino acid sequence of SEQ ID NO:54.

[0026] In one aspect, 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.

[0027] In one aspect, 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:55 or SEQ ID NO:56. In one aspect, the antigen-binding domain that specifically binds to human OX40 comprises a VL-CDR3 comprising the amino acid sequence of SEQ ID NO:52. In one aspect, the antigen-binding domain that specifically binds to human OX40 comprises a VL comprising the amino acid sequence of SEQ ID NO:55. In one aspect, the antigen-binding domain that specifically binds to human OX40 comprises a light chain comprising the amino acid sequence of SEQ ID NO:67. In one aspect, the antigen-binding domain that specifically binds to human OX40 comprises a light chain comprising the amino acid sequence of SEQ ID NO:68. In one aspect, the antigen-binding domain that specifically binds to human OX40 comprises a VL-CDR3 comprising the amino acid sequence SEQ ID NO:53. In one aspect, the antigen-binding domain that specifically binds to human OX40 comprises a VL comprising the amino acid sequence of SEQ ID NO:56. In one

aspect, the antigen-binding domain that specifically binds to human OX40 comprises a light chain comprising the amino acid sequence of SEQ ID NO:69. In one aspect, the antigen-binding domain that specifically binds to human OX40 comprises a light chain comprising the amino acid sequence of SEQ ID NO:70.

[0028] In one aspect, 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.

[0029] In one aspect, the antigen-binding domain that specifically binds to human OX40 comprises the VH and VL sequences set forth in SEQ ID NOs: 54 and 55 or SEQ ID NOs: 54 and 56, respectively.

[0030] In one aspect, the first heavy chain constant region comprises a F405L mutation, and the second heavy chain constant region comprises a K409R mutation, numbered according to the EU numbering system. In one aspect, the first heavy chain constant region comprises the amino acid sequence of SEQ ID NO: 108, and the second heavy chain constant region comprises the amino acid sequence of SEQ ID NO: 109. In one aspect, the first heavy chain constant region comprises the amino acid sequence of SEQ ID NO: 135, and the second heavy chain constant region comprises the amino acid sequence of SEQ ID NO: 136. In one aspect, the antigen-binding domain that specifically binds to human OX40 comprises a heavy chain comprising the amino acid sequence of SEQ ID NO:64. In one aspect, the antigen-binding domain that specifically binds to human OX40 comprises a heavy chain comprising the amino acid sequence of SEQ ID NO:123.

[0031] In one aspect, the first heavy chain constant region comprises a K409R mutation, and the second heavy chain constant region comprises a F405L mutation, numbered according to the EU numbering system. In one aspect, the first heavy chain constant region comprises the amino acid sequence of SEQ ID NO: 109, and the second heavy chain constant region comprises the amino acid sequence of SEQ ID NO: 108. In one aspect, the first heavy chain constant region comprises the amino acid sequence of SEQ ID NO: 136, and the second heavy chain constant region comprises the amino acid sequence of SEQ ID NO: 135. In one aspect, the antigen-binding domain that specifically binds to human OX40 comprises a heavy chain comprising the amino acid sequence of SEQ ID NO:61. In one aspect, the antigen-binding domain that specifically binds to human OX40 comprises a heavy chain comprising the amino acid sequence

of SEQ ID NO:120.

[0032] In one aspect, each heavy chain constant region is selected from the group consisting of human immunoglobulins IgG_1, IgG_2, IgG_3, IgG_4, IgA_1, and IgA_2.

[0033] In one aspect, each heavy chain constant region is human immunoglobulin IgG_1. In one aspect, the first heavy chain constant region and the second heavy chain constant region further comprise an identical mutation selected from the group consisting of N297A, N297Q, D265A, L234F/L235E/D265A, and a combination thereof, numbered according to the EU numbering system. In one aspect, the first heavy chain constant region and the second heavy chain constant region further comprise an identical mutation selected from the group consisting of D265A, P329A, and a combination thereof, numbered according to the EU numbering system.

[0034] In one aspect, the antibody is antagonistic to human OX40. In one aspect, the antibody deactivates, reduces, or inhibits an activity of human OX40. In one aspect, the antibody inhibits or reduces binding of human OX40 to human OX40 ligand. In one aspect, the antibody inhibits or reduces human OX40 signaling. In one aspect, the antibody inhibits or reduces human OX40 signaling induced by human OX40 ligand.

[0035] In one aspect, the antibody decreases CD4+ T cell proliferation induced by synovial fluid from rheumatoid arthritis patients. In one aspect, the antibody increases survival of NOG mice transplanted with human PBMCs. In one aspect, the antibody increases proliferation of regulatory T cells in a GVHD model.

[0036] In one aspect, the antibody further comprises a detectable label.

[0037] In one aspect, provided herein is a pharmaceutical composition comprising an antibody that specifically binds to OX40 (*e.g.*, human OX40) provided herein and a pharmaceutically acceptable excipient.

[0038] In one aspect, provided herein is a method of modulating an immune response in a subject comprising administering to the subject an effective amount of an antibody that specifically binds to OX40 (*e.g.*, human OX40) provided herein or a pharmaceutical composition provided herein. In one aspect, modulating an immune response comprises reducing or inhibiting the immune response in the subject.

[0039] In one aspect, provided herein is a method of treating an autoimmune or inflammatory disease or disorder in a subject comprising administering to the subject an effective amount of an antibody that specifically binds to OX40 (*e.g.*, human OX40) provided

herein or a pharmaceutical composition provided herein. In one aspect, the disease or disorder is selected from the group consisting of: transplant rejection, graft-versus-host disease, vasculitis, asthma, rheumatoid arthritis, dermatitis, inflammatory bowel disease, uveitis, lupus, colitis, diabetes, multiple sclerosis, and airway inflammation. In one aspect, provided herein is a method of treating infectious disease in a subject comprising administering to the subject an effective amount of an antibody that specifically binds to OX40 (*e.g.*, human OX40) provided herein or a pharmaceutical composition provided herein. In one aspect, the subject is human.

[0040] In one aspect, provided herein is a method for detecting OX40 in a sample comprising contacting the sample with an antibody that specifically binds to OX40 (*e.g.*, human OX40) provided herein.

[0041] In one aspect, provided herein is a kit comprising an antibody that specifically binds to OX40 (*e.g.*, human OX40) provided herein or a pharmaceutical composition provided herein and a) a detection reagent, b) an OX40 antigen, c) a notice that reflects approval for use or sale for human administration, or d) a combination thereof.

6. BRIEF DESCRIPTION OF THE FIGURES

[0042] **Figures 1A, 1B and 1C** are a set of graphs showing the binding of DuoBody® (Genmab A/S) pab2049 x isotype and an isotype control antibody to Jurkat cells expressing human OX40 (Figure 1A), activated Hut102 cells (Figure 1B) and activated primary CD4+ T cells (Figure 1C). The mean fluorescence intensity (MFI) is plotted against a range of antibody concentrations.

[0043] **Figure 2** is the result of an assay examining the effect of pab2049, pab2049 (K409, LFLEDA), DuoBody pab2049 x isotype, DuoBody pab2049 x isotype (K409, LFLEDA) and an isotype control antibody on primary human T cells following Staphylococcus Enterotoxin A (SEA) stimulation. IL-2 production at an antibody concentration of 20 μg/ml is plotted for each antibody tested. The mean values (bar) of IL-2 production are shown.

[0044] **Figures 3A and 3B**: Figure 3A depicts NF-κB-luciferase signal from Jurkat-huOX40-NF-κB-luciferase reporter cells triggered by multimeric OX40L, DuoBody pab2049 x isotype or an isotype control antibody. The relative light units (RLU) are plotted against a dose titration of OX40L or antibody concentrations. Figure 3B is the result of a reporter assay where Jurkat-huOX40-NF-κB-luciferase reporter cells were pre-incubated with DuoBody pab2049 x

isotype or an isotype control antibody before activated by multimeric OX40L. The % OX40L activity is plotted against a range of antibody concentrations.

[0045] **Figure 4** is a table summarizing the binding of the monoclonal anti-OX40 antibodies pab1949w, pab2049 and pab1928 to 1624-5 cells expressing human OX40 alanine mutants.

7. DETAILED DESCRIPTION

[0046] Provided herein are antagonist DuoBody antibodies that specifically bind to OX40 (*e.g.*, monovalent antibodies that contain only one human OX40-specific antigen-binding domain, and optionally a second antigen-binding domain that is not OX40-specific). For example, in one aspect, provided herein are antibodies that specifically bind to OX40 (*e.g.*, human OX40) and deactivate, reduce, or inhibit one or more OX40 activities. In a specific embodiment, the antibodies are isolated.

[0047] Also provided are isolated nucleic acids (polynucleotides), such as complementary DNA (cDNA), encoding such antibodies. Further provided are vectors (*e.g.*, expression vectors) and cells (*e.g.*, host cells) comprising nucleic acids (polynucleotides) encoding such antibodies. Also provided are methods of making such antibodies. In other aspects, provided herein are methods and uses, for example, for deactivating, reducing, or inhibiting an OX40 activity, and treating certain conditions, such as inflammatory or autoimmune diseases and disorders. Related compositions (*e.g.*, pharmaceutical compositions), kits, and detection methods are also provided.

7.1 Terminology

[0048] 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 and 5% to 10% below the value or range remain within the intended meaning of the recited value or range.

[0049] As used herein, the terms "antibody" and "antibodies" are terms of art and can be used interchangeably herein and refer to a molecule with an antigen-binding site that specifically binds an antigen.

[0050] Antibodies can include, for example, monoclonal antibodies, recombinantly produced antibodies, human antibodies, humanized antibodies, resurfaced 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, 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), bispecific antibodies, and multi-specific antibodies. 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, IgG_2, 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, *e.g.*, that is an immunoglobulin. In certain embodiments, an antibody described herein is an IgG_1, IgG_2, or IgG_4 antibody.

[0051] As used herein, the terms "antigen-binding domain," "antigen-binding region," "antigen-binding site," and similar terms refer to the portion of antibody molecules which comprises the amino acid residues that confer on the antibody molecule its specificity for the antigen (*e.g.*, the complementarity determining regions (CDR)). The antigen-binding region can be derived from any animal species, such as rodents (*e.g.*, mouse, rat, or hamster) and humans.

[0052] As used herein, the terms "variable region" or "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).

[0053] The terms "VL" and "VL domain" are used interchangeably to refer to the light chain variable region of an antibody.

[0054] The terms "VH" and "VH domain" are used interchangeably to refer to the heavy chain variable region of an antibody.

[0055] The term "Kabat numbering" and like terms are recognized in the art and refer to a system of numbering amino acid residues in the heavy and light chain variable regions of an antibody, or an antigen-binding portion thereof. In certain aspects, the CDRs of an antibody can be determined according to the Kabat numbering system (see, *e.g.*, Kabat EA & Wu TT (1971) Ann NY Acad Sci 190: 382-391 and Kabat EA *et al.*, (1991) Sequences of Proteins of Immunological Interest, Fifth Edition, U.S. Department of Health and Human Services, NIH Publication No. 91-3242). Using the Kabat numbering system, CDRs within an antibody heavy chain molecule are typically present at amino acid positions 31 to 35, which optionally can include one or two additional amino acids, following 35 (referred to in the Kabat numbering scheme as 35A and 35B) (CDR1), amino acid positions 50 to 65 (CDR2), and amino acid positions 95 to 102 (CDR3). Using the Kabat numbering system, CDRs within an antibody light chain molecule are typically present at amino acid positions 24 to 34 (CDR1), amino acid positions 50 to 56 (CDR2), and amino acid positions 89 to 97 (CDR3). In a specific embodiment, the CDRs of the antibodies described herein have been determined according to the Kabat numbering scheme.

[0056] As used herein, the term "constant region" or "constant domain" are interchangeable and have its meaning 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 the Fc receptor. The constant region of an immunoglobulin molecule generally has a more conserved amino acid sequence relative to an immunoglobulin variable domain.

[0057] 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.

[0058] 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. In specific embodiments, the light chain is a human light chain.

[0059] 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.

[0060] "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 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.

[0061] As used herein, a "conservative amino acid substitution" is one in which the amino acid residue is replaced with an amino acid residue having a similar side chain. Families of amino acid residues having side chains have been defined in the art. These families include amino acids with basic side chains (*e.g.*, lysine, arginine, histidine), acidic side chains (*e.g.*, aspartic acid, glutamic acid), uncharged polar side chains (*e.g.*, glycine, asparagine, glutamine, serine, threonine, tyrosine, cysteine, tryptophan), nonpolar side chains (*e.g.*, alanine, valine, leucine, isoleucine, proline, phenylalanine, methionine), beta-branched side chains (*e.g.*, threonine, valine, isoleucine) and aromatic side chains (*e.g.*, tyrosine, phenylalanine, tryptophan, histidine). In certain embodiments, one or more amino acid residues within a CDR(s) or within a framework region(s) of an antibody can be replaced with an amino acid residue with a similar side chain.

[0062] 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, 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). Antibody:antigen crystals can be studied using well known X-ray diffraction techniques and can 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). Mutagenesis mapping studies can 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 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.

[0063] As used herein, the terms "immunospecifically binds," "immunospecifically recognizes," "specifically binds," and "specifically 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 immunospecifically bind to an antigen bind to the antigen with a K_A that is at least 2 logs,

2.5 logs, 3 logs, 4 logs or greater than the K_A when the molecules bind non-specifically to another antigen. In the context of antibodies with an anti-OX40 antigen-binding domain and a second antigen-binding domain (*e.g.*, and a second antigen-binding domain that does not specifically bind to an antigen expressed by a human immune cell), the terms "immunospecifically binds," "immunospecifically recognizes," "specifically binds," and "specifically recognizes" refer to antibodies that have distinct specificities for more than one antigen (*i.e.*, OX40 and the antigen associated with the second antigen-binding domain).

[0064] In another specific embodiment, antigen-binding domains that immunospecifically bind to an antigen do not cross react with other proteins under similar binding conditions. In another specific embodiment, antigen-binding domains that immunospecifically bind to an OX40 antigen do not cross react with other non-OX40 proteins. In a specific embodiment, provided herein is an antibody containing an antigen-binding domain that binds to OX40 with higher affinity than to another unrelated antigen. In certain embodiments, provided herein is an antibody containing an antigen-binding domain that binds to OX40 (*e.g.*, human OX40) 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-OX40 antigen-binding domain described herein to an unrelated, non-OX40 protein is less than 10%, 15%, or 20% of the binding of the antigen-binding domain to OX40 protein as measured by, *e.g.*, a radioimmunoassay.

[0065] In a specific embodiment, provided herein is an antibody containing an antigen-binding domain that binds to human OX40 with higher affinity than to another species of OX40. In certain embodiments, provided herein is an antibody containing an antigen-binding domain that binds to human OX40 with a 5%, 10%, 15%, 20%, 25%, 30%, 35%, 40%, 45%, 50%, 55%, 60%, 65%, 70% or higher affinity than to another species of OX40 as measured by, *e.g.*, a radioimmunoassay, surface plasmon resonance, or kinetic exclusion assay. In a specific embodiment, an antibody containing an antigen-binding domain described herein, which binds to human OX40, will bind to another species of OX40 protein with less than 10%, 15%, or 20% of the binding of the antibody containing an antigen-binding domain to the human OX40 protein as measured by, *e.g.*, a radioimmunoassay, surface plasmon resonance, or kinetic exclusion assay.

[0066] As used herein, the terms "OX40 receptor" or "OX40" or "OX40 polypeptide" refer

to OX40 including, but not limited to, native OX40, an isoform of OX40, or an interspecies OX40 homolog of OX40. OX40 is also known as tumor necrosis factor receptor superfamily member 4 (TNFRSF4), ACT35, CD134, IMD16, and TXGP1L. GenBank™ accession numbers BC105070 and BC105072 provide human OX40 nucleic acid sequences. Refseq number NP_003318.1 provides the amino acid sequence of human OX40. The immature amino acid sequence of human OX40 is provided as SEQ ID NO:73. The mature amino acid sequence of human OX40 is provided as SEQ ID NO:72. Human OX40 is designated GeneID: 7293 by Entrez Gene. RefSeq numbers XM_005545122.1 and XP_005545179.1 provide predicted cynomolgus OX40 nucleic acid sequences and amino acid sequences, respectively. A soluble isoform of human OX40 has also been reported (Taylor L *et al.*, (2001) J Immunol Methods 255: 67-72). As used herein, the term "human OX40" refers to OX40 comprising the polypeptide sequence of SEQ ID NO:72.

[0067] As used herein, the terms "OX40 ligand" and "OX40L" refer to tumor necrosis factor ligand superfamily member 4 (TNFSF4). OX40L is otherwise known as CD252, GP34, TXGP1, and CD134L. GenBank™ accession numbers D90224.1 and AK297932.1 provide exemplary human OX40L nucleic acid sequences. RefSeq number NP_003317.1 and Swiss-Prot accession number P23510-1 provide exemplary human OX40L amino acid sequences for isoform 1. RefSeq number NP_001284491.1 and Swiss-Prot accession number P23510-2 provide exemplary human OX40L amino acid sequences for isoform 2. Human OX40L is designated GeneID: 7292 by Entrez Gene.

[0068] As used herein, the term "host cell" can be any type of cell, *e.g.*, a primary cell, a cell in culture, or a cell from a cell line. In specific embodiments, the term "host cell" refers to a cell transfected with a nucleic acid molecule and the progeny or potential progeny of such a cell. Progeny of such a cell may not be identical to the parent cell transfected with the nucleic acid molecule, *e.g.*, due to mutations or environmental influences that may occur in succeeding generations or integration of the nucleic acid molecule into the host cell genome.

[0069] 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. Examples of effective amounts are provided in Section 7.5, *infra*.

[0070] As used herein, the terms "subject" and "patient" are used interchangeably. The subject can be an animal. In some embodiments, the subject is a mammal such as a non-primate

(*e.g.*, cow, pig, horse, cat, dog, rat, etc.) or a primate (*e.g.*, monkey or human), most preferably a human. In some embodiments, the subject is a cynomolgus monkey. In certain embodiments, such terms refer to a non-human animal (*e.g.*, a non-human animal such as a pig, horse, cow, cat, or dog). In some embodiments, such terms refer to a pet or farm animal. In specific embodiments, such terms refer to a human.

[0071] As used herein, the binding between a test antibody and a first antigen is "substantially weakened" relative to the binding between the test antibody and a second antigen if the binding between the test antibody and the first antigen is reduced by at least 30%, 40%, 50%, 60%, 70%, or 80% relative to the binding between the test antibody and the second antigen, as measured in, *e.g.*, a flow cytometry analysis.

[0072] The determination of "percent identity" between two sequences (*e.g.,* amino acid sequences or nucleic acid sequences) can also 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. Such an algorithm is incorporated into the NBLAST and XBLAST programs of Altschul SF *et al.*, (1990) J Mol Biol 215: 403. 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. 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. 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.

[0073] 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.

[0074] As used herein, the term "antigen-binding domain that does not bind to an antigen expressed by a human immune cell" means that the antigen-binding domain does not bind to an antigen expressed by any human cell of hematopoietic origin that plays a role in the immune response. Immune cells include lymphocytes, such as B cells and T cells; natural killer cells; and myeloid cells, such as monocytes, macrophages, eosinophils, mast cells, basophils, and granulocytes. For example, such a binding domain would not bind to OX40, or any other members of the TNF receptor superfamily that are expressed by a human immune cell. However, the antigen-binding domain can bind to an antigen such as, but not limited to, an antigen expressed in other organisms and not humans (*i.e.*, a non-human antigen); an antigen that is not expressed by wild-type human cells; or a viral antigen, including, but not limited to, an antigen from a virus that does not infect human cells, or a viral antigen that is absent in an uninfected human immune cell.

7.2 Antibodies

[0075] The activation of OX40 signaling depends on receptor clustering to form higher order receptor complexes that efficiently recruit apical adapter proteins to drive intracellular signal transduction. Without being bound by theory, an anti-OX40 agonist antibody may mediate receptor clustering through bivalent antibody arms (*i.e.*, two antibody arms that each bind OX40 antigen) and/or through Fc-Fc receptor (FcR) co-engagement on accessory myeloid or lymphoid cells. Consequently, one approach for developing an anti-OX40 antagonist antibody is to select an antibody that competes with OX40 ligand (OX40L) for binding to OX40, diminish or eliminate the binding of the Fc region of an antibody to Fc receptors, and/or adopt a monovalent antibody format. The monovalent antibody format can include antibodies that are structurally monovalent, such as, but not limited to, anti-OX40 antibodies comprising a first antigen-binding domain that binds to OX40 (*e.g.*, human OX40) paired with, for example, an Fc region (*i.e.*, the monovalent antibody comprises only one antigen-binding arm). The monovalent antibody format can also include antibodies that are functionally monovalent, for example, antibodies comprising only one antigen-binding domain that binds to OX40 (*e.g.*, human OX40) that is

paired with a second-antigen binding domain that does not bind to an antigen expressed by a human immune cell (*i.e.*, the antibody comprises two antigen-binding domains, but only one antigen-binding domain binds to OX40).

[0076] Provided herein are antagonist antibodies which specifically bind to OX40 (*e.g.*, human OX40). The antibodies are DuoBody (Genmab A/S) anti-OX40 antagonists, each comprising one antigen-binding domain that binds to OX40.

7.2.1 Antigen-Binding Domains that Bind to OX40

[0077] In certain embodiments, an antigen-binding domain as described herein that binds to OX40 contains a combination of CDRs shown in a single row of Table 1 below.

Table 1: CDR sequences of exemplary antigen-binding domains that bind to human OX40*

OX40-Binding Sequence SEQ ID NO.					
VH CDR1	VH CDR2	VH CDR3	VL CDR1	VL CDR2	VL CDR3
47	48	49	50	51	52
47	48	49	50	51	53

*The VL CDRs in Table 1 are determined according to Kabat.

[0078] In certain embodiments, an antigen-binding domain as described herein that binds to OX40 contains a combination of a heavy chain variable domain and a light chain variable domain shown in a single row of Table 2 below.

Table 2: Heavy chain variable domain and light chain variable domain sequences of exemplary anti-OX40 antibodies

OX40 VH (SEQ ID NO:)	OX40 VL (SEQ ID NO:)
54	55
54	56

[0079] In a particular embodiment, an antigen-binding domain that binds to OX40 (*e.g.*, human OX40) as described herein comprises a light chain variable region (VL) comprising:

(a) a VL CDR1 comprising, consisting of, or consisting essentially of the amino acid sequence RSSQSLLHSNGYNYLD (SEQ ID NO:50),

(b) a VL CDR2 comprising, consisting of, or consisting essentially of the amino acid sequence LGSNRAS (SEQ ID NO:51), and

(c) a VL CDR3 comprising, consisting of, or consisting essentially of the amino acid sequence MQALQTPLT (SEQ ID NO:52) or MQALQTPLT (SEQ ID NO:53), as shown in Table 3.

Table 3. VL CDR Amino Acid Sequences *

Antibody	**VL CDR1 (SEQ ID NO:)**	**VL CDR2 (SEQ ID NO:)**	**VL CDR3 (SEQ ID NO:)**
pab1949w	RSSQSLLHSNGYNYLD (50)	LGSNRAS (51)	MQALQTPLT (53)
pab2049w	RSSQSLLHSNGYNYLD (50)	LGSNRAS (51)	MQGSKWPLT (52)

*The VL CDRs in Table 3 are determined according to Kabat.

[0080] In some embodiments, an antigen-binding domain that binds to OX40 comprises the VL framework regions described herein. In specific embodiments, the antigen-binding domain that binds to OX40 comprises the VL framework regions (FRs) set forth in Table 4.

Table 4. VL FR Amino Acid Sequences *

Antibody	**VL FR1 (SEQ ID NO:)**	**VL FR2 (SEQ ID NO:)**	**VL FR3 (SEQ ID NO:)**	**VL FR4 (SEQ ID NO:)**
pab1949w	DIVMTQSPLSLPV TPGEPASISC (89)	WYLQKPGQ SPQLLIY (91)	GVPDRFSGSGSGTDFT LKISRVEAEDVGVYYC (116)	FGGGTKVEI K (117)
pab2049w	DIVMTQSPLSLPV TPGEPASISC (89)	WYLQKPGQ SPQLLIY (91)	GVPDRFSGSGAGTDFT LKISRVEAEDVGIYYC (110)	FGGGTKLEI K (111)

*The VL framework regions described in Table 4 are determined based upon the boundaries of the Kabat numbering system for CDRs. In other words, the VL CDRs are determined by Kabat and the framework regions are the amino acid residues surrounding the CDRs in the variable region in the format FR1, CDR1, FR2, CDR2, FR3, CDR3, and FR4.

[0081] In another embodiment, an antigen-binding domain described herein, which specifically binds to OX40 (*e.g.*, human OX40), comprises a heavy chain variable region (VH) comprising:

(a) a VH CDR1 comprising, consisting of, or consisting essentially of the amino acid sequence GSAMH (SEQ ID NO:47),

(b) a VH CDR2 comprising, consisting of, or consisting essentially of the amino acid sequence RIRSKANSYATAYAASVKG (SEQ ID NO:48), and

(c) a VH CDR3 comprising, consisting of, or consisting essentially of the amino acid sequence GIYDSSGYDY (SEQ ID NO:49), as shown in Table 5.

Table 5. VH CDR Amino Acid Sequences *

Antibody	**VH CDR1 (SEQ ID NO:)**	**VH CDR2 (SEQ ID NO:)**	**VH CDR3 (SEQ ID NO:)**
pab1949w	GSAMH (47)	RIRSKANSYATAYAASVKG (48)	GIYDSSGYDY (49)
pab2049w	GSAMH (47)	RIRSKANSYATAYAASVKG (48)	GIYDSSGYDY (49)

*The VH CDRs in Table 5 are determined according to Kabat.

[0082] In some embodiments, an antigen-binding domain that binds to OX40 comprises the VH frameworks described herein. In specific embodiments, the antigen-binding domain that binds to OX40 comprises the VH framework regions (FRs) set forth in Table 6. In some embodiments, an antigen-binding domain that binds to OX40 comprises the four VL FRs set forth in Table 4 and the four VH FRs set forth in Table 6.

Table 6. VH FR Amino Acid Sequences *

Antibody	**VH FR1 (SEQ ID NO:)**	**VH FR2 (SEQ ID NO:)**	**VH FR3 (SEQ ID NO:)**	**VH FR4 (SEQ ID NO:)**
pab1949w / pab2049w	EVQLVESGGGLVQ PGGSLKLSCAASGF TFS (112)	WVRQASGK GLEWVG (113)	RFTISRDDSKNTAYL QMNSLKTEDTAVY YCTS (114)	WGQGTLVT VSS (115)

*The VH framework regions described in Table 6 are determined based upon the boundaries of the Kabat numbering system for CDRs. In other words, the VH CDRs are determined by Kabat and the framework regions are the amino acid residues surrounding the CDRs in the variable region in the format FR1, CDR1, FR2, CDR2, FR3, CDR3, and FR4.In certain embodiments, provided herein is an antigen-binding domain which specifically binds to OX40 (*e.g.*, human OX40) and comprises light chain variable region (VL) CDRs and heavy chain variable region (VH) CDRs of pab1949w, or pab2049w, for example as set forth in Tables 3 and 5 (*i.e.*, SEQ ID NOs: 47-52 or SEQ ID NOs: 47-51 and 53).

[0083] In certain embodiments, an OX40 antigen-binding domain comprises a light chain

variable framework region that is derived from an amino acid sequence encoded by a human gene, wherein the amino acid sequence is that of IGKV2-28*01 (SEQ ID NO:58).

[0084] In certain embodiments, the OX40 antigen-binding domain comprises a heavy chain variable framework region that is derived from an amino acid sequence encoded by a human gene, wherein the amino acid sequence is that of IGHV3-73*01 (SEQ ID NO:57).

[0085] In a specific embodiment, an antigen-binding domain that specifically binds to OX40 (*e.g.*, human OX40) comprises a VL domain comprising the amino acid sequence of SEQ ID NO:55 or 56. In a specific embodiment, an antigen-binding domain that specifically binds to OX40 (*e.g.*, human OX40) comprises a VL domain consisting of or consisting essentially of the amino acid sequence of SEQ ID NO:55 or 56.

[0086] In certain embodiments, an antigen-binding domain that specifically binds to OX40 (*e.g.,* human OX40) comprises a VH domain comprising the amino acid sequence of SEQ ID NO:54. In some embodiments, an antigen-binding domain that specifically binds to OX40 (*e.g.,* human OX40) comprises a VH domain consisting of or consisting essentially of the amino acid sequence of SEQ ID NO:54.

[0087] In certain embodiments, an antigen-binding domain that specifically binds to OX40 (*e.g.*, human OX40) comprises a VH domain and a VL domain, wherein the VH domain and the VL domain comprise the amino acid sequences of SEQ ID NO:54 and SEQ ID NO:55 or 56, respectively. In certain embodiments, an antigen-binding domain that specifically binds to OX40 (*e.g.*, human OX40) comprises a VH domain and a VL domain, wherein the VH domain and the VL domain consist of or consist essentially of the amino acid sequences of SEQ ID NO:54 and SEQ ID NO:55 or 56, respectively.

[0088] In specific aspects, provided herein is an antigen-binding domain comprising a light chain and heavy chain, *e.g.*, a separate light chain and heavy chain. With respect to the light chain, in a specific embodiment, the light chain of an antigen-binding domain described herein is a kappa light chain. In another specific embodiment, the light chain of an antigen-binding domain described herein is a lambda light chain. In yet another specific embodiment, the light chain of an antigen-binding domain described herein is a human kappa light chain or a human lambda light chain. In a particular embodiment, an antigen-binding domain described herein, which immunospecifically binds to an OX40 polypeptide (*e.g.*, human OX40) comprises a light chain wherein the amino acid sequence of the VL domain comprises the sequence set forth in

SEQ ID NO: 55 or 56, and wherein the constant region of the light chain comprises the amino acid sequence of a human kappa light chain constant region. In another particular embodiment, an antigen-binding domain described herein, which immunospecifically binds to OX40 (*e.g.*, human OX40) comprises a light chain wherein the amino acid sequence of the VL domain comprises the sequence set forth in SEQ ID NO: 55 or 56 and wherein the constant region of the light chain comprises the amino acid sequence of a human lambda light chain constant region. In a specific embodiment, an antigen-binding domain described herein, which immunospecifically binds to OX40 (*e.g.*, human OX40) comprises a light chain wherein the amino acid sequence of the VL domain comprises the sequence set forth in SEQ ID NO:55 or 56 and wherein the constant region of the light chain comprises the amino acid sequence of a human kappa or lambda light chain constant region. Non-limiting examples of human constant region sequences have been described in the art, *e.g.*, see U.S. Patent No. 5,693,780 and Kabat EA *et al.*, (1991) *supra*.

[0089] In a particular embodiment, an antigen-binding domain described herein, which specifically binds to OX40 (*e.g.*, human OX40) comprises a light chain comprising the amino acid sequence set forth in SEQ ID NO:67 or 69.

[0090] With respect to the heavy chain, in a specific embodiment, the heavy chain of an antigen-binding domain described herein can be an alpha (α), delta (δ), epsilon (ε), gamma (γ) or mu (μ) heavy chain. In another specific embodiment, the heavy chain of an antigen-binding domain described can comprise a human alpha (α), delta (δ), epsilon (ε), gamma (γ) or mu (μ) heavy chain. In a particular embodiment, an antigen-binding domain described herein, which immunospecifically binds to OX40 (*e.g.*, human OX40), comprises a heavy chain wherein the amino acid sequence of the VH domain can comprise the sequence set forth in SEQ ID NO:54 and wherein the constant region of the heavy chain comprises the amino acid sequence of a human gamma (γ) heavy chain constant region. In a specific embodiment, an antigen-binding domain described herein, which specifically binds to OX40 (*e.g.*, human OX40), comprises a heavy chain wherein the amino acid sequence of the VH domain comprises the sequence set forth in SEQ ID NO:54, and wherein the constant region of the heavy chain comprises the amino acid of a human heavy chain described herein or known in the art. Non-limiting examples of human constant region sequences have been described in the art, *e.g.*, see U.S. Patent No. 5,693,780 and Kabat EA *et al.*, (1991) *supra*.

[0091] In a particular embodiment, an antigen-binding domain described herein, which specifically binds to OX40 (*e.g.*, human OX40), comprises a heavy chain comprising the amino acid sequence set forth in SEQ ID NO:61. In another embodiment, an antigen-binding domain described herein, which specifically binds to OX40 (*e.g.*, human OX40), comprises a heavy chain comprising the amino acid sequence set forth in SEQ ID NO:62. In another embodiment, an antigen-binding domain described herein, which specifically binds to OX40 (*e.g.*, human OX40), comprises a heavy chain comprising the amino acid sequence set forth in SEQ ID NO:63. In another embodiment, an antigen-binding domain described herein, which specifically binds to OX40 (*e.g.*, human OX40), comprises a heavy chain comprising the amino acid sequence set forth in SEQ ID NO:64. In another embodiment, an antigen-binding domain described herein, which specifically binds to OX40 (*e.g.*, human OX40), comprises a heavy chain comprising the amino acid sequence set forth in SEQ ID NO:65. In another embodiment, an antigen-binding domain described herein, which specifically binds to OX40 (*e.g.*, human OX40), comprises a heavy chain comprising the amino acid sequence set forth in SEQ ID NO:71.

[0092] In a specific embodiment, an antigen-binding domain described herein, which immunospecifically binds to OX40 (*e.g.*, human OX40) comprises a VL domain and a VH domain comprising any amino acid sequences described herein, wherein the constant regions comprise the amino acid sequences of the constant regions of an IgG, IgE, IgM, IgD, IgA, or IgY immunoglobulin molecule, or a human IgG, IgE, IgM, IgD, IgA, or IgY immunoglobulin molecule. In another specific embodiment, an antigen-binding domain described herein, which immunospecifically binds to OX40 (*e.g.*, human OX40) comprises a VL domain and a VH domain comprising any amino acid sequences described herein, wherein the constant regions comprise the amino acid sequences of the constant regions of an 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. In a particular embodiment, the constant regions comprise the amino acid sequences of the constant regions of 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.

[0093] In another specific embodiment, an antigen-binding domain described herein, which immunospecifically binds to OX40 (*e.g.*, human OX40), comprises a VL domain and a VH

domain comprising any amino acid sequences described herein, wherein the constant regions comprise the amino acid sequences of the constant regions of a human IgG_1 (*e.g.*, allotypes G1m3, G1m17,1 or G1m17,1,2), human IgG_2, or human IgG_4. In a particular embodiment, an antigen-binding domain described herein, which immunospecifically binds to OX40 (*e.g.*, human OX40), comprises a VL domain and a VH domain comprising any amino acid sequences described herein, wherein the constant regions comprise the amino acid sequences of the constant region of a human IgG_1 (allotype Glm3). Non-limiting examples of human constant regions are described in the art, *e.g.*, *see* Kabat EA *et al.*, (1991) *supra*.

[0094] In another embodiment, an antigen-binding domain described herein, which specifically binds to OX40 (*e.g.*, human OX40), comprises a light chain comprising the amino acid sequence set forth in SEQ ID NO:67 and a heavy chain comprising the amino acid sequence set forth in SEQ ID NO:61, 62, 63, 64, 65, or 71. In another embodiment, an antigen-binding domain described herein, which specifically binds to OX40 (*e.g.*, human OX40), comprises a light chain comprising the amino acid sequence set forth in SEQ ID NO:69 and a heavy chain comprising the amino acid sequence set forth in SEQ ID NO:61, 62, 63, 64, 65, or 71.

[0095] In certain embodiments, an antigen-binding domain described herein, which immunospecifically binds to OX40 (*e.g.*, human OX40), comprises a VL domain having at least 70%, at least 75%, at least 80%, at least 85%, at least 90%, at least 95%, or at least 98% sequence identity to the amino acid sequence of the VL domain of pab1949w or pab2049w (*i.e.*, SEQ ID NO:55 or 56), *e.g.*, wherein the antigen-binding domain comprises VL CDRs that are identical to the VL CDRs of pab1949w or pab2049w.

[0096] In certain embodiments, an antigen-binding domain described herein, which immunospecifically binds to OX40 (*e.g.*, human OX40), comprises a VH domain having at least 70%, at least 75%, at least 80%, at least 85%, at least 90%, at least 95%, or at least 98% sequence identity to the amino acid sequence of the VH domain of pab1949w or pab2049w (*i.e.*, SEQ ID NO:54), *e.g.,* wherein the antigen-binding domain comprises VH CDRs that are identical to the VH CDRs of pab1949w or pab2049w.

[0097] In certain embodiments, an antigen-binding domain described herein, which immunospecifically binds to OX40 (*e.g.*, human OX40), comprises: (i) a VL domain having at least 70%, at least 75%, at least 80%, at least 85%, at least 90%, at least 95%, or at least 98% sequence identity to the amino acid sequence of the VL domain of pab1949w or pab2049w (*i.e.*,

SEQ ID NO:55 or 56); and (ii) a VH domain having at least 70%, at least 75%, at least 80%, at least 85%, at least 90%, at least 95%, or at least 98% sequence identity to the amino acid sequence of the VH domain of pab1949w or pab2049w (*i.e.*, SEQ ID NO:54), *e.g.*, wherein the antibody comprises VL CDRs and VH CDRs that are identical to the VL CDRs and VH CDRs of pab1949w or pab2049w.

[0098] In certain aspects, an antigen-binding domain described herein may be described by its VL domain alone, or its VH domain alone, or by its 3 VL CDRs alone, or its 3 VH CDRs alone. *See*, for example, Rader C *et al.*, (1998) PNAS 95: 8910-8915, which is incorporated herein by reference in its entirety, describing the humanization of the mouse anti-αvβ3 antibody by identifying a complementing light chain or heavy chain, respectively, from a human light chain or heavy chain library, resulting in humanized antibody variants having affinities as high or higher than the affinity of the original antibody. *See also* Clackson T *et al.*, (1991) Nature 352: 624-628, which is incorporated herein by reference in its entirety, describing methods of producing antibodies that bind a specific antigen by using a specific VL domain (or VH domain) and screening a library for the complementary variable domains. The screen produced 14 new partners for a specific VH domain and 13 new partners for a specific VL domain, which were strong binders, as determined by ELISA. *See also* Kim SJ & Hong HJ, (2007) J Microbiol 45: 572-577, which is incorporated herein by reference in its entirety, describing methods of producing antibodies that bind a specific antigen by using a specific VH domain and screening a library (*e.g.*, human VL library) for complementary VL domains; the selected VL domains in turn could be used to guide selection of additional complementary (*e.g.*, human) VH domains.

[0099] In certain aspects, the CDRs of an antigen-binding domain 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). Typically, when using the Kabat numbering convention, the Chothia CDR-H1 loop is present at heavy chain amino acids 26 to 32, 33, or 34, the Chothia CDR-H2 loop is present at heavy chain amino acids 52 to 56, and the Chothia CDR-H3 loop is present at heavy chain amino acids 95 to 102, while the Chothia CDR-L1 loop is present at light chain amino acids 24 to 34, the Chothia CDR-L2 loop is present at light chain amino acids 50 to 56, and the Chothia CDR-L3 loop is

present at light chain amino acids 89 to 97. The end of the Chothia CDR-H1 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).

[00100] In certain aspects, provided herein are antigen-binding domains that specifically bind to OX40 (*e.g.*, human OX40) and comprise the Chothia VL CDRs of a VL of pab2049w or pab1949w. In certain aspects, provided herein are antigen-binding domains that specifically bind to OX40 (*e.g.*, human OX40) and comprise the Chothia VH CDRs of a VH of pab2049w or pab1949w. In certain aspects, provided herein are antigen-binding domains that specifically bind to OX40 (*e.g.*, human OX40) and comprise the Chothia VL CDRs of a VL of pab2049w or pab1949w and comprise the Chothia VH CDRs of a VH of pab2049w or pab1949w. In certain embodiments, antigen-binding domains that specifically bind to OX40 (*e.g.*, human OX40) comprise one or more CDRs, in which the Chothia and Kabat CDRs have the same amino acid sequence. In certain embodiments, provided herein are antigen-binding domains that specifically bind to OX40 (*e.g.*, human OX40) and comprise combinations of Kabat CDRs and Chothia CDRs.

[00101] In certain aspects, the CDRs of an antigen-binding domain 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. According to the IMGT numbering scheme, VH-CDR1 is at positions 26 to 35, VH-CDR2 is at positions 51 to 57, VH-CDR3 is at positions 93 to 102, VL-CDR1 is at positions 27 to 32, VL-CDR2 is at positions 50 to 52, and VL-CDR3 is at positions 89 to 97. In a particular embodiment, provided herein are antigen-binding domains that specifically bind to OX40 (*e.g.*, human OX40) and comprise CDRs of pab2049w or pab1949w 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*).

[00102] In certain aspects, the CDRs of an antigen-binding domain can be determined according to MacCallum RM *et al.*, (1996) J Mol Biol 262: 732-745. *See also*, *e.g.*, 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). In a particular embodiment, provided herein are antigen-binding domains that specifically bind to OX40 (*e.g.*, human OX40) and comprise CDRs of pab2049w or pab1949w as determined by the method in MacCallum RM *et al.*

[00103] In certain aspects, the CDRs of an antibody can be determined according to the AbM numbering scheme, which refers 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.). In a particular embodiment, provided herein are antigen-binding domains that specifically bind to OX40 (*e.g.*, human OX40) and comprise CDRs of pab2049w or pab1949w as determined by the AbM numbering scheme.

[00104] In a specific embodiment, the position of one or more CDRs along the VH (*e.g.*, CDR1, CDR2, or CDR3) and/or VL (*e.g.*, CDR1, CDR2, or CDR3) region of an antigen-binding domain described herein may vary by one, two, three, four, five, or six amino acid positions so long as immunospecific binding to OX40 (*e.g.*, human OX40) is maintained (*e.g.*, substantially maintained, for example, at least 50%, at least 60%, at least 70%, at least 80%, at least 90%, at least 95%). For example, in one embodiment, the position defining a CDR of an antigen-binding domain described herein can vary by shifting the N-terminal and/or C-terminal boundary of the CDR by one, two, three, four, five, or six amino acids, relative to the CDR position of an antigen-binding domain described herein, so long as immunospecific binding to OX40 (*e.g.*, human OX40) is maintained (*e.g.*, substantially maintained, for example, at least 50%, at least 60%, at least 70%, at least 80%, at least 90%, at least 95%). In another embodiment, the length of one or more CDRs along the VH (*e.g.*, CDR1, CDR2, or CDR3) and/or VL (*e.g.*, CDR1, CDR2, or CDR3) region of an antigen-binding domain described herein may vary (*e.g.*, be shorter or longer) by one, two, three, four, five, or more amino acids, so long as immunospecific binding to OX40 (*e.g.*, human OX40) is maintained (*e.g.*, substantially maintained, for example, at least 50%, at least 60%, at least 70%, at least 80%, at least 90%, at least 95%).

[00105] In one embodiment, a VL CDR1, VL CDR2, VL CDR3, VH CDR1, VH CDR2, and/or VH CDR3 described herein may be one, two, three, four, five or more amino acids shorter than one or more of the CDRs described herein (*e.g.*, SEQ ID NOs:47-52, or SEQ ID NOs: 47-51 and 53) so long as immunospecific binding to OX40 (*e.g.*, human OX40) is maintained (*e.g.*, substantially maintained, for example, at least 50%, at least 60%, at least 70%, at least 80%, at least 90%, at least 95%). In another embodiment, a VL CDR1, VL CDR2, VL CDR3, VH

CDR1, VH CDR2, and/or VH CDR3 described herein may be one, two, three, four, five or more amino acids longer than one or more of the CDRs described herein (*e.g.*, SEQ ID NOs:47-52, or SEQ ID NOs: 47-51 and 53) so long as immunospecific binding to OX40 (*e.g.*, human OX40) is maintained (*e.g.*, substantially maintained, for example, at least 50%, at least 60%, at least 70%, at least 80%, at least 90%, at least 95%). In another embodiment, the amino terminus of a VL CDR1, VL CDR2, VL CDR3, VH CDR1, VH CDR2, and/or VH CDR3 described herein may be extended by one, two, three, four, five or more amino acids compared to one or more of the CDRs described herein (*e.g.*, SEQ ID NOs:47-52, or SEQ ID NOs: 47-51 and 53) so long as immunospecific binding to OX40 (*e.g.*, human OX40) is maintained (*e.g.*, substantially maintained, for example, at least 50%, at least 60%, at least 70%, at least 80%, at least 90%, at least 95%). In another embodiment, the carboxy terminus of a VL CDR1, VL CDR2, VL CDR3, VH CDR1, VH CDR2, and/or VH CDR3 described herein may be extended by one, two, three, four, five or more amino acids compared to one or more of the CDRs described herein (*e.g.*, SEQ ID NOs:47-52, or SEQ ID NOs: 47-51 and 53) so long as immunospecific binding to OX40 (*e.g.*, human OX40) is maintained (*e.g.*, substantially maintained, for example, at least 50%, at least 60%, at least 70%, at least 80%, at least 90%, at least 95%). In another embodiment, the amino terminus of a VL CDR1, VL CDR2, VL CDR3, VH CDR1, VH CDR2, and/or VH CDR3 described herein may be shortened by one, two, three, four, five or more amino acids compared to one or more of the CDRs described herein (*e.g.*, SEQ ID NOs:47-52, or SEQ ID NOs: 47-51 and 53) so long as immunospecific binding to OX40 (*e.g.*, human OX40) is maintained (*e.g.*, substantially maintained, for example, at least 50%, at least 60%, at least 70%, at least 80%, at least 90%, at least 95%). In one embodiment, the carboxy terminus of a VL CDR1, VL CDR2, VL CDR3, VH CDR1, VH CDR2, and/or VH CDR3 described herein may be shortened by one, two, three, four, five or more amino acids compared to one or more of the CDRs described herein (*e.g.*, SEQ ID NOs:47-52, or SEQ ID NOs: 47-51 and 53) so long as immunospecific binding to OX40 (*e.g.*, human OX40) is maintained (*e.g.*, substantially maintained, for example, at least 50%, at least 60%, at least 70%, at least 80%, at least 90%, at least 95%). Any method known in the art can be used to ascertain whether immunospecific binding to OX40 (*e.g.*, human OX40) is maintained, for example, the binding assays and conditions described in the "Examples" section (Section 8) provided herein.

[00106] In another particular embodiment, an antigen-binding domain described herein, which

immunospecifically binds to OX40 (*e.g.*, human OX40), comprises a heavy chain and a light chain, wherein (i) the heavy and light chains comprise a VH domain and a VL domain, respectively, wherein the VH CDR1, VH CDR2, VH CDR3, VL CDR1, VL CDR2, and VL CDR3 of the VH and VL domains comprise the amino acid sequences set forth in SEQ ID NOs:47-52, or SEQ ID NOs: 47-51 and 53, respectively; (ii) the light chain further comprises a constant light chain domain comprising the amino acid sequence of the constant domain of a human kappa light chain; and (iii) the heavy chain further comprises a constant heavy chain domain comprising the amino acid sequence of the constant domain of a human IgG_1 (optionally IgG_1 (allotype Glm3)) heavy chain.

[00107] In another particular embodiment, an antigen-binding domain described herein, which immunospecifically binds to OX40 (*e.g.*, human OX40), comprises a heavy chain and a light chain, wherein (i) the heavy and light chains comprise a VH domain and a VL domain, respectively comprising the amino acid sequences set forth in SEQ ID NOs: 54 and 55, or SEQ ID NOs: 54 and 56, respectively; (ii) the light chain further comprises a constant domain comprising the amino acid sequence of the constant domain of a human kappa light chain; and (iii) the heavy chain further comprises a constant domain comprising the amino acid sequence of the constant domain of a human IgG_1 (optionally IgG_1 (allotype Glm3)) heavy chain.

[00108] In another particular embodiment, an antigen-binding domain described herein, which immunospecifically binds to OX40 (*e.g.*, human OX40), comprises a light chain and a heavy chain, wherein (i) the light chain comprises a VL domain comprising the VL CDR1, VL CDR2, and VL CDR3 amino acid sequences set forth in SEQ ID NOs: 50-51 and 53, or SEQ ID NOs: 50-52 (*e.g.*, those listed in Table 3); (ii) the heavy chain comprises a VH domain comprising the VH CDR1, VH CDR2, and VH CDR3 amino acid sequences set forth in SEQ ID NOs: 47-49 (*e.g.*, those listed in Table 5); (iii) the light chain further comprises a constant light chain domain comprising the amino acid sequence of the constant domain of a human kappa light chain; and (iv) the heavy chain further comprises a constant heavy chain domain comprising the amino acid sequence of the constant domain of a human IgG_4 heavy chain.

[00109] In another particular embodiment, an antigen-binding domain described herein, which immunospecifically binds to OX40 (*e.g.*, human OX40), comprises a light chain and a heavy chain, wherein (i) the light chain comprises a VL domain comprising the amino acid sequence of SEQ ID NO: 55 or 56; (ii) the heavy chain comprises a VH domain comprising the amino acid

sequence of SEQ ID NO: 54; (iii) the light chain further comprises a constant domain comprising the amino acid sequence of the constant domain of a human kappa light chain; and (iv) the heavy chain further comprises a constant domain comprising the amino acid sequence of the constant domain of a human IgG_4 heavy chain.

[00110] In another particular embodiment, an antigen-binding domain described herein, which immunospecifically binds to OX40 (*e.g.*, human OX40), comprises a light chain and a heavy chain, wherein (i) the light chain comprises a VL domain comprising the VL CDR1, VL CDR2, and VL CDR3 amino acid sequences set forth in SEQ ID NOs: 50-51 and 53, or SEQ ID NOs: 50-52 (*e.g.*, those listed in Table 3); (ii) the heavy chain comprises a VH domain comprising the VH CDR1, VH CDR2, and VH CDR3 amino acid sequences set forth in SEQ ID NOs: 47-49 (*e.g.*, those listed in Table 5); (iii) the light chain further comprises a constant light chain domain comprising the amino acid sequence of the constant domain of a human kappa light chain; and (iv) the heavy chain further comprises a constant heavy chain domain comprising the amino acid sequence of the constant domain of a human IgG_2 heavy chain.

[00111] In another particular embodiment, an antibody described herein, which immunospecifically binds to OX40 (*e.g.*, human OX40), comprises a light chain and a heavy chain, wherein (i) the light chain comprises a VL domain comprising the amino acid sequence of SEQ ID NO: 55 or 56; (ii) the heavy chain comprises a VH domain comprising the amino acid sequence of SEQ ID NO: 54; (iii) the light chain further comprises a constant domain comprising the amino acid sequence of the constant domain of a human kappa light chain; and (iv) the heavy chain further comprises a constant domain comprising the amino acid sequence of the constant domain of a human IgG_2 heavy chain.

[00112] In another specific embodiment, an antibody provided herein, which specifically binds to OX40 (*e.g.*, human OX40), comprises (a) a heavy chain comprising the amino acid sequence of SEQ ID NO: 59 with an amino acid substitution of N to A or Q at amino acid position 297, numbered according to the EU numbering system; and (b) a light chain comprising the amino acid sequence of SEQ ID NO: 67 or 69.

[00113] In another specific embodiment, an antibody provided herein, which specifically binds to OX40 (*e.g.*, human OX40), comprises (a) a heavy chain comprising the amino acid sequence of SEQ ID NO: 59 with an amino acid substitution selected from the group consisting of: S to E at amino acid position 267, L to F at amino acid position 328, and both S to E at amino

acid position 267 and L to F at amino acid position 328, numbered according to the EU numbering system; and (b) a light chain comprising the amino acid sequence of SEQ ID NO: 67 or 69.

[00114] In specific embodiments, an antigen-binding domain described herein, which immunospecifically binds to OX40 (*e.g.*, human OX40), comprises framework regions (*e.g.*, framework regions of the VL domain and/or VH domain) that are human framework regions or derived from human framework regions. Non-limiting examples of human framework regions are described in the art, *e.g.*, *see* Kabat EA *et al.*, (1991) *supra*). In certain embodiment, an antigen-binding domain described herein comprises framework regions (*e.g.*, framework regions of the VL domain and/or VH domain) that are primate (*e.g.*, non-human primate) framework regions or derived from primate (*e.g.*, non-human primate) framework regions.

[00115] For example, CDRs from antigen-specific non-human antibodies, typically of rodent origin (*e.g.*, mouse or rat), are grafted onto homologous human or non-human primate acceptor frameworks. In one embodiment, the non-human primate acceptor frameworks are from Old World apes. In a specific embodiment, the Old World ape acceptor framework is from *Pan troglodytes*, *Pan paniscus* or *Gorilla gorilla*. In a particular embodiment, the non-human primate acceptor frameworks are from the chimpanzee *Pan troglodytes*. In a particular embodiment, the non-human primate acceptor frameworks are Old World monkey acceptor frameworks. In a specific embodiment, the Old World monkey acceptor frameworks are from the genus *Macaca*. In a certain embodiment, the non-human primate acceptor frameworks are derived from the cynomolgus monkey *Macaca cynomolgus*. Non-human primate framework sequences are described in U.S. Patent Application Publication No. US 2005/0208625.

[00116] In another aspect, provided herein are antibodies that contain antigen-binding domains that bind the same or an overlapping epitope of OX40 (*e.g.*, an epitope of human OX40) as an antibody described herein (*e.g.*, pab1949w or pab2049w). In certain embodiments, the epitope of an antibody 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, 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). 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). 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 antigen-binding domain is determined using alanine scanning mutagenesis studies. In addition, antigen-binding domains that recognize and bind to the same or overlapping epitopes of OX40 (*e.g.*, human OX40) 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 OX40. 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 (Cheung RC *et al.*, (1990) Virology 176: 546-52); and direct labeled RIA. (Moldenhauer G *et al.*, (1990) Scand J Immunol 32: 77-82). Typically, such an assay involves the use of purified antigen (*e.g.*, OX40, such as human OX40) 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.

[00117] In one embodiment, a competition assay is performed using surface plasmon resonance (BIAcore®), *e.g.*, by an 'in tandem approach' such as that described by Abdiche YN *et al.*, (2009) Analytical Biochem 386: 172-180, whereby OX40 antigen is immobilized on the chip surface, for example, a CM5 sensor chip and the anti-OX40 antibodies are then run over the chip. To determine if an antibody competes with an anti-OX40 antigen-binding domain described herein, the antibody containing the anti-OX40 antigen binding domain is first run over the chip surface to achieve saturation and then the potential, competing antibody is added. Binding of the competing antibody can then be determined and quantified relative to a non-competing control.

[00118] In certain aspects, competition binding assays can be used to determine whether an antibody is competitively blocked, *e.g.*, in a dose dependent manner, by another antibody for example, an antibody binds essentially the same epitope, or overlapping epitopes, as a reference antibody, when the two antibodies recognize identical or sterically overlapping epitopes in competition binding assays such as competition ELISA assays, which can be configured in all number of different formats, using either labeled antigen or labeled antibody. In a particular embodiment, an antibody can be tested in competition binding assays with an antibody described herein (*e.g.*, antibody pab1949w or pab2049w), or a chimeric or Fab antibody thereof, or an antibody comprising VH CDRs and VL CDRs of an antibody described herein (*e.g.*, pab1949w or pab2049w).

[00119] In another aspect, provided herein are antigen-binding domains that compete (*e.g.*, in a dose dependent manner) for binding to OX40 (*e.g.*, human OX40) with an antigen-binding domain described herein, as determined using assays known to one of skill in the art or described herein (*e.g.*, ELISA competitive assays or surface plasmon resonance). In another aspect, provided herein are antigen-binding domains that competitively inhibit (*e.g.*, in a dose dependent manner) an antigen-binding domain described herein (*e.g.*, pab1949w or pab2049w) from binding to OX40 (*e.g.*, human OX40), as determined using assays known to one of skill in the art or described herein (*e.g.*, ELISA competitive assays, or suspension array or surface plasmon resonance assay). In specific aspects, provided herein is an antigen-binding domain which competes (*e.g.*, in a dose dependent manner) for specific binding to OX40 (*e.g.*, human OX40), with an antigen-binding domain comprising the amino acid sequences described herein (*e.g.*, VL and/or VH amino acid sequences of pab1949w or pab2049w), as determined using assays known to one of skill in the art or described herein (*e.g.*, ELISA competitive assays, or suspension array or surface plasmon resonance assay).

[00120] In certain embodiments, provided herein is an antigen-binding domain that competes with an antigen-binding domain described herein for binding to OX40 (*e.g.*, human OX40) to the same extent that the antigen-binding domain described herein self-competes for binding to OX40 (*e.g.*, human OX40). In some embodiments, provided herein is a first antigen-binding domain that competes with an antigen-binding domain described herein for binding to OX40 (*e.g.*, human OX40), wherein the first antigen-binding domain competes for binding in an assay comprising the following steps: (a) incubating OX40-transfected cells with the first antigen-binding domain in unlabeled form in a container; and (b) adding an antigen-binding domain described herein in labeled form in the container and incubating the cells in the container; and (c) detecting the binding of the antigen-binding domain described herein in labeled form to the cells. In certain embodiments, provided herein is a first antigen-binding domain that competes with an antigen-binding domain described herein for binding to OX40 (*e.g.*, human OX40), wherein the competition is exhibited as reduced binding of the first antigen-binding domain to OX40 by more than 80% (*e.g.*, 85%, 90%, 95%, or 98%, or between 80% to 85%, 80% to 90%, 85% to 90%, or 85% to 95%).

[00121] In specific aspects, provided herein is an antigen-binding domain which competes (*e.g.*, in a dose dependent manner) for specific binding to OX40 (*e.g.*, human OX40), with an

antigen-binding domain comprising a VH domain and a VL domain, respectively comprising the amino acid sequences set forth in SEQ ID NOs: 54 and 55, or SEQ ID NOs: 54 and 56, respectively.

[00122] In specific aspects, provided herein is an antigen-binding domain which competes (*e.g.*, in a dose dependent manner) for specific binding to OX40 (*e.g.*, human OX40), with an antigen-binding domain comprising (i) a VL domain comprising a VL CDR1, VL CDR2, and VL CDR3 having the amino acid sequences of the VL CDRs listed in Table 3; and (ii) a VH domain comprising a VH CDR1, VH CDR2, and VH CDR3 having the amino acid sequences of the CDRs listed in Table 5.

[00123] In a specific embodiment, an antigen-binding domain described herein is one that is competitively blocked (*e.g.*, in a dose dependent manner) by an antigen-binding domain comprising a VH domain and a VL domain, respectively comprising the amino acid sequences set forth in SEQ ID NOs: 54 and 55, or SEQ ID NOs: 54 and 56, respectively, for specific binding to OX40 (*e.g.*, human OX40).

[00124] In another specific embodiment, an antigen-binding domain described herein is one that is competitively blocked (*e.g.*, in a dose dependent manner) by an antigen-binding domain comprising (i) a VL domain comprising a VL CDR1, VL CDR2, and VL CDR3 having the amino acid sequences of the CDRs listed in Table 3; and (ii) a VH domain comprising a VH CDR1, VH CDR2, and VH CDR3 having the amino acid sequences of the CDRs listed in Table 5.

[00125] In specific aspects, provided herein is an antigen-binding domain, which immunospecifically binds to the same epitope as that of pab1949w or pab2049w for specific binding to OX40 (*e.g.*, human OX40). Assays known to one of skill in the art or described herein (*e.g.*, X-ray crystallography, hydrogen/deuterium exchange coupled with mass spectrometry (*e.g.*, liquid chromatography electrospray mass spectrometry), alanine scanning, ELISA assays, etc.) can be used to determine if two antigen-binding domains bind to the same epitope.

[00126] In a specific embodiment, an antigen-binding domain described herein immunospecifically binds to the same epitope as that bound by pab1949w or pab2049w or an epitope that overlaps the epitope.

[00127] In another specific embodiment, an antigen-binding domain described herein,

immunospecifically binds to the same epitope as that of an antigen-binding domain comprising (i) a VL domain comprising a VL CDR1, VL CDR2, and VL CDR3 having the amino acid sequences of the CDRs listed in Table 3 and (ii) a VH domain comprising a VH CDR1, VH CDR2, and VH CDR3 having the amino acid sequences of the CDRs listed in Table 5.

[00128] In a specific aspect, the binding between an antigen-binding domain described herein and a variant OX40 is substantially weakened relative to the binding between the antigen-binding domain and a human OX40 sequence of SEQ ID NO: 72, wherein the variant OX40 comprises the sequence of SEQ ID NO: 72 except for an amino acid mutation (*e.g.*, substitution) selected from the group consisting of: N60A, R62A, R80A, L88A, P93A, P99A, P115A, and a combination thereof, numbered according to SEQ ID NO: 72. In some embodiments, the variant OX40 comprises the sequence of SEQ ID NO: 72 except for any one mutation selected from the group consisting of: N60A, R62A, R80A, L88A, P93A, P99A, and P115A, numbered according to SEQ ID NO: 72. In some embodiments, the variant OX40 comprises the sequence of SEQ ID NO: 72 except for any two, three, four, five, six, or seven mutations selected from the group consisting of: W58A, N60A, R62A, R80A, L88A, P93A, P99A, and P115A, numbered according to SEQ ID NO: 72. In some embodiments, the variant OX40 comprises the sequence of SEQ ID NO: 72 except for the amino acid mutations W58A, N60A, R62A, R80A, L88A, P93A, P99A, and P115A, numbered according to SEQ ID NO: 72. In some embodiments, the variant OX40 comprises the sequence of SEQ ID NO: 72 except for the amino acid mutations N60A, R62A, R80A, L88A, and P93A, numbered according to SEQ ID NO: 72.

[00129] In a specific aspect, an antigen-binding domain described herein specifically binds to an epitope of a human OX40 sequence comprising, consisting essentially of, or consisting of a residue of SEQ ID NO: 72 selected from the group consisting of: 60, 62, 80, 88, 93, 99, 115, and a combination thereof. In some embodiments, the epitope comprises, consists essentially of, or consists of any one residue selected from the group consisting of: 60, 62, 80, 88, 93, 99, and 115 of SEQ ID NO: 72. In some embodiments, the epitope comprises, consists essentially of, or consists of any two, three, four, five, six, or seven residues selected from the group consisting of: 58, 60, 62, 80, 88, 93, 99, and 115 of SEQ ID NO: 72. In some embodiments, the epitope comprises, consists essentially of, or consists of residues 58, 60, 62, 80, 88, 93, 99, and 115 of SEQ ID NO: 72. In some embodiments, the epitope comprises, consists essentially of, or consists of residues 60, 62, 80, 88, and 93 of SEQ ID NO: 72.

[00130] In a specific embodiment, an antigen-binding domain described herein specifically binds to an epitope of SEQ ID NO: 72 comprising, consisting essentially of, or consisting of a residue selected from the group consisting of: 60, 62, 80, 88, 93, 99, 115, and a combination thereof. In some embodiments, the epitope comprises, consists essentially of, or consists of any one residue selected from the group consisting of: 60, 62, 80, 88, 93, 99, and 115 of SEQ ID NO: 72. In some embodiments, the epitope comprises, consists essentially of, or consists of any two, three, four, five, six, or seven residues selected from the group consisting of: 58, 60, 62, 80, 88, 93, 99, and 115 of SEQ ID NO: 72. In some embodiments, the epitope comprises, consists essentially of, or consists of residues 58, 60, 62, 80, 88, 93, 99, and 115 of SEQ ID NO: 72. In some embodiments, the epitope comprises, consists essentially of, or consists of residues 60, 62, 80, 88, and 93 of SEQ ID NO: 72.

[00131] In a specific aspect, an antigen-binding domain described herein specifically binds to at least one residue of SEQ ID NO: 72 selected from the group consisting of: 58, 60, 62, 80, 88, 93, 99, 115, and a combination thereof. In some embodiments, an antigen-binding domain described herein specifically binds to any one residue selected from the group consisting of: 60, 62, 80, 88, 93, 99, and 115 of SEQ ID NO: 72. In some embodiments, an antigen-binding domain described herein specifically binds to any two, three, four, five, six, or seven residues selected from the group consisting of: 58, 60, 62, 80, 88, 93, 99, and 115 of SEQ ID NO: 72. In some embodiments, an antigen-binding domain described herein specifically binds to residues 58, 60, 62, 80, 88, 93, 99, and 115 of SEQ ID NO: 72. In some embodiments, an antigen-binding domain described herein specifically binds to residues 60, 62, 80, 88, and 93 of SEQ ID NO: 72.

[00132] In a specific aspect, an antigen-binding domain described herein exhibits, as compared to binding to a human OX40 sequence of SEQ ID NO: 72, reduced or absent binding to a protein identical to SEQ ID NO: 72 except for the presence of an amino acid mutation (*e.g.*, substitution) selected from the group consisting of: N60A, R62A, R80A, L88A, P93A, P99A, P115A, and a combination thereof, numbered according to SEQ ID NO: 72. In some embodiments, the protein is identical to SEQ ID NO: 72 except for the presence of an amino acid mutation comprising any one mutation selected from the group consisting of: N60A, R62A, R80A, L88A, P93A, P99A, and P115A, numbered according to SEQ ID NO: 72. In some embodiments, the protein is identical to SEQ ID NO: 72 except for the presence of an amino acid

mutation comprising any two, three, four, five, six, or seven mutations selected from the group consisting of: W58A, N60A, R62A, R80A, L88A, P93A, P99A, and P115A, numbered according to SEQ ID NO: 72. In some embodiments, the protein is identical to SEQ ID NO: 72 except for the presence of an amino acid substitution comprising the mutations W58A, N60A, R62A, R80A, L88A, P93A, P99A, and P115A, numbered according to SEQ ID NO: 72. In some embodiments, the protein is identical to SEQ ID NO: 72 except for the presence of an amino acid substitution comprising the mutations N60A, R62A, R80A, L88A, and P93A, numbered according to SEQ ID NO: 72.

7.2.2 Constant Region Mutations and Modifications

[00133] 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, with numbering 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.

[00134] 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. 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.

[00135] 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, with numbering 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, which are incorporated herein by reference.

[00136] In a specific embodiment, one, two, or more amino acid mutations (*i.e.*, 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 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 (*i.e.*, 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 (*i.e.*, 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), with numbering 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 the EU numbering system. *See* U.S. Patent No. 7,658,921, which is incorporated herein by reference. 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). 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.

[00137] 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. 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. *See*, *e.g.*, U.S. Patent Nos. 5,585,097 and 8,591,886 for a description of mutations that delete or inactivate the constant domain. 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). 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.

[00138] 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.

[00139] 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*). In some embodiments, one or more amino acid residues within amino acid positions 231 to 238, numbered according to the EU numbering system, 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. This approach is described further in International Publication No. WO 94/29351. 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.

[00140] In certain embodiments, an antibody described herein comprises the constant domain of an IgG_1 with a mutation (*e.g.*, substitution) at position 267, 328, or a combination thereof, numbered according to the EU numbering system. In certain embodiments, an antibody described herein comprises the constant domain of an IgG_1 with a mutation (*e.g.*, substitution) selected from the group consisting of S267E, L328F, and a combination thereof, numbered according to the EU numbering system. In certain embodiments, an antibody described herein comprises the constant domain of an IgG_1 with a S267E/L328F mutation (*e.g.*, substitution), numbered according to the EU numbering system. In certain embodiments, an antibody described herein comprising the constant domain of an IgG_1 with a S267E/L328F mutation (*e.g.*, substitution) has an increased binding affinity for FcγRIIA, FcγRIIB, or FcγRIIA and FcγRIIB, numbered according to the EU numbering system.

[00141] 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.

[00142] In certain embodiments, an antibody described herein comprises the constant region of an IgG_2 antibody and the cysteine at amino acid residue 127 of the heavy chain, numbered according to Kabat, is substituted for serine.

[00143] Antibodies with reduced fucose content have been reported to have an increased affinity for Fc receptors, such as, *e.g.*, FcγRIIIa. Accordingly, in certain embodiments, the antibodies described herein have reduced fucose content or no fucose content. Such antibodies can be produced using techniques known to one skilled in the art. For example, the antibodies can be expressed in cells deficient or lacking the ability of fucosylation. In a specific example, cell lines with a knockout of both alleles of α1,6-fucosyltransferase can be used to produce

antibodies with reduced fucose content. The Potelligent® system (Lonza) is an example of such a system that can be used to produce antibodies with reduced fucose content. Alternatively, antibodies with reduced fucose content or no fucose content can be produced by, *e.g.*: (i) culturing cells under conditions which prevent or reduce fucosylation; (ii) posttranslational removal of fucose (*e.g.*, with a fucosidase enzyme); (iii) post-translational addition of the desired carbohydrate, *e.g.*, after recombinant expression of a non-glycosylated glycoprotein; or (iv) purification of the glycoprotein so as to select for antibodies thereof which are not fucsoylated. *See*, *e.g.*, Longmore GD & Schachter H (1982) Carbohydr Res 100: 365-92 and Imai-Nishiya H *et al.*, (2007) BMC Biotechnol. 7: 84 for methods for producing antibodies thereof with no fucose content or reduced fucose content.

[00144] Engineered glycoforms may be useful for a variety of purposes, including but not limited to enhancing or reducing effector function. Methods for generating engineered glycoforms in an antibody described herein include but are not limited to those disclosed, *e.g.*, in Umaña P *et al.*, (1999) Nat Biotechnol 17: 176-180; Davies J *et al.*, (2001) Biotechnol Bioeng 74: 288-294; Shields RL *et al.*, (2002) J Biol Chem 277: 26733-26740; Shinkawa T *et al.*, (2003) J Biol Chem 278: 3466-3473; Niwa R *et al.*, (2004) Clin Cancer Res 1: 6248-6255; Presta LG *et al.*, (2002) Biochem Soc Trans 30: 487-490; Kanda Y *et al.*, (2007) Glycobiology 17: 104-118; U.S. Patent Nos. 6,602,684; 6,946,292; and 7,214,775; U.S. Patent Publication Nos. US 2007/0248600; 2007/0178551; 2008/0060092; and 2006/0253928; International Publication Nos. WO 00/61739; WO 01/292246; WO 02/311140; and WO 02/30954; Potillegent™ technology (Biowa, Inc. Princeton, N.J.); and GlycoMAb® glycosylation engineering technology (Glycart biotechnology AG, Zurich, Switzerland). *See also*, *e.g.*, Ferrara C *et al.*, (2006) Biotechnol Bioeng 93: 851-861; International Publication Nos. WO 07/039818; WO 12/130831; WO 99/054342; WO 03/011878; and WO 04/065540.

[00145] In certain embodiments, the technology used to engineer the Fc domain of an antibody described herein is the Xmab® Technology of Xencor (Monrovia, CA). *See*, *e.g.*, U.S. Patent Nos. 8,367,805; 8,039,592; 8,124,731; 8,188,231; U.S. Patent Publication No. 2006/0235208; International Publication Nos. WO 05/077981; WO 11/097527; and Richards JO *et al.*, (2008) Mol Cancer Ther 7: 2517-2527.

[00146] 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

IgG1 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. In a particular embodiment, the amino acids corresponding to positions L234, L235, and D265 in a human IgG1 heavy chain are F, E, and A; or A, A, and A, respectively, numbered according to the EU numbering system.

[00147] 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.

7.2.3 DuoBody Anti-OX40 Antagonist Antibodies

[00148] The DuoBody (Genmab A/S) technology can be used to produce a heterodimeric protein by combining one half of a first homodimeric protein (*e.g.*, one heavy and light chain pair of a first antibody) with one half of a second homodimeric protein (*e.g.*, one heavy and light chain pair of a second antibody, or one polypeptide of a homodimeric fragment of the second antibody containing a constant region with CH3 domain residues). *See*, *e.g.*, International Publication Nos. WO 2011/131746, WO 2011/147986, WO 2008/119353, WO 2013/060867, and Labrijn AF *et al.*, (2013) PNAS 110(13): 5145-5150.

[00149] In a specific aspect, an antibody as described herein (*e.g.*, an anti-OX40-monovalent antibody) which immunospecifically binds to OX40 (*e.g.*, human OX40), comprises: (a) a first antigen-binding domain that specifically binds to OX40 (*e.g.*, human OX40), as described herein, comprising a first heavy chain constant region; and (b) a second antigen-binding domain that does not bind to an antigen expressed by a human immune cell (*i.e.,* the second antigen-binding domain does not bind to OX40 or any other antigen expressed by a human immune cell), as described herein, comprising a second heavy chain constant region; wherein each heavy chain constant region comprises a mutation at a residue selected from the group consisting of residues 366, 368, 370, 399, 405, 407, and 409, numbered according to the EU numbering system, and wherein the first heavy chain constant region and the second heavy chain constant region contain different mutations. In certain embodiments, the antigen to which the second antigen-binding domain specifically binds is not naturally expressed by a human immune cell. In certain embodiments, the immune cell is selected from the group consisting of a T cell (*e.g.*, a CD4+ T cell or a CD8+ T cell), a B cell, a natural killer cell, a dendritic cell, a macrophage, and an

eosinophil. In certain embodiments, the antigen-binding domain that specifically binds to OX40 (*e.g.*, human OX40) comprises a first VH and a first VL, and the second antigen-binding domain comprises a second VH and a second VL. In certain embodiments, the antigen-binding domain that specifically binds to OX40 (*e.g.*, human OX40) comprises a first heavy chain and a first light chain, and the second antigen-binding domain comprises a second heavy chain and a second light chain. In certain embodiments, the antibody is for administration to a sample or subject in which the second antigen-binding domain is non-reactive (*i.e.*, the antigen to which the second antigen-binding domain binds is not present in the sample or subject). In certain embodiments, the second antigen-binding domain does not specifically bind to an antigen on a cell expressing OX40 (*e.g.*, human OX40). In certain embodiments, the second antigen-binding domain does not specifically bind to an antigen that is naturally expressed by a cell that expresses OX40 (*e.g.*, human OX40). In certain embodiments, the antibody functions as a monovalent antibody in a sample or subject, wherein the first antigen-binding domain of the antibody specifically binds to OX40 (*e.g.*, human OX40), while the second antigen-binding domain is non-reactive in the sample or subject (*e.g.*, due to the absence of antigen to which the second antigen-binding domain specifically binds in the sample or subject). In certain embodiments, the second antigen-binding domain specifically binds to a non-human antigen (*i.e.*, an antigen expressed in other organisms and not humans). In certain embodiments, the second antigen-binding domain specifically binds to a viral antigen. In certain embodiments, the viral antigen is from a virus that does not infect humans (*i.e.*, a non-human virus). In certain embodiments, the viral antigen is absent in a human immune cell (*e.g.*, the human immune cell is uninfected with the virus associated with the viral antigen). In certain embodiments, the viral antigen is a HIV antigen. In certain embodiments, the second antigen-binding domain specifically binds to chicken albumin or hen egg lysozyme. In certain embodiments, the second antigen-binding domain specifically binds to an antigen that is not expressed by (*i.e.*, is absent from) wild-type cells (*e.g.*, wild-type human cells). In certain embodiments, the second antigen-binding domain specifically binds to a tumor-associated antigen that is not expressed by (*i.e.*, is absent from) normal cells (*e.g.*, wild-type cells, *e.g.*, wild-type human cells). In certain embodiments, the tumor-associated antigen is not expressed by (*i.e.*, is absent from) human cells. In certain embodiments, the second antigen-binding domain comprises a heavy chain comprising a mutation selected from the group consisting of: N297A, D265A, L234F, L235E, N297Q,

P331S, and a combination thereof, numbered according to the EU numbering system. In certain embodiments, the mutation is N297A or D265A, numbered according to the EU numbering system. In certain embodiments the mutation is L234F and L235E, numbered according to the EU numbering system. In certain embodiments, the mutation is L234F, L234E, and D265A, numbered according to the EU numbering system. In certain embodiments, the mutation is L234F, L234E, and N297Q, numbered according to the EU numbering system. In certain embodiments, the mutation is L234F, L235E, and P331S, numbered according to the EU numbering system. In certain embodiments, the mutation is D265A and N297Q, numbered according to the EU numbering system. In certain embodiments, the mutation is L234F, L235E, D265A, N297Q, and P331S, numbered according to the EU numbering system. In certain embodiments, the second antigen-binding domain comprises a heavy chain comprising a mutation selected from the group consisting of: D265A, P329A, and a combination thereof, numbered according to the EU numbering system. In certain embodiments, the heavy chain constant regions of the first and second antigen-binding domains are selected from the group consisting of immunoglobulins IgG_1, IgG_2, IgG_3, IgG_4, IgA_1, and IgA_2. In certain embodiments, the immunoblobulins are human immunoglobulins. Human immunoglobulins containing mutations (*e.g.*, substitutions) are also referred to as human immunoglobulins herein. In certain embodiments, the heavy chain constant regions of the first and second antigen-binding domains are the same isotype. When the first and second antigen-binding domains are the same isotype, the sequences associated with the second antigen-binding domain are also described herein as "isotype" sequences (*e.g.*, isotype VH or isotype HC). In certain embodiments, the heavy chain constant regions of the first and second antigen-binding domains are IgG_1. In certain embodiments, the heavy chain constant regions of the first and second antigen-binding domains are human IgG_1. In certain embodiments, the first antigen-binding domain comprises a first IgG_1 heavy chain constant region and the second antigen-binding domain comprises a second IgG_1 heavy chain constant region, wherein the first and second heavy chains comprise an identical mutation selected from the group consisting of N297A, N297Q, D265A, L234F/L235E/D265A, and a combination thereof, numbered according to the EU numbering system. In certain embodiments, the first antigen-binding domain comprises a first IgG_1 heavy chain constant region and the second antigen-binding domain comprises a second IgG_1 heavy chain constant region, wherein the first and second heavy chains comprise an identical mutation selected from

the group consisting of D265A, P329A, and a combination thereof, numbered according to the EU numbering system. In certain embodiments, the first antigen-binding domain comprises a first IgG_2 heavy chain constant region and the second antigen-binding domain comprises a second IgG_2 heavy chain constant region, wherein the first and second heavy chain constant regions comprise a C127S mutation, numbered according to Kabat. In certain embodiments, the first antigen-binding domain comprises a first IgG_4 heavy chain constant region and the second antigen-binding domain comprises a second IgG_4 heavy chain constant region, wherein the first and second heavy chain constant regions comprise a S228P mutation, numbered according to the EU numbering system.

[00150] In another specific aspect, an antibody as described herein (*e.g.*, an anti-OX40-monovalent antibody or a bispecific antibody) which immunospecifically binds to OX40 (*e.g.*, human OX40), comprises: (a) an antigen-binding domain that specifically binds to OX40 (*e.g.*, human OX40), as described herein, wherein the antigen binding domain comprises a first heavy chain constant region; and (b) a heavy chain or fragment thereof, as described herein, wherein the heavy chain or fragment thereof comprises a second heavy chain constant region; wherein each of the first and second heavy chain constant regions comprises a mutation at a residue selected from the group consisting of residues 366, 368, 370, 399, 405, 407, and 409, numbered according to the EU numbering system, and wherein the first heavy chain constant region and the second heavy chain constant region contain different mutations. Such an antibody can optionally comprise a first light chain or fragment thereof and a second light chain or fragment thereof. The first light chain can comprise a first light chain constant domain and a first light chain variable domain. The second light chain can comprise a second light chain constant domain and a second light chain variable domain. In some embodiments, the fragment of the second heavy chain is an Fc fragment. In some embodiments, the heavy chain or second heavy chain comprises a constant domain and a variable domain. In certain embodiments, the second heavy chain or fragment thereof is from an antigen-binding domain that specifically binds to a non-human antigen (*i.e.*, an antigen expressed in other organisms and not humans). In certain embodiments, the second heavy chain or fragment thereof is from an antigen-binding domain that specifically binds to a viral antigen. In certain embodiments, the viral antigen is from a virus that does not infect humans (*i.e.*, a non-human virus). In certain embodiments, the viral antigen is absent in a human immune cell (*e.g.*, the human immune cell is uninfected with the virus associated with the viral

antigen). In certain embodiments, the viral antigen is a HIV antigen. In certain embodiments, the second heavy chain or fragment thereof is from an antigen-binding domain that specifically binds to chicken albumin or hen egg lysozyme. In certain embodiments, the second heavy chain or fragment thereof is from an antigen-binding domain that specifically binds to an antigen that is not expressed by (*i.e.*, is absent from) wild-type cells (*e.g.*, wild-type human cells). In certain embodiments, the second heavy chain or fragment thereof is from an antigen-binding domain that specifically binds to a tumor-associated antigen that is not expressed by (*i.e.*, is absent from) normal cells (*e.g.*, wild-type cells, *e.g.*, wild-type human cells). In certain embodiments, the tumor-associated antigen is not expressed by (*i.e.*, is absent from) human cells. In certain embodiments, the second heavy chain comprises a mutation selected from the group consisting of: N297A, D265A, L234F, L235E, N297Q, P331S, and a combination thereof, numbered according to the EU numbering system. In certain embodiments, the mutation is N297A or D265A, numbered according to the EU numbering system. In certain embodiments the mutation is L234F and L235E, numbered according to the EU numbering system. In certain embodiments, the mutation is L234F, L234E, and D265A, numbered according to the EU numbering system. In certain embodiments, the mutation is L234F, L234E, and N297Q, numbered according to the EU numbering system. In certain embodiments, the mutation is L234F, L235E, and P331S, numbered according to the EU numbering system. In certain embodiments, the mutation is D265A and N297Q, numbered according to the EU numbering system. In certain embodiments, the mutation is L234F, L235E, D265A, N297Q, and P331S, numbered according to the EU numbering system. In certain embodiments, the second heavy chain comprises a mutation selected from the group consisting of: D265A, P329A, and a combination thereof, numbered according to the EU numbering system. In certain embodiments, the first and second heavy chain constant regions are selected from the group consisting of immunoglobulins IgG_1, IgG_2, IgG_3, IgG_4, IgA_1, and IgA_2. In certain embodiments, the immunoblobulins are human immunoglobulins. In certain embodiments, the first and second heavy chain constant regions are the same isotype. When the first and second heavy chain constant regions are the same isotype, the sequences associated with the second heavy chain are also described herein as "isotype" sequences (*e.g.*, isotype VH or isotype HC). In certain embodiments, the first and second heavy chain constant regions are IgG_1. In certain embodiments, the first and second heavy chain constant regions are human IgG_1. In certain

embodiments, the first antigen-binding domain comprises a first IgG_1 heavy chain constant region and the second antigen-binding domain comprises a second IgG_1 heavy chain constant region, wherein the first and second heavy chains comprise an identical mutation selected from the group consisting of N297A, N297Q, D265A, L234F/L235E/D265A, and a combination thereof, numbered according to the EU numbering system. In certain embodiments, the first antigen-binding domain comprises a first IgG_1 heavy chain constant region and the second antigen-binding domain comprises a second IgG_1 heavy chain constant region, wherein the first and second heavy chains comprise an identical mutation selected from the group consisting of D265A, P329A, and a combination thereof, numbered according to the EU numbering system. In certain embodiments, the first antigen-binding domain comprises a first IgG_2 heavy chain constant region and the second antigen-binding domain comprises a second IgG_2 heavy chain constant region, wherein the first and second heavy chain constant regions comprise a C127S mutation, numbered according to Kabat. In certain embodiments, the first antigen-binding domain comprises a first IgG_4 heavy chain constant region and the second antigen-binding domain comprises a second IgG_4 heavy chain constant region, wherein the first and second heavy chain constant regions comprise a S228P mutation, numbered according to the EU numbering system.

[00151] In the above aspects directed to an antibody comprising an antigen-binding domain that specifically binds to OX40 (*e.g.*, human OX40) and either a second antigen-binding domain or a second heavy chain or fragment thereof, the antigen-binding domain that specifically binds to OX40 (*e.g.*, human OX40) can comprise any of the anti-OX40 sequences described herein. In certain embodiments, the antigen-binding domain that specifically binds to OX40 (*e.g.*, 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:47); a VH-CDR2 comprising the amino acid sequence of RIRSKANSYATAYAASVKG (SEQ ID NO:48); and a VH-CDR3 comprising the amino acid sequence of GIYDSSGYDY (SEQ ID NO:49); and (b) a first light chain variable domain (VL) comprising a VL-CDR1 comprising the amino acid sequence of RSSQSLLHSNGYNYLD (SEQ ID NO:50); a VL-CDR2 comprising the amino acid sequence of LGSNRAS (SEQ ID NO:51); and a VL-CDR3 comprising the amino acid sequence of MQGSKWPLT (SEQ ID NO:52) or MQALQTPLT (SEQ ID NO:53). In certain embodiments, the antigen-binding domain that

specifically binds to OX40 (*e.g.*, human OX40) specifically binds to the same epitope of OX40 as an antibody comprising a VH comprising the amino acid sequence of SEQ ID NO:54 and a VL comprising the amino acid sequence of SEQ ID NO:55 or SEQ ID NO:56. In certain embodiments, the antigen-binding domain that specifically binds to OX40 (*e.g.*, human OX40) exhibits, as compared to binding to a human OX40 sequence of SEQ ID NO:72, reduced or absent binding to a protein identical to SEQ ID NO:72 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, numbered according to SEQ ID NO: 72. In certain embodiments, the antigen-binding domain that specifically binds to OX40 (*e.g.*, human OX40) comprises a VH and a VL, wherein the VH comprises the amino acid sequence of SEQ ID NO:54. In certain embodiments, the antigen-binding domain that binds to OX40 comprises a VH and a VL, wherein the VL comprises the amino acid sequence SEQ ID NO:55 or SEQ ID NO:56. In certain embodiments, the antigen-binding domain that specifically binds to OX40 (*e.g.*, 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:54. In certain embodiments, the antigen-binding domain that specifically binds to OX40 (*e.g.*, human OX40) comprises a VH comprising the amino acid sequence of SEQ ID NO:54. In certain embodiments, the antigen-binding domain that specifically binds to OX40 (*e.g.*, human OX40) comprises a VH comprising an amino acid sequence derived from a human IGHV3-73 germline sequence (*e.g.*, IGHV3-73*01, *e.g.*, having the amino acid sequence of SEQ ID NO:57). In certain embodiments, the antigen-binding domain that specifically binds to OX40 (*e.g.*, 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:55 or SEQ ID NO:56. In certain embodiments, the antigen-binding domain that specifically binds to OX40 (*e.g.*, human OX40) comprises a VL-CDR3 comprising the amino acid sequence SEQ ID NO:52. In certain embodiments, the antigen-binding domain that specifically binds to OX40 (*e.g.*, human OX40) comprises a VL comprising the amino acid sequence of SEQ ID NO:55. In certain embodiments, the antigen-binding domain that specifically binds to OX40 (*e.g.*, human OX40) comprises a light chain comprising the amino acid sequence of SEQ ID NO:67. In certain embodiments, the antigen-binding domain that specifically binds to OX40 (*e.g.*, human OX40) comprises a light chain comprising the amino acid sequence of SEQ ID NO:68. In certain

embodiments, the antigen-binding domain that specifically binds to OX40 (*e.g.*, human OX40) comprises a VL-CDR3 comprising the amino acid sequence SEQ ID NO:53. In certain embodiments, the antigen-binding domain that specifically binds to OX40 (*e.g.*, human OX40) comprises a VL comprising the amino acid sequence of SEQ ID NO:56. In certain embodiments, the antigen-binding domain that specifically binds to OX40 (*e.g.*, human OX40) comprises a light chain comprising the amino acid sequence of SEQ ID NO:69. In certain embodiments, the antigen-binding domain that specifically binds to OX40 (*e.g.*, human OX40) comprises a light chain comprising the amino acid sequence of SEQ ID NO:70. In certain embodiments, the antigen-binding domain that specifically binds to OX40 (*e.g.*, human OX40) comprises a VL comprising an amino acid sequence derived from a human IGKV2-28 germline sequence (*e.g.*, IGKV2-28*01, *e.g.*, having the amino acid sequence of SEQ ID NO:58). In certain embodiments, the antigen-binding domain that specifically binds to OX40 (*e.g.*, human OX40) comprises the VH and VL sequences set forth in SEQ ID NOs: 54 and 55, or SEQ ID NOs: 54 and 56, respectively. In certain embodiments, the antigen-binding domain that specifically binds to OX40 (*e.g.*, human OX40) comprises a mutation selected from the group consisting of: N297A, D265A, L234F, L235E, N297Q, P331S, and a combination thereof, numbered according to the EU numbering system. In certain embodiments, the mutation is N297A or D265A, numbered according to the EU numbering system. In certain embodiments the mutation is L234F and L235E, numbered according to the EU numbering system. In certain embodiments, the mutation is L234F, L234E, and D265A, numbered according to the EU numbering system. In certain embodiments, the mutation is L234F, L234E, and N297Q, numbered according to the EU numbering system. In certain embodiments, the mutation is L234F, L235E, and P331S, numbered according to the EU numbering system. In certain embodiments, the mutation is D265A and N297Q, numbered according to the EU numbering system. In certain embodiments, the mutation is L234F, L235E, D265A, N297Q, and P331S, numbered according to the EU numbering system. In certain embodiments, the antigen-binding domain that specifically binds to OX40 (*e.g.*, human OX40) comprises a mutation selected from the group consisting of: D265A, P329A, and a combination thereof, numbered according to the EU numbering system.

[00152] In certain embodiments of the above aspects directed to an antibody comprising an antigen-binding domain that specifically binds to OX40 (*e.g.*, human OX40) and either a second

antigen-binding domain or a second heavy chain or fragment thereof, the first heavy chain constant region comprises a F405L or a K409R mutation, the second heavy chain constant region comprises a F405L or a K409R mutation, numbered according to the EU numbering system, and the first heavy chain constant region and the second heavy chain constant region contain different mutations. In certain embodiments, the first heavy chain constant region comprises a F405L mutation, and the second heavy chain constant region comprises a K409R mutation, numbered according to the EU numbering system. In certain embodiments, the first heavy chain constant region comprises the amino acid sequence of SEQ ID NO:108, and the second heavy chain constant region comprises the amino acid sequence of SEQ ID NO:109. In certain embodiments, the antigen-binding domain that specifically binds to OX40 (*e.g.*, human OX40) comprises a heavy chain comprising the amino acid sequence of SEQ ID NO:64.

[00153] In certain embodiments of the above aspects directed to an antibody comprising an antigen-binding domain that specifically binds to OX40 (*e.g.*, human OX40) and either a second antigen-binding domain or a second heavy chain or fragment thereof, the first heavy chain constant region comprises a K409R mutation, and the second heavy chain constant region comprises a F405L mutation, numbered according to the EU numbering system. In certain embodiments, the first heavy chain constant region comprises the amino acid sequence of SEQ ID NO:109, and the second heavy chain constant region comprises the amino acid sequence of SEQ ID NO:108. In certain embodiments, the antigen-binding domain that specifically binds to OX40 (*e.g.*, human OX40) comprises a heavy chain comprising the amino acid sequence of SEQ ID NO:61.

[00154] In certain embodiments, an antibody provided herein that immunospecifically binds to OX40 contains a combination of a heavy chain and a light chain for the anti-OX40 antigen-binding domain as shown in a single row of Table 7 below.

Table 7. Heavy chain (HC) and light chain (LC) sequences of anti-OX40 antigen-binding domains of exemplary anti-OX40 DuoBody antibodies

Antibody	OX40 HC (SEQ ID NO:)	OX40 LC (SEQ ID NO:)
pab2049w (F405L)	64	67
pab2049w (F405L/N297A)	65	67
pab2049w (F405L/L234F/L235E/D265A)	71	67
pab2049w (K409R)	61	67
pab2049w (K409R/N297A)	62	67

Antibody	OX40 HC (SEQ ID NO:)	OX40 LC (SEQ ID NO:)
pab2049w (K409R/L234F/L235E/D265A)	63	67
pab2049w (F405L) without heavy chain terminal lysine	123	67
pab2049w (F405L/N297A) without heavy chain terminal lysine	124	67
pab2049w (F405L/L234F/L235E/D265A) without heavy chain terminal lysine	83	67
pab2049w (K409R) without heavy chain terminal lysine	120	67
pab2049w (K409R/N297A) without heavy chain terminal lysine	121	67
pab2049w (K409R/L234F/L235E/D265A) without heavy chain terminal lysine	122	67

[00155] In certain embodiments, an antibody described herein (*e.g.*, an anti-OX40-monovalent antibody) is antagonistic to OX40 (*e.g.*, human OX40). In certain embodiments, the antibody deactivates, reduces, or inhibits an activity of OX40 (*e.g.*, human OX40). In certain embodiments, the antibody inhibits or reduces binding of human OX40 to OX40 ligand (*e.g.*, human OX40 ligand). In certain embodiments, the antibody inhibits or reduces OX40 (*e.g.*, human OX40) signaling. In certain embodiments, the antibody inhibits or reduces OX40 (*e.g.*, human OX40) activity (*e.g.*, OX40 signaling) induced by OX40 ligand (*e.g.*, human OX40 ligand). In certain embodiments, an antagonistic antibody described herein inhibits or reduces T cell proliferation. In certain embodiments, an antagonistic antibody described herein inhibits or reduces T cell proliferation. In certain embodiments, an antagonistic antibody described herein inhibits or reduces production of cytokines (*e.g.*, inhibits or reduces production of IL-2, TNFα, IFNγ, IL-4, IL-10, IL-13, or a combination thereof) by stimulated T cells. In certain embodiments, an antagonistic antibody described herein inhibits or reduces production of IL-2 by SEA-stimulated T cells. In certain embodiments, an antagonistic antibody described herein blocks the interaction of OX40 and OX40L (*e.g.*, blocks the binding of OX40L and OX40 to one another, *e.g.*, blocks the binding of human OX40 ligand and human OX40)).

[00156] In certain embodiments, an antagonistic antibody described herein, which immunospecifically binds to OX40 (*e.g.*, human OX40), decreases OX40 (*e.g.*, human OX40) 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 OX40 (*e.g.*, human OX40) activity without any antibody or with an unrelated antibody (*e.g.*, an antibody that does not immunospecifically bind to OX40). In certain embodiments, an antagonistic antibody described herein, which immunospecifically binds to OX40 (*e.g.*, human OX40), decreases OX40 (*e.g.*, human OX40) 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 OX40 (*e.g.*, human OX40) activity without any antibody or with an unrelated antibody (*e.g.*, an antibody that does not immunospecifically bind to OX40). Non-limiting examples of OX40 (*e.g.*, human OX40) activity can include OX40 (*e.g.*, human OX40) signaling, cell proliferation, cell survival, and cytokine production (*e.g.*, IL-2, TNF-α, IFN-γ, IL-4, IL-10, and/or IL-13). In certain embodiments, an antagonistic antibody described herein, which immunospecifically binds to OX40 (*e.g.*, human OX40), inhibits, reduces, or inactivates an OX40 (*e.g.*, human OX40) activity. In specific embodiments, OX40 activity is assessed as described in the Examples, *infra*.

[00157] In certain aspects, an antagonistic antibody described herein, which immunospecifically binds to OX40 (*e.g.*, human OX40), inhibits, reduces, or deactivates the cellular proliferation of cells that express OX40 and that respond to OX40 signaling (*e.g.*, cells that proliferate in response to OX40 stimulation and OX40 signaling, such as T cells). Cell proliferation assays are described in the art, such as a ^{3}H-thymidine incorporation assay, BrdU incorporation assay, or CFSE assay, and can be readily carried out by one of skill in the art. In specific embodiments, T cells (*e.g.*, $CD4^{+}$ or $CD8^{+}$ effector T cells) stimulated with a T cell mitogen or T cell receptor complex stimulating agent (*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), in the presence of an antagonistic antibody described herein, which immunospecifically binds to OX40 (*e.g.*, human OX40), have decreased cellular proliferation relative to T cells only stimulated with the T cell mitogen or T cell receptor complex stimulating agent, such as phytohaemagglutinin (PHA) and/or phorbol myristate acetate (PMA), or a TCR complex stimulating antibody, such as an anti-CD3 antibody and anti-CD28 antibody.

[00158] In certain aspects, an antagonistic antibody described herein, which

immunospecifically binds to OX40 (*e.g.*, human OX40), decreases the survival of cells (*e.g.*, T cells, such as CD4 and CD8 effector T cells). In a specific embodiment, T cells (*e.g.*, $CD4^+$ or $CD8^+$ effector T cells) stimulated with a T cell mitogen or T cell receptor complex stimulating agent (*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) in the presence of an antagonistic antibody described herein, which immunospecifically binds to OX40 (*e.g.*, human OX40), have decreased survival relative to T cells only stimulated with the T cell mitogen. Cell survival assays are described in the art (*e.g.*, a trypan blue exclusion assay) and can be readily carried out by one of skill in the art.

[00159] In specific embodiments, an antagonistic antibody described herein, which immunospecifically binds to OX40 (*e.g.*, human OX40), decreases cell survival (*e.g.*, T cells, such as CD4 and CD8 effector T cells) 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 or known to one of skill in the art (*e.g.*, a trypan blue exclusion assay), without any antibody or with an unrelated antibody (*e.g.*, an antibody that does not immunospecifically bind to OX40). In specific embodiments, an antagonistic antibody described herein, which immunospecifically binds to OX40 (*e.g.*, human OX40), decreases cell survival (*e.g.*, T cells, such as CD4 and CD8 effector T cells) 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 or known to one of skill in the art (*e.g.*, a trypan blue exclusion assay), relative to OX40 (*e.g.*, human OX40) activity without any antibody or with an unrelated antibody (*e.g.*, an antibody that does not immunospecifically bind to OX40).

[00160] In some embodiments, T cells (*e.g.*, $CD4^+$ or $CD8^+$ effector T cells) stimulated with a T cell mitogen (*e.g.*, an anti-CD3 antibody or phorbol ester) in the presence of an antagonistic antibody described herein, which immunospecifically binds to OX40 (*e.g.*, human OX40), have decreased cell survival 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 T cells only stimulated with the T cell mitogen or T cell receptor complex stimulating agent (*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), as assessed by methods described herein or known to one of skill in the art (*e.g.*, a trypan blue exclusion assay). In some embodiments, T cells (*e.g.*, $CD4^+$ or $CD8^+$ effector T cells) stimulated with a T cell mitogen or T cell receptor complex stimulating agent (*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) in the presence of an antagonistic antibody described herein, which immunospecifically binds to OX40 (*e.g.*, human OX40), have decreased cell survival 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% relative to T cells only stimulated with the T cell mitogen, as assessed by methods described herein or known to one of skill in the art (*e.g.*, a trypan blue exclusion assay).

[00161] In certain embodiments, an antagonistic antibody described herein, which immunospecifically binds to OX40 (*e.g.*, human OX40), does not protect effector T cells (*e.g.*, $CD4^+$ and $CD8^+$ effector T cells) from activation-induced cell death.

[00162] In specific embodiments, an antagonistic antibody described herein, which immunospecifically binds to OX40 (*e.g.*, human OX40), inhibits, reduces, or deactivates cytokine production (*e.g.*, IL-2, TNF-α, IFN-γ, IL-4, IL-10, and/or IL-13) 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 or known to one of skill in the art, relative to cytokine production in the presence or absence of OX40L (*e.g.*, human OX40L) stimulation without any antibody or with an unrelated antibody (*e.g.*, an antibody that does not immunospecifically bind to OX40). In specific embodiments, an antagonistic antibody described herein, which immunospecifically binds to OX40 (*e.g.*, human OX40), inhibits or reduces cytokine production (*e.g.*, IL-2, TNF-α, IFN-γ, IL-4, IL-10, and/or IL-13) 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 or known to one of skill in the art, relative to cytokine production in the presence or absence of OX40L (*e.g.*, human OX40L) stimulation without any antibody or with an unrelated antibody (*e.g.*, an antibody that does not immunospecifically bind to OX40).

[00163] In certain embodiments, T cells (*e.g.*, $CD4^+$ or $CD8^+$ effector T cells) stimulated with

a T cell mitogen or T cell receptor complex stimulating agent (*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) in the presence of an antagonistic antibody described herein, which immunospecifically binds to OX40 (*e.g.*, human OX40), have decreased cytokine production (*e.g.*, IL-2, TNF-α, IFN-γ, IL-4, IL-10, and/or IL-13) 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% relative to T cells only stimulated with the T cell mitogen or T cell receptor complex stimulating agent (*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), as assessed by methods described herein or known to one of skill in the art (*e.g.*, an ELISA assay). In some embodiments, T cells (*e.g.*, $CD4^+$ or $CD8^+$ effector T cells) stimulated with a T cell mitogen or T cell receptor complex stimulating agent (*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) in the presence of an antagonistic antibody described herein, which immunospecifically binds to OX40 (*e.g.*, human OX40), have decreased cytokine production (*e.g.*, IL-2, TNF-α, IFN-γ, IL-4, IL-10, and/or IL-13) 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 T cells only stimulated with the T cell mitogen or T cell receptor complex stimulating agent (*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), as assessed by methods described herein or known to one of skill in the art (*e.g.*, an ELISA assay).

[00164] In specific embodiments, an antagonistic antibody described herein, which immunospecifically binds to OX40 (*e.g.*, human OX40), decreases IL-2 production in response to Staphylococcus Enterotoxin A (SEA) stimulation 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 or known to one of skill in the art, relative to IL-2 production without any antibody or with an unrelated antibody (*e.g.*, an antibody that does not immunospecifically bind to OX40).

[00165] In certain embodiments, T cells (*e.g.*, $CD4^+$ or $CD8^+$ T cells) stimulated with Staphylococcus Enterotoxin A (SEA) stimulation in the presence of an antagonistic antibody described herein, which immunospecifically binds to OX40 (*e.g.*, human OX40), have decreased 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 T cells only stimulated with SEA, as assessed by methods described herein or known to one of skill in the art (*e.g.*, an ELISA assay).

[00166] An antibody provided herein that binds to OX40 can be fused or conjugated (*e.g.*, covalently or noncovalently linked) to a detectable label or substance. Examples of detectable labels or substances 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 labeled antibodies can be used to detect OX40 (*e.g.*, human OX40) protein. *See*, *e.g.*, Section 7.5.2, *infra*.

7.3 Antibody Production

[00167] An antibody as described herein is generated according to the DuoBody technology platform (Genmab A/S) as described, *e.g.*, in International Publication Nos. WO 2011/131746, WO 2011/147986, WO 2008/119353, and WO 2013/060867, and in Labrijn AF *et al.*, (2013) PNAS 110(13): 5145-5150.

[00168] The DuoBody technology is used to create a heterodimeric protein from two homodimeric proteins, and requires that each of the homodimeric proteins includes a heavy chain constant region with a single point mutation in the CH3 domain. The point mutations allow for a stronger interaction between the CH3 domains in the resultant heterodimeric protein than between the CH3 domains in either of the homodimeric proteins. The single point mutation in each homodimeric protein is at residue 366, 368, 370, 399, 405, 407, or 409 in the CH3 domain of the heavy chain constant region, numbered according to the EU numbering system, as described, *e.g.*, in International Publication No. WO 2011/131746. Moreover, the single point mutation is located at a different residue in one homodimeric protein as compared to the other homodimeric protein. For example, one homodimeric protein can comprise the mutation F405L (*i.e.*, a mutation from phenylalanine to leucine at residue 405), while the other homodimeric

protein can comprise the mutation K409R (*i.e.*, a mutation from lysine to arginine at residue 409), numbered according to the EU numbering system. The heavy chain constant regions of the homodimeric proteins can be an IgG_1, IgG_2, IgG_3, IgG_4, IgA_1, or IgA_2 isotype (*e.g.*, a human IgG_1 isotype).

[00169] Additionally, the methods described herein employ, 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.

[00170] Antibodies that bind OX40 can, in some instances contain, IgG4 and IgG1, IgG4 and IgG2, IgG4 and IgG2, IgG4 and IgG3, or IgG1 and IgG3 chain heterodimers. Such heterodimeric heavy chain antibodies, can routinely be engineered by, for example, modifying selected amino acids forming the interface of the CH3 domains in human IgG4 and the IgG1 or IgG3 so as to favor heterodimeric heavy chain formation.

[00171] In particular embodiments, an antibody can be a chimeric antibody or a humanized antibody. In certain embodiments, an antibody can be an $F(ab')_2$ fragment. A $F(ab')_2$ fragment contains the two antigen-binding arms of a tetrameric antibody molecule linked by disulfide bonds in the hinge region.

[00172] In a certain aspect, provided herein is a method of making an antibody or an antigen-binding fragment which immunospecifically binds to OX40 (*e.g.*, human OX40) comprising culturing a cell or cells described herein. In a certain aspect, provided herein is a method of making an antibody or antigen-binding fragment which immunospecifically binds to OX40 (*e.g.*,

human OX40) comprising expressing (*e.g.*, recombinantly expressing) the antibody or antigen-binding fragment 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 obtained from the cell or host cell.

[00173] Antigen-binding fragments of antibodies can be prepared, *e.g.*, from monoclonal antibodies, 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). 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. Monoclonal antibodies described herein can, for example, be made by the hybridoma method as described in Kohler G & Milstein C (1975) Nature 256: 495 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*).

[00174] Further, the antibodies or antigen-binding fragments thereof described herein can also be generated using various phage display methods known in the art. In phage display methods, proteins 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 antibody or fragment 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.

[00175] As described in the above references, after phage selection, the antibody coding regions from the phage can be isolated and used to generate antibodies, including human antibodies, 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 antibodies 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.

[00176] In one aspect, to generate 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 antibodies, *e.g.,* IgG, using techniques known to those of skill in the art.

[00177] 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.

[00178] 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. See also U.S. Application Publication No. US 2005/0042664 A1 (Feb. 24, 2005), which is incorporated by reference herein in its entirety.

[00179] In particular embodiments, a human antibody comprises an antigen-binding domain described herein which binds to the same epitope of OX40 (*e.g.*, human OX40) as an anti-OX40 antigen-binding fragment thereof described herein. In particular embodiments, a human antibody comprises an antigen-binding domain which competitively blocks (*e.g.*, in a dose-dependent manner) any one of the anti-OX40 antigen-binding domains described herein, (*e.g.*,

pab1949w or pab2049w) from binding to OX40 (*e.g.*, human OX40). 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.*, OX40). 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. 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).

[00180] Human antibodies or antigen-binding fragments which specifically bind to OX40 (*e.g.*, human OX40) 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.

[00181] 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 immunospecifically bind to a target antigen (*e.g.*, OX40 (*e.g.*, human OX40)). 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.

7.3.1 Polynucleotides

[00182] In certain aspects, provided herein are polynucleotides comprising a nucleotide sequence encoding an antibody described herein or a fragment thereof (*e.g.*, a variable light chain region and/or variable heavy chain region) that immunospecifically binds to a OX40 (*e.g.*, human OX40) 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.

[00183] 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.

[00184] In particular aspects, provided herein are polynucleotides comprising nucleotide sequences encoding antibodies, which immunospecifically bind to an OX40 polypeptide (*e.g.*, human OX40) and comprises an amino acid sequence as described herein, as well as antibodies that compete with such antibodies for binding to an OX40 polypeptide (*e.g.*, in a dose-dependent manner), or which binds to the same epitope as that of such antibodies.

[00185] 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 or light chain variable domain comprising the VL CDRs of antibodies described herein (*see*, *e.g.*, Table 3). The polynucleotides can comprise nucleotide sequences encoding a heavy chain or heavy chain variable domain comprising the VH CDRs of antibodies described herein (*see*, *e.g.*, Table 5). In specific embodiments, a polynucleotide described herein encodes a VL domain comprising the amino acid sequence set forth in SEQ ID NO: 55 or 56. In specific embodiments, a polynucleotide described herein encodes a VH domain comprising the amino acid sequence set forth in SEQ ID NO: 54.

[00186] In particular embodiments, provided herein are polynucleotides comprising a nucleotide sequence encoding an anti-OX40 antigen-binding domain comprising three VL chain CDRs, *e.g.*, containing VL CDR1, VL CDR2, and VL CDR3 of any one of antibodies described herein (*e.g.*, see Table 3). In specific embodiments, provided herein are polynucleotides comprising three VH chain CDRs, *e.g.*, containing VH CDR1, VH CDR2, and VH CDR3 of any one of antibodies described herein (*e.g.*, see Table 5). In specific embodiments, provided herein are polynucleotides comprising a nucleotide sequence encoding an anti-OX40 antigen-binding domain comprising three VL chain CDRs, *e.g.*, containing VL CDR1, VL CDR2, and VL CDR3 of any one of antibodies described herein (*e.g.*, see Table 3) and three VH chain CDRs, *e.g.*, containing VH CDR1, VH CDR2, and VH CDR3 of any one of antibodies described herein (*e.g.*, see Table 5).

[00187] In certain embodiments, a polynucleotide described herein comprises a nucleotide sequence encoding an antibody or antigen-binding domain provided herein comprising a light chain variable region comprising an amino acid sequence described herein (*e.g.*, 55 or 56), wherein the antibody or antigen-binding domain immunospecifically binds to OX40 (*e.g.*, human OX40).

[00188] In certain embodiments, a polynucleotide described herein comprises a nucleotide sequence encoding an antibody or antigen-binding domain provided herein comprising a heavy chain variable region comprising an amino acid sequence described herein (*e.g.*, SEQ ID NO:54), wherein the antibody or antigen-binding domain immunospecifically binds to OX40 (*e.g.*, human OX40).

[00189] In specific aspects, provided herein is a polynucleotide comprising a nucleotide sequence encoding an antibody comprising a light chain and a heavy chain, *e.g.*, a separate light chain and heavy chain. With respect to the light chain, in a specific embodiment, a polynucleotide provided herein comprises a nucleotide sequence encoding a kappa light chain. In another specific embodiment, a polynucleotide provided herein comprises a nucleotide sequence encoding a lambda light chain. In yet another specific embodiment, a polynucleotide provided herein comprises a nucleotide sequence encoding an antibody described herein comprising a human kappa light chain or a human lambda light chain. In a particular embodiment, a polynucleotide provided herein comprises a nucleotide sequence encoding an antibody, which immunospecifically binds to OX40 (*e.g.*, human OX40), wherein the antibody comprises a light chain, wherein the amino acid sequence of the VL domain can comprise the amino acid sequence set forth in SEQ ID NO: 55 or 56 and wherein the constant region of the light chain comprises the amino acid sequence of a human kappa light chain constant region. In another particular embodiment, a polynucleotide provided herein comprises a nucleotide sequence encoding an antibody, which immunospecifically binds to OX40 (*e.g.*, human OX40), and comprises a light chain, wherein the amino acid sequence of the VL domain can comprise the amino acid sequence set forth in SEQ ID NO: 55 or 56, and wherein the constant region of the light chain comprises the amino acid sequence of a human lambda light chain constant region. For example, human constant region sequences can be those described in U.S. Patent No. 5,693,780.

[00190] In a particular embodiment, a polynucleotide provided herein comprises a nucleotide sequence encoding an antibody described herein, which immunospecifically binds to OX40 (*e.g.*, human OX40), wherein the antibody comprises a heavy chain, wherein the amino acid sequence of the VH domain can comprise the amino acid sequence set forth in SEQ ID NO: 54, and wherein the constant region of the heavy chain comprises the amino acid sequence of a human gamma (γ) heavy chain constant region.

[00191] In a certain embodiment, a polynucleotide provided herein comprises a nucleotide sequence(s) encoding a VH domain and/or a VL domain of an antibody described herein (such as SEQ ID NO: 54 for the VH domain and/or SEQ ID NO: 55 or 56 for the VL domain), which immunospecifically binds to OX40 (*e.g.*, human OX40).

[00192] In yet another specific embodiment, a polynucleotide provided herein comprises a nucleotide sequence encoding an antibody described herein, which immunospecifically binds OX40 (*e.g.*, human OX40), wherein the antibody comprises a VL domain and a VH domain comprising any amino acid sequences described herein, and wherein the constant regions comprise the amino acid sequences of the constant regions of a human IgG_1 (*e.g.*, allotype 1, 17, or 3), human IgG_2, or human IgG_4.

[00193] In a specific embodiment, provided herein are polynucleotides comprising a nucleotide sequence encoding an anti-OX40 antibody or domain thereof, designated herein, *see*, *e.g.*, Tables 1-7.

[00194] Also provided herein are polynucleotides encoding an anti-OX40 antibody or a fragment thereof 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-OX40 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. 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.

[00195] In certain embodiments, an optimized polynucleotide sequence encoding an anti-OX40 antibody described herein or a fragment thereof (*e.g.*, VL domain or VH domain) can hybridize to an antisense (*e.g.*, complementary) polynucleotide of an unoptimized polynucleotide sequence encoding an anti-OX40 antibody described herein or a fragment thereof (*e.g.*, VL

domain or VH domain). In specific embodiments, an optimized nucleotide sequence encoding an anti-OX40 antibody described herein or a fragment hybridizes under high stringency conditions to antisense polynucleotide of an unoptimized polynucleotide sequence encoding an anti-OX40 antibody described herein or a fragment thereof. In a specific embodiment, an optimized nucleotide sequence encoding an anti-OX40 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-OX40 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.

[00196] 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.

[00197] 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 (*e.g.*, SEQ ID NO: 54) and/or VL domain (*e.g.*, SEQ ID NO: 55 or 56) provided herein.

[00198] 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.

7.3.2 Cells and Vectors

[00199] In certain aspects, provided herein are cells (*e.g.*, host cells) expressing (*e.g.*, recombinantly) antibodies described herein which specifically bind to OX40 (*e.g.*, human OX40) and related polynucleotides and expression vectors. Provided herein are vectors (*e.g.*, expression vectors) comprising polynucleotides comprising nucleotide sequences encoding anti-OX40 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-OX40 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 in a host cell.

[00200] Recombinant expression of an antibody or fragment thereof described herein (*e.g.*, a heavy or light chain of an antibody described herein) that specifically binds to OX40 (*e.g.*, human OX40) involves construction of an expression vector containing a polynucleotide that encodes the antibody or fragment. Once a polynucleotide encoding an antibody or fragment thereof (*e.g.*, heavy or light chain variable domains) 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 domain 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) and variable domains 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.

[00201] 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 (*e.g.*, an antibody comprising the CDRs of pab1949w or pab2049w) or a fragment thereof. Thus, provided herein are host cells containing a polynucleotide encoding an antibody described herein (*e.g.*, an antibody comprising the CDRs of pab1949w or pab2049w) or fragments thereof (*e.g.*, a heavy or light chain thereof, or fragment thereof), 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 (*e.g.*, an antibody comprising the CDRs of pab1949w or pab2049w), 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 (*e.g.*, an antibody comprising the CDRs of pab1949w or pab2049w), 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 (*e.g.*, an antibody comprising the CDRs of pab1949w or pab2049w), 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 (*e.g.*, an antibody comprising the CDRs of pab1949w or pab2049w), 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 (*e.g.*, an antibody comprising the CDRs of pab1949w or pab2049w). 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-OX40 antibody described herein (*e.g.*, antibody comprising the CDRs pab1949w or pab2049w). In certain embodiments, provided herein is a population of host cells comprising such first host cell and such second host cell.

[00202] 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-OX40 antibody described herein (*e.g.*, antibody comprising the CDRs of pab1949w or pab2049w), and a second vector comprising a polynucleotide encoding a heavy chain/heavy

chain variable region of an anti-OX40 antibody described herein (*e.g.*, antibody comprising the CDRs of pab1949w or pab2049w).

[00203] 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 (*e.g.*, an antibody comprising the CDRs of any one of antibodies pab1949w or pab2049w) 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: 101-105; and Cockett MI *et al.*, (1990) Biotechnology 8: 662-667). 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 immunospecifically bind OX40 (*e.g.*, human OX40) is regulated by a constitutive promoter, inducible promoter or tissue specific promoter.

[00204] 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-OX40 antibodies described herein are produced in mammalian cells, such as CHO cells.

[00205] In a specific embodiment, the antibodies described herein 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 with reduced fucose content. The Potelligent® system (Lonza) is an example of such a system that can be used to produce antibodies with reduced fucose content.

[00206] For long-term, high-yield production of recombinant proteins, stable expression cells can be generated. For example, cell lines which stably express an anti-OX40 antibody described herein can be engineered.

[00207] 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.

[00208] In specific embodiments, an antibody 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. When the antibody or fragment 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 or fragment 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 or fragment have less than about 30%, 20%, 10%, or 5% (by dry weight) of chemical precursors or compounds other than the antibody or fragment of interest. In a specific embodiment, antibodies described herein are isolated or purified.

7.4 Pharmaceutical Compositions

[00209] Provided herein are compositions comprising an 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.

[00210] Pharmaceutical compositions as described herein that comprise an antibody described herein can be useful in reducing, inhibiting, or deactivating an OX40 activity and treating a condition, such as an inflammatory or autoimmune disease or disorder or an infectious disease. Pharmaceutical compositions as described herein that comprise an antibody described herein can be useful in reducing, inhibiting, or deactivating an OX40 activity and treating a condition selected from the group consisting of infections (viral, bacterial, fungal and parasitic), endotoxic shock associated with infection, arthritis, rheumatoid arthritis, asthma, chronic obstructive pulmonary disease (COPD), pelvic inflammatory disease, Alzheimer's Disease, inflammatory bowel disease, Crohn's disease, ulcerative colitis, Peyronie's Disease, coeliac disease, gallbladder disease, Pilonidal disease, peritonitis, psoriasis, vasculitis, surgical adhesions, stroke, Type I Diabetes, lyme disease, arthritis, meningoencephalitis, uveitis, autoimmune uveitis, immune mediated inflammatory disorders of the central and peripheral nervous system such as multiple sclerosis, lupus (such as systemic lupus erythematosus) and Guillain-Barr syndrome, dermatitis, Atopic dermatitis, autoimmune hepatitis, fibrosing alveolitis, Grave's disease, IgA nephropathy, idiopathic thrombocytopenic purpura, Meniere's disease, pemphigus, primary biliary cirrhosis, sarcoidosis, scleroderma, Wegener's granulomatosis, pancreatitis, trauma (surgery), graft-versus-host disease, transplant rejection, heart disease (*i.e.*, cardiovascular disease) including ischaemic diseases such as myocardial infarction as well as atherosclerosis, intravascular coagulation, bone resorption, osteoporosis, osteoarthritis, periodontitis, hypochlorhydia, and neuromyelitis optica.

[00211] The compositions to be used for *in vivo* administration can be sterile. This is readily accomplished by filtration through, *e.g.,* sterile filtration membranes.

7.5 Uses and Methods

7.5.1 Therapeutic Uses and Methods

[00212] In one aspect, presented herein are methods for modulating one or more immune functions or responses in a subject, comprising to a subject in need thereof administering an antibody that binds to OX40 described herein (*e.g.*, an anti-OX40 antagonistic antibody, *e.g.*, an anti-OX40-monovalent antibody) or a composition comprising such an antibody.

[00213] In one aspect, the methods for modulating one or more immune functions or responses in a subject as presented herein are methods for deactivating, reducing, or inhibiting one or more immune functions or responses in a subject, comprising to a subject in need thereof

administering an anti-OX40 antagonistic antibody or a composition thereof as described herein. In a specific embodiment, presented herein are methods for preventing and/or treating diseases in which it is desirable to deactivate, reduce, or inhibit one or more immune functions or responses, comprising administering to a subject in need thereof an anti-OX40 antagonistic antibody described herein or a composition thereof. In a certain embodiment, presented herein are methods of treating an autoimmune or inflammatory disease or disorder comprising administering to a subject in need thereof an effective amount of an anti-OX40 antagonistic antibody or a composition thereof as described herein. In a certain embodiment, presented herein are methods of treating an infectious disease comprising administering to a subject in need thereof an effective amount of an anti-OX40 antagonistic antibody or a composition thereof as described herein. In certain embodiments, the subject is a human. In certain embodiments, the disease or disorder is selected from the group consisting of: infections (viral, bacterial, fungal and parasitic), endotoxic shock associated with infection, arthritis, rheumatoid arthritis, asthma, chronic obstructive pulmonary disease (COPD), pelvic inflammatory disease, Alzheimer's Disease, inflammatory bowel disease, Crohn's disease, ulcerative colitis, Peyronie's Disease, coeliac disease, gallbladder disease, Pilonidal disease, peritonitis, psoriasis, vasculitis, surgical adhesions, stroke, Type I Diabetes, lyme disease, arthritis, meningoencephalitis, uveitis, autoimmune uveitis, immune mediated inflammatory disorders of the central and peripheral nervous system such as multiple sclerosis, lupus (such as systemic lupus erythematosus) and Guillain-Barr syndrome, dermatitis, Atopic dermatitis, autoimmune hepatitis, fibrosing alveolitis, Grave's disease, IgA nephropathy, idiopathic thrombocytopenic purpura, Meniere's disease, pemphigus, primary biliary cirrhosis, sarcoidosis, scleroderma, Wegener's granulomatosis, pancreatitis, trauma (surgery), graft-versus-host disease, transplant rejection, heart disease (*i.e.*, cardiovascular disease) including ischaemic diseases such as myocardial infarction as well as atherosclerosis, intravascular coagulation, bone resorption, osteoporosis, osteoarthritis, periodontitis, hypochlorhydia, and neuromyelitis optica. In certain embodiments, the disease or disorder is selected from the group consisting of: transplant rejection, graft-versus-host disease, vasculitis, asthma, rheumatoid arthritis, dermatitis, inflammatory bowel disease, uveitis, lupus, colitis, diabetes, multiple sclerosis, and airway inflammation.

[00214] In another embodiment, an anti-OX40 antagonistic antibody is administered to a patient diagnosed with an autoimmune or inflammatory disease or disorder to decrease the

proliferation and/or effector function of one or more immune cell populations (*e.g.*, T cell effector cells, such as $CD4^+$ and $CD8^+$ T cells) in the patient.

[00215] In a specific embodiment, an anti-OX40 antagonistic antibody described herein deactivates or reduces or inhibits one or more immune functions or responses in a subject by at least 99%, at least 98%, at least 95%, at least 90%, at least 85%, at least 80%, at least 75%, at least 70%, at least 60%, at least 50%, at least 45%, at least 40%, at least 45%, at least 35%, at least 30%, at least 25%, at least 20%, or at least 10%, or in the range of between 10% to 25%, 25% to 50%, 50% to 75%, or 75% to 95% relative to the immune function in a subject not administered the anti-OX40 antagonistic antibody described herein using assays well known in the art, *e.g.*, ELISPOT, ELISA, and cell proliferation assays. In a specific embodiment, the immune function is cytokine production (*e.g.*, IL-2, TNF-α, IFN-γ, IL-4, IL-10, and/or IL-13 production). In another embodiment, the immune function is T cell proliferation/expansion, which can be assayed, *e.g.*, by flow cytometry to detect the number of cells expressing markers of T cells (*e.g.*, CD3, CD4, or CD8). In another embodiment, the immune function is antibody production, which can be assayed, *e.g.*, by ELISA. In some embodiments, the immune function is effector function, which can be assayed, *e.g.*, by a cytotoxicity assay or other assays well known in the art. In another embodiment, the immune function is a Th1 response. In another embodiment, the immune function is a Th2 response. In another embodiment, the immune function is a memory response.

[00216] In specific embodiments, non-limiting examples of immune functions that can be reduced or inhibited by an anti-OX40 antagonistic antibody or composition thereof as described herein are proliferation/expansion of effector lymphocytes (*e.g.*, decrease in the number of effector T lymphocytes), and stimulation of apoptosis of effector lymphocytes (*e.g.*, effector T lymphocytes). In particular embodiments, an immune function reduced or inhibited by an anti-OX40 antagonistic antibody or composition thereof as described herein is proliferation/expansion in the number of or activation of $CD4^+$ T cells (*e.g.*, Th1 and Th2 helper T cells), $CD8^+$ T cells (*e.g.*, cytotoxic T lymphocytes, alpha/beta T cells, and gamma/delta T cells), B cells (*e.g.*, plasma cells), memory T cells, memory B cells, tumor-resident T cells, $CD122^+$ T cells, natural killer (NK) cells), macrophages, monocytes, dendritic cells, mast cells, eosinophils, basophils or polymorphonucleated leukocytes. In one embodiment, an anti-OX40 antagonistic antibody or composition thereof as described herein deactivates or reduces or

inhibits the proliferation/expansion or number of lymphocyte progenitors. In some embodiments, an anti-OX40 antagonistic antibody or composition thereof as described herein decreases the number of $CD4^+$ T cells (*e.g.*, Th1 and Th2 helper T cells), $CD8^+$ T cells (*e.g.*, cytotoxic T lymphocytes, alpha/beta T cells, and gamma/delta T cells), B cells (*e.g.*, plasma cells), memory T cells, memory B cells, tumor-resident T cells, $CD122^+$ T cells, natural killer cells (NK cells), macrophages, monocytes, dendritic cells, mast cells, eosinophils, basophils or polymorphonucleated leukocytes by approximately at least 99%, at least 98%, at least 95%, at least 90%, at least 85%, at least 80%, at least 75%, at least 70%, at least 60%, at least 50%, at least 45%, at least 40%, at least 45%, at least 35%, at least 30%, at least 25%, at least 20%, or at least 10%, or in the range of between 10% to 25%, 25% to 50%, 50% to 75%, or 75% to 95% relative a negative control (*e.g.*, number of the respective cells not treated, cultured, or contacted with an anti-OX40 antagonistic antibody or composition thereof as described herein).

[00217] In certain embodiments, any of the methods herein (*e.g.*, methods of treating an infectious disease, or methods of treating an autoimmune or inflammatory disease or disorder) comprise administration to a subject of an antibody as described herein and a checkpoint targeting agent. In certain embodiments, the checkpoint targeting agent is an antibody (*e.g.*, an anti-PD-1 antibody, an anti-PD-L1 antibody, an anti-PD-L2 antibody, an anti-CTLA-4 antibody, an anti-TIM-3 antibody, an anti-LAG-3 antibody, an anti-CEACAM1 antibody, an anti-GITR antibody, or an anti-OX40 antibody). In certain embodiments, the checkpoint targeting agent is an antagonist or agonist antibody.

7.5.1.1 Routes of Administration & Dosage

[00218] An antibody or composition described herein can be delivered to a subject by a variety of routes.

[00219] 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.

[00220] The precise dose to be employed in a composition will also depend on the route of administration, and the seriousness of the disease, 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.

[00221] In certain embodiments, an *in vitro* assay is employed to help identify optimal dosage ranges. Effective doses may be extrapolated from dose response curves derived from *in vitro* or animal model test systems.

[00222] Generally, human antibodies have a longer half-life within the human body than antibodies from other species due to the immune response to the foreign polypeptides. Thus, lower dosages of human antibodies and less frequent administration is often possible.

7.5.2 Detection & Diagnostic Uses

[00223] An anti-OX40 antibody described herein (see, *e.g.*, Section 7.2) can be used to assay OX40 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 described herein. Alternatively, a second antibody that recognizes an anti-OX40 antibody described herein can be labeled and used in combination with an anti-OX40 antibody to detect OX40 protein levels.

[00224] Assaying for the expression level of OX40 protein is intended to include qualitatively or quantitatively measuring or estimating the level of a OX40 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). OX40 polypeptide expression level in the first biological sample can be measured or estimated and compared to a standard OX40 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" OX40 polypeptide level is known, it can be used repeatedly as a standard for comparison.

[00225] 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 OX40. 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.

[00226] An anti-OX40 antibody 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, including combinations thereof, versus a different agent or antibody. 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.

[00227] In one embodiment, an anti-OX40 antibody can be used in immunohistochemistry of biopsy samples.

[00228] In another embodiment, an anti-OX40 antibody can be used to detect levels of OX40, or levels of cells which contain OX40 on their membrane surface, which levels can then be linked to certain disease symptoms. Anti-OX40 antibodies 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-OX40 antibodies described herein can 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-OX40 antibody can 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-OX40 antibody to OX40 (*e.g.*, human OX40). 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-OX40 antibody under conditions that allow for the formation of a complex between the antibody and OX40. Any complexes formed between the antibody and OX40 are detected and compared in the sample and the control. In light of the specific binding of the antibodies described herein for OX40, the antibodies thereof can be used to specifically detect OX40 expression on the surface of cells. The antibodies described herein can also be used to purify OX40 via immunoaffinity purification.

[00229] 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, OX40 or OX40/OX40L complexes. The system or test kit may comprise a labeled component, *e.g.*, a labeled antibody, and one or more additional immunochemical reagents. *See*, *e.g.*, Section 7.6 below for more on kits.

7.6 Kits

[00230] Provided herein are kits comprising one or more antibodies described herein 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. 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.

[00231] Also provided herein 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 OX40 antigen (*e.g.*, human OX40) that can be used as a control. In another specific embodiment, the kits described herein further comprise a control antibody which does not react with a OX40 antigen. In another specific embodiment, kits described herein contain one or more elements for detecting the binding of an antibody to a OX40 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 OX40 antigen. The OX40 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 OX40 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 OX40 antigen can be detected by binding of the said reporter-labeled antibody.

[00232] The following examples are offered by way of illustration and not by way of limitation.

8. EXAMPLES

[00233] The examples in this Section (*i.e.*, Section 8) are offered by way of illustration, and not by way of limitation.

8.1 Example 1: Characterization of anti-OX40-monovalent antibodies

[00234] In this example, two anti-OX40-monovalent antibodies were constructed using the Genmab DuoBody technology: DuoBody pab2049 x isotype and DuoBody pab1949 x isotype both of which comprise an OX40-binding arm and an isotype control arm. The SEQ ID NOs corresponding to the heavy chain and light chain sequences of these DuoBody antibodies are listed in Table 8.

Table 8. Heavy chain (HC) and light chain (LC) sequences of OX40 binding arm of anti-OX40 x isotype DuoBody antibodies

Antibody	OX40 HC (SEQ ID NO:)	OX40 LC (SEQ ID NO:)
pab2049 x isotype	61	68
pab1949 x isotype	61	70
pab2049 x isotype without heavy chain terminal lysine	120	68
pab1949 x isotype without heavy chain terminal lysine	120	70

8.1.1 Binding of anti-OX40-monovalent antibody to OX40-expressing cells

[00235] The binding characteristics of DuoBody pab2049 x isotype to OX40-expressing cells were analyzed by flow cytometry. Briefly, cells ectopically expressing human OX40 were generated by transduction of lentiviral vectors (EF1a promoter) into Jurkat cells. Stable clones were generated via single-cell sorting (FACS ARIA Fusion). Expression of OX40 was verified by flow cytometry. Hut102 cells (human T cell lymphoma, ATCC) were incubated for 72 hours in RPMI media, supplemented with 1 µg/ml phytohaemagglutinin (PHA) and 10% heat-inactivated FBS, at 37°C and 5% CO_2 to induce OX40 expression. For primary CD4+ T cells, PBMCs isolated via Ficoll gradient from healthy donor buffy coats (Research Blood Components, LLC) were activated with CD3-CD28 Dynabeads® (Life Technologies) for 3 days in RPMI media, supplemented with 10% heat-inactivated FBS, at 37°C and 5% CO_2. For binding analysis, stable Jurkat cells expressing human OX40 (Jurkat-huOX40), activated Hut102 cells, or activated primary CD4+ T cells were incubated with test antibodies (10-point dose titration, 0.5-10,000 ng/ml) diluted in FACS buffer (PBS, 2 mM EDTA, 0.5% BSA, pH 7.2) for 30 minutes at 4°C. Samples were washed two times in FACS buffer and then incubated with APC-conjugated mouse anti-human kappa detection antibody (Life Technologies, HP6062, 1:100 dilution in FACS buffer) for 30 minutes at 4°C. Samples were then washed two times and analyzed using the LSRFortessa flow cytometer (BD Biosciences). FACS plots were analyzed using a combination of FACS DIVA and WEHI Weasel software. Data were plotted with Graphpad Prism software.

[00236] DuoBody pab2049 x isotype bound to Jurkat cells expressing human OX40 (Figure 1A), activated Hut102 cells (Figure 1B) and activated primary CD4+ T cells (Figure 1C) in a dose-dependent manner.

8.1.2 Effect of anti-OX40-monovalent antibody on human T cells following Staphylococcus Enterotoxin A (SEA) stimulation

[00237] The activation of OX40 signaling depends on receptor clustering to form higher order receptor complexes that efficiently recruit apical adapter proteins to drive intracellular signal transduction. Without being bound by theory, an anti-OX40 agonist antibody may mediate receptor clustering through bivalent antibody arms and/or through Fc-Fc receptor (FcR) co-engagement on accessory myeloid or lymphoid cells. Consequently, one approach for developing an anti-OX40 antagonist antibody is to select an antibody that competes with OX40 ligand (OX40L) for binding to OX40, diminish or eliminate the binding of the Fc region of the antibody to Fc receptors, and/or adopt a monovalent antibody format (containing only one OX40-specific antigen-binding domain, and optionally a second antigen-binding domain that is not OX40-specific). The monovalent DuoBody pab2049 x isotype was tested in a human PBMC functional assay to examine whether it still retained agonistic activity. In addition, the impact of diminishing Fc receptor interaction was tested by introducing L234F/L235E/D265A substitutions into the Fc regions of the bivalent antibody pab2049 and the monovalent DuoBody pab2049 x isotype, numbered according to the EU numbering system. The L234F/L235E/D265A substitutions (referred to as LFLEDA), which abrogates the binding of Fc region to FcγR and C1q, have been described in U.S. Patent Publication No. US 2015/0175707 (herein incorporated by reference). A bivalent IgG_1 antibody pab2049 was included in the study as a positive control.

[00238] Briefly, PBMCs isolated via Ficoll gradient from healthy donor buffy coats (Research Blood Components, LLC) were incubated in RPMI media, supplemented with 100 ng/ml SEA superantigen (Sigma-Aldrich) and 10% heat-inactivated FBS, at 37°C and 5% CO_2 with 20 μg/ml of pab2049, pab2049 (K409R, LFLEDA), DuoBody pab2049 x isotype, DuoBody pab2049 x isotype (LFLEDA) or an isotype control antibody for 5 days. pab2049 (K409R, LFLEDA) is an antibody that contains K409R and L234F/L235E/D265A substitutions in both heavy chain constant regions, numbered according to the EU numbering system. Following incubation, cell-free supernatant was assayed for IL-2 production using an AlphaLISA immunoassay (Perkin-Elmer). Data were collected using the EnVision® Multilabel Plate Reader (Perkin-Elmer) and the concentration of IL-2 was determined using an IL-2 standard curve. All values were interpolated and plotted using Graphpad Prism software.

[00239] In contrast to the positive control bivalent antibody pab2049, the monovalent

DuoBody pab2049 x isotype induced much less IL-2 production in this primary human PBMC assay (Figure 2). Eliminating Fc receptor interaction by incorporating the LFLEDA substitutions further diminished the agonistic activity on human PBMCs (Figure 2).

8.1.3 Effect of anti-OX40-monovalent antibody on OX40 NF-κB-luciferase reporter cell line

[00240] An OX40 reporter assay was developed to first confirm the minimal agonistic activity of DuoBody pab2049 x isotype shown in section 8.1.2, and second examine the ability of DuoBody pab2049 x isotype to antagonize OX40L-induced signaling through OX40 receptors.

[00241] Cells ectopically expressing OX40 as well as NF-κB-luciferase (Nano luciferase, NanoLuc®) reporter were generated by transduction of lentiviral vectors (EF1a promoter) into Jurkat cells. Stable clones were generated via single-cell sorting (FACS ARIA Fusion). Expression of OX40 was verified by flow cytometry. To evaluate agonistic activity, Jurkat-huOX40-NF-κB-luciferase cells were incubated with increasing concentrations of DuoBody pab2049 x isotype or multimeric OX40L (10-point dose titration, 0.5-10,000 ng/ml) for 2 hours in RPMI media, supplemented with 10% heat-inactivated FBS, at 37°C and 5% CO2. For detection of luciferase activity, samples were incubated with prepared Nano-Glo® Luciferase Assay Substrate (Promega, 1:1 v/v) in passive lysis buffer for 5 minutes at room temperature. Data were collected using the EnVision® Multilabel Plate Reader (Perkin-Elmer). Values were plotted using Graphpad Prism software.

[00242] While multimeric OX40L induced NF-κB-luciferase activity over a wide range of concentrations, minimal luciferase signal was observed after incubation with the monovalent DuoBody pab2049 x isotype (Figure 3A).

[00243] Next, DuoBody pab2049 x isotype was assessed for its ability to block OX40L-induced NF-κB signaling. Jurkat-huOX40-NF-κB-luciferase cells were incubated with increasing concentrations of DuoBody pab2049 x isotype or an isotype control antibody (10-point dose titration, 0.5-10,000 ng/ml) for 30 minutes. Samples were then washed two times with RPMI, resuspended in 1 µg/ml of multimeric OX40L and incubated for additional 2 hours at 37°C. Luciferase activity was detected and analyzed as described above. To determine % OX40L activity, the RLU value for OX40L (1 µg/ml) without addition of antibody was established as 100% activity. Relative values for DuoBody pab2049 x isotype and the isotype control were calculated accordingly.

[00244] As shown in Figure 3B, pre-incubation of Jurkat-huOX40-NF-κB-luciferase reporter cells with increasing concentrations of DuoBody pab2049 x isotype significantly reduced OX40L-induced NF-κB-luciferase activity in a dose-dependent manner.

8.2 Example 2: Epitope mapping of anti-OX40 antibodies

[00245] This example characterizes the epitope of the anti-OX40 antibodies pab1949w, pab2049 and a reference anti-OX40 antibody pab1928. The antibody pab1928 was generated based on the variable regions of the antibody Hu106-122 provided in U.S. Patent Publication No. US 2013/0280275 (herein incorporated by reference). pab1928 comprises a heavy chain of the amino acid sequence of SEQ ID NO: 106 and a light chain of the amino acid sequence of SEQ ID NO: 107.

8.2.1 Epitope mapping – alanine scanning

[00246] The binding characteristics of pab1949w, pab2049 and the reference antibody pab1928 were assessed by alanine scanning. Briefly, the QuikChange HT Protein Engineering System from Agilent Technologies (G5901A) was used to generate human OX40 mutants with alanine substitutions in the extracellular domain. The human OX40 mutants were expressed on the surface of 1624-5 cells using standard techniques of transfection followed by transduction as described above.

[00247] Cells expressing correctly folded human OX40 mutants, as evidenced by binding to a polyclonal anti-OX40 antibody in flow cytometry, were further selected for a sub-population that expressed human OX40 mutants that did not bind the monoclonal anti-OX40 antibody pab1949w, pab2049, or pab1928. Cells that exhibited specific antibody binding were separated from the non-binding cell population by preparative, high-speed FACS (FACSAriaII, BD Biosciences). Antibody reactive or non-reactive cell pools were expanded again in tissue culture and, due to the stable expression phenotype of retrovirally transduced cells, cycles of antibody-directed cell sorting and tissue culture expansion were repeated, up to the point that a clearly detectable anti-OX40 antibody (pab1949w, pab2049, or pab1928) non-reactive cell population was obtained. This anti-OX40 antibody non-reactive cell population was subjected to a final, single-cell sorting step. After several days of cell expansion, single cell sorted cells were again tested for binding to a polyclonal anti-OX40 antibody and non-binding to monoclonal antibody pab1949w, pab2049 or pab1928 using flow cytometry. Briefly, 1624-5 cells expressing individual human OX40 alanine mutants were incubated with the monoclonal anti-OX40

antibody pab1949w, pab2049 or pab1928. For each antibody, two antibody concentrations were tested (pab1949w: 2 μg/ml and 0.5 μg/ml; pab2049: 1.8 μg/ml and 0.3 μg/ml; pab1928: 1.1 μg/ml and 0.4 μg/ml). The polyclonal anti-OX40 antibody (AF3388, R&D systems) conjugated with APC was diluted at 1:2000. Fc receptor block (1:200; BD Cat no. 553142) was added, and the samples were incubated for 20 minutes at 4°C. After washing, the cells were incubated with a secondary anti-IgG antibody if necessary for detection (PE conjugated; BD Cat no. 109-116-097) for 20 min at 4°C. The cells were then washed and acquired using a flow cytometer (BD Biosciences).

[00248] To connect phenotype (polyclonal anti-OX40 antibody +, monoclonal anti-OX40 antibody -) with genotype, sequencing of single cell sorted human OX40 mutants was performed. Figure 4 is a table showing the human OX40 alanine mutants that still bind the polyclonal anti-OX40 antibody but do not bind the monoclonal anti-OX40 antibody pab1949w, pab2049, or pab1928. All the residues are numbered according to the mature amino acid sequence of human OX40 (SEQ ID NO: 72). "+" indicates binding and "-" indicates loss of binding based on flow cytometry analysis.

* * *

[00249] 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.

[00250] All references (*e.g.*, publications or patents or patent applications) cited herein are incorporated herein by reference in their entirety 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.

[00251] Other embodiments are within the following claims.

WHAT IS CLAIMED:

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

(A) a first antigen-binding domain that specifically binds to human OX40; comprising:

(i) a first heavy chain comprising

(a) a first heavy chain constant region comprising a F405L or a K409R mutation, numbered according to the EU numbering system; and

(b) 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:47); a VH-CDR2 comprising the amino acid sequence of RIRSKANSYATAYAASVKG (SEQ ID NO:48); and a VH-CDR3 comprising the amino acid sequence of GIYDSSGYDY (SEQ ID NO:49); and

(ii) a first light chain comprising

(a) a first light chain constant region; and

(b) a first light chain variable domain (VL) comprising a VL–CDR1 comprising the amino acid sequence of RSSQSLLHSNGYNYLD (SEQ ID NO:50); a VL-CDR2 comprising the amino acid sequence of LGSNRAS (SEQ ID NO:51); and a VL-CDR3 comprising the amino acid sequence of MQGSKWPLT (SEQ ID NO:52) or MQALQTPLT (SEQ ID NO:53); and

(B) a second antigen-binding domain that does not specifically bind to an antigen expressed by a human immune cell; comprising:

(i) a second heavy chain comprising

(a) a second heavy chain constant region comprising a F405L or a K409R mutation, numbered according to the EU numbering system; and

(b) a second heavy chain variable domain; and

(ii) a second light chain comprising

(a) a second light chain constant region; and

(b) a second light chain variable domain,

wherein the first heavy chain constant region and the second heavy chain constant region contain different mutations.

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

(A) a first antigen-binding domain that specifically binds to the same epitope of human OX40 as an antibody comprising a VH comprising the amino acid sequence of SEQ ID NO:54 and a VL comprising the amino acid sequence of SEQ ID NO:55 or 56, wherein the first antigen-binding domain comprises a first heavy chain constant region comprising a F405L or a K409R mutation, numbered according to the EU numbering system; and

(B) a second antigen-binding domain that does not specifically bind to an antigen expressed by a human immune cell, comprising a second heavy chain constant region comprising a F405L or a K409R mutation, numbered according to the EU numbering system;

wherein the first heavy chain constant region and the second heavy chain constant region contain different mutations.

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

(A) a first antigen-binding domain that specifically binds to human OX40 and exhibits, as compared to binding to a human OX40 sequence of SEQ ID NO:72, reduced or absent binding to a protein identical to SEQ ID NO:72 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, numbered according to SEQ ID NO:72, wherein the first antigen-binding domain comprises a first heavy chain constant region comprising a F405L or a K409R mutation, numbered according to the EU numbering system; and

(B) a second antigen-binding domain that does not specifically bind to an antigen expressed by a human immune cell, comprising a second heavy chain constant region comprising a F405L or a K409R mutation, numbered according to the EU numbering system;

wherein the first heavy chain constant region and the second heavy chain constant region

contain different mutations.

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

(A) a first antigen-binding domain that specifically binds to human OX40 comprising a VH and a VL, wherein the VH comprises the amino acid sequence of SEQ ID NO:54, wherein the first antigen-binding domain comprises a first heavy chain constant region comprising a F405L or a K409R mutation, numbered according to the EU numbering system; and

(B) a second antigen-binding domain that does not specifically bind to an antigen expressed by a human immune cell, comprising a second heavy chain constant region comprising a F405L or a K409R mutation, numbered according to the EU numbering system;

wherein the first heavy chain constant region and the second heavy chain constant region contain different mutations.

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

(A) a first antigen-binding domain that specifically binds to human OX40 comprising a VH and a VL, wherein the VL comprises the amino acid sequence of SEQ ID NO:55 or SEQ ID NO:56, wherein the first antigen-binding domain comprises a first heavy chain constant region comprising a F405L or a K409R mutation, numbered according to the EU numbering system; and

(B) a second antigen-binding domain that does not specifically bind to an antigen expressed by a human immune cell, comprising a second heavy chain constant region comprising a F405L or a K409R mutation, numbered according to the EU numbering system;

wherein the first heavy chain constant region and the second heavy chain constant region contain different mutations.

6. The antibody of any one of claims 1-5, wherein the second antigen-binding domain specifically binds to a non-human antigen.

7. The antibody of any one of claims 1-6, wherein the second antigen-binding domain

specifically binds to a viral antigen.

8. The antibody of claim 7, wherein the viral antigen is a HIV antigen.

9. The antibody of any one of claims 1-6, wherein the second antigen-binding domain specifically binds to chicken albumin or hen egg lysozyme.

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

(A) an antigen-binding domain that specifically binds to human OX40; comprising:

(i) a first heavy chain comprising

(a) a first heavy chain constant region comprising a F405L or a K409R mutation, numbered according to the EU numbering system; and

(b) 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:47); a VH-CDR2 comprising the amino acid sequence of RIRSKANSYATAYAASVKG (SEQ ID NO:48); and a VH-CDR3 comprising the amino acid sequence of GIYDSSGYDY (SEQ ID NO:49); and

(ii) a light chain comprising

(a) a light chain constant region; and

(b) a light chain variable domain (VL) comprising a VL–CDR1 comprising the amino acid sequence of RSSQSLLHSNGYNYLD (SEQ ID NO:50); a VL-CDR2 comprising the amino acid sequence of LGSNRAS (SEQ ID NO:51); and a VL-CDR3 comprising the amino acid sequence of MQGSKWPLT (SEQ ID NO:52) or MQALQTPLT (SEQ ID NO:53); and

(B) a second heavy chain comprising a second heavy chain constant region comprising a F405L or a K409R mutation, or a fragment thereof comprising the F405L or K409R mutation, numbered according to the EU numbering system;

wherein the first heavy chain constant region, and the second heavy chain constant region or the fragment contain different mutations.

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

(A) an antigen-binding domain that specifically binds to the same epitope of human OX40 as an antibody comprising a VH comprising the amino acid sequence of SEQ ID NO:54 and a VL comprising the amino acid sequence of SEQ ID NO:55 or 56, wherein the antigen-binding domain comprises a first heavy chain constant region comprising a F405L or a K409R mutation, numbered according to the EU numbering system; and

(B) a heavy chain comprising a second heavy chain constant region comprising a F405L or a K409R mutation, or a fragment thereof comprising the F405L or K409R mutation, numbered according to the EU numbering system;

wherein the first heavy chain constant region, and the second heavy chain constant region or the fragment contain different mutations.

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

(A) an antigen-binding domain that specifically binds to human OX40 and exhibits, as compared to binding to a human OX40 sequence of SEQ ID NO:72, reduced or absent binding to a protein identical to SEQ ID NO:72 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, numbered according to SEQ ID NO:72, wherein the antigen-binding domain comprises a first heavy chain constant region comprising a F405L or a K409R mutation, numbered according to the EU numbering system; and

(B) a heavy chain comprising a second heavy chain constant region comprising a F405L or a K409R mutation, or a fragment thereof comprising the F405L or K409R mutation, numbered according to the EU numbering system;

wherein the first heavy chain constant region, and the second heavy chain constant region or the fragment contain different mutations.

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

(A) an antigen-binding domain that specifically binds to human OX40 comprising a VH and a VL, wherein the VH comprises the amino acid sequence of SEQ ID NO:54, wherein the antigen-binding domain comprises a first heavy chain constant region comprising a F405L or a K409R mutation, numbered according to the EU numbering system; and

(B) a heavy chain comprising a second heavy chain constant region comprising a F405L or a K409R mutation, or a fragment thereof comprising the F405L or K409R mutation, numbered according to the EU numbering system;

wherein the first heavy chain constant region, and the second heavy chain constant region or the fragment contain different mutations.

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

(A) an antigen-binding domain that specifically binds to human OX40 comprising a VH and a VL, wherein the VL comprises the amino acid sequence of SEQ ID NO:55 or SEQ ID NO:56, wherein the antigen-binding domain comprises a first heavy chain constant region comprising a F405L or a K409R mutation, numbered according to the EU numbering system; and

(B) a heavy chain comprising a second heavy chain constant region comprising a F405L or a K409R mutation, or a fragment thereof comprising the F405L or K409R mutation, numbered according to the EU numbering system;

wherein the first heavy chain constant region, and the second heavy chain constant region or the fragment contain different mutations.

15. The antibody of any one of claims 10-14, wherein the fragment of the heavy chain comprising a second heavy chain constant region is an Fc fragment.

16. The antibody of any one of claims 10-15, wherein the heavy chain comprising a second heavy chain constant region or fragment thereof is from an antigen-binding domain that specifically binds to a non-human antigen.

17. The antibody of any one of claims 10-16, wherein the heavy chain comprising a second

heavy chain constant region or fragment thereof is from an antigen-binding domain that specifically binds to a viral antigen.

18. The antibody of claim 17, wherein the viral antigen is a HIV antigen.

19. The antibody of any one of claims 10-16, wherein the heavy chain comprising a second heavy chain constant region or fragment thereof is from an antigen-binding domain that specifically binds to chicken albumin or hen egg lysozyme.

20. The antibody of any one of claims 1-19, wherein 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:54.

21. The antibody of any one of claims 1-20, wherein the antigen-binding domain that specifically binds to human OX40 comprises a VH comprising the amino acid sequence of SEQ ID NO:54.

22. The antibody of any one of claims 1-19, wherein 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.

23. The antibody of any one of claims 1-22, wherein 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:55 or SEQ ID NO:56.

24. The antibody of any one of claims 1-23, wherein the antigen-binding domain that specifically binds to human OX40 comprises a VL-CDR3 comprising the amino acid sequence of SEQ ID NO:52.

25. The antibody of any one of claims 1-24, wherein the antigen-binding domain that specifically binds to human OX40 comprises a VL comprising the amino acid sequence of SEQ ID NO:55.

26. The antibody of any one of claims 1-25, wherein the antigen-binding domain that specifically

binds to human OX40 comprises a light chain comprising the amino acid sequence of SEQ ID NO:67.

27. The antibody of any one of claims 1-25, wherein the antigen-binding domain that specifically binds to human OX40 comprises a light chain comprising the amino acid sequence of SEQ ID NO:68.

28. The antibody of any one of claims 1-23, wherein the antigen-binding domain that specifically binds to human OX40 comprises a VL-CDR3 comprising the amino acid sequence SEQ ID NO:53.

29. The antibody of any one of claims 1-23 or 28, wherein the antigen-binding domain that specifically binds to human OX40 comprises a VL comprising the amino acid sequence of SEQ ID NO:56.

30. The antibody of any one of claims 1-23 or 28-29, wherein the antigen-binding domain that specifically binds to human OX40 comprises a light chain comprising the amino acid sequence of SEQ ID NO:69.

31. The antibody of any one of claims 1-23 or 28-29, wherein the antigen-binding domain that specifically binds to human OX40 comprises a light chain comprising the amino acid sequence of SEQ ID NO:70.

32. The antibody of any one of claims 1-22, wherein 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.

33. The antibody of any one of claims 1-21, wherein the antigen-binding domain that specifically binds to human OX40 comprises the VH and VL sequences set forth in SEQ ID NOs: 54 and 55 or SEQ ID NOs: 54 and 56, respectively.

34. The antibody of any one of claims 1-33, wherein the first heavy chain constant region comprises a F405L mutation, and wherein the second heavy chain constant region comprises a K409R mutation, numbered according to the EU numbering system.

35. The antibody of any one of claims 1-34, wherein the first heavy chain constant region comprises the amino acid sequence of SEQ ID NO: 108, and wherein the second heavy chain constant region comprises the amino acid sequence of SEQ ID NO: 109.

36. The antibody of any one of claims 1-34, wherein the first heavy chain constant region comprises the amino acid sequence of SEQ ID NO: 135, and wherein the second heavy chain constant region comprises the amino acid sequence of SEQ ID NO: 136.

37. The antibody of any one of claims 1-35, wherein the antigen-binding domain that specifically binds to human OX40 comprises a heavy chain comprising the amino acid sequence of SEQ ID NO:64.

38. The antibody of any one of claims 1-34, wherein the antigen-binding domain that specifically binds to human OX40 comprises a heavy chain comprising the amino acid sequence of SEQ ID NO:123.

39. The antibody of any one of claims 1-33, wherein the first heavy chain constant region comprises a K409R mutation, and wherein the second heavy chain constant region of the comprises a F405L mutation, numbered according to the EU numbering system.

40. The antibody of any one of claims 1-33 or 39, wherein the first heavy chain constant region comprises the amino acid sequence of SEQ ID NO: 109, and wherein the second heavy chain constant region comprises the amino acid sequence of SEQ ID NO: 108.

41. The antibody of any one of claims 1-33 or 39, wherein the first heavy chain constant region comprises the amino acid sequence of SEQ ID NO: 136, and wherein the second heavy chain constant region comprises the amino acid sequence of SEQ ID NO: 135.

42. The antibody of any one of claims 1-33 or 39-40, wherein the antigen-binding domain that specifically binds to human OX40 comprises a heavy chain comprising the amino acid sequence of SEQ ID NO:61.

43. The antibody of any one of claims 1-33 or 39, wherein the antigen-binding domain that specifically binds to human OX40 comprises a heavy chain comprising the amino acid sequence of SEQ ID NO:120.

44. The antibody of any one of claims 1-43, wherein each heavy chain constant region is selected from the group consisting of human immunoglobulins IgG_1, IgG_2, IgG_3, IgG_4, IgA_1, and IgA_2.

45. The antibody of any one of claims 1-44, wherein each heavy chain constant region is human immunoglobulin IgG_1.

46. The antibody of claim 45, wherein the first heavy chain constant region and the second heavy chain constant region further comprise an identical mutation selected from the group consisting of N297A, N297Q, D265A, L234F/L235E/D265A, and a combination thereof, numbered according to the EU numbering system.

47. The antibody of any one of claims 1-46, wherein the antibody is antagonistic to human OX40.

48. The antibody of any one of claims 1-47, wherein the antibody deactivates, reduces, or inhibits an activity of human OX40.

49. The antibody of any one of claims 1-48, wherein the antibody inhibits or reduces binding of human OX40 to human OX40 ligand.

50. The antibody of any one of claims 1-49, wherein the antibody inhibits or reduces human OX40 signaling.

51. The antibody of any one of claims 1-50, wherein the antibody inhibits or reduces human OX40 signaling induced by human OX40 ligand.

52. The antibody of any one of claims 1-51, wherein the antibody decreases CD4+ T cell proliferation induced by synovial fluid from rheumatoid arthritis patients.

53. The antibody of any one of claims 1-52, wherein the antibody increases survival of NOG mice transplanted with human PBMCs.

54. The antibody of any one of claims 1-53, wherein the antibody increases proliferation of regulatory T cells in a GVHD model.

55. The antibody of any one of claims 1-54, further comprising a detectable label.

56. A pharmaceutical composition comprising the antibody of any one of claims 1-55, and a pharmaceutically acceptable excipient.

57. A method of modulating an immune response in a subject, the method comprising administering to the subject an effective amount of the antibody of any one of claims 1-55, or the pharmaceutical composition of claim 56.

58. The method of claim 57, wherein modulating an immune response comprises reducing or inhibiting the immune response in the subject.

59. A method of treating an autoimmune or inflammatory disease or disorder in a subject, the method comprising administering to the subject an effective amount of the antibody of any one of claims 1-55, or the pharmaceutical composition of claim 56.

60. The method of claim 59, wherein the disease or disorder is selected from the group consisting of: transplant rejection, graft-versus-host disease, vasculitis, asthma, rheumatoid arthritis, dermatitis, inflammatory bowel disease, uveitis, lupus, colitis, diabetes, multiple sclerosis, and airway inflammation.

61. A method of treating an infectious disease in a subject comprising administering to the subject an effective amount of the antibody of any one of claims 1-55, or the pharmaceutical composition of claim 56.

62. The method of any one of claims 57-61, wherein the subject is human.

63. A method for detecting OX40 in a sample comprising contacting the sample with the antibody of any one of claims 1-55.

64. A kit comprising the antibody of any one of claims 1-55 or the pharmaceutical composition of claim 56 and a) a detection reagent, b) an OX40 antigen, c) a notice that reflects approval for use or sale for human administration, or d) a combination thereof.

65. The isolated antibody of claim 10, wherein the second heavy chain further comprises a second heavy chain variable domain and wherein the isolated antibody that specifically binds to human OX40 further comprises a second light chain comprising a second light chain constant region and a second light chain variable region.

66. The isolated antibody of any one of claims 11-14, wherein the heavy chain further comprises a second heavy chain variable domain and wherein the isolated antibody that specifically binds to human OX40 further comprises a second light chain comprising a second light chain constant region and a second light chain variable region.

67. The isolated antibody of any one of claims 10-14, wherein the antibody is a bispecific antibody.

Figure 1A

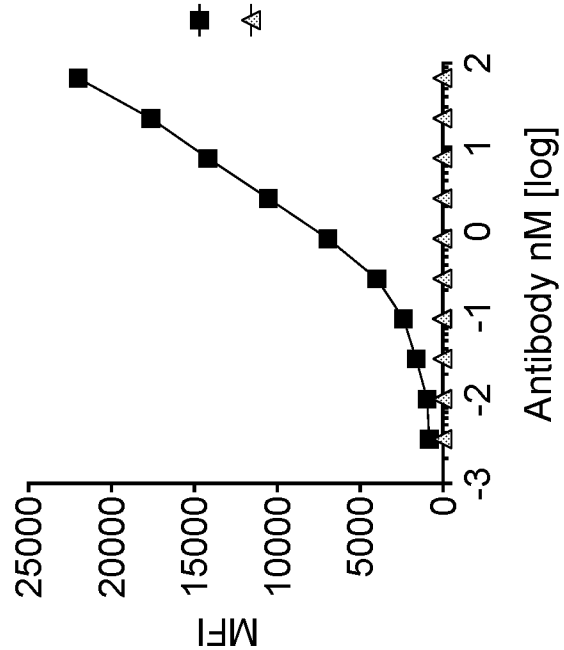


Figure 1B

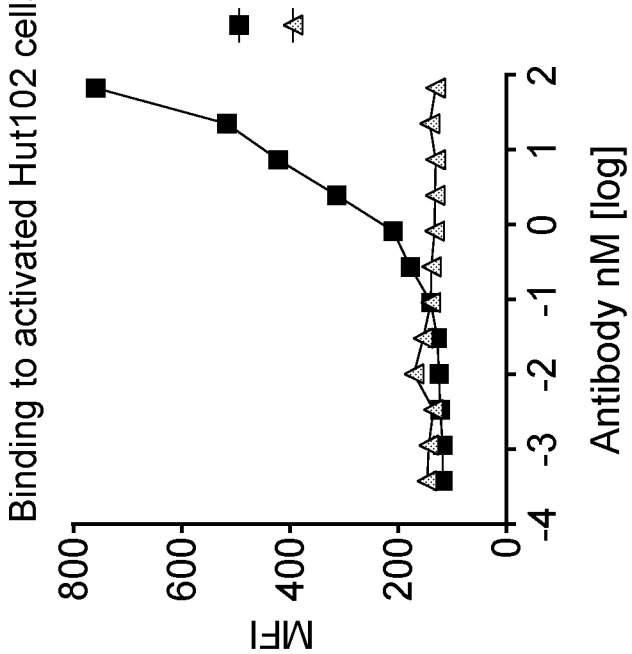
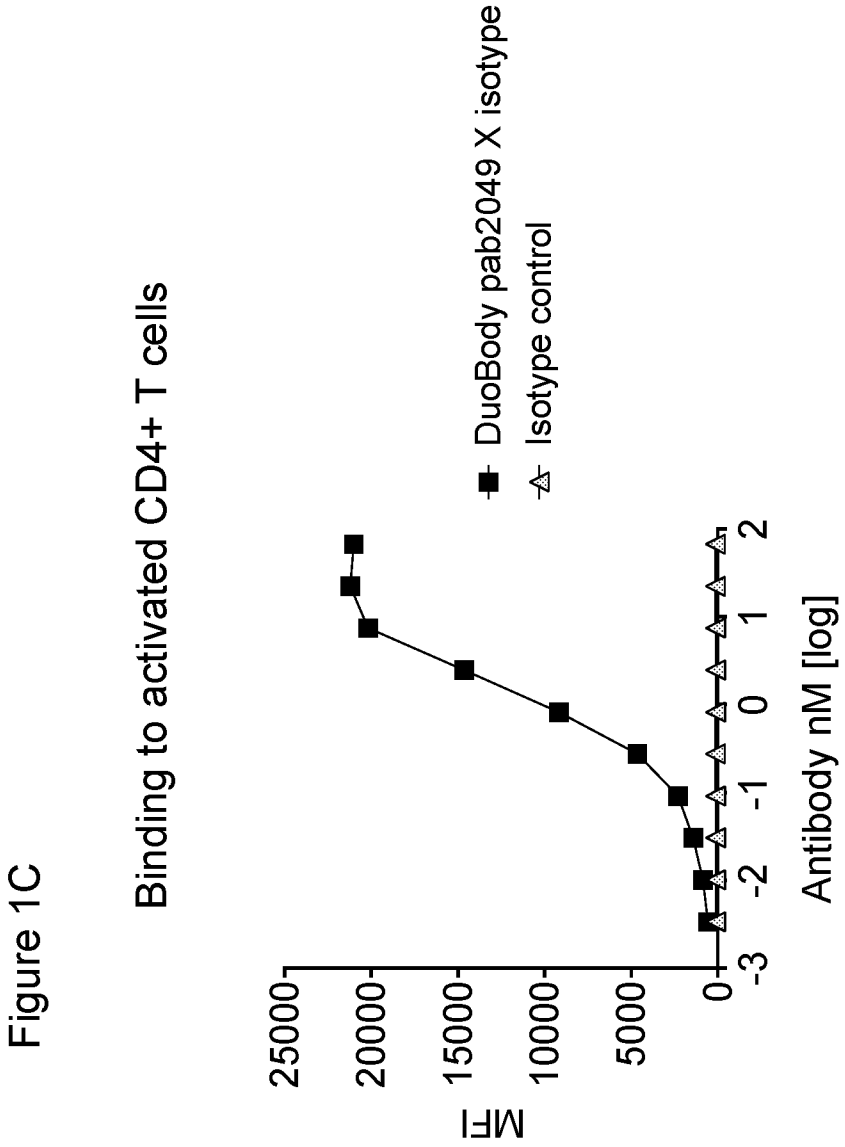


Figure 1C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DuoBody pab2049 X isotype
Isotype control
MFI
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20000
15000
10000
5000
0
-3
-2
-1
0
1
2
Antibody nM [log]

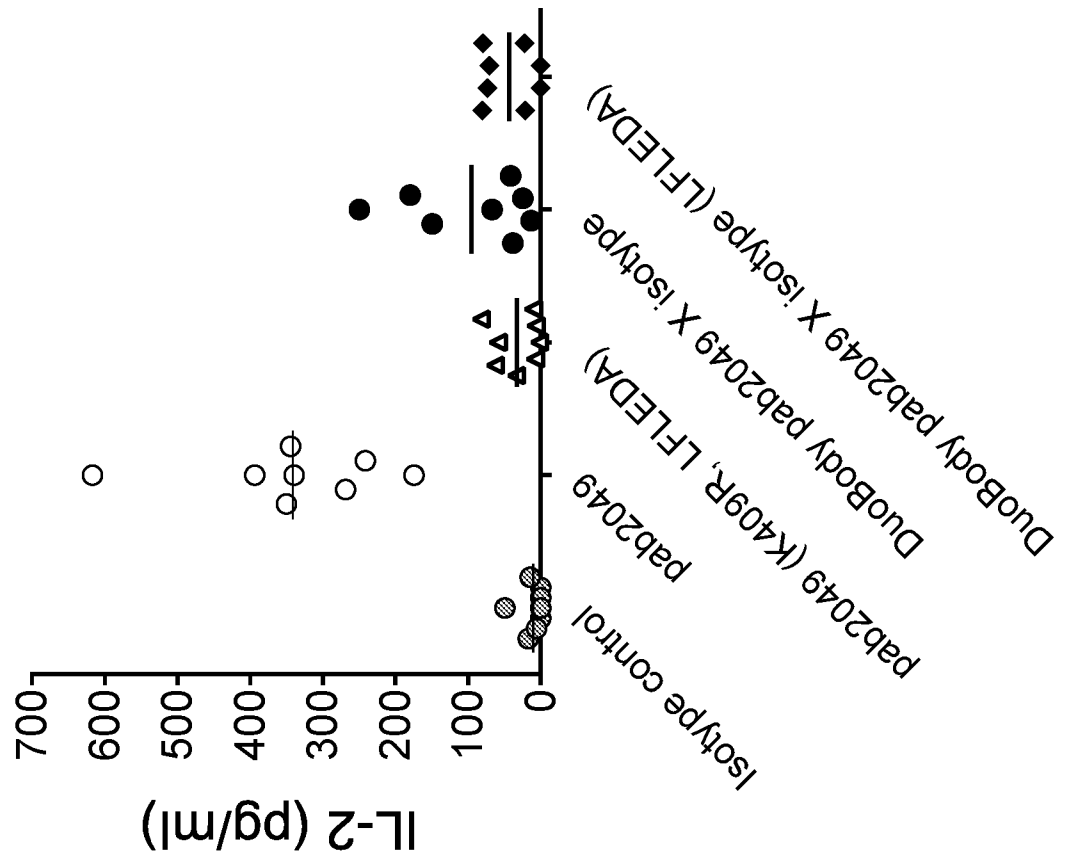
IL-2 (pg/ml)
0
100
200
300
400
500
600
700
Isotype control
pab2049
pab2049 (K409R, LFLEDA)
DuoBody pab2049 X isotype
DuoBody pab2049 X isotype (LFLEDA)

Figure 2

Figure 3A

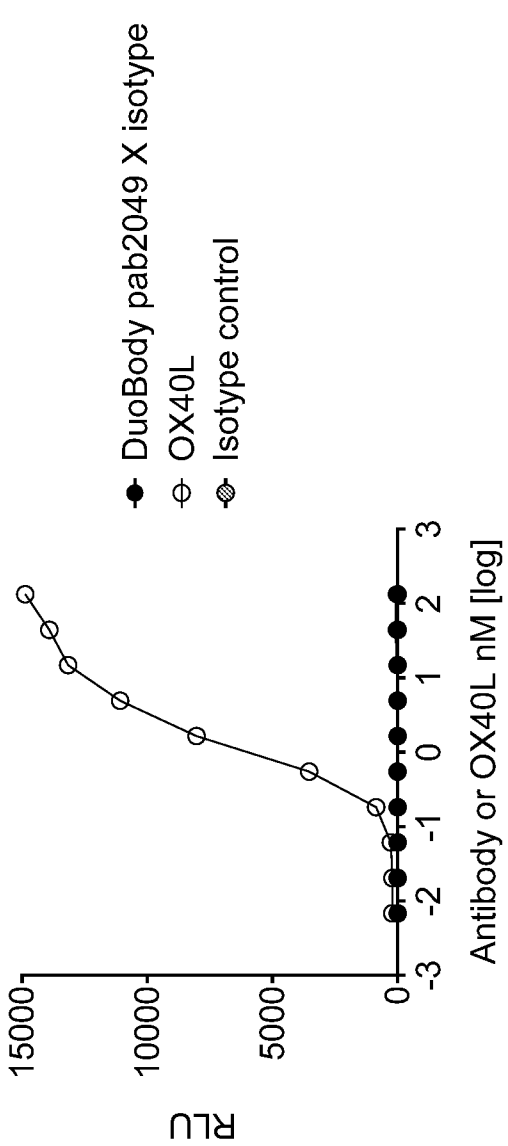

Figure 3B

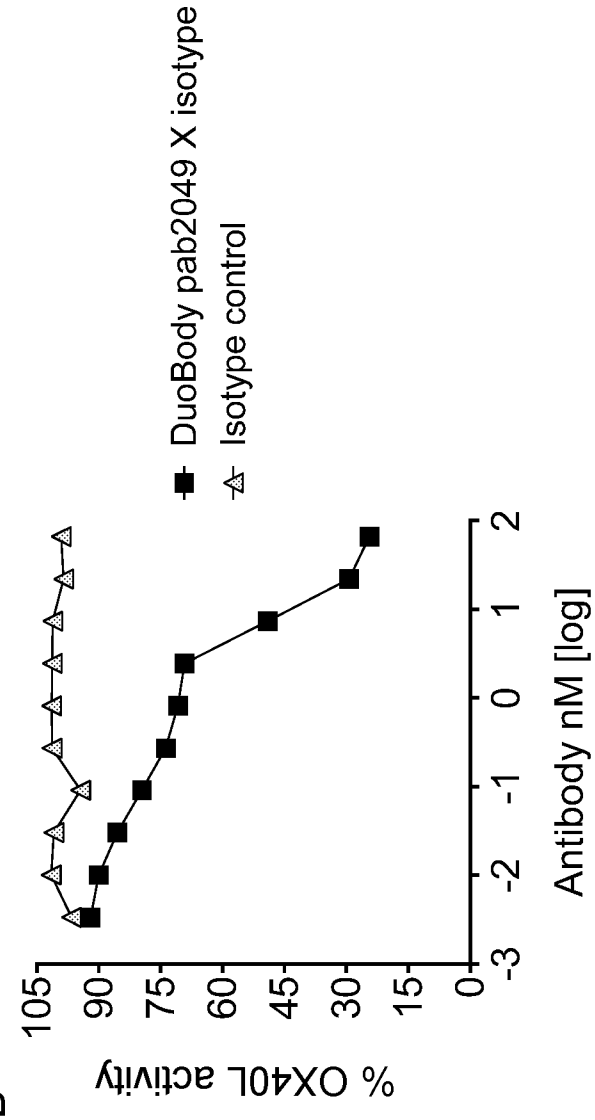

Figure 4

	pab1949w	pab2049	pab1928
W58A	-	+	-
N60A	-	-	+
R62A	-	-	+
R80A	-	-	+
L88A	-	-	+
P93A	-	-	+
P99A	-	+	+
P115A	-	+	+

INTERNATIONAL SEARCH REPORT

International application No.
PCT/US16/64794

A. CLASSIFICATION OF SUBJECT MATTER
IPC - C07K 16/28; A61K 39/395; G01N 33/68 (2017.01)
CPC - C07K 16/2878, 16/2875; A61K 39/3955; G01N 33/6863

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)
See Search History document

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched
See Search History document

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)
See Search History document

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
A	US 2013/0280275 A1 (LIU, YJ et al.) October 24, 2013; abstract; paragraph [0076]	1-5, 6/1-5, 10-14, 15/10-14, 65, 66/11-14, 67/10-14
A	WO 2011/131746 A2 (GENMAB A/S) October 27, 2011; page 35, lines 24-25	1-5, 6/1-5, 10-14, 15/10-14, 65, 66/11-14, 67/10-14
A	WO 2010/054007 A1 (FABRUS LLC) May 14, 2010; Claim 73	2, 4, 6/2, 6/4, 11, 13, 15/11, 15/13, 66/11, 66/13, 67/11, 67/13
A	US 2009/0137003 A1 (TOLSTRUP, AB, et al.) May 28, 2009; page 159	1, 6/1, 10, 15/10, 65, 67/10
A	WO 2015/095423 A2 (GENENTECH, INC.) June 25, 2015; paragraph [0057]	3, 6/3, 12, 15/12, 66/12, 67/12
A	WO 2003/030833 A2 (AMGEN INC.) April 17, 2003; Table 4	5, 6/5, 14, 15/14, 66/14, 67/14

☐ Further documents are listed in the continuation of Box C. ☐ See patent family annex.

* Special categories of cited documents:
"A" document defining the general state of the art which is not considered to be of particular relevance
"E" earlier application or patent but published on or after the international filing date
"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)
"O" document referring to an oral disclosure, use, exhibition or other means
"P" document published prior to the international filing date but later than the priority date claimed
"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention
"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone
"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art
"&" document member of the same patent family

Date of the actual completion of the international search	Date of mailing of the international search report
03 February 2017 (03.02.2017)	27 FEB 2017
Name and mailing address of the ISA/ Mail Stop PCT, Attn: ISA/US, Commissioner for Patents P.O. Box 1450, Alexandria, Virginia 22313-1450 Facsimile No. 571-273-8300	Authorized officer Shane Thomas PCT Helpdesk: 571-272-4300 PCT OSP: 571-272-7774

INTERNATIONAL SEARCH REPORT

International application No.
PCT/US16/64794

Box No. II Observations where certain claims were found unsearchable (Continuation of item 2 of first sheet)

This international search report has not been established in respect of certain claims under Article 17(2)(a) for the following reasons:

1. ☐ Claims Nos.:
because they relate to subject matter not required to be searched by this Authority, namely:

2. ☐ Claims Nos.:
because they relate to parts of the international application that do not comply with the prescribed requirements to such an extent that no meaningful international search can be carried out, specifically:

3. ☒ Claims Nos.: 7-9, 16-64
because they are dependent claims and are not drafted in accordance with the second and third sentences of Rule 6.4(a).

Box No. III Observations where unity of invention is lacking (Continuation of item 3 of first sheet)

This International Searching Authority found multiple inventions in this international application, as follows:

1. ☐ As all required additional search fees were timely paid by the applicant, this international search report covers all searchable claims.

2. ☐ As all searchable claims could be searched without effort justifying additional fees, this Authority did not invite payment of additional fees.

3. ☐ As only some of the required additional search fees were timely paid by the applicant, this international search report covers only those claims for which fees were paid, specifically claims Nos.:

4. ☐ No required additional search fees were timely paid by the applicant. Consequently, this international search report is restricted to the invention first mentioned in the claims; it is covered by claims Nos.:

Remark on Protest

☐ The additional search fees were accompanied by the applicant's protest and, where applicable, the payment of a protest fee.

☐ The additional search fees were accompanied by the applicant's protest but the applicable protest fee was not paid within the time limit specified in the invitation.

☐ No protest accompanied the payment of additional search fees.