

TITLE OF THE INVENTION

[0001] Anti-OX40L Antibodies and Methods of Use

CROSS-REFERENCE TO RELATED APPLICATIONS

[0002] Priority is claimed to U.S. Provisional Patent Applications Nos. 63/592,351 filed on 23 October 2023 and 63/642,002 filed on 3 May 2024, each of which are hereby incorporated by reference in their entirety.

SEQUENCE LISTING

[0003] The instant application contains a Sequence Listing which has been electronically submitted in XML format and is hereby incorporated by reference in its entirety. Said XML copy, created on 22 October 2024, is named 27050030WO01SEQLXML.xml and is 69,368 bytes in size.

TECHNICAL FIELD OF THE INVENTION

[0004] This disclosure generally relates to the fields of medicine and immunology. More specifically, this disclosure relates to anti-OX40L antibodies and antigen-binding fragments thereof that are useful in the treatment of a variety of autoimmune disorders.

BACKGROUND OF THE INVENTION

[0005] OX40 ligand (OX40L), also known as TNFSF4, CD252, Gp34, and CD134L, is a member of the tumor necrosis factor superfamily of proteins. The members of this family are transmembrane proteins that are predominantly expressed in immune cells and regulate a wide variety of cellular functions. OX40L binds to OX40, a member of the tumor necrosis factor receptor superfamily of proteins.

[0006] OX40 is expressed in a number of immune cell types (e.g., activated CD4⁺ T cells, activated CD8⁺ T cells, NK cells) and provides co-stimulatory functions during T cell activation, mediating the survival and expansion of both CD4⁺ and CD8⁺ T cells in both hyperproliferative and autoimmune disorders. While the mechanism by which OX40-OX40L binding affects T cell activation is not completely understood, agonism of the OX40-OX40L signaling pathway may contribute to tumor regression and prolonged survival. Conversely, there is evidence that antagonism of this pathway inhibits autoreactive T cells and ameliorates some autoimmune conditions.

[0007] Amlitelimab is a non-depleting, fully human IgG4 anti-OX40L monoclonal antibody developed to pursue this latter strategy (i.e., treating hidradenitis suppurativa, graft-versus-host disease, mild-to-moderate psoriasis, atopic dermatitis, asthma, and possibly other immune disorders). While amlitelimab appears to show promise in the treatment of moderate to severe atopic dermatitis in adults whose disease cannot adequately be controlled with topical medications, alternatives are still needed to exploit antagonizing the OX40-OX40L signaling pathway for other immune disorders.

BRIEF SUMMARY OF THE INVENTION

[0008] The present disclosure relates to methods, compositions of matter, and articles of manufacture that may be used in the treatment of autoimmune disorders as well as Atopic Dermatitis and the amelioration of symptoms thereof. To that end, provided herein are recombinantly produced antibodies and antigen-binding fragments thereof that specifically bind to OX40L. In particular, eighteen anti-OX40L antibodies annotated by internal designation numbers mcp22835 through mcp22846 and mcp32753 through mcp32758 are disclosed herein. Amino acid sequences of various regions of these antibodies are set forth in SEQ ID NOs: 1-63.

[0009] In particular embodiments, the inventions disclosed herein encompass an antibody or antigen-binding fragment thereof that specifically binds to OX40L, wherein the antibody or antigen-binding fragment thereof comprises three CDRs of a heavy chain variable region of one of the heavy chain variable region sequences set forth in one of odd-numbered SEQ ID NOs: 1-24 and comprises three CDRs of a light chain variable region having an amino acid sequence identical to one of the corresponding light chain variable region sequences set forth in one of even-numbered SEQ ID NOs: 1-24 (e.g., three CDRs of a heavy chain variable region set forth as SEQ ID NO: 13 and three CDRs of a light chain variable region set forth as SEQ ID NO: 14).

[0010] In some embodiments, the anti-OX40L antibody or antigen-binding fragment thereof comprises (a) a heavy chain variable region comprising residues 31-35 for CDR-H1, residues 50-66 for CDR-H2, and residues 99-116 for CDR-H3; and (b) a light chain variable region comprising residues 24-34 for CDR-L1, residues 50-56 for CDR-L2, and residues 89-97 for CDR-L3; wherein the CDR numbering is according to Kabat. In some embodiments, the six CDRs of the anti-OX40L antibody or antigen-binding fragment thereof are defined by reference to SEQ ID NOs. (e.g., SEQ ID NOs: 55-60).

[0011] In some embodiments, the anti-OX40L antibody or antigen-binding fragment thereof comprises a heavy chain variable region having an amino acid sequence that is at least 90%, 95%, 98%, 99%, or 100% identical to one of the heavy chain variable region sequences disclosed herein and a light chain variable region having an amino acid sequence that is at least 90%, 95%, 98%, 99%, or 100% identical to one of the light chain variable region sequences disclosed herein. In some embodiments, the anti-OX40L antibody or antigen-binding fragment thereof comprises a heavy chain variable region having an amino acid sequence identical to one of the heavy chain variable region sequences set forth in one of odd-numbered SEQ ID NOs: 1-24 and comprises a light chain variable region having an amino acid sequence identical to one of the corresponding light chain variable region sequences set forth in one of even-numbered SEQ ID NOs: 1-24 (e.g., a heavy chain variable region set forth as SEQ ID NO: 13 and a light chain variable region set forth as SEQ ID NO: 14).

[0012] In some embodiments, the anti-OX40L antibody further comprises a heavy chain constant region having an amino acid sequence that is at least 90%, 95%, 98%, 99%, or 100% identical to a heavy chain constant region of an anti-OX40L antibody disclosed herein and further comprises a light chain constant region having an amino acid sequence that is at least 90%, 95%, 98%, 99%, or 100% identical to a light chain constant region of an anti-OX40L antibody disclosed herein. In particular embodiments, the anti-OX40L antibody further comprises a heavy chain constant region having an amino acid sequence set forth as SEQ ID NO: 61 and a light chain constant region set forth as SEQ ID NO: 63 or a heavy chain constant region set forth as SEQ ID NO: 62 and a light chain constant region set forth as SEQ ID NO: 63. In some embodiments, the heavy chain constant region further comprises a C-terminal lysine residue.

[0013] In some embodiments, the anti-OX40L antibody comprises a heavy chain having an amino acid sequence that is at least 90%, 95%, 98%, 99%, or 100% identical to a heavy chain disclosed in one of the odd-numbered sequences set forth in SEQ ID NOs: 25-48 or any one of SEQ ID NOs: 49-54 and a light chain having an amino acid sequence that is at least 90%, 95%, 98%, 99%, or 100% identical to a light chain disclosed in one of the even-numbered sequences set forth in SEQ ID NOs: 25-48 (e.g., a heavy chain set forth as SEQ ID NO: 50 and a light chain set forth as SEQ ID NO: 38 or a heavy chain set forth as SEQ ID NO: 53 and a light chain set forth as SEQ ID NO: 38).

[0014] In some embodiments, the anti-OX40L antibody or antigen-binding fragment thereof is a Fab fragment, Fab' fragment, F(ab')₂ fragment, Fab'-SH fragment, Fv fragment, linear

antibody, or single-chain antibody. In other embodiments, the antibody is a full-length antibody. In yet other embodiments, the heavy chain variable region is fused to a heavy chain constant region and the light chain variable region is fused to a light chain constant region. In still yet other embodiments, the heavy chain constant region is selected from the group consisting of IgA1, IgA2, IgD, IgE, IgG1, IgG2, IgG3, IgG4, and IgM.

[0015] In some embodiments, the anti-OX40L antibody or antigen-binding fragment thereof is an Fc variant that comprises one or more amino acid substitutions to block antibody binding to FcγRI, FcγRII, or FcγRIIIa. In particular embodiments, the Fc variant comprises an amino acid substitution at a position selected from the group consisting of 234, 235, 252, 254, 256, 428, and 434, and wherein the Fc variant further comprises an amino acid deletion at position 447, and wherein amino acid numbering is according to the EU index. Examples of such embodiments of these FC variants include L234A, L235A, M428L, and N434S substitutions wherein K447 is deleted or L234A, L235A, M252Y, S254T, and T256E substitutions wherein K447 is deleted, wherein amino acid numbering is according to the EU index.

[0016] In some embodiments, the antibody or antigen-binding fragment thereof is formulated as a pharmaceutical composition. In some embodiments, the pharmaceutical composition may comprise one or more pharmaceutically acceptable carriers, diluents, or excipients.

[0017] The instant disclosure also encompasses isolated nucleic acids encoding part or all of the anti-OX40L antibodies and antigen-binding fragments thereof disclosed herein. In some embodiments, the foregoing nucleic acids may be incorporated into an expression vector. In some embodiments, the foregoing nucleic acids may be incorporated into a host cell or incorporated into an expression vector and then into a host cell. The instant disclosure also includes methods of manufacturing the anti-OX40L antibodies and antigen-binding fragments thereof disclosed herein using the aforementioned nucleic acids, expression vectors, and host cells. In particular embodiments, the methods comprise cultivating a host cell under conditions such that the antibody is expressed and recovered.

[0018] The instant disclosure also encompasses articles of manufacture useful for treating an autoimmune disorder or Atopic Dermatitis comprising a receptacle comprising an anti-OX40L antibody or antigen-binding fragment thereof, or pharmaceutical composition comprising the same, as well as instructional materials for using the same to treat the autoimmune disorder or Atopic Dermatitis.

[0019] The instant disclosure also encompasses methods of treating an autoimmune disorder or Atopic Dermatitis comprising administering to a subject a therapeutically effective amount of an anti-OX40L antibody or antigen-binding fragment thereof. Prior to administration, the antibody or antigen-binding fragment thereof may be formulated as a pharmaceutical composition.

[0020] The foregoing is a summary and thus contains, by necessity, simplifications, generalizations, and omissions of detail; consequently, those skilled in the art will appreciate that the summary is illustrative only and is not intended to be in any way limiting. Other aspects, features, and advantages of the subject matter described herein will become apparent in the teachings set forth herein. The summary is provided to introduce a selection of concepts in a simplified form that are further described below in the Detailed Description of the Invention. This summary is not intended to identify key features or essential features of the claimed subject matter, nor is it intended to be used as an aid in determining the scope of the claimed subject matter.

BRIEF SUMMARY OF THE SEVERAL VIEWS OF THE DRAWINGS

[0021] Figures 1A-1C show cell-based OX40L cross-reactivity antibody binding assay for human and cynomolgus OX40L. Figures 1A-1C show results of binding assays examining the cross-reactivity of 3 anti-OX40L antibodies (mcp22842, mcp32754, and mcp32757) compared to 1 benchmark antibody (1c_IgG4), mouse anti-rat OX40L, rat anti-mouse OS40L, and isotype control antibodies in serial dilution as depicted along the x-axis. Antibody cross-reactivity was assessed in ExpiCHO-S control cells (Fig. 1A) transiently transfected ExpiCHO-S cells expressing human (Fig. 1B) OX40L, and transiently transfected ExpiCHO-S cells expressing cynomolgus (Fig. 1C) OX40L.

[0022] Figures 2A-2C show cell-based OX40L cross-reactivity antibody binding assay for mouse and rat OX40L. Figures 2A-2C show results of binding assays examining the cross-reactivity of 3 anti-OX40L antibodies (mcp22842, mcp32754, and mcp32757) compared to 1 benchmark antibody (1c_IgG4), mouse anti-rat OX40L, rat anti-mouse OS40L, and isotype control antibodies in serial dilution as depicted along the x-axis. Antibody cross-reactivity was assessed in ExpiCHO-S control cells (Fig. 2A), transiently transfected ExpiCHO-S cells expressing rat (Fig. 2B) OX40L, and transiently transfected ExpiCHO-S cells expressing mouse (Fig. 2C) OX40L.

[0023] Figure 3 is an OX40 reporter assay for soluble OX40L stimulation. Figure 3 shows antagonism of three anti-OX40L antibodies and a benchmark antibody in the presence of soluble OX40L to prevent signaling through the OX40 receptor.

[0024] Figures 4A and 4B show the results of antibody-dependent cell cytotoxicity (ADCC) experiments. Figures 4A and 4B show the assessment of two anti-OX40L antibodies compared to two benchmarks and an isotype control to induce Fc receptor-mediated ADCC as reported in RLU values (Fig. 4A) and FOI values (Fig. 4B).

[0025] Figures 5A-5F show the results of complement dependent cytotoxicity (CDC) experiments. Figures 5A-5F show the results of assessing the CDC activity of anti-OX40L antibodies on CHO-K1 expressing OX40L cells. CDC activity was assessed in the absence of human pooled sera (no Sera), the presence of human pooled sera (Sera), and in the presence of inactivated pooled sera (Inact. Sera). The figures show a comparison of CDC activity in human pooled sera for the 2 anti-OX40L antibodies, 2 benchmark antibodies, and an isotype control antibody (Fig. 5A), and the results for each assessed molecule: amlitelimabHsIgG4PE (Fig. 5B), amlitelimabHsIgG1PE (Fig. 5C), mcp32754 (Fig. 5D), mcp32757 (Fig. 5E), and huIgG1 Isotype (Fig. 5F).

[0026] Figures 6A-6F show the results of a T cell activation assay. Figures 6A, 6C, and 6E show the results of assessing the efficacy of two anti-OX40L antibodies at inhibiting soluble OX40L-evoked primary T cell action, which is measured as IL-2 secretion. The two anti-OX40L antibodies are compared against a benchmark antibody and an isotype control antibody. Figures 6B, 6D, and 6F show the results of assessing the ability of two anti-OX40L antibodies at inducing non-specific T cell activation, which is measured as IL-2 secretion. The assessment is made in the absence of soluble OX40L and with OX40L expression on T cells. The results are grouped based on donor tcepbmc0074 (6A and 6B), tcepbmc0075 (6C and 6D), and tcepbmc0076 (6E and 6F).

[0027] Figures 7A and 7B illustrate antibody dependent cellular phagocytosis (ADCP). Figures 7A and 7B show the results of assessing the ability of two anti-OX40L antibodies to induce Fc receptor-mediated ADCP through the FcγRI. The anti-OX40L antibodies are compared to two benchmark antibodies, and an isotype control antibody. The results are illustrated as RLU values (Fig. 7A) and FOI values (Fig. 7B).

[0028] Figures 8A-8I illustrate cytokine release inhibition by anti-OX40L antibodies in human PBMCs. Figures 8A-8C show the effect of anti-OX40L blocking antibodies on normalized IL-9 secretion in human PBMCs stimulated with recombinant human thymic stromal lymphopoietin

(TSLP) and Staphylococcal enterotoxin B (SEB). Figures 8D-8F show the effect of anti-OX40L blocking antibodies on normalized IL-13 secretion in human PBMCs stimulated with TSLP and SEB. Figures 8G-8I show the effect of anti-OX40L blocking antibodies on normalized IL-5 secretion in human PBMCs stimulated with TSLP and SEB. In figures 8A-8I dose response curves in human PBMCs are presented as mean ($\pm$ SD) percentage of cytokine response (y-axis; % response) per donor, in the presence or absence of increasing concentrations (x-axis) of amlitelimab, mcp32754, and mcp32757. Data are normalized to the corresponding cytokine levels in cells stimulated in the absence of an anti-OX40L blocking antibody (No Ab, 100% response).

[0029] Figure 9 illustrates the results of an *in vitro* IgG recycling assay. Figure 9 (left panel) shows the recycled IgG concentration found in the supernatant from Madin-Darby Canine Kidney (MDCK-II) cells expressing human FcRn for 3 anti-OX40L antibodies compared to a benchmark antibody, and an isotype control antibody. Figure 9 (right panel) shows the recycled IgG concentration found in the supernatant from MDCK-II cells expressing cynomolgus monkey FcRn for 3 anti-OX40L antibodies compared to a benchmark antibody, and an isotype control antibody.

[0030] Figures 10A and 10B illustrate a pharmacokinetic (PK) study. Figures 10A and 10B show the results of pharmacokinetic studies to assess half-life of three different antibodies mcp32754 and mcp32757 (Fig. 10A) and mcp22841 (Fig. 10B) in accordance with the inventions disclosed herein.

[0031] Figures 11A-11C illustrate target binding kinetics. Figure 11 shows the results from surface plasmon resonance (SPR) kinetic experiments comparing the kinetics of binding to human OX40L (top panel) and cynomolgus OX40L (bottom panel) for anti-OX40L antibodies mcp32754 (Fig. 11A) and mcp32757 (Fig. 11B) compared to the benchmark antibody (Fig. 11C).

[0032] Figure 12 illustrates the apparent affinities for OX40L. Figure 12 shows results for binding affinity constants determined for each of mcp32757 and mcp32754 to both human OX40L and cynomolgus OX40L compared to the binding affinity constants determined for the benchmark antibody to both human OX40L and cynomolgus OX40L.

DETAILED DESCRIPTION OF THE INVENTION

[0033] While the present inventions may be embodied in many different forms, disclosed herein are specific illustrative embodiments thereof that exemplify the principles of inventions described herein. It should be emphasized that the inventions described herein are not limited to

the specific embodiments illustrated. Moreover, any section headings used herein are for organizational purposes only and are not to be construed as limiting the subject matter described.

[0034] Unless otherwise defined herein, scientific, and technical terms used in connection with the present invention shall have the meanings that are commonly understood by those of ordinary skill in the art. Further, unless otherwise required by context, singular terms shall include pluralities and plural terms shall include the singular. More specifically, as used in this specification and the appended claims, the singular forms “a,” “an” and “the” include plural referents unless the context clearly dictates otherwise. Thus, for example, reference to “a protein” includes a plurality of proteins; reference to “a cell” includes mixtures of cells, and the like. In addition, ranges provided in the specification and appended claims include both end points and all points between the end points. Therefore, a range of 1.0 to 2.0 includes 1.0, 2.0, and all points between 1.0 and 2.0.

[0035] Generally, nomenclature used in connection with, and techniques of, cell and tissue culture, molecular biology, immunology, microbiology, genetics and protein and nucleic acid chemistry and hybridization described herein are those well-known and commonly used in the art. The methods and techniques of the present invention are generally performed according to conventional methods well known in the art and as described in various general and more specific references that are cited and discussed throughout the present specification unless otherwise indicated. Enzymatic reactions and purification techniques are performed according to manufacturer's specifications, as commonly accomplished in the art, or as described herein. The nomenclature used in connection with, and the laboratory procedures and techniques of, analytical chemistry, synthetic organic chemistry, and medicinal and pharmaceutical chemistry described herein are those well-known and commonly used in the art.

[0036] Exemplary Embodiments of the Invention

[0037] In particular embodiments, the inventions disclosed herein encompass an antibody or antigen-binding fragment thereof that specifically binds OX40L, wherein the antibody or antigen-binding fragment thereof comprises three CDRs of a heavy chain variable region of one of the heavy chain variable region sequences set forth in one of odd-numbered SEQ ID NOs: 1-24 and comprises three CDRs of a light chain variable region having an amino acid sequence identical to one of the corresponding light chain variable region sequences set forth in one of even-numbered SEQ ID NOs: 1-24. For example, the inventions disclosed herein encompass an antibody or

antigen-binding fragment thereof that specifically binds OX40L, wherein the antibody or antigen-binding fragment thereof comprise three CDRs of a heavy chain variable region set forth as SEQ ID NO: 13 and three CDRs of a light chain variable region set forth as SEQ ID NO: 14.

[0038] In particular embodiments, the inventions disclosed herein encompass an antibody or antigen-binding fragment thereof, wherein the antibody or fragment thereof comprises: (a) CDR-H1 comprising residues 31-35, CDR-H2 comprising residues 50-66, and CDR-H3 comprising residues 99-116 of the heavy chain variable region (V_H); and (b) CDR-L1 comprising residues 24-34, CDR-L2 comprising residues 50-56, and CDR-L3 comprising residues 89-97 of the light chain variable region (V_L), wherein the CDR numbering is according to Kabat. In some embodiments, the six CDRs of the anti-OX40L antibody or antigen-binding fragment thereof are defined by reference to SEQ ID NOs. (e.g., SEQ ID NOs: 55-60).

[0039] In particular embodiments, the anti-OX40L antibody or antigen-binding fragment thereof comprises a heavy chain variable region having an amino acid sequence that is at least 90%, 95%, 98%, 99%, or 100% identical to one of the heavy chain variable region sequences disclosed herein and a light chain variable region having an amino acid sequence that is at least 90%, 95%, 98%, 99%, or 100% identical to one of the light chain variable region sequences disclosed herein. In particular embodiments, the anti-OX40L antibody or antigen-binding fragment thereof comprises a heavy chain variable region having an amino acid sequence identical to one of the heavy chain variable region sequences set forth in one of odd-numbered SEQ ID NOs: 1-24 and comprises a light chain variable region having an amino acid sequence identical to one of the corresponding light chain variable region sequences set forth in one of even-numbered SEQ ID NOs: 1-24 (e.g., a heavy chain variable region set forth as SEQ ID NO: 13 and a light chain variable region set forth as SEQ ID NO: 14).

[0040] In particular embodiments, the anti-OX40L antibody further comprises a heavy chain constant region having an amino acid sequence that is at least 90%, 95%, 98%, 99%, or 100% identical to a heavy chain constant region of an anti-OX40L antibody disclosed herein and further comprises a light chain constant region having an amino acid sequence that is at least 90%, 95%, 98%, 99%, or 100% identical to a light chain constant region of an anti-OX40L antibody disclosed herein. In particular embodiments, the anti-OX40L antibody further comprises a heavy chain constant region having an amino acid sequence set forth as SEQ ID NO: 61 and a light chain constant region set forth as SEQ ID NO: 63 or a heavy chain constant region set forth as SEQ ID NO: 62

and a light chain constant region set forth as SEQ ID NO: 63. In particular embodiments, the heavy chain constant region further comprises a C-terminal lysine residue.

[0041] In particular embodiments, the anti-OX40L antibody comprises a heavy chain having an amino acid sequence that is at least 90%, 95%, 98%, 99%, or 100% identical to a heavy chain disclosed in one of the odd-numbered sequences set forth in SEQ ID NOs: 25-48 or any one of SEQ ID NOs: 49-54 and a light having an amino acid sequence that is at least 90%, 95%, 98%, 99%, or 100% identical to a light chain disclosed in one of the even-numbered sequences set forth in SEQ ID NOs: 25-48 (e.g., a heavy chain set forth as SEQ ID NO: 50 and a light chain set forth as SEQ ID NO: 38 or a heavy chain set forth as SEQ ID NO: 53 and a light chain set forth as SEQ ID NO: 38).

[0042] In particular embodiments, the anti-OX40L antibody or antigen-binding fragment thereof is a Fab fragment, Fab' fragment, F(ab')₂ fragment, Fab'-SH fragment, Fv fragment, linear antibody, or single-chain antibody. In other embodiments, the antibody is a full-length antibody. In yet other embodiments, the heavy chain variable region is fused to a heavy chain constant region and the light chain variable region is fused to a light chain constant region. In still yet other embodiments, the heavy chain constant region is selected from the group consisting of IgA1, IgA2, IgD, IgE, IgG1, IgG2, IgG3, IgG4, and IgM.

[0043] In particular embodiments, the anti-OX40L antibody or antigen-binding fragment thereof is an Fc variant that comprises one or more amino acid substitutions to reduce antibody binding to FcγRI, FcγRII, or FcγRIIIa. In particular embodiments, the Fc variant comprises an amino acid substitution at a position selected from the group consisting of 234, 235, 252, 254, 256, 428, and 434, and wherein the Fc variant further comprises an amino acid deletion at position 447, and wherein amino acid numbering is according to the EU index. Examples of such embodiments of these FC variants include L234A, L235A, M428L, and N434S substitutions wherein K447 is deleted or L234A, L235A, M252Y, S254T, and T256E substitutions wherein K447 is deleted, wherein amino acid numbering is according to the EU index.

[0044] In particular embodiments, the inventions disclosed herein encompass a pharmaceutical composition comprising an antibody or antigen-binding fragment thereof described in the preceding paragraphs of this section and one or more pharmaceutically acceptable carriers, diluents, or excipients. In particular embodiments, the pharmaceutical composition comprises at least one additional antibody that binds to OX40L.

[0045] In particular embodiments, the inventions disclosed herein encompass a nucleic acid encoding a heavy chain variable region having an amino acid sequence that is identical to a heavy chain variable region described herein. In particular embodiments, the inventions disclosed herein encompass a nucleic acid encoding a light chain variable region having an amino acid sequence that is identical to a light chain variable region described herein. In particular embodiments, the inventions disclosed herein encompass a nucleic acid encoding a heavy chain having an amino acid sequence that is identical to a heavy chain described herein or a nucleic acid encoding a light chain having an amino acid sequence that is identical to the light chain described herein.

[0046] In some embodiments, the inventions disclosed herein encompass a vector comprising at least one of (a) a nucleic acid encoding a heavy chain variable region having an amino acid sequence that is identical to a heavy chain variable region described herein; and (b) a nucleic acid encoding a light chain variable region having an amino acid sequence that is identical to a light chain variable region described herein. In particular embodiments, the inventions disclosed herein encompass a vector comprising at least one of (a) a nucleic acid encoding a heavy chain having an amino acid sequence that is identical to a heavy chain described herein; and (b) a nucleic acid encoding a light chain having an amino acid sequence that is identical to a light chain described herein.

[0047] In some embodiments, the inventions disclosed herein encompass a host cell comprising at least one of (a) a nucleic acid encoding a heavy chain variable region having an amino acid sequence that is identical to a heavy chain variable region described herein; and (b) a nucleic acid encoding a light chain variable region having an amino acid sequence that is identical to a light chain variable region described herein. In other embodiments, the inventions disclosed herein encompass a host cell comprising at least one of (a) a nucleic acid encoding a heavy chain having an amino acid sequence that is identical to a heavy chain described herein; and (b) a nucleic acid encoding a light chain having an amino acid sequence that is identical to a light chain described herein.

[0048] In particular embodiments, the inventions disclosed herein encompass a process for producing an antibody or antigen-binding fragment thereof described in the preceding paragraphs of this section comprising (a) cultivating the host cell described in the preceding paragraphs under conditions such that the antibody or antigen-binding fragment thereof is expressed; and (b) recovering the expressed antibody or antigen-binding fragment thereof.

[0049] In particular embodiments, the inventions disclosed herein encompass an article of manufacture useful for treating an autoimmune disorder comprising a receptacle comprising the antibody or antigen-binding fragment thereof described in the preceding paragraphs of this section, or a pharmaceutical composition comprising the same and instructional materials for using the same to treat the autoimmune disorder.

[0050] In particular embodiments, the inventions disclosed herein encompass a method of treating or preventing an autoimmune disorder comprising administering to a subject a therapeutically effective amount of the antibody or antigen-binding fragment thereof described in the preceding paragraphs of this section or a pharmaceutical composition comprising the same. In some embodiments, the autoimmune disorder is caused by autoreactive T cells. In some embodiments, the autoimmune disorder is selected from the group consisting of Alopecia or Alopecia Areata (AA), Ankylosing spondylitis, Autoimmune Lymphoproliferative Syndrome (ALPS), Celiac disease, Crohn's Disease, Graft-versus-host disease, Graves' Disease, Guillain-Barre' syndrome, Hypersensitivity Vasculitis (HV), Inflammatory bowel disease, Myasthenia Gravis Neutropenia, Rheumatoid arthritis (RA), Sarcoidosis, Systemic lupus erythematosus (SLE), and Systemic sclerosis (scleroderma).

[0051] In some embodiments of the methods of treating or preventing an autoimmune disorder disclosed herein, the antibody or antigen-binding fragment thereof is administered via intravenous or subcutaneous injection. In some embodiments, the antibody or antigen-binding fragment thereof is administered via injection and the method comprises administering at least one injection at a dose of at least about 20 mg of the antibody or antigen-binding fragment thereof. In some embodiments, the antibody or antigen-binding fragment thereof is a disease modifying drug. In some embodiments, the antibody or antigen-binding fragment thereof is administered at least twice with at least one interval of 2 to 6 months. In some embodiments, the antibody or antigen-binding fragment thereof is administered at least twice with at least one interval of around 3 months.

[0052] In some embodiments of the methods of treating or preventing an autoimmune disorder disclosed herein, each dose of the antibody or antigen-binding fragment thereof is between about 20 mg and about 1000 mg; or each dose of the antibody or antigen-binding fragment thereof is about 75 mg, about 100 mg, about 150 mg, about 300 mg, or about 600 mg; or the dose of the antibody or antigen-binding fragment thereof is about 75 mg; or the dose of the antibody or antigen-binding fragment thereof is about 100 mg; or the dose of the antibody or antigen-binding fragment thereof is about 150 mg; or the dose of the antibody or antigen-binding fragment thereof

is about 300 mg; or the dose of the antibody or antigen-binding fragment thereof is about 600 mg; or the dose of the antibody or antigen-binding fragment thereof is between about 0.45 mg/kg and about 12 mg/kg.

[0053] In some embodiments of the methods of treating or preventing an autoimmune disorder disclosed herein, the antibody or antigen-binding fragment thereof is administered via injection and the minimum blood serum concentration reached by the antibody or antigen-binding fragment thereof between any two injections (C_{min}) is at least 2.5 $\mu\text{g/mL}$; or the maximum blood serum concentration reached by the antibody or antigen-binding fragment thereof after administration of an injection and prior to administration of a subsequent injection (C_{max}) is at least about 1.5 $\mu\text{g/mL}$; or blood serum concentrations of the antibody or antigen-binding fragment thereof during treatment range between about 1.5 $\mu\text{g/mL}$ and about 550 $\mu\text{g/mL}$; or the area under the serum concentration-time curve (AUC) following the first (AUC extrapolated to infinity [$AUC_{0-\infty}$]) injection may be at least around 100,000 $\text{ng}\cdot\text{day/mL}$ to at least around 15,500,000 $\text{ng}\cdot\text{day/mL}$.

[0054] In some embodiments, the methods of treating or preventing an autoimmune disorder disclosed herein comprise an induction phase and a maintenance phase. In some embodiments, the induction dose of the antibody or antigen-binding fragment thereof is between about 20 mg and about 1000 mg or the maintenance dose of the antibody or antigen-binding fragment thereof is between about 20 mg and about 600 mg or the antibody or antigen-binding fragment thereof is administered via injection and the interval between two or more induction phase injections is between about 2 weeks and about 24 weeks, and the interval between two or more maintenance phase injections is between about 2 weeks and about 52 weeks.

[0055] In some embodiments of the methods of treating or preventing an autoimmune disorder disclosed herein, the first injection of the antibody or antigen-binding fragment thereof can be a loading dose, wherein the loading dose of the antibody or antigen-binding fragment thereof is up to three times the mass of the treatment dose or the loading dose of the antibody or antigen-binding fragment thereof is between about 100 mg and about 600 mg. In such embodiments, the second and additional injections of the antibody or antigen-binding fragment thereof can be treatment doses of at least 5 treatment injections, wherein the treatment dose of the antibody or antigen-binding fragment is between about 50 mg and about 300 mg.

[0056] In particular embodiments, the inventions disclosed herein encompass a method of treating Atopic Dermatitis in a subject in need thereof comprising administering a therapeutically

effective amount of the antibody or antigen-binding fragment thereof described in the preceding paragraphs of this section or a pharmaceutical composition comprising the same.

[0057] In some embodiments of the methods of treating Atopic Dermatitis disclosed herein, the antibody or antigen-binding fragment thereof is administered via intravenous or subcutaneous injection. In some embodiments, the antibody or antigen-binding fragment thereof is administered via injection and the method comprises administering at least one injection at a dose of at least about 20 mg of the antibody or antigen-binding fragment thereof. In some embodiments, the antibody or antigen-binding fragment thereof is a disease modifying drug. In some embodiments, the antibody or antigen-binding fragment thereof is a disease modifying drug and the subject achieves an IGA-AD score of 0 or 1 for at least six months or the antibody or antigen-binding fragment thereof is administered at least twice with at least one interval of 2 to 6 months or the antibody or antigen-binding fragment thereof is administered at least twice with at least one interval of around 3 months.

[0058] In some embodiments of the methods of treating Atopic Dermatitis disclosed herein, each dose of the antibody or antigen-binding fragment thereof is between about 20 mg and about 1000 mg; or each dose of the antibody or antigen-binding fragment thereof is about 75 mg, about 100 mg, about 150 mg, about 300 mg, or about 600 mg; or the dose of the antibody or antigen-binding fragment thereof is about 75 mg; or the dose of the antibody or antigen-binding fragment thereof is about 100 mg; or the dose of the antibody or antigen-binding fragment thereof is about 150 mg; or the dose of the antibody or antigen-binding fragment thereof is about 300 mg; or the dose of the antibody or antigen-binding fragment thereof is about 600 mg; or the dose of the antibody or antigen-binding fragment thereof is between about 0.45 mg/kg and about 12 mg/kg.

[0059] In some embodiments of the methods of treating Atopic Dermatitis disclosed herein, the antibody or antigen-binding fragment thereof is administered via injection and the minimum blood serum concentration reached by the antibody or antigen-binding fragment thereof between any two injections (C_{min}) is at least 2.5 $\mu\text{g/mL}$; or the maximum blood serum concentration reached by the antibody or antigen-binding fragment thereof after administration of an injection and prior to administration of a subsequent injection (C_{max}) is at least about 1.5 $\mu\text{g/mL}$; or blood serum concentrations of the antibody or antigen-binding fragment thereof during treatment range between about 1.5 $\mu\text{g/mL}$ and about 550 $\mu\text{g/mL}$; or the area under the serum concentration-time curve (AUC) following the first (AUC extrapolated to infinity [$AUC_{0-\infty}$]) injection may be at least around 100,000 $\text{ng}\cdot\text{day/mL}$ to at least around 15,500,000 $\text{ng}\cdot\text{day/mL}$.

[0060] In some embodiments, the methods of treating Atopic Dermatitis disclosed herein comprise an induction phase and a maintenance phase. In some embodiments, the induction dose of the antibody or antigen-binding fragment thereof is between about 20 mg and about 1000 mg or the maintenance dose of the antibody or antigen-binding fragment thereof is between about 20 mg and about 600 mg or the antibody or antigen-binding fragment thereof is administered via injection and the interval between two or more induction phase injections is between about 2 weeks and about 24 weeks, and the interval between two or more maintenance phase injections is between about 2 weeks and about 52 weeks.

[0061] In some embodiments of the methods of treating Atopic Dermatitis disclosed herein, the first injection of the antibody or antigen-binding fragment thereof can be a loading dose, wherein the loading dose of the antibody or antigen-binding fragment thereof is up to three times the mass of the treatment dose or the loading dose of the antibody or antigen-binding fragment thereof is between about 100 mg and about 600 mg. In such embodiments, the second and additional injections of the antibody or antigen-binding fragment thereof can be treatment doses of at least 5 treatment injections, wherein the treatment dose of the antibody or antigen-binding fragment is between about 50 mg and about 300 mg.

[0062] In some embodiments of the methods of treating Atopic Dermatitis disclosed herein, the subject is at least 6 years of age and/or less than 100 years of age; or the atopic dermatitis is moderate-to-severe atopic dermatitis; or the atopic dermatitis is moderate-to-severe atopic dermatitis and wherein the subject is a patient candidate for systemic therapy; or the atopic dermatitis is moderate-to-severe atopic dermatitis and wherein the subject is a patient whose disease is not adequately controlled with topical prescription therapies or a patient for whom topical prescription therapies are not advisable; or the atopic dermatitis is resistant, non-responsive, or inadequately responsive to treatment by either topical corticosteroids and/or systemic therapies; or either topical corticosteroids and/or systemic therapies are not advisable for the subject; or the subject has had an inadequate response to, was intolerant to, or is refractory to one or more topical corticosteroids.

[0063] In some embodiments of the methods of treating Atopic Dermatitis disclosed herein, the methods further comprise administering a therapeutically effective amount of one or more topical corticosteroids. In some embodiments, the topical corticosteroid is selected from the group consisting of betamethasone dipropionate, clobetasol propionate, dexamethasone, methylprednisolone, methylprednisolone aceponate, mometasone furoate, diflorasone diacetate,

halobetasol propionate, amcinonide, augmented betamethasone dipropionate, fluocinonide, halcinonide, triamcinolone acetonide, betamethasone valerate, clocortolone pivalate, desoximetasone, fluocinolone acetonide, flurandrenolide, fluticasone propionate, hydrocortisone butyrate, hydrocortisone probutate, hydrocortisone valerate, prednicarbate, alclometasone dipropionate, desonide, gentamicin sulphate, hydrocortisone, and hydrocortisone acetate.

[0064] In some embodiments of the methods of treating Atopic Dermatitis disclosed herein, administering the antibody or antigen-binding fragment thereof results in at least one improvement selected from the group consisting of a decrease from baseline in validated investigator global assessment atopic dermatitis (vIGA-AD) score of at least 2 points, or achieving clear or almost clear skin (vIGA-AD 0/1) from baseline, or a decrease from baseline in eczema area and severity index (EASI) score of at least 50%, or a decrease from baseline in EASI score of at least 75%, or (e) achieving EASI-75, or achieving EASI-90, or achieving an improvement of at least 3 points in pruritus numerical rating scale (NRS) score, or achieving an improvement of at least 4 points in pruritus NRS score, or a decrease from baseline in affected BSA (Body Surface Area) score of at least 60%, or a decrease from baseline in affected BSA score of at least 70%.

[0065] In some embodiments of the methods of treating Atopic Dermatitis disclosed herein, administering the anti-OX40L antibody or antigen-binding fragment thereof results in a decrease in serum levels of at least one biomarker selected from the group consisting of IL-4, IL-5, IL-9, IL-13, IL-22, IL-17A, IL-31, IgE, TARC, MDC, PARC, CTACK, eosinophils, and OX40L; or a decrease in serum levels of at least one biomarker selected from the group consisting of IL-4, IL-5, IL-9, IL-13, IL-22, IL-17A, IL-31, IgE, TARC, MDC, PARC, CTACK, eosinophils, and OX40L, wherein the decrease is maintained for at least 12 weeks or at least 24 weeks following the final dose; or the atopic dermatitis has been assessed by determining a baseline EASI score; or the atopic dermatitis has been assessed by determining a baseline EASI score and wherein the post-administration EASI score is maintained, without additional administration of an anti-OX40L antibody or antigen-binding fragment thereof for at least 2 months, at least 3 months, at least 4 months, at least 5 months, or at least 6 months after administration of the last injection; or at least about 4, 8, 15, 22, 25, 29, 36, 43, 50, 57, 64, 71, 85, 113, 169, or 253 days; or the atopic dermatitis has been assessed by determining a baseline EASI score and wherein the post-administration EASI and/or further post-administration EASI is determined at least around 113 days after administering a first injection of the antibody or antigen-binding fragment thereof and wherein the post-administration EASI and/or further post-administration EASI is EASI50, EASI75, EASI90 or

EASI100; or the atopic dermatitis has been assessed by determining a baseline EASI score and wherein the post-administration EASI and/or further post-administration EASI is determined at least around 169 days after administering a first injection of the antibody or antigen-binding fragment thereof and wherein the post-administration EASI and/or further post-administration EASI is EASI50, EASI75, EASI90 or EASI100; or the atopic dermatitis has been assessed by determining a baseline EASI score and wherein the post-administration EASI and/or further post-administration EASI is determined at least around 253 days after administering a first injection of the antibody or antigen-binding fragment thereof and wherein the post-administration EASI and/or further post-administration EASI is EASI50, EASI75, EASI90 or EASI100; or the atopic dermatitis has been assessed by determining a baseline EASI score and wherein the atopic dermatitis is treated as evidenced by a reduction in the EASI score by at least 40% after the third injection as a treatment dose and wherein the reduction in EASI score is persistent for at least 2 months, at least 3 months, at least 4 months, at least 5 months, or at least 6 months after administration of the last injection as a treatment dose.

[0066] In some embodiments of the methods of treating Atopic Dermatitis disclosed herein, the Atopic Dermatitis has been assessed by determining a baseline vIGA-AD score, wherein the post-administration vIGA-AD score is 0 or 1; or the post-administration vIGA-AD score is maintained, without additional administration of an anti-OX40L antibody, or antigen-binding fragment thereof, for at least 2 months, at least 3 months, at least 4 months, at least 5 months, or at least 6 months after administration of the last injection; or at least about 4, 8, 15, 22, 25, 29, 36, 43, 50, 57, 64, 71, 85, 113, 169 or 253 days; or the post-administration vIGA-AD and/or further post-administration vIGA-AD is determined at least around 113 days after administering a first injection of the antibody or antigen-binding fragment thereof and wherein the post-administration vIGA-AD and/or further post-administration vIGA-AD is a vIGA-AD score of 0 or 1, and/or reduced at least 2 points relative to the baseline vIGA-AD score; or the post-administration vIGA-AD and/or further post-administration vIGA-AD is determined at least around 169 days after administering a first injection of the antibody or antigen-binding fragment thereof and wherein the post-administration vIGA-AD and/or further post-administration vIGA-AD is a vIGA-AD score of 0 or 1, and/or reduced at least 2 points relative to the baseline vIGA-AD score; or the post-administration vIGA-AD and/or further post-administration vIGA-AD is determined at least around 253 days after administering a first injection of the antibody or antigen-binding fragment thereof and wherein the post-administration vIGA-AD and/or further post-administration vIGA-

AD is a vIGA-AD score of 0 or 1, and/or reduced at least 2 points relative to the baseline vIGA-AD score; or the atopic dermatitis is treated as evidenced by a reduction in the vIGA-AD score by at least 2 points after the third injection as a treatment dose and wherein the reduction in vIGA-AD score is persistent for at least 2 months, at least 3 months, at least 4 months, at least 5 months, or at least 6 months after administration of the last injection as a treatment dose.

[0067] In particular embodiments, the inventions disclosed herein encompass an antibody or antigen-binding fragment thereof described in the preceding paragraphs of this section or a pharmaceutical composition comprising the same for use in the treatment of an autoimmune disease or Atopic Dermatitis.

[0068] In particular embodiments, the inventions disclosed herein encompass an antibody or antigen-binding fragment thereof described in the preceding paragraphs of this section or a pharmaceutical composition comprising the same for use in the manufacture of a medicament for the treatment of an autoimmune disease or Atopic Dermatitis.

[0069] Anti-OX40L antibodies

[0070] The present disclosure is directed to anti-OX40L antibodies and their use in the diagnosis, theragnosis, treatment and/or prophylaxis of an autoimmune disease and symptoms thereof. As used herein, the term “antibody” or “immunoglobulin” are used interchangeably and in the broadest sense and cover both intact molecules and immunologically-reactive fragments thereof. These terms cover, for example, synthetic antibodies, monoclonal antibodies, oligoclonal or polyclonal antibodies, multiclonal antibodies, recombinantly produced antibodies, monovalent antibodies, multivalent antibodies, human antibodies, humanized antibodies, chimeric antibodies, CDR-grafted antibodies, primatized antibodies, Fab fragments, F(ab') fragments, F(ab')₂ fragments, F(ab)c fragments, single-chain FvFc (scFvFc), single-chain Fvs (scFv), anti-idiotypic (anti-Id) antibodies, and any other immunologically-reactive/antigen-binding fragments thereof.

[0071] As used herein, the term “anti-OX40L antibody(ies)” describes antibodies that specifically recognize, bind to, or otherwise associate with an OX40L molecule present on a cell (e.g., a T cell) or an OX40L molecule that is solubilized. As used herein terms, “specifically recognize”, “bind”, and “binds” are intended to mean, unless indicated otherwise, the ability of a protein or molecule to form a chemical bond or attractive interaction with another protein or molecule, which results in proximity of the two proteins or molecules as determined by common methods known in the art.

[0072] Human antibodies are grouped into five distinct classes that can be distinguished biochemically and depending on the amino acid sequence of the constant domain of their heavy chains, can readily be assigned to the appropriate class. For historical reasons, the major classes of intact antibodies are termed IgA, IgD, IgE, IgG, and IgM. In humans, the IgG and IgA classes may be further divided into recognized subclasses (isotypes), i.e., IgG1, IgG2, IgG3, IgG4, IgA1, and IgA2 depending on structure and certain biochemical properties. It will be appreciated that the IgG isotypes in humans are named in order of their abundance in serum with IgG1 being the most abundant.

[0073] While all five classes of antibodies (i.e., IgA, IgD, IgE, IgG, and IgM) and all isotypes (i.e., IgG1, IgG2, IgG3, IgG4, IgA1, and IgA2), as well as variations thereof, are within the scope of the present disclosure, preferred embodiments belonging to the IgG class are discussed in some detail solely for the purposes of illustration. It will be understood that such disclosure is, however, merely demonstrative of exemplary compositions and methods and is not in any way limiting.

[0074] In this respect, human IgG immunoglobulins comprise two identical light polypeptide chains of molecular weight approximately 23,000 Daltons, and two identical heavy chains of molecular weight 53,000-70,000 depending on the isotype. Heavy-chain constant domains that correspond to the different classes of antibodies are denoted by the corresponding lower case Greek letter α , δ , ϵ , γ , and μ , respectively. The light chains of the antibodies from any vertebrate species can be assigned to one of two clearly distinct types, called kappa (κ) and lambda (λ), based on the amino acid sequences of their constant domains. Those skilled in the art will appreciate that the subunit structures and three-dimensional configurations of different classes of immunoglobulins are well known.

[0075] The four chains are joined by disulfide bonds in a "Y" configuration wherein the light chains bracket the heavy chains starting at the mouth of the "Y" and continuing through the variable region to the dual ends of the "Y". Each light chain is linked to a heavy chain by one covalent disulfide bond while two disulfide linkages in the hinge region join the heavy chains. The respective heavy and light chains also have regularly spaced intrachain disulfide bridges the number of which may vary based on the isotype of IgG.

[0076] Each heavy chain has at one end a variable region (V_H) followed by a number of constant regions. Each light chain has a variable region at one end (V_L) and a constant region at its other end; the constant region of the light chain is aligned with the first constant region of the heavy chain, and the light chain variable region is aligned with the variable region of the heavy

chain. The variable regions of both the light (V_L) and heavy (V_H) chain portions determine antigen recognition and specificity. Conversely, the constant regions of the light chain (C_L) and the heavy chain (C_{H1} , C_{H2} or C_{H3}) confer and regulate important biological properties such as secretion, transplacental mobility, circulation half-life, complement binding, and the like. By convention, the numbering of the constant region regions increases as they become more distal from the antigen binding site or amino-terminus of the antibody. Thus, the amino or N-terminus of the antibody comprises the variable region and the carboxy or C-terminus comprises the constant region. Thus, the C_{H3} and C_L regions actually comprise the carboxy-terminus of the heavy and light chain, respectively.

[0077] Portions of the variable regions of both the heavy chain and light chain differ extensively in sequence among immunoglobulins and these hypervariable sites largely define the binding and specificity of a particular antibody. These hypervariable sites manifest themselves in three segments, known as complementarity determining regions (CDRs). The more highly conserved portions of variable regions flanking the CDRs are termed framework regions (FRs). More specifically, in naturally occurring monomeric IgG antibodies, the six CDRs present on each arm of the antibody are short, non-contiguous sequences of amino acids that are specifically positioned to form the antigen-binding site as the antibody assumes its three-dimensional configuration *in vivo* or *in vitro*. CDRs encompass amino acid residues identified using any sequence or structure-based method or nomenclature system known in the art and as described below.

[0078] By way of example, CDRs may be defined using the nomenclature described by Kabat et al. (1991, NIH Publication 91-3242, National Technical Information Service, Springfield, Va.), specifically, residues 31-35 (CDR-H1), 50-66 (CDR-H2), and 99-116 (CDR-H3) in the heavy chain variable region and residues 24-34 (CDR-L1), 50-56 (CDR-L2), and 89-97 (CDR-L3) in the light chain variable region.

[0079] CDRs vary considerably from antibody to antibody (and by definition will not exhibit homology with the Kabat consensus sequences). Maximal alignment of framework residues frequently requires the insertion of spacer residues in the numbering system, to be used for the Fv region. In addition, the identity of certain individual residues at any given Kabat site number may vary from antibody chain to antibody chain due to interspecies or allelic divergence.

[0080] One skilled in the art can readily define, identify, derive and/or enumerate the CDRs as defined by Kabat et al. for each respective antibody heavy and light chain sequence set forth herein.

Accordingly, each of the subject CDRs and antibodies comprising CDRs defined by all such nomenclature are expressly included within the scope of the instant disclosure.

[0081] The framework regions comprise the remainder of the heavy and light chain variable regions and are thus comprised of a non-contiguous sequence between about 100-120 amino acids in length. For example, using the nomenclature of Kabat et al., framework region 1 corresponds to the region of the variable region encompassing amino acids 1-30; framework region 2 corresponds to the region of the variable region encompassing amino acids 36-49; framework region 3 corresponds to the region of the variable region encompassing amino acids 66-94, and framework region 4 corresponds to the region of the variable region from amino acids 103 to the end of the variable region.

[0082] The framework regions show less inter-molecular variability in amino acid sequence and largely adopt a β -sheet conformation and the CDRs form loops which connect, and in some cases form part of, the β -sheet structure. Thus, these framework regions act to form a scaffold that provides for positioning the six CDRs in correct orientation by inter-chain, non-covalent interactions. The antigen-binding site formed by the positioned CDRs defines a surface complementary to the epitope on the immunoreactive antigen. This complementary surface promotes the non-covalent binding of the antibody to the immunoreactive antigen epitope.

[0083] All or part of the heavy and light chain variable regions may be recombined or engineered using standard recombinant and expression techniques to provide or improve one or more properties of the resultant antibody. That is, the heavy or light chain variable region from a first antibody (or any portion thereof) may be mixed and matched with any selected portion of the heavy or light chain variable region from a second antibody. For example, in one embodiment, the entire light chain variable region comprising the three light chain CDRs of a first antibody may be paired with the entire heavy chain variable region comprising the three heavy chain CDRs of a second antibody. Moreover, in other embodiments, individual heavy and light chain CDRs derived from various antibodies may be mixed and matched to provide the desired antibody having optimized characteristics. Thus, an exemplary antibody may comprise three light chain CDRs from a first antibody, two heavy chain CDRs derived from a second antibody and a third heavy chain CDR from a third antibody.

[0084] With the aforementioned structural considerations in mind, those skilled in the art will appreciate that the antibodies in accordance with the invention disclosed herein may comprise any one of a number of functional embodiments. In this respect, compatible antibodies may comprise

any immunoreactive antibody (as the term is defined herein) that provides the desired physiological response in a subject. While any of the disclosed antibodies may be used in conjunction with the present teachings, certain embodiments of the invention will comprise chimeric, humanized, or human monoclonal antibodies or immunoreactive fragments thereof. Moreover, it will be understood that such configurations are not mutually exclusive and that compatible individual antibodies may comprise one or more of the functional aspects disclosed herein. For example, a compatible antibody may comprise a single chain diabody with humanized variable regions or a fully human full length antibody with Fc modifications that alter the glycosylation pattern to modulate serum half-life. Other exemplary embodiments are readily apparent to those skilled in the art and may easily be discernible as being within the scope of the invention.

[0085] Antibodies produced by naive libraries (either natural or synthetic) can be of moderate affinity (K_a of about 10^6 to 10^7 M^{-1}), but affinity maturation can also be mimicked *in vitro* by constructing and reselecting from secondary libraries as described in the art. For example, mutations can be introduced at random *in vitro* by using error-prone polymerase.

[0086] Additionally, affinity maturation can be performed by randomly mutating one or more CDRs, e.g., using PCR with primers carrying random sequences spanning the CDR of interest, in selected individual Fv clones and screening for higher affinity clones. Another approach is to recombine the V_H or V_L regions selected by phage display with repertoires of naturally occurring variable region variants obtained from unimmunized donors and screen for higher affinity in several rounds of chain reshuffling. This technique allows the production of antibodies and antibody fragments with a dissociation constant K_d (k_{off}/k_{on}) of about 10^{-9} M or less.

[0087] Regardless of the type of antibody (e.g., chimeric, humanized, etc.), those skilled in the art will appreciate that immunoreactive or antigen-binding fragments of the same may also be used. In the broadest sense, an antibody fragment comprises at least a portion of an intact antibody (e.g., a naturally occurring immunoglobulin). More particularly the term "fragment" refers to a part or portion of an antibody or antibody chain comprising fewer amino acid residues than an intact or complete antibody or antibody chain. As used herein, the term "antigen-binding fragment" refers to a polypeptide fragment of an immunoglobulin or antibody that binds antigen or competes with intact antibody (i.e., with the intact antibody from which they were derived) for antigen binding (i.e., specific binding). As used herein, antigen-binding fragments included an antibody light chain (V_L), an antibody heavy chain (V_H), a single chain antibody (scFv), a $F(ab')_2$ fragment,

a Fab fragment, an Fd fragment, an Fv fragment, single region antibody fragments, linear antibodies, single-chain antibody molecules, and multispecific antibodies formed from antibody fragments.

[0088] Those skilled in the art will also appreciate that antibody fragments can be obtained via chemical or enzymatic treatment of an intact or complete antibody or antibody chain or by recombinant means. In this regard, while various antibody fragments are defined in terms of the digestion of an intact antibody, one of skill in the art will appreciate that such fragments may be synthesized *de novo* either chemically or by using recombinant DNA methodology.

[0089] By way of example, papain digestion of antibodies produces two identical antigen-binding fragments, called Fab fragments, each with a single antigen-binding site, and a residual Fc fragment, whose name reflects its ability to crystallize readily. Pepsin treatment yields an F(ab')₂ fragment that has two antigen-binding sites and is still capable of cross-linking antigen. The Fab fragment also contains the constant region of the light chain and the first constant region (C_{H1}) of the heavy chain. Fab' fragments differ from Fab fragments by the addition of a few residues at the carboxy terminus of the heavy-chain C_{H1} region including one or more cysteines from the antibody hinge region. Fab'-SH is the designation herein for Fab' in which the cysteine residue(s) of the constant regions bear at least one free thiol group. F(ab')₂ antibody fragments originally were produced as pairs of Fab' fragments that have hinge cysteines between them. Other chemical couplings of antibody fragments are also known.

[0090] By way of further example, an Fv fragment is an antibody fragment that contains a complete antigen recognition and binding site. This region is made up of a dimer of one heavy and one light chain variable region in tight association, which can be covalent in nature, for example in scFv. It is in this configuration that the three CDRs of each variable region interact to define an antigen binding site on the surface of the V_H-V_L dimer. Collectively, the six CDRs or a subset thereof confer antigen binding specificity to the antibody. However, even a single variable region (or half of an Fv comprising only three CDRs specific for an antigen) has the ability to recognize and bind antigen, although usually at a lower affinity than the entire binding site.

[0091] In some embodiments an anti-OX40L antibody fragment, for example, is one that comprises the Fc region, retains at least one of the biological functions normally associated with the Fc region when present in an intact antibody, such as FcRn binding, antibody half-life modulation, ADCC function and complement binding. In one embodiment, an antibody fragment is a monovalent antibody that has an *in vivo* half-life substantially similar to an intact antibody.

For example, such an antibody fragment may comprise an antigen-binding arm linked to an Fc sequence capable of conferring *in vivo* stability to the fragment.

[0092] In addition to various modifications, substitutions, additions, or deletions to the variable or binding regions of anti-OX40L antibodies disclosed herein, those skilled in the art will appreciate that selected embodiments may also comprise substitutions or modifications of the constant region (i.e., the Fc region). More particularly, it is contemplated that anti-OX40L antibodies disclosed herein may contain one or more additional amino acid residue substitutions, mutations and/or modifications, which result in a compound with preferred characteristics including, but not limited to, altered pharmacokinetics, increased serum half-life, increase binding affinity, reduced immunogenicity, increased production, altered Fc ligand binding, enhanced or reduced ADCC or CDC activity, altered glycosylation and/or disulfide bonds and modified binding specificity.

[0093] As used herein, the term "Fc region" defines a C-terminal region of an immunoglobulin heavy chain, including native sequence Fc regions and variant Fc regions. Although the boundaries of the Fc region of an immunoglobulin heavy chain might vary, the human IgG heavy chain Fc region is usually defined to stretch from an amino acid residue at position Cys226, or from Pro230, to the carboxyl-terminus thereof. The C-terminal lysine (residue 447 according to the EU numbering system) of the Fc region may be removed, for example, during production or purification of the antibody, or by recombinantly engineering the nucleic acid encoding a heavy chain of the antibody. Accordingly, a composition of intact antibodies may comprise antibody populations with all K447 residues removed, antibody populations with no K447 residues removed, and antibody populations having a mixture of antibodies with and without the K447 residue. A functional Fc region possesses an effector function of a native sequence Fc region. Exemplary effector functions include C1q binding; CDC; Fc receptor binding; ADCC; phagocytosis; down regulation of cell surface receptors (e.g., B cell receptor; BCR), etc. Such effector functions generally require the Fc region to be combined with a binding region (e.g., an antibody variable region) and can be assessed using various assays as disclosed, for example, in definitions herein.

[0094] As used herein, the term "Fc receptor" or FcR, describes a receptor that binds to the Fc region of an antibody. In some embodiments, an FcR is a native human FcR. In some embodiments, an FcR is one that binds an IgG antibody (a gamma receptor) and includes receptors of the FcγRI, FcγRII, and FcγRIII subclasses, including allelic variants and alternatively spliced

forms of those receptors. FcγII receptors include FcγRIIA (an activating receptor) and FcγRIIB (an inhibiting receptor), which have similar amino acid sequences that differ primarily in the cytoplasmic regions thereof.

[0095] Activating receptor FcγRIIA contains an immunoreceptor tyrosine-based activation motif (ITAM) in its cytoplasmic region. Inhibiting receptor FcγRIIB contains an immunoreceptor tyrosine-based inhibition motif (ITIM) in its cytoplasmic region. Methods of measuring binding to FcRn are known in the art.

[0096] As used herein, "complement dependent cytotoxicity" or CDC refers to the lysing of a target cell in the presence of complement. The complement activation pathway is initiated by the binding of the first component of the complement system (C1q) to a molecule, an antibody for example, complexed with a cognate antigen. To assess complement activation, a CDC assay may be performed.

[0097] "Antibody-dependent cell-mediated cytotoxicity" or ADCC refers to a form of cytotoxicity in which secreted Ig bound onto Fc receptors (FcRs) present on certain cytotoxic cells (e.g., Natural Killer (NK) cells, neutrophils, and macrophages) enables these cytotoxic effector cells to bind specifically to an antigen-bearing target cell and subsequently kill the target cell with cytotoxins. Lysis of the target cell is extracellular, requires direct cell-to-cell contact, and does not involve complement.

[0098] "Antibody-dependent cellular phagocytosis" or ADCP is a mechanism by which antibody-opsonized target cells activate the FcγRs on the surface of macrophages to induce phagocytosis, resulting in internalization and degradation of the target cell through phagosome acidification. ADCP can be measured by, e.g., by labeling target cells with a pH sensitive dye that only fluoresces in mature phagosomes.

[0099] Variants of anti-OX40L antibodies disclosed herein that have altered FcR binding affinity or ADCC activity are those that have either enhanced or diminished FcR binding activity and/or ADCC activity compared to a parent or unmodified antibody or comprising a native sequence Fc region. A variant antibody that displays increased binding to an FcR binds at least one FcR with better affinity than the parent or unmodified antibody comprising a native sequence Fc region. A variant antibody that displays decreased binding to an FcR, binds at least one FcR with worse affinity than the parent or unmodified antibody comprising a native sequence Fc region. Such variants which display decreased binding to an FcR may possess little or no appreciable binding to an FcR, e.g., 0-20% binding to the FcR compared to a native sequence IgG Fc region,

e.g., as determined by techniques well known in the art. In some embodiments, the anti-OX40L antibodies disclosed herein have enhanced ADCC activities.

[00100] As to FcRn, anti-OX40L antibodies disclosed herein can also encompass Fc variants with modifications to the constant region that provide half-lives (e.g., serum half-lives) in a mammal, preferably a human, of greater than 5 days, greater than 10 days, greater than 15 days, preferably greater than 20 days, greater than 25 days, greater than 30 days, greater than 35 days, greater than 40 days, greater than 45 days, greater than 2 months, greater than 3 months, greater than 4 months, or greater than 5 months. The increased half-lives of the antibodies (or Fc containing molecules) of the present invention in a mammal, preferably a human, results in a higher serum titer of antibodies or antibody fragments in the mammal, and thus, reduces the frequency of the administration of antibodies or antibody fragments and/or reduces the concentration of antibodies or antibody fragments to be administered. Antibodies having increased *in vivo* half-lives can be generated by techniques known to those of skill in the art. For example, antibodies with increased *in vivo* half-lives can be generated by modifying (e.g., substituting, deleting, or adding) amino acid residues identified as involved in the interaction between the Fc region and the FcRn receptor. Binding to human FcRn *in vivo* and serum half-life of human FcRn high affinity binding polypeptides can be assayed, e.g., in transgenic mice or transfected human cell lines expressing human FcRn, or in primates to which the polypeptides with a variant Fc region are administered.

[00101] In some embodiments, variants of anti-OX40L antibodies disclosed herein can encompass an Fc region with modifications that improve their half-lives (e.g., serum half-lives) in a human. Studies have shown that some Fc mutation that enhances FcRn binding results in increased binding to rheumatoid factor (RF), whereas some Fc mutation combinations enhance FcRn binding and prolong antibody half-life without increased binding to RF, e.g., N434A/Y436T/Q438R/S440E (ACT1), N434A/Y436V/Q438R/S440E (ACT2), M428L/N434A/Y436T/Q438R/S440E (ACT3), M428L/N434A/Y436V/Q438R/S440E (ACT4), M428L/N434A/Q438R/S440E (ACT5) (positions numbered according to EU Index numbering). In some embodiments, the anti-OX40L antibodies disclosed herein comprise an Fc region comprising any set of mutations selected from ACT1, ACT2, ACT3, ACT4, or ACT5. In some embodiments, antibodies in accordance with the instant inventions comprise one or more mutations: L234A, L235A, M252Y, S254T, T256E, M248L, N434S, and K447del.

[00102] In still other embodiments, glycosylation patterns or compositions of the anti-OX40L antibodies disclosed herein may be modified. More particularly, preferred embodiments may comprise one or more engineered glycoforms, i.e., an altered glycosylation pattern or altered carbohydrate composition that is covalently attached to a molecule comprising an Fc region. Engineered glycoforms may be useful for a variety of purposes, including but not limited to enhancing or reducing effector function, increasing the affinity of the antibody for a target antigen or facilitating production of the antibody. In cases where reduced effector function is desired, it will be appreciated that the antibody may be engineered to express in an aglycosylated form. Such carbohydrate modifications can be accomplished by, for example, altering one or more sites of glycosylation within the antibody sequence. That is, one or more amino acid substitutions can be made that result in elimination of one or more variable region framework glycosylation sites to thereby eliminate glycosylation at that site. Conversely, enhanced effector functions or improved binding may be imparted to the Fc containing molecule by adding one or more additional glycosylation sites.

[00103] Additionally, or alternatively, an Fc variant can be made that has an altered glycosylation composition, such as a hypofucosylated antibody having reduced amounts of fucosyl residues or an antibody having increased bisecting GlcNAc structures. These and similar altered glycosylation patterns have been demonstrated to increase the ADCC ability of antibodies. Engineered glycoforms may be generated by any method known to one skilled in the art, for example by using engineered or variant expression strains, by co-expression with one or more enzymes (for example N-acetylglucosaminyltransferase III (GnTII 1)), by expressing a molecule comprising an Fc region in various organisms or cell lines from various organisms or by modifying carbohydrate(s) after the molecule comprising Fc region has been expressed.

[00104] In some embodiments, variants of the anti-OX40L antibodies described herein have reduced fucosylation. In some embodiments, such variants comprise an Fc region comprising N-glycoside-linked sugar chains bound to the Fc region, wherein the sugar chains do not contain fucose. In some embodiments, such variants have increased ADCC activities, compared to the same antibodies comprising N-glycoside-linked sugar chains that comprise fucose.

[00105] The amino acid sequences of the antibodies described in the foregoing paragraphs are provided below:

mAb mcp22835 VH (SEQ ID NO: 1)

EVQVLESGGGLVQPGGSLRLSCAASGFRFSSYAMNWVRQAPGKGLEWVSIISGSGGFTYYADSVKGRFTISR
DNSKKTLYLQMHSLRAEDTAVYYCAKDYIVAAGTFDPWGQGTLTVTVSS

mAb mcp22835 VL (SEQ ID NO: 2)

AIQLTQSPSSLSASVGDRTITCRASQGISSALAWYQQKPGKTPKLLIYDASSLESGVPSRFSGSGSGTDFTLTIS
NLQPEDFATYYCQQFKNYPYTFGQGTKLEIK

mAb mcp22836 VH (SEQ ID NO: 3)

EVQLVQSGAEVKKPGESLKISCKSGSHSFTKSWIAWVRQMPGKGLEWMGIIYPDDSNTRYSPSFQGGQVTM
SADKSIRTAYLQWSSLKASDTAIYYCARGLAMITGDVWGKGTTVTVSS

mAb mcp22836 VL (SEQ ID NO: 4)

DIQMTQSPSSLSASVGDRTITCRASQGIHDLGWYQQKPGKAPKRLIFAASSLEDGVPSRFSGSGSGTDFTL
TISSLQPEDFATYYCLQHHSYPYTFGQGTKLYIK

mAb mcp22837 VH (SEQ ID NO: 5)

QVQLVQSGAEVKKPGASVKVSCKASGYTFNNYDINWVRQAAGQGLEWMGWMNPNIGKAGYSQKFQGR
VTMTRDSTRTAYMELSSLRSEDTAVYYCARQAYDLWSGHHSSWFDPWGQGTLTVTVSS

mAb mcp22837 VL (SEQ ID NO: 6)

DIQLTQSPSFLSASVGDRTFTCRASQGISSYLAWYHQKPGKVPKLLIYASTLQSGVPSRFSGSGSGTEFTLTIS
ALQPEDFATYYCQVNSYPFTFGPGTKVAFK

mAb mcp22838 VH (SEQ ID NO: 7)

EVQLVESGGGLVQPGGSLRLSCAASGFTFSNAWMSWVRQAPGKGLDWVGRIKSKTDGGKIDYAAPVKGRF
TISRDDSKNTLYLQMNSLKTEDTAVYYCNTEKYFEFWTGYSDDFDIWGQGTMTVTVSS

mAb mcp22838 VL (SEQ ID NO: 8)

DIQMTQSPSSLSASVGDRTITCRASQDISNHLAWYQQKPGKVPKLLIYPASTLHSGVPSRFSGSGSGTIFTLT
ISSLQPEDVATFYCQKYNAPLTFGGGTKVGVK

mAb mcp22839 VH (SEQ ID NO: 9)

QVKLVQSGAEVKKPGASVKVSCKASDYTFSSYGISWVRQAPGRGLEWMGWISAHSGNTNYEEKFQGRVT
MNKDTSTSTAYMELRRLRSDDTAVYYCVRDLGYDSSGGYYPNWFDSWGQGTLTVTVSS

mAb mcp22839 VL (SEQ ID NO: 10)

DIQMTQSPSSVSASVGDRTITCRASQGIRTWLAWYQQKPGKAPKALIYAASRLRGGVPSRFSGSGSGTDFT
LTISSLQPEDFATYYCQQGNSFPLTFGGGTKVEIK

mAb mcp22840 VH (SEQ ID NO: 11)

EVQLLESGGGLVQPGGSLRLSCAASGFTFSSYAMTWVRQAPGKGLEWVSAISGSGGSTKYADSVKGRFTISR
DNSKTTLYLKMNSLRAEDTAVYWCVKDLGFGELTDVWGKGTTVTVSS

mAb mcp22840 VL (SEQ ID NO: 12)

DIQMTQSPSSLSASVGDRVITITCRASQGIRNDLDWYQQKPGKAPKRLIYAASSLQSGVPSRFSGSGSGTEFTL
TISSLQPEDFATYYCLQHSSFPWTFGQGTEVEIK

mAb mcp22841 // mcp32754 // mcp32756 // mcp32757 // vrc3576 VH (SEQ ID NO: 13)
QIHLVESGGGVVQPGRSLRLSCVASGFTFSNYGIHWVRQSPGKGLEWVALIWYDGTNRYNTDTVRGRFTIS
RDN SKNTLYLQMNSLRAEDTAVYYCARDETA AVGRRGNYYYYMDVWGKGTTVTVSS

mAb mcp22841 // mcp32754 // mcp32756 // mcp32757 // vrc3576 VL (SEQ ID NO: 14)
DIQMTQSPSSLSASVGDRVITITCRTSQSISNFLNWYQQKPGKAPKLLIYAESDLQSGVPSRFSGSGSGTDFTLA
ISSLQPEDFATYYCQGY SAPFTFGPGTKVDIK

mAb mcp22842 VH (SEQ ID NO: 15)
EVKLVESGGGLVKPGGSLRLSCVASGFTFSNAWMNWVRQAPGKGLEWVGRIKSKTDGGAIDYAAPVKGRF
SISRDDSENTLSLQMNSLKTEDTAVYYCTTGTYFEIFTGYYNVDYWGQGT LVTVSS

mAb mcp22842 VL (SEQ ID NO: 16)
DIQMTQSPSSLSASVGDRVITITCRASQGISNHLVWYQQKPGKVPKLLIFAASLTQSGVPSRFSGSGSGTDFTL
TISSLQPEDVATYYCQKYN SAPLTFGGGTKVDIK

mAb mcp22843 VH (SEQ ID NO: 17)
EVQLVESGGGLVKPGGSLRLSCAASGFTFSYAWMSWVRQAPGKGLEWIGRIKSKTDSGTIDYAE PVKGRFTI
SRDDSRDTLYLQMNGLKTEDTAVYFCTTMTYFEILTGYSHLDYWGQ GALVTVSS

mAb mcp22843 VL (SEQ ID NO: 18)
GIQMTQSPSSLSASVGDRVITITCRASQDISSHLVWYQQKPGKVPKLLIYAASALQPGVPSRFSGSGSGTDFTL
TISSLQPEDVATYYCQKFNSAPYTFGQGTKLEIK

mAb mcp22844 VH (SEQ ID NO: 19)
EVQLVESGGGLVQPGGSLRLSCAASGFTFRNYAMTWVRQTPGKGLEWVSDISGSGGSTKYADSVRGRFTIS
RDN SKKMLYLQMNSLRAEDTALYYCVKDIGFSAYEDYWGQGT LVTVSS

mAb mcp22844 VL (SEQ ID NO: 20)
DIQMTQSPSSLSASVGDRITITCRASQDIRDDLWYQQKPGKAPKRLIYAASSLQSGVPSRFSGSGSGTEFTLT
ISSLQPEDFATYSCLQHNRYPWTFGQGTKVEIK

mAb mcp22845 VH (SEQ ID NO: 21)
QVQLQESGPGLVKPSSETLSICTVSGGSISTYFWSWIRQPAGKGLEWIGRIYSSWR TNYNPSLKS RV TMSVDT
SKNQFSLKLRSVTAADTAVYYCAREEMSKDFYYYYMDVWGKGTTVTVSS

mAb mcp22845 VL (SEQ ID NO: 22)
EVVLTQSPGTLSPGERATLSCRASQSVSSSYLAWYQQKPGQAPRLIYGPSSRATGIPDRFSGSGSGTDFT
LTISRLEPEDFAVYFCQY GSSITFGQGTRLENK

mAb mcp22846 // mcp32753 // mcp32755 // mcp32758 // vrc3521 VH (SEQ ID NO: 23)

EVQLLESGGGLVQPGGSLRLSCAASGFTFNYYAMTWVRQGPKGLEWVSLISGSGGLTKYADFEGRFTISR
DNSKKTLYLQMNSLRVEDTAVYYCAKDGINMVRGVIPMDVWGRGTTVTVSS

mAb mcp22846 // mcp32753 // mcp32755 // mcp32758 // vrc3270 VL (SEQ ID NO: 24)
AIQVTQSPSSLSASVGDRVTITCRASQGIRNDLGWYQQKPGKAPKLLIYAASSLQSGVPSRFSGSGSGTDFTLT
ISSLPEDFATYYCLQDYNYPYTFGQGTKLEIK

mAb mcp22835 Heavy Chain (SEQ ID NO: 25)
EVQVLESGGGLVQPGGSLRLSCAASGFRFSYAMNWWVRQAPKGLEWVSIISGSGGFTYYADSVKGRFTISR
DNSKKTLYLQMHSLRAEDTAVYYCAKDYIVAAGTFDPWGQGTTLTVSSASTKGPSVFPLAPSSKSTSGGTAA
LGCLVKDYFPEPVTVSWNSGALTSGVHTFPAVLQSSGLYSLSSVVPSSSLGTQTYICNVNHKPSNTKVDKK
VEPKSCDKHTHTCPPCPAPELLGGPSVFLFPPKPKDTLMISRTPEVTCVVDVSHEDPEVKFNWYVDGVEVHN
AKTKPREEQYASTYRVVSVLTVLHQDWLNGKEYKCKVSNKALPAPIEKTISKAKGQPREPQVYTLPPSREEMT
KNQVSLTCLVKGFYPDSIAVEWESNGQPENNYKTPPVLDSDGSFFLYSKLTVDKSRWQQGNVFSCSVLHEA
LHSHYTKQKSLSLSPG

mAb mcp22835 Light Chain (SEQ ID NO: 26)
AIQLTQSPSSLSASVGDRVTITCRASQGISSALAWYQQKPGKTPKLLIYDASSLESQVPSRFSGSGSGTDFTLTIS
NLQPEDFATYYCQKFNYPYTFGQGTKLEIKRTVAAPSVFIFPPSDEQLKSGTASVVCLLNNFYPREAKVQWK
VDNALQSGNSQESVTEQDSKSTYLSSTLTLSKADYEKHKVYACEVTHQGLSSPVTKSFNRGEC

mAb mcp22836 Heavy Chain (SEQ ID NO: 27)
EVQLVQSGAEVKKPGESLKISCKGSGHSFTKSWIAWVRQMPKGLEWMGIYPPDDSNTRYSPSFQGGQVTM
SADKSIRTAYLQWSSLKASDTAIYYCARGLAMITGDVWGKGTTLTVSSASTKGPSVFPLAPSSKSTSGGTAAL
GCLVKDYFPEPVTVSWNSGALTSGVHTFPAVLQSSGLYSLSSVVPSSSLGTQTYICNVNHKPSNTKVDKKV
EPKSCDKHTHTCPPCPAPELLGGPSVFLFPPKPKDTLMISRTPEVTCVVDVSHEDPEVKFNWYVDGVEVHNA
KTKPREEQYASTYRVVSVLTVLHQDWLNGKEYKCKVSNKALPAPIEKTISKAKGQPREPQVYTLPPSREEMTK
NQVSLTCLVKGFYPDSIAVEWESNGQPENNYKTPPVLDSDGSFFLYSKLTVDKSRWQQGNVFSCSVLHEAL
HSHYTKQKSLSLSPG

mAb mcp22836 Light Chain (SEQ ID NO: 28)
DIQMTQSPSSLSASVGDRVTITCRASQGIHDLGWYQQKPGKAPKRLIFAASSLEDGVPSRFSGSGSGTDFTL
TISSLPEDFATYYCLQHHSYPYTFGQGTKLYIKRTVAAPSVFIFPPSDEQLKSGTASVVCLLNNFYPREAKVQ
WKVDNALQSGNSQESVTEQDSKSTYLSSTLTLSKADYEKHKVYACEVTHQGLSSPVTKSFNRGEC

mAb mcp22837 Heavy Chain (SEQ ID NO: 29)
QVQLVQSGAEVKKPGASVKVSCKASGYTFNYYDINWVRQAAGQGLEWMGWMNPNIGKAGYSQKFQGR
VTMTRDTSTRAYMELSLRSEDVAVYYCARQAYDLWSGHSSWFDPWGQGTTLTVSSASTKGPSVFPLAP
SSKSTSGGTAALGCLVKDYFPEPVTVSWNSGALTSGVHTFPAVLQSSGLYSLSSVVPSSSLGTQTYICNVNH
KPSNTKVDKKVEPKSCDKHTHTCPPCPAPELLGGPSVFLFPPKPKDTLMISRTPEVTCVVDVSHEDPEVKFNW
YVDGVEVHNAKTKPREEQYASTYRVVSVLTVLHQDWLNGKEYKCKVSNKALPAPIEKTISKAKGQPREPQVY
TLPPSREEMTKNQVSLTCLVKGFYPDSIAVEWESNGQPENNYKTPPVLDSDGSFFLYSKLTVDKSRWQQGN
VFSCSVLHEALHSHYTKQKSLSLSPG

mAb mcp22837 Light Chain (SEQ ID NO: 30)

DIQLTQSPSFLSASVGDRVTFTCRASQGISSYLAWYHQKPGKVPKLLIYASTLQSGVPSRFSGSGSGTEFTLTIS
ALQPEDFATYYCQQVNSYPFTFGPGTKVAFKRTVAAPSVFIFPPSDEQLKSGTASVVCLLNNFYPREAKVQWK
VDNALQSGNSQESVTEQDSKDYSLSTLTLSKADYEKHKVYACEVTHQGLSSPVTKSFNRGEC

mAb mcp22838 Heavy Chain (SEQ ID NO: 31)

EVQLVESGGGLVQPGGSLRLSCAASGFTFSNAWMSWVRQAPGKGLDWVGRIKSKTDGGKIDYAAPVKGRF
TISRDDSKNTLYLQMNSLKTEDTAVYYCNTEKYFEFWTGYSDDFDIWGQGTMTVSSASTKGPSVFPLAPSS
KSTSGGTAALGCLVKDYFPEPVTVSWNSGALTSGVHTFPAVLQSSGLYSLSSVTVPSSSLGTQTYICNVNHK
PSNTKVDKKVEPKSCDKTHTCPPCPAPELLGGPSVFLFPPKPKDTLMISRTPEVTCVVDVSHEDPEVKFNWY
VDGVEVHNAKTKPREEQASTYRVVSVLTVLHQDWLNGKEYKCKVSNKALPAPIEKTISKAKGQPREPQVY
LPPSREEMTKNQVSLTCLVKGFYPSDIAVEWESNGQPENNYKTPPVLDSDGSFFLYSKLTVDKSRWQQGN
VFSCSVLHEALHSHYTQKSLSLSPG

mAb mcp22838 Light Chain (SEQ ID NO: 32)

DIQMTQSPSSLSASVGDRVTITCRASQDISNHLAWYQQKPGKVPKLLIYPASTLHSGVPSRFSGSGSGTIFTLT
SSLQPEDVATFYCQKYNAPLTFGGGTKVGVKRTVAAPSVFIFPPSDEQLKSGTASVVCLLNNFYPREAKVQW
KVDNALQSGNSQESVTEQDSKDYSLSTLTLSKADYEKHKVYACEVTHQGLSSPVTKSFNRGEC

mAb mcp22839 Heavy Chain (SEQ ID NO: 33)

QVKLVQSGAEVKKPGASVKVSKASDYTFSSYGISWVRQAPGRGLEWMGWISAHSGNTNYYEKFQGRVT
MNKDTSTSTAYMELRRLRSDDTAVYYCVRDLGYDSSGGYYPNWFDSWGQGTLTVSSASTKGPSVFPLAPS
SKSTSGGTAALGCLVKDYFPEPVTVSWNSGALTSGVHTFPAVLQSSGLYSLSSVTVPSSSLGTQTYICNVNHK
PSNTKVDKKVEPKSCDKTHTCPPCPAPELLGGPSVFLFPPKPKDTLMISRTPEVTCVVDVSHEDPEVKFNWY
VDGVEVHNAKTKPREEQASTYRVVSVLTVLHQDWLNGKEYKCKVSNKALPAPIEKTISKAKGQPREPQVY
LPPSREEMTKNQVSLTCLVKGFYPSDIAVEWESNGQPENNYKTPPVLDSDGSFFLYSKLTVDKSRWQQGN
VFSCSVLHEALHSHYTQKSLSLSPG

mAb mcp22839 Light Chain (SEQ ID NO: 34)

DIQMTQSPSSVSASVGDRVTITCRASQGIRTWLAWYQQKPGKAPKALIYAASRLRGGVPSRFSGSGSGTDFT
LTISLQPEDFATYYCQQGNSFPLTFGGGTKVEIKRTVAAPSVFIFPPSDEQLKSGTASVVCLLNNFYPREAKVQ
WKVDNALQSGNSQESVTEQDSKDYSLSTLTLSKADYEKHKVYACEVTHQGLSSPVTKSFNRGEC

mAb mcp22840 Heavy Chain (SEQ ID NO: 35)

EVQLLESGGGLVQPGGSLRLSCAASGFTFSYAMTWVRQAPGKGLEWVSAISGSGGSTKYADSVKGRFTISR
DNSKTTLYLKMNSLRAEDTAVYWCVKDLGFGELTDVWGKGTITVSSASTKGPSVFPLAPSSKSTSGGTAAL
GCLVKDYFPEPVTVSWNSGALTSGVHTFPAVLQSSGLYSLSSVTVPSSSLGTQTYICNVNHKPSNTKVDKKV
EPKSCDKTHTCPPCPAPELLGGPSVFLFPPKPKDTLMISRTPEVTCVVDVSHEDPEVKFNWYVDGVEVHNA
KTKPREEQASTYRVVSVLTVLHQDWLNGKEYKCKVSNKALPAPIEKTISKAKGQPREPQVYTLPPSREEMTK
NQVSLTCLVKGFYPSDIAVEWESNGQPENNYKTPPVLDSDGSFFLYSKLTVDKSRWQQGNVFSCSVLHEAL
HSHYTQKSLSLSPG

mAb mcp22840 Light Chain (SEQ ID NO: 36)

DIQMTQSPSSLSASVGDRVITITCRASQGIRNDLDWYQQKPGKAPKRLIYAASSLQSGVPSRFSGSGSGTEFTL
TISSLQPEDFATYYCLQHSSFPWTFGGQTEVEIKRTVAAPSVFIFPPSDEQLKSGTASVVCLLNNFYPREAKVQ
WKVDNALQSGNSQESVTEQDSKDYSLSTLTLSKADYEKHKVYACEVTHQGLSSPVTKSFNRGEC

mAb mcp22841 Heavy Chain (SEQ ID NO: 37)

QIHLVESGGGVVQPGRLSLSCVASGFTFSNYGIHWVRQSPGKGLEWVALIWYDGTNRYNTDTRGRFTIS
RDNSKNTLYLQMNSLRAEDTAVYYCARDATAAVGRRGNYYYYMDVWGKGTITVTVSSASTKGPSVFPLAPSS
KSTSGGTAALGCLVKDYFPEPVTVSWNSGALTSGVHTFPAVLQSSGLYSLSSVTVPSSSLGTQTYICNVNHK
PSNTKVDKKVEPKSCDKTHTCPPCPAPELLGGPSVFLFPPKPKDTLMISRTPEVTCVVDVSHEDPEVKFNWY
VDGVEVHNAKTKPREEQYASTYRVVSVLTVLHQDWLNGKEYKCKVSNKALPAPIEKTISKAKGQPREPQVYT
LPPSREEMTKNQVSLTCLVKGFYPSDIAVEWESNGQPENNYKTPPVLDSDGSFFLYSKLTVDKSRWQQGN
VFSCSVLHEALHSHYTQKSLSLSPG

mAb mcp22841 // mcp32754 // mcp32756 // mcp32757 // Light Chain exc3674 (SEQ ID NO: 38)

DIQMTQSPSSLSASVGDRVITICRTSQSISNFWYQQKPGKAPKLLIYAESDLQSGVPSRFSGSGSGTDFTLA
ISSLQPEDFATYYCQYGSAFTFGPGTKVDIKRTVAAPSVFIFPPSDEQLKSGTASVVCLLNNFYPREAKVQW
KVDNALQSGNSQESVTEQDSKDYSLSTLTLSKADYEKHKVYACEVTHQGLSSPVTKSFNRGEC

mAb mcp22842 Heavy Chain (SEQ ID NO: 39)

EVKLVESGGGLVKPGGSLRLSCVASGFTFSNAWMNVWRQAPGKGLEWVGRIKSKTDGGAIDYAAPVKGRF
SISRDDSENTLSLQMNSLKTEDTAVYYCTGTGYFEIFTGYNVVDYWGQGLTVTVSSASTKGPSVFPLAPSSKST
SGGTAALGCLVKDYFPEPVTVSWNSGALTSGVHTFPAVLQSSGLYSLSSVTVPSSSLGTQTYICNVNHKPSN
TKVDKKVEPKSCDKTHTCPPCPAPELLGGPSVFLFPPKPKDTLMISRTPEVTCVVDVSHEDPEVKFNWYVDG
VEVHNAKTKPREEQYASTYRVVSVLTVLHQDWLNGKEYKCKVSNKALPAPIEKTISKAKGQPREPQVYTLPPS
REEMTKNQVSLTCLVKGFYPSDIAVEWESNGQPENNYKTPPVLDSDGSFFLYSKLTVDKSRWQQGNVFSC
SVLHEALHSHYTQKSLSLSPG

mAb mcp22842 Light Chain (SEQ ID NO: 40)

DIQMTQSPSSLSASVGDRVITVTCRASQGISNHLVWYQQKPGKVPKLLIFAASTLQSGVPSRFSGSGSGTDFTL
TISSLQPEDVATYYCQKYNAPLTFGGGTQVDIKRTVAAPSVFIFPPSDEQLKSGTASVVCLLNNFYPREAKVQ
WKVDNALQSGNSQESVTEQDSKDYSLSTLTLSKADYEKHKVYACEVTHQGLSSPVTKSFNRGEC

mAb mcp22843 Heavy Chain (SEQ ID NO: 41)

EVQLVESGGGLVKPGGSLRLSCAASGFTFSYAWMSWVRQAPGKGLEWIGRIKSKTDSGTIDYAEPVKGRFTI
SRDDSRDTLYLQMNGLKTEDTAVYFCTTMTYFEILTGYSHLDYWGQALVTVSSASTKGPSVFPLAPSSKSTS
GGTAALGCLVKDYFPEPVTVSWNSGALTSGVHTFPAVLQSSGLYSLSSVTVPSSSLGTQTYICNVNHKPSNT
KVDKKVEPKSCDKTHTCPPCPAPELLGGPSVFLFPPKPKDTLMISRTPEVTCVVDVSHEDPEVKFNWYVDG
VEVHNAKTKPREEQYASTYRVVSVLTVLHQDWLNGKEYKCKVSNKALPAPIEKTISKAKGQPREPQVYTLPPS
REEMTKNQVSLTCLVKGFYPSDIAVEWESNGQPENNYKTPPVLDSDGSFFLYSKLTVDKSRWQQGNVFSC
SVLHEALHSHYTQKSLSLSPG

mAb mcp22843 Light Chain (SEQ ID NO: 42)

GIQMTQSPSSLSASVGDRTITCRASQDISSHLVWYQQKPGKVPKLLIYAASALQPGVPSRFSGSGSGTDFTL
TISSLQPEDVATYYCQKFNSAPYTFGQGTLKLEIKRTVAAPSVFIFPPSDEQLKSGTASVCLNNFYPRKAVQ
WKVDNALQSGNSQESVTEQDSKSTYLSSTLTLSKADYEKHKVYACEVTHQGLSSPVTKSFNRGEC

mAb mcp22844 Heavy Chain (SEQ ID NO: 43)

EVQLVESGGGLVQPGGSLRLSCAASGFTFRNYAMTWVRQTPGKGLEWVSDISGSGGSTKYADSVRGRFTIS
RDNSKKMLYLQMNSLR AEDTALYYCVKDIGFSAYEDYWGQGLTVTVSSASTKGPSVFPLAPSSKSTSGGTAA
LGCLVKDYFPEPVTVSWNSGALTSGVHTFPAVLQSSGLYSLSSVTVPSSSLGTQTYICNVNHKPSNTKVDKK
VEPKSCDKHTHTCPPCPAPELLGGPSVFLFPPKPKDTLMISRTPEVTCVVDVSHEDPEVKFNWYVDGVEVHN
AKTKPREEQYASTYRVVSVLTVLHQDWLNGKEYKCKVSNKALPAPIEKTISKAKGQPREPQVYTLPPSREEMT
KNQVSLTCLVKGFYPSDIAVEWESNGQPENNYKTPPVLDSDGSFFLYSKLTVDKSRWQQGNVFCFSLHEA
LHSHYTQKSLSLSPG

mAb mcp22844 Light Chain (SEQ ID NO: 44)

DIQMTQSPSSLSASVGDRLTITCRASQDIRDDLWYQQKPGKAPKRLIYAASSLQSGVPSRFSGSGSGTEFTLT
ISSLPEDFATYSCQLHNRYPWTFGQGTKEIKRTVAAPSVFIFPPSDEQLKSGTASVCLNNFYPRKAVQ
WKVDNALQSGNSQESVTEQDSKSTYLSSTLTLSKADYEKHKVYACEVTHQGLSSPVTKSFNRGEC

mAb mcp22845 Heavy Chain (SEQ ID NO: 45)

QVQLQESGPGLVKPSETLSICTVSGGSISTYFWSWIRQPAGKGLEWIGRIYSSWRTNYPNPSLKSRTVMSVDT
SKNQFSLKLRSVTAADTAVYYCAREEMSKDFYYYYMDVWGKTTTVTVSSASTKGPSVFPLAPSSKSTSGGTA
ALGCLVKDYFPEPVTVSWNSGALTSGVHTFPAVLQSSGLYSLSSVTVPSSSLGTQTYICNVNHKPSNTKVDK
KVEPKSCDKHTHTCPPCPAPELLGGPSVFLFPPKPKDTLMISRTPEVTCVVDVSHEDPEVKFNWYVDGVEVH
NAKTKPREEQYASTYRVVSVLTVLHQDWLNGKEYKCKVSNKALPAPIEKTISKAKGQPREPQVYTLPPSREEM
TKNQVSLTCLVKGFYPSDIAVEWESNGQPENNYKTPPVLDSDGSFFLYSKLTVDKSRWQQGNVFCFSLHE
ALHSHYTQKSLSLSPG

mAb mcp22845 Light Chain (SEQ ID NO: 46)

EVVLTQSPGTLSMSPGERATLSCRASQSVSSSYLAWYQQKPGQAPRLLIYGPSRATGIPDRFSGSGSGTDFT
LTISRLEPEDFAVYFCQYQYSSITFGQGTRLENKRTVAAPSVFIFPPSDEQLKSGTASVCLNNFYPRKAVQ
WKVDNALQSGNSQESVTEQDSKSTYLSSTLTLSKADYEKHKVYACEVTHQGLSSPVTKSFNRGEC

mAb mcp22846 Heavy Chain (SEQ ID NO: 47)

EVQLLES GGGLVQPGGSLRLSCAASGFTFNYYAMTWVRQGP G KGLEWVSLISGSGGLTKYADFVEGRFTISR
DNSKKTLYLQMNSLRVEDTAVYYCAKDGINMVRGVIPMDVWGRGTTTVTVSSASTKGPSVFPLAPSSKSTSG
GTAALGCLVKDYFPEPVTVSWNSGALTSGVHTFPAVLQSSGLYSLSSVTVPSSSLGTQTYICNVNHKPSNTK
VDKKVEPKSCDKHTHTCPPCPAPELLGGPSVFLFPPKPKDTLMISRTPEVTCVVDVSHEDPEVKFNWYVDGV
EVHNAKTKPREEQYASTYRVVSVLTVLHQDWLNGKEYKCKVSNKALPAPIEKTISKAKGQPREPQVYTLPPSR
EEMTKNQVSLTCLVKGFYPSDIAVEWESNGQPENNYKTPPVLDSDGSFFLYSKLTVDKSRWQQGNVFCFSL
VLHEALHSHYTQKSLSLSPG

mAb mcp22846 // mcp32753 // mcp32755 // mcp32758 Light Chain exc3701 (SEQ ID NO: 48)

AIQVTQSPSSLSASVGDRVITITCRASQGIRNDLGWYQQKPGKAPKLLIYAASSLQSGVPSRFSGSGSGTDFTLT
ISSLQPEDFATYYCLQDYNYPYTFGQGTLEIKRTVAAPSVFIFPPSDEQLKSGTASVCLLNNFYPREAKVQW
KVDNALQSGNSQESVTEQDSKDYSLSTLTLSKADYEKHKVYACEVTHQGLSSPVTKSFNRGEC

mAb mcp32753 Heavy Chain exc36681 (SEQ ID NO: 49)

EVQLLESGGGLVQPGGSLRLSCAASGFTFNYYAMTWVRQGPGLGLEWVSLISGSGGLTKYADFVEGRFTISR
DNSKKTLYLQMNSLRVEDTAVYYCAKDGINMVRGVIPMDVWGRGTTTVSSASTKGPSVFPLAPSSKSTSGG
TAALGCLVKDYFPEPVTWSWNSGALTSGVHTFPAVLQSSGLYSLSSVTVPSSSLGTQTYICNVNHKPSNTKVD
KKVEPKSCDKTHTCPPCPAPEAAGGPSVFLFPPKPKDTLMISRTPEVTCVVDVSHEDPEVKFNWYVDGVEV
HNAKTKPREEQYNSTYRVVSVLTVLHQDWLNGKEYKCKVSNKALPAPIEKTISKAKGQPREPQVYTLPPSREE
MTKNQVSLTCLVKGFYPSDIAVEWESNGQPENNYKTPPVLDSDGSFFLYSKLTVDKSRWQQGNVFSCSVLH
EALHSHYTQKSLSLSPG

mAb mcp32754 Heavy Chain exc36682 (SEQ ID NO: 50)

QIHLVESGGGVVQPGSLRLSCVASGFTFSNYGIHWVRQSPGKLEWVALIWDGTNRYNTDVRGRFTISR
DNSKNTLYLQMNSLRAEDTAVYYCARDETAAGVRRGNYYYYMDVWGKGTTVTVSSASTKGPSVFPLAPSSKS
TSGGTAALGCLVKDYFPEPVTWSWNSGALTSGVHTFPAVLQSSGLYSLSSVTVPSSSLGTQTYICNVNHKPSN
TKVDKKVEPKSCDKTHTCPPCPAPEAAGGPSVFLFPPKPKDTLMISRTPEVTCVVDVSHEDPEVKFNWYVD
GVEVHNAKTKPREEQYNSTYRVVSVLTVLHQDWLNGKEYKCKVSNKALPAPIEKTISKAKGQPREPQVYTLPP
SREEMTKNQVSLTCLVKGFYPSDIAVEWESNGQPENNYKTPPVLDSDGSFFLYSKLTVDKSRWQQGNVFSCS
VLHEALHSHYTQKSLSLSPG

mAb mcp32755 Heavy Chain exc36683 (SEQ ID NO: 51)

EVQLLESGGGLVQPGGSLRLSCAASGFTFNYYAMTWVRQGPGLGLEWVSLISGSGGLTKYADFVEGRFTISR
DNSKKTLYLQMNSLRVEDTAVYYCAKDGINMVRGVIPMDVWGRGTTTVTVSSASTKGPSVFPLAPSSKSTSGG
TAALGCLVKDYFPEPVTWSWNSGALTSGVHTFPAVLQSSGLYSLSSVTVPSSSLGTQTYICNVNHKPSNTKVD
KKVEPKSCDKTHTCPPCPAPELLGGPSVFLFPPKPKDTLYITREPEVTCVVDVSHEDPEVKFNWYVDGVEVH
NAKTKPREEQYASTYRVVSVLTVLHQDWLNGKEYKCKVSNKALPAPIEKTISKAKGQPREPQVYTLPPSREEMT
KNQVSLTCLVKGFYPSDIAVEWESNGQPENNYKTPPVLDSDGSFFLYSKLTVDKSRWQQGNVFSCSVMH
LHNHYTQKSLSLSPG

mAb mcp32756 Heavy Chain exc36684 (SEQ ID NO: 52)

QIHLVESGGGVVQPGSLRLSCVASGFTFSNYGIHWVRQSPGKLEWVALIWDGTNRYNTDVRGRFTISR
DNSKNTLYLQMNSLRAEDTAVYYCARDETAAGVRRGNYYYYMDVWGKGTTVTVSSASTKGPSVFPLAPSSKS
TSGGTAALGCLVKDYFPEPVTWSWNSGALTSGVHTFPAVLQSSGLYSLSSVTVPSSSLGTQTYICNVNHKPSN
TKVDKKVEPKSCDKTHTCPPCPAPELLGGPSVFLFPPKPKDTLYITREPEVTCVVDVSHEDPEVKFNWYVDG
EVHNAKTKPREEQYASTYRVVSVLTVLHQDWLNGKEYKCKVSNKALPAPIEKTISKAKGQPREPQVYTLPPSRE
EMTKNQVSLTCLVKGFYPSDIAVEWESNGQPENNYKTPPVLDSDGSFFLYSKLTVDKSRWQQGNVFSCSV
HEALHNHYTQKSLSLSPG

mAb mcp32757 Heavy Chain exc36685 (SEQ ID NO: 53)

QIHLVESGGGVVQPGSLRLSCVASGFTFSNYGIHWVRQSPGKLEWVALIWDGTNRYNTDVRGRFTISR
DNSKNTLYLQMNSLRAEDTAVYYCARDETAAGVRRGNYYYYMDVWGKGTTVTVSSASTKGPSVFPLAPSSKS
TSGGTAALGCLVKDYFPEPVTWSWNSGALTSGVHTFPAVLQSSGLYSLSSVTVPSSSLGTQTYICNVNHKPSN

TKVDKKVEPKSCDKTHTCPPCPAPEAAGGPSVFLFPPKPKDTLYITREPEVTCVVVDVSHEDPEVKFNWYVDG
VEVHNAKTKPREEQYNSTYRVVSVLTVLHQDWLNGKEYKCKVSNKALPAPIEKTISKAKGQPREPQVYTLPPS
REEMTKNQVSLTCLVKGFYPSDIAVEWESNGQPENNYKTTTPVLDSDGSFFLYSKLTVDKSRWQQGNVFSCS
VMHEALHNHYTQKSLSLSPG

mAb mcp32758 Heavy Chain exc36686 (SEQ ID NO: 54)

EVQLLESGGGLVQPGGSLRLSCAASGFTFNYYAMTWVRQGPGLVSLISGSGGLTKYADFEVGRFTISR
DNSKKTLYLQMNSLRVEDTAVYYCAKDGIMVIRGVIPMDVWGRGTTVTSSASTKGPSVFPLAPSSKSTSGG
TAALGCLVKDYFPEPVTWSWNSGALTSGVHTFPAVLQSSGLYSLSSVVTVPSSSLGTQTYICNVNHKPSNTKVD
KKVEPKSCDKTHTCPPCPAPEAAGGPSVFLFPPKPKDTLYITREPEVTCVVVDVSHEDPEVKFNWYVDGVEVH
NAKTKPREEQYNSTYRVVSVLTVLHQDWLNGKEYKCKVSNKALPAPIEKTISKAKGQPREPQVYTLPPSREEM
TKNQVSLTCLVKGFYPSDIAVEWESNGQPENNYKTTTPVLDSDGSFFLYSKLTVDKSRWQQGNVFSCSVMHE
ALHNHYTQKSLSLSPG

mAb mcp32754 // mcp32757 CDR-H1 (SEQ ID NO: 55)

NYGIH

mAb mcp32754 // mcp32757 CDR-H2 (SEQ ID NO: 56)

LIWYDGTNRYNTDTRVG

mAb mcp32754 // mcp32757 CDR-H3 (SEQ ID NO: 57)

DETAAGRRGNYYYYMDV

mAb mcp32754 // mcp32757 CDR-L1 (SEQ ID NO: 58)

RTSQSISNFLN

mAb mcp32754 // mcp32757 CDR-L2 (SEQ ID NO: 59)

AESDLQS

mAb mcp32754 // mcp32757 CDR-L3 (SEQ ID NO: 60)

QQGYSAPFT

mAb mcp32754 CH (SEQ ID NO: 61)

ASTKGPSVFPLAPSSKSTSGGTAALGCLVKDYFPEPVTWSWNSGALTSGVHTFPAVLQSSGLYSLSSVVTVPSSSL
GTQTYICNVNHKPSNTKVDKKVEPKSCDKTHTCPPCPAPEAAGGPSVFLFPPKPKDTLMISRTPEVTCVVVDV
SHEDPEVKFNWYVDGVEVHNAKTKPREEQYNSTYRVVSVLTVLHQDWLNGKEYKCKVSNKALPAPIEKTISK
AKGQPREPQVYTLPPSREEMTKNQVSLTCLVKGFYPSDIAVEWESNGQPENNYKTTTPVLDSDGSFFLYSKLTV
DKSRWQQGNVFSCSVLHEALHSHYTQKSLSLSPG

mAb mcp32757 CH (SEQ ID NO: 62)

ASTKGPSVFPLAPSSKSTSGGTAALGCLVKDYFPEPVTWSWNSGALTSGVHTFPAVLQSSGLYSLSSVVTVPSS
SLGTQTYICNVNHKPSNTKVDKKVEPKSCDKTHTCPPCPAPEAAGGPSVFLFPPKPKDTLYITREPEVTCVVVD
VSHEDPEVKFNWYVDGVEVHNAKTKPREEQYNSTYRVVSVLTVLHQDWLNGKEYKCKVSNKALPAPIEKTIS
KAKGQPREPQVYTLPPSREEMTKNQVSLTCLVKGFYPSDIAVEWESNGQPENNYKTTTPVLDSDGSFFLYSKL
TVDKSRWQQGNVFSCSVLHEALHSHYTQKSLSLSPG

mAb mcp32754 // mcp32757 CL (SEQ ID NO: 63)
RTVAAPSVFIFPPSDEQLKSGTASVVCLLNNFYPREAKVQWKVDNALQSGNSQESVTEQDSKDSSTLSSTLT
LSKADYEEKHKVYACEVTHQGLSSPVTKSFNRGEC

[00106] Characterization of Anti-OX40L antibodies

[00107] Anti-OX40L antibodies disclosed herein exhibit one or more desirable characteristics. Thus, anti-OX40L antibody-producing cells (e.g., human B cells) may be selected, cloned, and further screened for these desirable characteristics including, for example, robust growth, high antibody production, or desirable antibody characteristics. Hybridomas can be expanded *in vivo* in syngeneic animals, in animals that lack an immune system, e.g., nude mice, or in cell culture *in vitro*. Methods of selecting, cloning, and expanding hybridomas and/or colonies, each of which produces a discrete antibody species, are well known to those of ordinary skill in the art.

[00108] For example, anti-OX40L antibodies disclosed herein may be characterized by their epitope specificity. Anti-OX40L antibodies disclosed herein will associate with, or bind to, discrete epitopes or determinants presented by the selected target(s). As used herein, the term "epitope" refers to that portion of the target antigen capable of being recognized and specifically bound by a particular antibody. Epitopes can be formed both from contiguous amino acids and noncontiguous amino acids juxtaposed by tertiary folding of a protein. Epitopes formed from contiguous amino acids are typically retained upon protein denaturing, whereas epitopes formed by tertiary folding are typically lost upon protein denaturing. An epitope typically includes at least 3, and more usually, at least 5 to 10 amino acids in a unique spatial conformation. More specifically, the skilled artisan will appreciate the term epitope includes any protein determinant capable of specific binding to an immunoglobulin or T-cell receptor or otherwise interacting with a molecule. Epitopic determinants generally consist of chemically active surface groupings of molecules such as amino acids or carbohydrate or sugar side chains and generally have specific three-dimensional structural characteristics, as well as specific charge characteristics. Additionally, an epitope may be linear or conformational. In a linear epitope, all of the points of interaction between the protein and the interacting molecule (such as an antibody) occur linearly along the primary amino acid sequence of the protein. In a conformational epitope, the points of interaction occur across amino acid residues on the protein that are linearly separated from one another.

[00109] The term "epitope" refers to the amino acid residues, of an antigen, that are bound by an antibody. An epitope can be a linear epitope, a conformational epitope, or a hybrid epitope. An epitope can be determined according to different experimental techniques, also called "epitope mapping techniques." It is understood that the determination of an epitope may vary based on the different epitope mapping techniques used and may also vary with the different experimental conditions used, e.g., due to the conformational changes or cleavages of the antigen induced by specific experimental conditions. Epitope mapping techniques are known in the art, including, but not limited to, X-ray crystallography, nuclear magnetic resonance (NMR) spectroscopy, electron microscopy, site-directed mutagenesis, species swap mutagenesis, alanine-scanning mutagenesis, hydrogen-deuterium exchange (HDX), and cross-blocking assays.

[00110] Once a desired epitope on an antigen is determined, it is possible to generate antibodies to that epitope, e.g., by immunizing with a peptide comprising the epitope using techniques known in the art. Alternatively, during the discovery process, the generation and characterization of antibodies may elucidate information about desirable epitopes. From this information, it is then possible to competitively screen antibodies for binding to the same epitope. An approach to achieve this is to conduct competition studies to find antibodies that competitively bind with one another, i.e., the antibodies compete for binding to the antigen. A high throughput process for binning antibodies based upon their cross-competition is known in the art.

[00111] As used herein, the term "binning" refers to a method to group antibodies based on their antigen binding characteristics. The grouping is somewhat arbitrary, depending on how different the observed binding patterns of the antibodies tested. Thus, while the technique is a useful tool for categorizing antibodies, the bins do not always directly correlate with epitopes and such initial determinations of epitope binding should be further confirmed by other art recognized methodology.

[00112] With this caveat one can determine whether a selected antibody (or fragment thereof) binds to the same epitope or cross-competes for binding with a second antibody by using methods known in the art. If the second antibody is able to bind OX40L, then the second antibody binds to a different epitope than the first antibody. However, if the second antibody is not able to bind, then the second antibody binds to the same epitope, an overlapping epitope, or an epitope that is in close proximity to the epitope bound by the first antibody. As known in the art, the desired data can be obtained using solid phase direct or indirect radioimmunoassay (RIA), solid phase direct or

indirect enzyme immunoassay (EIA), sandwich competition assay, surface plasmon resonance, bio-layer interferometry, or flow cytometric methodology.

[00113] As used herein, the term "compete" means competition between antibodies as determined by an assay in which the antibody or immunologically-reactive fragment under test prevents or inhibits specific binding of a reference antibody to a common antigen. Typically, such an assay involves the use of purified antigen bound to a solid surface or cells bearing either of these, an unlabeled test immunoglobulin and a labeled reference immunoglobulin. Competitive inhibition is 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. Antibodies identified by competition assay (competing antibodies) include antibodies binding to the same epitope as the reference antibody and antibodies binding to an adjacent epitope sufficiently proximal to the epitope bound by the reference antibody for steric hindrance to occur. Additional details regarding methods for determining competitive binding are provided in the Examples herein. 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 40%, 45%, 50%, 55%, 60%, 65%, 70% or 75%. In some instances, binding is inhibited by at least 80%, 85%, 90%, 95%, or 97% or more.

[00114] Anti-OX40L antibodies disclosed herein may also be characterized by their binding affinity. In some embodiments, anti-OX40L antibodies specifically bind to a target antigen, i.e., the dissociation constant K_d (k_{off}/k_{on}) is $\leq 10^{-8}M$. The antibody specifically binds its antigen with high affinity when the K_d is $\leq 5 \times 10^{-9}M$, and with very high affinity when the K_d is $\leq 5 \times 10^{-10}M$. In particular embodiments, the antibody has a K_d of $\leq 10^{-9}M$ and an off-rate of about $1 \times 10^{-4}/sec$. In other embodiments, the off-rate is $\leq 1 \times 10^{-5}/sec$. In other embodiments, the antibodies will bind with a K_d of between about $10^{-8}M$ and $10^{-10}M$, and in yet other embodiments, antibodies bind with a $K_d \leq 2 \times 10^{-10}M$. Still other selected embodiments comprise antibodies that have a disassociation constant or K_d (k_{off}/k_{on}) of less than $10^{-2}M$, less than $5 \times 10^{-2}M$, less than $10^{-3}M$, less than $5 \times 10^{-3}M$, less than $10^{-4}M$, less than $5 \times 10^{-4}M$, less than $10^{-5}M$, less than $5 \times 10^{-5}M$, less than $10^{-6}M$, less than $5 \times 10^{-6}M$, less than $10^{-7}M$, less than $5 \times 10^{-7}M$, less than $10^{-8}M$, less than $5 \times 10^{-8}M$, less than $10^{-9}M$, less than $5 \times 10^{-9}M$, less than $10^{-10}M$, less than $5 \times 10^{-10}M$, less than $10^{-11}M$, less than $5 \times 10^{-11}M$, less than $10^{-12}M$, less than $5 \times 10^{-12}M$, less than $10^{-13}M$, less than $5 \times 10^{-13}M$, less than $10^{-14}M$, less than $5 \times 10^{-14}M$, less than $10^{-15}M$, or less than $5 \times 10^{-15}M$.

[00115] In another embodiment, an anti-OX40L antibody that specifically binds to its antigen has an association rate constant or k_{on} rate $((Ab) + \text{antigen } (Ag) \xrightarrow{k_{on}} Ab-Ag)$ of at least $10^5 M^{-1}s^{-1}$, at least $2 \times 10^5 M^{-1}s^{-1}$, at least $5 \times 10^5 M^{-1}s^{-1}$, at least $10^6 M^{-1}s^{-1}$, at least $5 \times 10^6 M^{-1}s^{-1}$, at least $10^7 M^{-1}s^{-1}$, at least $5 \times 10^7 M^{-1}s^{-1}$, or at least $10^8 M^{-1}s^{-1}$.

[00116] In another embodiment, an anti-OX40L antibody that specifically binds to its antigen has a disassociation rate constant or k_{off} rate $((Ab) + \text{antigen } (Ag) \xleftarrow{k_{off}} Ab-Ag)$ of less than $10^{-1} s^{-1}$, less than $5 \times 10^{-1} s^{-1}$, less than $10^{-2} s^{-1}$, less than $5 \times 10^{-2} s^{-1}$, less than $10^{-3} s^{-1}$, less than $5 \times 10^{-3} s^{-1}$, less than $10^{-4} s^{-1}$, less than $5 \times 10^{-4} s^{-1}$, less than $10^{-5} s^{-1}$, less than $5 \times 10^{-5} s^{-1}$, less than $10^{-6} s^{-1}$, less than $5 \times 10^{-6} s^{-1}$, less than $10^{-7} s^{-1}$, less than $5 \times 10^{-7} s^{-1}$, less than $10^{-8} s^{-1}$, less than $5 \times 10^{-8} s^{-1}$, less than $10^{-9} s^{-1}$, less than $5 \times 10^{-9} s^{-1}$ or less than $10^{-10} s^{-1}$.

[00117] In another embodiment, an anti-OX40L antibody that specifically binds to its antigen will have an affinity constant or K_a (k_{on}/k_{off}) of at least $10^2 M^{-1}$, at least $5 \times 10^2 M^{-1}$, at least $10^3 M^{-1}$, at least $5 \times 10^3 M^{-1}$, at least $10^4 M^{-1}$, at least $5 \times 10^4 M^{-1}$, at least $10^5 M^{-1}$, at least $5 \times 10^5 M^{-1}$, at least $10^6 M^{-1}$, at least $5 \times 10^6 M^{-1}$, at least $10^7 M^{-1}$, at least $5 \times 10^7 M^{-1}$, at least $10^8 M^{-1}$, at least $5 \times 10^8 M^{-1}$, at least $10^9 M^{-1}$, at least $5 \times 10^9 M^{-1}$, at least $10^{10} M^{-1}$, at least $5 \times 10^{10} M^{-1}$, at least $10 nM^{-1}$, at least $5 \times 10 nM^{-1}$, at least $10^{12} M^{-1}$, at least $5 \times 10^{12} M^{-1}$, at least $10^{13} M^{-1}$, at least $5 \times 10^{13} M^{-1}$, at least $10^{14} M^{-1}$, at least $5 \times 10^{14} M^{-1}$, at least $10^{15} M^{-1}$ or at least $5 \times 10^{15} M^{-1}$.

[00118] Anti-OX40L antibodies disclosed herein may also be characterized by their thermal stability as reflected by their respective melting point (T_m). The T_m of the Fab region of an antibody can be a good indicator of the thermal stability of an antibody and may further provide an indication of the shelf life. T_m is merely the temperature of 50% unfolding for a given region or sequence. A lower T_m indicates more aggregation/less stability, whereas a higher T_m indicates less aggregation/more stability. Thus, antibodies or fragments or derivatives having higher T_m are preferable. Moreover, using art-recognized techniques it is possible to alter the composition of antibodies or regions thereof to increase or optimize molecular stability. Thermal melting temperatures (T_m) of a protein region (e.g., a Fab region) can be measured using any standard method known in the art, e.g., by differential scanning calorimetry.

[00119] In some embodiments, the Fab region of an anti-OX40L antibody disclosed herein has a T_m value higher than at least $50^\circ C$, $55^\circ C$, $60^\circ C$, $65^\circ C$, $70^\circ C$, $75^\circ C$, $80^\circ C$, $85^\circ C$, $90^\circ C$, $95^\circ C$, $100^\circ C$, $105^\circ C$, $110^\circ C$, $115^\circ C$, or $120^\circ C$. In another embodiment, the Fab region of an anti-OX40L antibody disclosed herein has a T_m value higher than at least about $50^\circ C$, about $55^\circ C$, about $60^\circ C$,

about 65°C, about 70°C, about 75°C, about 80°C, about 85°C, about 90°C, about 95°C, about 100°C, about 105°C, about 110°C, about 115°C, or about 120°C.

[00120] Anti-OX40L antibodies disclosed herein may also be characterized by their isoelectric point (pI), which is generally defined as the pH at which a polypeptide carries no net charge. It is known in the art that protein solubility is typically lowest when the pH of the solution is equal to the isoelectric point (pI) of the protein. Therefore, it is possible to optimize solubility by altering the number and location of ionizable residues in the antibody to adjust the pI. For example, the pI of a polypeptide can be manipulated by making the appropriate amino acid substitutions (e.g., by substituting a charged amino acid such as a lysine, for an uncharged residue such as alanine). Without wishing to be bound by any particular theory, amino acid substitutions of an antibody that result in changes of the pI of the antibody may improve solubility and/or the stability of the antibody. One skilled in the art would understand which amino acid substitutions would be most appropriate for a particular antibody to achieve a desired pI. The pI of a protein may be determined by a variety of methods including but not limited to, isoelectric focusing and various computer algorithms.

[00121] In some embodiments, the pI of an anti-OX40L antibody disclosed herein is higher than about 6.5, about 7.0, about 7.5, about 8.0, about 8.5, or about 9.0. In another embodiment, the pI of an anti-OX40L antibody disclosed herein is higher than 6.5, 7.0, 7.5, 8.0, 8.5, or 9.0. In yet another embodiment, substitutions resulting in alterations in the pI of an anti-OX40L antibody disclosed herein will not significantly diminish its binding affinity. As discussed in more detail below, it is specifically contemplated that the substitution(s) of the Fc region that result in altered binding to FcγR may also result in a change in the pI. In a preferred embodiment, substitution(s) of the Fc region are specifically chosen to effect both the desired alteration in FcγR binding and any desired change in pI. As used herein, the pI value is defined as the pI of the predominant charge form.

[00122] Nucleic Acids and Anti-OX40L Antibody Expression

[00123] Provided herein are also nucleic acids encoding a heavy chain or light chain, or a V_H or V_L, of the anti-OX40L antibodies disclosed herein, and vectors comprising one or more such nucleic acids. The terms “nucleic acid” or “polynucleotide”, as used interchangeably herein, refer to polymers of nucleotides, including single-stranded and/or double-stranded nucleotide-containing molecules, such as DNA, cDNA, and RNA molecules, incorporating native, modified,

and/or analogs of, nucleotides. Polynucleotides of the present disclosure may also include substrates incorporated therein, for example, by DNA or RNA polymerase or a synthetic reaction. In some embodiments, provided herein are nucleic acids encoding a V_H or V_L of the anti-OX40L antibody described herein, e.g., any one of the antibodies identified by internal designation numbers mcp22835 through mcp22846 and mcp32753 through mcp32758. In some embodiments, the nucleic acids encode a V_H or V_L comprising any one of SEQ ID NOs: 1-24. In some embodiments, the nucleic acids encode a complete heavy chain or light chain any one of SEQ ID NOs: 25-54.

[00124] DNA encoding the anti-OX40L antibodies disclosed herein may be readily isolated and sequenced using conventional procedures (e.g., by using oligonucleotide probes that are capable of binding specifically to genes encoding antibody heavy and light chains). Isolated and subcloned hybridoma cells (or phage or yeast derived colonies) may serve as a preferred source of such DNA. More particularly, the isolated DNA (which may be modified) can be used to clone constant and variable region sequences for the manufacture of antibodies.

[00125] One exemplary method entails extraction of RNA from selected cells, conversion to cDNA, and amplification by PCR using antibody specific primers. Suitable primers are well known in the art, and as exemplified herein, are readily available from numerous commercial sources. It will be appreciated that, to express a recombinant human or non-human antibody isolated by screening of a combinatorial library, the DNA encoding the antibody is cloned into a recombinant expression vector and introduced into host cells including mammalian cells, insect cells, plant cells, yeast, and bacteria. In yet other embodiments, antibodies are introduced into and expressed by simian COS cells, NS0 cells, Chinese Hamster Ovary (CHO) cells or myeloma cells that do not otherwise produce the desired construct. As will be discussed in more detail below, transformed cells expressing the desired antibody may be grown in relatively large quantities to provide clinical and commercial supplies of the antibody.

[00126] However the nucleic acid encoding an anti-OX40L antibody disclosed herein is obtained or derived, it should be understood that the inventions disclosed herein encompass nucleic acid molecules and sequences encoding anti-OX40L antibodies, fusion proteins, or antigen-binding fragments or derivatives thereof. The inventions disclosed herein further encompass nucleic acids or nucleic acid molecules (e.g., polynucleotides) that hybridize under high stringency, or alternatively, under intermediate or lower stringency hybridization conditions (e.g., as defined below), to polynucleotides complementary to nucleic acids having a polynucleotide

sequence that encodes an antibody of the invention or a fragment or variant thereof. The term nucleic acid molecule or isolated nucleic acid molecule, as used herein, is intended to include at least DNA molecules and RNA molecules. A nucleic acid molecule may be single-stranded or double-stranded, but preferably is double-stranded DNA. Moreover, the present invention comprises any vehicle or construct, incorporating such antibody-encoding polynucleotide including, without limitation, vectors, plasmids, host cells, cosmids, or viral constructs.

[00127] As used herein, the term “isolated nucleic acid” refers to a nucleic acid that was (i) amplified *in vitro*, for example by polymerase chain reaction (PCR), (ii) recombinantly produced by cloning, (iii) purified, for example by cleavage and gel-electrophoretic fractionation, or (iv) synthesized, for example by chemical synthesis. An isolated nucleic acid is a nucleic acid that is available for manipulation by recombinant DNA techniques.

[00128] More specifically, nucleic acids that encode anti-OX40L antibodies described herein, polynucleotides sufficient for use as hybridization probes, PCR primers or sequencing primers for identifying, analyzing, mutating, or amplifying a polynucleotide encoding a polypeptide, anti-sense nucleic acids for inhibiting expression of a polynucleotide, and complementary sequences of the foregoing are also encompassed herein. Such nucleic acids can be any length. They can be, for example, 5, 10, 15, 20, 25, 30, 35, 40, 45, 50, 75, 100, 125, 150, 175, 200, 250, 300, 350, 400, 450, 500, 750, 1,000, 1,500, 3,000, 5,000 or more nucleotides in length, and/or can comprise one or more additional sequences, for example, regulatory sequences, and/or be part of a larger nucleic acid, for example, a vector. These nucleic acids can be single-stranded or double-stranded and can comprise RNA and/or DNA nucleotides, and artificial variants thereof (e.g., peptide nucleic acids).

[00129] The inventions disclosed herein also include nucleic acids that hybridize to other nucleic acids under particular hybridization conditions. Methods for hybridizing nucleic acids are well known in the art. For example, a moderately stringent hybridization condition uses a prewashing solution containing 5x sodium chloride/sodium citrate (SSC), 0.5% SDS, 1.0 mM EDTA (pH 8.0), hybridization buffer of about 50% formamide, 6xSSC, and a hybridization temperature of 55°C (or other similar hybridization solutions, such as one containing about 50% formamide, with a hybridization temperature of 42°C), and washing conditions of 60°C, in 0.5xSSC, 0.1 % SDS. A stringent hybridization condition hybridizes in 6xSSC at 45°C, followed by one or more washes in 0.1xSSC, 0.2% SDS at 68°C. One of skill in the art can manipulate the hybridization and/or washing conditions to increase or decrease the stringency of hybridization such that nucleic acids comprising nucleotide sequences that are at least 65, 70, 75, 80, 85, 90, 95,

98 or 99% identical to each other typically remain hybridized to each other. More generally, for the purposes of the instant disclosure the term “substantially identical” with regard to a nucleic acid sequence refers to a sequence of nucleotides exhibiting at least about 85%, or 90%, or 95%, or 97% sequence identity to the reference nucleic acid sequence. The basic parameters affecting the choice of hybridization conditions and guidance for devising suitable conditions are well known, and can be readily determined by those having ordinary skill in the art based on, for example, the length and/or base composition of the nucleic acid.

[00130] It will further be appreciated that nucleic acids may be present alone or in combination with other nucleic acids, which may be homologous or heterologous. In preferred embodiments, a nucleic acid is functionally linked to expression control sequences that may be homologous or heterologous with respect to that nucleic acid. In this context, the term “homologous” means that a nucleic acid is also functionally linked to the expression control sequence naturally and the term “heterologous” means that a nucleic acid is not functionally linked to the expression control sequence naturally.

[00131] A nucleic acid, such as a nucleic acid expressing RNA and/or protein or peptide, and an expression control sequence are functionally linked to one another, if they are covalently linked to one another in such a way that expression or transcription of the nucleic acid is under the control or under the influence of the expression control sequence. If the nucleic acid is to be translated into a functional protein, then, with an expression control sequence functionally linked to a coding sequence, induction of the expression control sequence results in transcription of the nucleic acid, without causing a frame shift in the coding sequence or the coding sequence not being capable of being translated into the desired protein or peptide. As used herein, the term “expression control sequence” includes promoters, ribosome binding sites, enhancers and other control elements that regulate transcription of a gene or translation of mRNA. In particular embodiments, the expression control sequences can be regulated. The exact structure of expression control sequences may vary as a function of the species or cell type, but generally comprises 5'-untranscribed and 5'- and 3'-untranslated sequences which are involved in initiation of transcription and translation, respectively, such as TATA box, capping sequence, CAAT sequence, and the like. More specifically, 5'-untranscribed expression control sequences comprise a promoter region that includes a promoter sequence for transcriptional control of the functionally linked nucleic acid. Expression control sequences may also comprise enhancer sequences or upstream activator sequences.

[00132] As used herein, the term “promoter” or “promoter region” relates to a nucleic acid sequence which is located upstream (5') to the nucleic acid sequence being expressed and controls expression of the sequence by providing a recognition and binding site for RNA-polymerase. The promoter region may include further recognition and binding sites for further factors that are involved in the regulation of transcription of a gene. A promoter may control the transcription of a prokaryotic or eukaryotic gene. Furthermore, a promoter may be inducible and may initiate transcription in response to an inducing agent or may be constitutive if transcription is not controlled by an inducing agent. A gene that is under the control of an inducible promoter is not expressed or only expressed to a small extent if an inducing agent is absent. In the presence of the inducing agent the gene is switched on or the level of transcription is increased. This is mediated, in general, by binding of a specific transcription factor.

[00133] Promoters for use in the production of anti-OX40L antibodies disclosed herein include promoters for SP6, T3 and T7 polymerase, human U6 RNA promoter, CMV promoter, and artificial hybrid promoters thereof (e.g., CMV) where a part or parts are fused to a part or parts of promoters of genes of other cellular proteins such as, e.g., human GAPDH (glyceraldehyde-3-phosphate dehydrogenase), and may include (an) additional intron(s).

[00134] As used herein, the term “expression” is used in its most general meaning and comprises the production of RNA or of RNA and protein/peptide. It also comprises partial expression of nucleic acids. Furthermore, expression may be carried out transiently or stably.

[00135] In a preferred embodiment, a nucleic acid molecule is present in a vector, where appropriate with a promoter, which controls expression of the nucleic acid. As used herein, the term “vector” is used here in its most general meaning and comprises any intermediary vehicle for a nucleic acid that enables the nucleic acid, for example, to be introduced into prokaryotic and/or eukaryotic cells and, where appropriate, to be integrated into a genome. Vectors of this kind are preferably replicated and/or expressed in the cells. Vectors may comprise plasmids, phagemids, bacteriophages or viral genomes. As used herein, the term “plasmid” generally relates to a construct of extrachromosomal genetic material, usually a circular DNA duplex, which can replicate independently of chromosomal DNA.

[00136] It will be appreciated by those of skill in the art, that many conventional techniques in molecular biology, microbiology, and recombinant DNA technology are optionally used. Such conventional techniques relate to vectors, host cells and recombinant methods as defined herein.

In addition, essentially any polynucleotide (including, e.g., labeled or biotinylated polynucleotides) can be obtained from any of a variety of commercial sources.

[00137] The inventions disclosed herein also encompass recombinant host cells allowing recombinant expression of antibodies of the invention or portions thereof. Anti-OX40L antibodies disclosed herein produced by expression in such recombinant host cells are referred to herein as recombinant antibodies. The inventions disclosed herein also encompass progeny cells of such host cells, and anti-OX40L antibodies produced by the same.

[00138] As used herein, the term “recombinant host cell” or “host cell” means a cell into which a recombinant expression vector has been introduced. It should be understood that recombinant host cell and host cell mean not only the particular subject cell but also the progeny of such a cell. Because certain modifications may occur in succeeding generations due to either mutation or environmental influences, such progeny may not, in fact, be identical to the parent cell, but are still included within the scope of the term host cell as used herein. Such cells may comprise a vector as described above.

[00139] The inventions disclosed herein also encompass methods for making anti-OX40L antibodies disclosed herein. According to one embodiment, such a method comprises culturing a cell transfected or transformed with a vector as described above, and isolating the antibody.

[00140] As indicated above, expression of an antibody preferably comprises expression vector(s) containing a polynucleotide that encodes the anti-OX40L antibody. Methods that are well known to those skilled in the art can be used to construct expression vectors comprising antibody 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. Particular embodiments provide replicable vectors comprising a nucleotide sequence encoding an anti-OX40L antibody disclosed herein operably linked to a promoter. In preferred embodiments, such vectors may include a nucleotide sequence encoding the heavy chain of an antibody molecule (or fragment thereof), a nucleotide sequence encoding the light chain of an antibody (or fragment thereof), or both the heavy and light chain.

[00141] Using art recognized molecular biology techniques and current protein expression methodology, substantial quantities of the anti-OX40L antibodies disclosed herein may be produced. More specifically, nucleic acid molecules encoding such antibodies may be integrated into well-known and commercially available protein production systems comprising various types of host cells to provide preclinical, clinical, or commercial quantities of the desired pharmaceutical

product. In preferred embodiments the nucleic acid molecules encoding the antibodies are engineered into vectors or expression vectors that provide for efficient integration into the selected host cell and subsequent high expression levels of the antibody.

[00142] Preferably nucleic acid molecules encoding anti-OX40L antibodies disclosed herein and vectors comprising these nucleic acid molecules can be used for transfection of a suitable mammalian, plant, bacterial or yeast host cell though it will be appreciated that prokaryotic systems may also be used. Transfection can be by any known method for introducing polynucleotides into a host cell. Methods for the introduction of heterologous polynucleotides into mammalian cells are well known in the art and include dextran-mediated transfection, calcium phosphate precipitation, polybrene-mediated transfection, protoplast fusion, electroporation, encapsulation of the polynucleotide(s) in liposomes, and direct microinjection of the DNA into nuclei. In addition, nucleic acid molecules may be introduced into mammalian cells by viral vectors. Methods of transforming mammalian cells are well known in the art. Methods of transforming plant cells are also well known in the art, including, e.g., agrobacterium-mediated transformation, biolistic transformation, direct injection, electroporation, and viral transformation. Methods of transforming bacterial and yeast cells are also well known in the art.

[00143] Moreover, the host cell may be co-transfected with two expression vectors of the invention, for example, the first vector encoding a heavy chain polypeptide and the second vector encoding a light chain polypeptide. The two vectors may contain identical selectable markers that enable substantially equal expression of heavy and light chain polypeptides. Alternatively, a single vector may be used which encodes, and is capable of expressing, both heavy and light chain polypeptides. In such situations, the light chain is preferably placed before the heavy chain to avoid an excess of toxic free heavy chain. The coding sequences for the heavy and light chains may comprise cDNA or genomic DNA.

[00144] A variety of host-expression vector systems, many commercially available, may be used to express anti-OX40L antibodies disclosed herein. Such host-expression systems represent vehicles by which the coding sequences of interest may be expressed and subsequently purified, but also represent cells which may, when transformed or transfected with the appropriate nucleotide coding sequences, express a molecule of the invention in situ. Such systems include, but are not limited to, microorganisms such as bacteria (e.g., *E. coli*, *B. subtilis*, *streptomyces*) transformed with recombinant bacteriophage DNA, plasmid DNA or cosmid DNA expression vectors containing antibody coding sequences; yeast (e.g., *Saccharomyces*, *Pichia*) transfected

with recombinant yeast expression vectors containing antibody coding sequences; insect cell antibody coding sequences; plant cell systems (e.g., *Nicotiana*, *Arabidopsis*, duckweed, corn, wheat, potato, etc.) infected with recombinant virus expression vectors (e.g., cauliflower mosaic virus, CaMV; tobacco mosaic virus, TMV) or transfected with recombinant plasmid expression vectors (e.g., Ti plasmid) containing antibody coding sequences; or mammalian cell systems (e.g., COS, CHO, BHK, 293, 3T3 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).

[00145] In bacterial systems, a number of expression vectors may be advantageously selected depending upon the use intended for the molecule being expressed. For example, when a large quantity of such a protein is to be produced, for the generation of pharmaceutical compositions, vectors which direct the expression of high levels of fusion protein products that are readily purified may be desirable.

[00146] In an insect system, *Autographa californica* nuclear polyhedrosis virus (AcNPV) may be used as a vector to express foreign genes. The virus grows in *Spodoptera frugiperda* cells. The coding sequences may be cloned individually into non-essential regions (for example, the polyhedrin gene) of the virus and placed under control of an AcNPV promoter (for example, the polyhedrin promoter).

[00147] In mammalian host cells, a number of viral-based expression systems may be used to introduce the desired nucleotide sequence. In cases where an adenovirus is used as an expression vector, the coding sequence of interest may be ligated to an adenovirus transcription/translation control complex, e.g., the late promoter and tripartite leader sequence. This chimeric gene may then be inserted in the adenovirus genome by *in vitro* or *in vivo* recombination. Insertion in a non-essential region of the viral genome (e.g., region E1 or E3) will result in a recombinant virus that is viable and capable of expressing the molecule in infected hosts. Specific initiation signals may also be required for efficient translation of inserted coding sequences. These signals include the ATG initiation codon and adjacent sequences. Furthermore, the initiation codon must be in phase with the reading frame of the desired coding sequence to ensure translation of the entire insert. These exogenous translational control signals and initiation codons can be of a variety of origins, both natural and synthetic. The efficiency of expression may be enhanced by the inclusion of appropriate transcription enhancer elements, transcription terminators, and the like. Thus, compatible mammalian cell lines available as hosts for expression are well known in the art and

include many immortalized cell lines available from the American Type Culture Collection (ATCC). These include, *inter alia*, Chinese hamster ovary (CHO) cells, NS0 cells, SP2 cells, HEK-293T cells, 293 Freestyle cells (Life Technologies), NIH-3T3 cells, HeLa cells, baby hamster kidney (BHK) cells, African green monkey kidney cells (COS), human hepatocellular carcinoma cells (e.g., Hep G2), A549 cells, and a number of other cell lines.

[00148] For long-term, high-yield production of recombinant proteins stable expression is preferred. Accordingly, cell lines that stably express the selected antibody may be engineered using standard art recognized techniques. Rather than using expression vectors that contain viral origins of replication, host cells can be transformed with DNA controlled by appropriate expression control elements (e.g., promoter, enhancer, sequences, transcription terminators, polyadenylation sites, etc.), and a selectable marker. Following the introduction of the foreign DNA, engineered cells may be allowed to grow for 1 to 2 days in an enriched media, and then are switched to a selective media. The selectable marker in the recombinant plasmid confers resistance to the selection and allows cells to stably integrate the plasmid into their chromosomes and grow to form foci which in turn can be cloned and expanded into cell lines. This method may advantageously be used to engineer cell lines which express the molecule. Such engineered cell lines may be particularly useful in screening and evaluation of compositions that interact directly or indirectly with the molecule.

[00149] A number of selection systems are well known in the art and may be used including, but not limited to, the herpes simplex virus thymidine kinase, hypoxanthineguanine phosphoribosyltransferase, and adenine phosphoribosyltransferase genes can be employed in *tk*-, *hgprt*- or *aprt*- cells, respectively. Also, antimetabolite resistance can be used as the basis of selection for the following genes: *dhfr*, which confers resistance to methotrexate; *gpt*, which confers resistance to mycophenolic acid; *neo*, which confers resistance to the aminoglycoside G-418; and *hygro*, which confers resistance to hygromycin.

[00150] Methods commonly known in the art of recombinant DNA technology may be routinely applied to select the desired recombinant clone. It will be appreciated by those of skill in the art that one particularly preferred method of establishing a stable, high yield cell line comprises the glutamine synthetase gene expression system (the GS system), which provides an efficient approach for enhancing expression under certain conditions.

[00151] In addition, a host cell strain may 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 may be important for the function and/or purification of the protein. Different host cells have characteristic and specific mechanisms for the post-translational processing and modification of proteins and gene products. As known in the art, appropriate cell lines or host systems can be chosen to ensure the desired modification and processing of the expressed polypeptide. To this end, eukaryotic host cells that possess the cellular machinery for proper processing of the primary transcript, glycosylation, and phosphorylation of the gene product are particularly effective. Accordingly, some preferred mammalian host lines include, but are not limited to, CHO, VERO, BHK, HeLa, COS, NS0, MDCK, 293, 3T3, and W138. Depending on the anti-OX40L antibody and the selected production system, those of skill in the art may easily select and optimize appropriate host cells for efficient expression of the antibody.

[00152] Those of skill in the art will appreciate that anti-OX40L antibodies disclosed herein may be chemically synthesized using techniques known in the art. For example, a peptide corresponding to a polypeptide fragment of the invention can be synthesized by use of a peptide synthesizer. If desired, non-genetically encoded amino acids or synthetic amino acids can be substituted or added into a polypeptide sequence.

[00153] Representative non-genetically encoded amino acids include but are not limited to 2-amino adipic acid; 3-amino adipic acid; β -aminopropionic acid; 2-aminobutyric acid; 4-aminobutyric acid (piperidinic acid); 6-aminocaproic acid; 2-aminoheptanoic acid; 2-aminoisobutyric acid; 3-aminoisobutyric acid; 2-aminopimelic acid; 2,4-diaminobutyric acid; desmosine; 2,2'-diaminopimelic acid; 2,3-diaminopropionic acid; N-ethylglycine; N-ethylasparagine; hydroxylysine; allo-hydroxylysine; 3-hydroxyproline; 4-hydroxyproline; isodesmosine; allo-isoleucine; N-methylglycine (sarcosine); N-methylisoleucine; N-methylvaline; norvaline; norleucine; and ornithine.

[00154] Representative synthetic amino acids include, for example, those molecules in which free amino groups have been derivatized to form amine hydrochlorides, p-toluene sulfonyl groups, carbobenzoxy groups, t-butyloxycarbonyl groups, chloroacetyl groups or formyl groups. Free carboxyl groups may be derivatized to form salts, methyl and ethyl esters or other types of esters or hydrazides. Free hydroxyl groups may be derivatized to form O-acyl or O-alkyl derivatives. The imidazole nitrogen of histidine may be derivatized to form N-im-benzylhistidine.

[00155] Anti-OX40L antibodies disclosed herein also can be produced transgenically through the generation of a mammal or plant that is transgenic for the immunoglobulin heavy and light

chain sequences (or fragments or derivatives or variants thereof) of interest and production of the desired compounds in a recoverable form. For example, anti-OX40L antibodies can be produced in, and recovered from, e.g., the milk of goats, cows, or other mammals.

[00156] Non-human transgenic animals or plants may be produced by introducing one or more nucleic acid molecules encoding an anti-OX40L antibody into the animal or plant by standard transgenic techniques. The transgenic cells used for making the transgenic animal can be embryonic stem cells or somatic cells or a fertilized egg. The transgenic non-human organisms can be chimeric, nonchimeric heterozygotes, and nonchimeric homozygotes. In some embodiments, the transgenic non-human animals have a targeted disruption and replacement by a targeting construct that encodes, for example, a heavy chain and/or a light chain of interest. While anti-OX40L antibodies disclosed herein may be produced in any transgenic animal, particularly preferred embodiments include mice, rats, sheep, pigs, goats, cattle, and horses. In particular embodiments, the non-human transgenic animal expresses the desired pharmaceutical product in blood, milk, urine, saliva, tears, mucus, and other bodily fluids from which it is readily obtainable using art recognized purification techniques.

[00157] It is likely that antibodies expressed by different cell lines or in transgenic animals will have different glycosylation patterns from each other. However, the instant inventions encompass anti-OX40L antibodies encoded by the nucleic acid molecules provided herein, or comprising the amino acid sequences provided herein, regardless of the glycosylation state of the molecule, and more generally, regardless of the presence or absence of post-translational modification(s). The instant inventions also encompass anti-OX40L antibodies that are differentially modified during or after translation, e.g., by glycosylation, acetylation, phosphorylation, amidation, derivatization by known protecting/blocking groups, proteolytic cleavage, linkage to an antibody molecule or other cellular ligand, etc. Any of numerous chemical modifications may be carried out by known techniques, including but not limited, to specific chemical cleavage by cyanogen bromide, trypsin, chymotrypsin, papain, V8 protease, acetylation, formylation, oxidation, reduction, metabolic synthesis in the presence of tunicamycin, etc. Various post-translational modifications are also encompassed by the invention include, for example, e.g., N-linked or O-linked carbohydrate chains, processing of N-terminal or C-terminal ends, attachment of chemical moieties to the amino acid backbone, chemical modifications of N-linked or O-linked carbohydrate chains, and addition or deletion of an N-terminal methionine residue as a result of prokaryotic host cell expression. Moreover, anti-OX40L antibodies disclosed herein may also be modified with a detectable label,

such as an enzymatic, fluorescent, radioisotopic or affinity label to allow for their detection and isolation.

[00158] Once anti-OX40L antibodies disclosed herein have been produced by recombinant expression or any one of the other techniques disclosed herein, they may be purified by any method known in the art for purification of immunoglobulins, or more generally by any other standard technique for the purification of proteins. In this respect the anti-OX40L antibody may be isolated. As used herein, an “isolated anti-OX40L antibody” is one that has been identified and separated and/or recovered from a component of its natural environment. Contaminant components of its natural environment are materials that would interfere with diagnostic or therapeutic uses of the antibody and may include enzymes, hormones, and other proteinaceous or non-proteinaceous solutes. Isolated anti-OX40L antibodies include antibodies *in situ* within recombinant cells because at least one component of the antibody's natural environment will not be present.

[00159] When using recombinant techniques, anti-OX40L antibodies disclosed herein can be produced intracellularly, in the periplasmic space, or directly secreted into the medium. If the desired molecule is produced intracellularly, as a first step, the particulate debris, either host cells or lysed fragments, may be removed, for example, by centrifugation or ultrafiltration. Where the antibody is secreted into the medium, supernatants from such expression systems are generally first concentrated using a commercially available protein concentration filter. A protease inhibitor such as PMSF may be included in any of the foregoing steps to inhibit proteolysis and antibiotics may be included to prevent the growth of adventitious contaminants.

[00160] Compositions comprising anti-OX40L antibodies disclosed herein prepared from cells can be purified using, for example, hydroxyapatite chromatography, gel electrophoresis, dialysis, and affinity chromatography. The suitability of protein A as an affinity ligand depends on the species and isotype of any immunoglobulin Fc region that is present in the selected construct. Protein A can be used to purify antibodies that are based on human IgG1, IgG2 or IgG4 heavy chains. Protein G is recommended for all mouse isotypes and for human IgG3. The matrix to which the affinity ligand is attached is most often agarose, but other matrices are available. Mechanically-stable matrices such as controlled pore glass or poly(styrenedivinyl)benzene allow for faster flow rates and shorter processing times than can be achieved with agarose. Where the antibody comprises a C_H3 region, the BAKERBOND ABX™ resin is useful for purification. Other techniques for protein purification such as fractionation on an ion-exchange column, ethanol precipitation, reverse phase HPLC, chromatography on silica, chromatography on heparin,

SEPHAROSE® chromatography on an anion or cation exchange resin (such as a polyaspartic acid column), chromatofocusing, SDS-PAGE and ammonium sulfate precipitation are also available depending on the antibody to be recovered.

[00161] By way of illustration of the foregoing, cDNA sequences encoding an anti-OX40L antibody heavy chain and light chain may be cloned and engineered into an appropriate expression vector. The engineered immunoglobulin expression vector may then be stably transfected into a mammalian (e.g., human) cell. As one skilled in the art will appreciate, mammalian expression of antibodies will result in glycosylation, typically at highly conserved N-glycosylation sites in the Fc region. Positive clones may be expanded into serum-free culture medium for antibody production in bioreactors. Medium, into which an antibody has been secreted, may be purified by conventional techniques. For example, the medium may be conveniently applied to a Protein A or G SEPHAROSE® FF column that has been equilibrated with a compatible buffer, such as phosphate buffered saline. The column is washed to remove nonspecific binding components. The bound antibody is eluted, for example, by pH gradient and antibody fractions are detected, such as by SDS-PAGE, and then pooled. The antibody may be concentrated and/or sterile filtered using common techniques. Soluble aggregate and multimers may be effectively removed by common techniques, including size exclusion, hydrophobic interaction, ion exchange, or hydroxyapatite chromatography. The product may be immediately frozen, for example, at -70°C, or may be lyophilized.

[00162] Pharmaceutical Compositions and Therapeutic Uses

[00163] Also provided herein are methods of treating an autoimmune disorder by administering to a subject a therapeutically effective amount of one or more anti-OX40L antibodies described herein, or a pharmaceutical composition comprising one or more (e.g., two or three) anti-OX40L antibodies described herein in combination with another anti-OX40L antibody or therapy.

[00164] As used interchangeably herein, “treatment” or “treating” or “treat” refers to all processes wherein there may be a slowing, interrupting, arresting, controlling, stopping, alleviating, or ameliorating symptoms or complications, or reversing of the progression of an autoimmune disorder, but does not necessarily indicate a total elimination of all symptoms.

[00165] A wide variety of autoimmune disorders may be treated using one or more anti-OX40L antibodies described herein, or a pharmaceutical composition comprising the same. A non-exhaustive list of contemplated autoimmune disorders includes Alopecia or Alopecia Areata (AA),

Ankylosing spondylitis, Autoimmune Lymphoproliferative Syndrome (ALPS), Celiac disease, Crohn's Disease, Graft-versus-host disease, Graves' Disease, Guillain-Barre' syndrome, Hypersensitivity Vasculitis (HV), Inflammatory bowel disease, Myasthenia Gravis Neutropenia, Rheumatoid arthritis (RA), Sarcoidosis, Systemic lupus erythematosus (SLE), Systemic sclerosis (scleroderma). Antibodies and antigen-binding fragments thereof disclosed herein may also be used to treat Atopic Dermatitis.

[00166] In some embodiments, methods of preventing or treating an autoimmune disorder or Atopic Dermatitis comprise administering to a subject a pharmaceutical composition comprising one or more (e.g., two or three) anti-OX40L antibodies described herein. In some embodiments, such methods comprise administering to a subject a pharmaceutical composition comprising two or three anti-OX40L antibodies or antigen-binding fragments thereof that bind different epitopes present on OX40L.

[00167] Also provided are anti-OX40L antibodies or antigen-binding fragments or pharmaceutical compositions comprising one or more (e.g., two or three) anti-OX40L antibodies or antigen-binding fragments for use in therapy. In some embodiments anti-OX40L antibodies or antigen-binding fragments thereof or pharmaceutical compositions comprise one or more (e.g., two or three) anti-OX40L antibodies or antigen-binding fragments, for use in the treatment or prevention of an autoimmune disorder or Atopic Dermatitis. Further provided herein are uses of anti-OX40L antibodies or antigen-binding fragments described herein in the manufacture of a medicament for the treatment or prevention of an autoimmune disorder or Atopic Dermatitis.

[00168] Depending on the form of anti-OX40L antibody, mode of intended delivery, and numerous other variables, anti-OX40L antibodies disclosed herein may be formulated as desired using art recognized techniques. Various pharmaceutically acceptable carriers, which include vehicles, adjuvants, and diluents, are readily available from numerous commercial sources. Moreover, an assortment of pharmaceutically acceptable auxiliary substances, such as pH adjusting and buffering agents, tonicity adjusting agents, stabilizers, wetting agents, and the like, are also available. Certain non-limiting exemplary carriers include saline, buffered saline, dextrose, water, glycerol, ethanol, and combinations thereof.

[00169] In some embodiments, anti-OX40L antibodies may be administered to a subject neat or with a minimum of additional components. In other embodiments, anti-OX40L antibodies may be formulated to contain suitable pharmaceutically acceptable carriers comprising excipients and auxiliaries that are well known in the art and are relatively inert substances that facilitate

administration or which aid processing of the active compounds into preparations that are pharmaceutically optimized for delivery. For example, an excipient can give form or consistency or act as a diluent to improve the pharmacokinetics of the antibody. Suitable excipients include but are not limited to stabilizing agents, wetting, and emulsifying agents, salts for varying osmolality, encapsulating agents, buffers, and skin penetration enhancers.

[00170] Anti-OX40L antibodies disclosed herein may be formulated for enteral, parenteral, or topical administration. Indeed, all three types of formulation may be used simultaneously to achieve systemic administration of the active ingredient. Excipients as well as formulations for parenteral and non-parenteral drug delivery are known in the art. Suitable formulations for parenteral administration include aqueous solutions of the active compounds in water-soluble form, for example, water-soluble salts. In addition, suspensions of the active compounds as appropriate for oily injection suspensions may be administered. Suitable lipophilic solvents or vehicles include fatty oils, for example, sesame oil, or synthetic fatty acid esters, for example, ethyl oleate or triglycerides. Aqueous injection suspensions may contain substances that increase the viscosity of the suspension and include, for example, sodium carboxymethyl cellulose, sorbitol, and/or dextran. Optionally, the suspension may also contain stabilizers. Liposomes can also be used to encapsulate the agent for delivery into the cell.

[00171] In general, anti-OX40L antibodies disclosed herein may be administered *in vivo* to a subject in need thereof, by various routes, including, but not limited to, oral, intravenous, intra-arterial, subcutaneous, parenteral, intranasal, intramuscular, intracardiac, intraventricular, intratracheal, buccal, rectal, intraperitoneal, intradermal, topical, transdermal, and intrathecal, or otherwise by implantation or inhalation. Compositions may be formulated into preparations in solid, semi-solid, liquid, or gaseous forms; including, but not limited to, tablets, capsules, powders, granules, ointments, solutions, suppositories, enemas, injections, inhalants, and aerosols. The appropriate formulation and route of administration may be selected according to the intended application and therapeutic regimen. In preferred embodiments, anti-OX40L antibodies disclosed herein are either administered intravenously or subcutaneously.

[00172] Similarly, the particular dosage regimen, i.e., dose, timing, and repetition, will depend on the particular individual and that individual's medical history. Empirical considerations such as pharmacokinetics (e.g., half-life, clearance rate, etc.) will contribute to the determination of the dosage. Frequency of administration may be determined and adjusted over the course of therapy. Alternatively, sustained continuous release formulations of a subject therapeutic composition may

be appropriate. Various formulations and devices for achieving sustained release are known in the art.

[00173] Pharmaceutical compositions are administered in therapeutically effective amount in order to treat an autoimmune disorder or Atopic Dermatitis in a subject. As used herein, the term “therapeutically effective amount” means that amount of an anti-OX40L antibody or pharmaceutical composition comprising the same that will elicit the biological or medical response in the subject that is sought by a medical doctor or other clinician.

[00174] With regard to autoimmune disorders, a “therapeutically effective amount” is intended to include an amount sufficient to address one or more symptoms commonly observed in autoimmune disorders, which include but are not limited to fatigue, joint pain and swelling, skin problems (e.g., rashes), abdominal pain or digestive issues, recurring fever, and swollen glands.

[00175] With regard to Atopic Dermatitis, a “therapeutically effective amount” is intended to include an amount sufficient to address one or more symptoms commonly observed in a subject suffering from this condition, which include but are not limited to pruritus, redness, swelling, cracking, crusting, and scaling.

[00176] In some embodiments, a therapeutically effective amount of an anti-OX40L antibody or pharmaceutical composition comprising the same has a beneficial effect but does not cure an autoimmune disorder or Atopic Dermatitis. In certain embodiments, therapy may encompass the administration of multiple doses of an anti-OX40L antibody or pharmaceutical composition comprising the same at a certain frequency to achieve a therapeutic effect.

[00177] A therapeutically effective amount is typically dependent on the weight of the subject being treated, his or her physical condition, the extensiveness of the condition to be treated, and the age of the subject being treated. In general, anti-OX40L antibodies disclosed herein may be administered in an amount in the range of about 10 ng/kg body weight to about 1000 mg/kg body weight per dose. In certain embodiments, antibodies may be administered in an amount in the range of about 50 µg/kg body weight to about 500 mg/kg body weight per dose. In other embodiments, antibodies may be administered in an amount in the range of about 100 µg/kg body weight to about 1000 mg/kg body weight per dose. In other embodiments, antibodies may be administered in an amount in the range of about 100 µg/kg body weight to about 200 mg/kg body weight per dose. In other embodiments, antibodies may be administered in an amount in the range of about 0.45 mg/kg body weight to about 12.5 mg/kg body weight per dose. In other embodiments, antibodies may be administered in a dose of at least about 100 µg/kg body weight, at least about

250 µg/kg body weight, at least about 750 µg/kg body weight, at least about 3 mg/kg body weight, at least about 5 mg/kg body weight, or at least about 10 mg/kg body weight.

[00178] In some embodiments, dosage regimens comprise a loading dose and one or more maintenance doses as part of an induction phase and maintenance phase respectively. In some embodiments, the induction dose is between about 20 mg and about 1000 mg. In some embodiments, the maintenance dose is between about 20 mg and about 600 mg. In some embodiments, the interval between two or more induction phase injections is between about 2 weeks and about 24 weeks, and the interval between two or more maintenance phase injections is between about 2 weeks and about 52 weeks.

[00179] In particular embodiments, the first injection of the antibody or antigen-binding fragment thereof can be a loading dose, wherein the loading dose of the antibody or antigen-binding fragment thereof is up to three times the mass of the treatment dose, or the loading dose of the antibody or antigen-binding fragment thereof is between about 100 mg and about 600 mg; and the second and additional injections of the antibody or antigen-binding fragment thereof can be treatment doses of at least 5 treatment injections, wherein the treatment dose of the antibody or antigen-binding fragment is between about 50 mg and about 300 mg

[00180] Other dosing regimens may be predicated on Body Surface Area (BSA) calculations. As is well known in the art, a subject's BSA is calculated using the subject's height and weight and provides a measure of a subject's size as represented by the surface area of his or her body. In some embodiments, anti-OX40L antibodies disclosed herein are administered in dosages from 10 mg/m² to 800 mg/m². In other embodiments, antibodies are administered in dosages from 50 mg/m² to 500 mg/m² and even more preferably at dosages of 100 mg/m², 150 mg/m², 200 mg/m², 250 mg/m², 300 mg/m², 350 mg/m², 400 mg/m² or 450 mg/m².

[00181] Escalation for an individual subject can occur at the discretion of a clinician in the absence of any clinically significant occurrence that the clinician might reasonably believe would present an undue safety risk for the subject, such as, for example, infusion reactions, acute anaphylaxis, and serum sickness.

[00182] Anti-OX40L antibodies disclosed herein are usually administered to a subject on multiple occasions in accordance with regimens known in the art. In some instances, two or more antibodies with different binding specificities may be administered simultaneously, in which case the dosage of each antibody administered falls within the ranges indicated. Intervals between single dosages can be weekly, monthly, or yearly. Intervals can also be irregular depending upon

levels of antibody in the blood and other clinical indicia. In some embodiments, the dosage is adjusted to achieve a plasma antibody concentration of about 1–1000 µg/mL or about 1.5–25 µg/mL. In other embodiments, the dosage is adjusted so that minimum blood serum concentration reached between any two injections (C_{min}) is at least 2.5 µg/mL or the maximum blood serum concentration reached after administration of an injection and prior to administration of a subsequent injection (C_{max}) is at least about 1.5 µg/mL or blood serum concentrations of the during treatment range between about 1.5 µg/mL and about 550 µg/mL or the area under the serum concentration-time curve (AUC) following the first (AUC extrapolated to infinity [AUC_{0-inf}]) injection may be at least around 100,000 ng*day/mL to at least around 15,500,000 ng*day/mL. Alternatively, antibodies can be administered as a sustained release formulation, in which case less frequent administration is required.

[00183] Dosage and frequency will vary depending on the half-life of the antibody in the subject. In general, human antibodies show the longest half-life, followed by humanized antibodies, chimeric antibodies, and nonhuman antibodies. The dosage and frequency of administration can vary depending on whether the treatment is prophylactic or therapeutic. In prophylactic applications, a relatively low dosage is administered at relatively infrequent intervals over a long period of time. Some subjects continue to receive treatment for the rest of their lives. In therapeutic applications, a relatively high dosage at relatively short intervals is sometimes required until progression of the disease is reduced or terminated, until a partial or complete response is achieved, and/or until the subject shows lessening or amelioration of symptoms of disease. Thereafter, the patient can be administered a prophylactic regime.

[00184] The duration of a therapeutic regimen depends on a variety of factors that are readily appreciated by one of skill in the art. A clinician can observe the therapy's effects closely and make any adjustments as needed. When agents are used in combination, the two or more therapeutic agents are administered simultaneously or sequentially in any order, i.e., an anti-OX40L antibody disclosed herein is administered prior to administering a second therapeutic agent, concurrently with a second therapeutic agent, or subsequent to administration of a second therapeutic agent. For example, a combination therapy may be performed by administering a first therapeutic agent prior to (e.g., 1 minute, 5 minutes, 15 minutes, 30 minutes, 45 minutes, 1 hour, 2 hours, 4 hours, 6 hours, 12 hours, 24 hours, 48 hours, 72 hours, 96 hours, 1 week, 2 weeks, 3 weeks, 4 weeks, 5 weeks, 6 weeks, 8 weeks, or 12 weeks before), concurrently with, or subsequent to (e.g., 1 minute, 5 minutes, 15 minutes, 30 minutes, 45 minutes, 1 hour, 2 hours, 4 hours, 6 hours, 12 hours, 24 hours,

48 hours, 72 hours, 96 hours, 1 week, 2 weeks, 3 weeks, 4 weeks, 5 weeks, 6 weeks, 8 weeks, or 12 weeks after) administering a second therapeutic agent. Exemplary second therapeutic agents for treating Atopic Dermatitis, for example, include any number of corticosteroids, such as alclometasone dipropionate, amcinonide, augmented betamethasone dipropionate, betamethasone dipropionate, betamethasone valerate, clobetasol propionate, clocortolone pivalate, desonide, dexamethasone, desoximetasone, diflorasone diacetate, fluocinolone acetonide, fluocinonide, flurandrenolide, fluticasone propionate, gentamicin sulphate, halcinonide, halobetasol propionate, hydrocortisone, hydrocortisone acetate, hydrocortisone butyrate, hydrocortisone probutate, hydrocortisone valerate, methylprednisolone, methylprednisolone aceponate, mometasone furoate, prednicarbate, or triamcinolone acetonide.

[00185] The dosage, frequency, and mode of administration of each component of a combination therapy can be controlled independently. For example, one therapeutic agent may be administered orally three times per day, while the second therapeutic agent may be administered intravenously once per day. Combination therapy may be given in on-and-off cycles that include rest periods. The compounds may also be admixed or otherwise formulated together such that one administration delivers both therapeutic agents. In this case, each therapeutic agent is generally present in an amount of 1-95% by weight of the total weight of the composition. Alternatively, therapeutic agents can be formulated separately and in individual dosage amounts. Combinations of therapeutic agents for treatment can be provided as components of a pharmaceutical pack.

[00186] Preferably, combination therapies elicit a synergistic therapeutic effect, i.e., an effect greater than the sum of their individual effects or therapeutic outcomes, such as those described above. For example, a synergistic therapeutic effect may be an effect of at least about two-fold greater than sum of the therapeutic effects elicited by the single agents of a given combination, or at least about five-fold greater, or at least about ten-fold greater, or at least about twenty-fold greater, or at least about fifty-fold greater, or at least about one hundred-fold greater. A synergistic therapeutic effect may also be observed as an increase in therapeutic effect of at least 10% compared to the sum of the therapeutic effects elicited by the single agents of a given combination, or at least 20%, or at least 30%, or at least 40%, or at least 50%, or at least 60%, or at least 70%, or at least 80%, or at least 90%, or at least 100%, or more. A synergistic effect is also an effect that permits reduced dosing of therapeutic agents when they are used in combination.

[00187] The effectiveness of any particular therapeutic regimen can be evaluated by those of skill in the art using established methodologies. For example, there are a multitude of outcomes

measures known in the art that pertain to evaluating the severity and treatment of Atopic Dermatitis: Eczema Area and Severity Index (EASI; Investigator's Global Assessment scale (IGA or IGA-AD); Scoring Atopic Dermatitis (SCORAD); Patient Global Assessment of Disease Status (PGADS); Pruritus Numerical Rating Scale (NRS) Dermatology Life Quality Index (DLQI); EuroQol 5-Dimensions questionnaire (EQ-5D); Hospital Anxiety and Depression Scale (HADS); Patient-Oriented Eczema Measure (POEM). Indicia of therapeutic effectiveness include, but are not limited to, a decrease from baseline in IGA score of at least 2 points; achieving clear or almost clear skin (IGA-AD 0/1) from baseline; a decrease from baseline in eczema area and severity index (EASI) score of at least 50%; a decrease from baseline in EASI score of at least 75%; achieving EASI50, EASI75, EASI90, or EASI100; achieving an improvement of at least 3 points in pruritus NRS score; achieving an improvement of at least 4 points in pruritus NRS score; a decrease from baseline in SCORAD index score of at least 50%; a decrease from baseline in SCORAD index score of at least 55%; a decrease from baseline in affected BSA score of at least 60%; or a decrease from baseline in affected BSA score of at least 70%. Clinical indicia of therapeutically effective dosages and treatment regimens may also include observing a reduction in one or more biomarkers, such as IL-4, IL-5, IL-9, IL-13, IL-22, IL-17A, IL-31, IgE, TARC, MDC, PARC, CTACK, eosinophils, or OX40L. The effectiveness of any particular therapeutic regimen may be evaluated days, weeks, months, or years after an initial dose, a final dose, or any intervening dose administered to the subject.

[00188] Articles of Manufacture

[00189] The inventions disclosed herein also encompass pharmaceutical packs and kits comprising one or more containers and comprising one or more doses of an anti-OX40L antibody disclosed herein. In certain embodiments, a unit dosage is provided wherein the unit dosage contains a predetermined amount of a composition comprising, for example, an anti-OX40L antibody disclosed herein, with or without one or more additional agents. For other embodiments, such a unit dosage is supplied in single-use prefilled syringe for injection. In still other embodiments, the composition contained in the unit dosage may comprise saline, sucrose, or the like; a buffer, such as phosphate, or the like; and/or be formulated within a stable and effective pH range. Alternatively, in certain embodiments, the composition may be provided as a lyophilized powder that may be reconstituted upon addition of an appropriate liquid, for example, sterile water. In certain preferred embodiments, the composition comprises one or more substances that inhibit

protein aggregation, including, but not limited to, sucrose and arginine. Any label on, or associated with, the container(s) indicates that the enclosed composition is used for diagnosis or treatment.

[00190] The present invention also provides kits for producing single-dose or multi-dose administration units of an anti-OX40L antibody disclosed herein and, optionally, one or more other diagnostic or therapeutic agents. The kit comprises a container and a label or package insert on or associated with the container. Suitable containers include, for example, bottles, vials, syringes, etc. The containers may be formed from a variety of materials such as glass or plastic. The container holds a composition that is effective for treating the condition and may have a sterile access port (for example the container may be an intravenous solution bag or a vial having a stopper pierceable by a hypodermic injection needle). Such kits will generally contain in a suitable container a pharmaceutically acceptable formulation of the anti-OX40L antibody and, optionally, one or more other diagnostic or therapeutic agents in the same or different containers. The kits may also contain other pharmaceutically acceptable formulations, either for diagnosis or combined therapy.

[00191] More specifically the kits may have a single container that contains the anti-OX40L antibody with or without additional components, or they may have distinct containers for each desired agent. Where combined therapeutics are provided for conjugation, a single solution may be pre-mixed, either in a molar equivalent combination, or with one component in excess of the other. Alternatively, the anti-OX40L antibody and any optional diagnostic or therapeutic agent of the kit may be maintained separately within distinct containers prior to administration to a subject. The kits may also comprise a second/third container means for containing a sterile, pharmaceutically acceptable buffer or other diluent such as bacteriostatic water for injection (BWFI), phosphate-buffered saline (PBS), Ringer's solution, and dextrose solution.

[00192] When the components of the kit are provided in one or more liquid solutions, the liquid solution is preferably an aqueous solution, with a sterile aqueous solution being particularly preferred. However, the components of the kit may be provided as dried powder(s). When reagents or components are provided as a dry powder, the powder can be reconstituted by the addition of a suitable solvent. It is envisioned that the solvent may also be provided in another container.

[00193] As indicated briefly above the kits may also contain a means by which to administer the antibody and any optional components to the subject, e.g., one or more needles or syringes, or even an eye dropper, pipette, or other such like apparatus, from which the formulation may be injected or introduced into the subject. Such kits will also typically include a means for containing the vials, or such like, and other component in close confinement for commercial sale, such as,

e.g., injection or blow-molded plastic containers into which the desired vials and other apparatus are placed and retained. Any label or package insert indicates that the anti-OX40L antibody composition is used for treating cancer, for example colorectal cancer.

[00194] In other preferred embodiments, anti-OX40L antibodies disclosed herein may be used in conjunction with, or comprise, diagnostic or therapeutic devices useful in the diagnosis or treatment of proliferative disorders. For example, in one embodiment, anti-OX40L antibodies may be combined with certain diagnostic devices or instruments that may be used to detect, monitor, quantify or profile cells or marker compounds involved in the etiology or manifestation of an autoimmune disease.

EXAMPLES

[00195] The following examples have been included to illustrate aspects of the inventions disclosed herein. In light of the present disclosure and the general level of skill in the art, those of skill appreciate that the following examples are intended to be exemplary only and that numerous changes, modifications, and alterations may be employed without departing from the scope of the disclosure.

[00196] Example 1

[00197] Antibody Characterization Using Antigen-on-Bead Antibody Binding Assay

[00198] A mixture of human OX40 Ligand (Acro Biosystems) and recombinant cynomolgus OX40 Ligand (Elabscience) antigens-on-beads were prepared in order to ensure a robust median fluorescence intensity, in 10x the volume of PBS + 2% FBS used to wash the beads. The bead mixture was centrifuged at 10000xg for 1 minute at room temperature to pellet beads, and then re-suspended in PBS + 2% FBS.

[00199] The bead mixture was then incubated with 25 nM anti-OX40L antibodies or isotype control in PBS + 2% FBS in duplicate for 30 minutes at room temperature on a THERMOMIXER® at 1000 rpm. Following incubation, beads were pelleted by centrifugation at 600xg for 5 minutes, washed in excess volume of PBS + 2% FBS, and pelleted again.

[00200] Beads were then resuspended in a solution of 75 nM ALEXA FLUOR® F(ab')₂ 488 Goat anti-human IgG Fc Gamma in PBS + 2% FBS for 30 minutes at room temperature on a THERMOMIXER® at 1000 rpm. Following incubation, beads were washed with excess volume

of PBS + 2% FBS, pelleted by centrifugation at 600xg for 5 minutes and resuspended in PBS + 2% FBS.

[00201] Samples were loaded onto an INTELLICYT® IQUE®3 Flow Cytometer and measured using FORECYT® Software. The average median fluorescence intensity (MFI) was calculated across all isotype replicates for each antigen-on-bead type, and the fold over isotype (FOI) by dividing the sample MFI by the average isotype MFI for each antigen-on-bead type. The results for human OX40-conjugated beads are shown in Table 1.

[00202] Table 1: Binding to hOX40L (Bead Assay)

Antibody ID	Mean FOI	SD	Positive Control Mean FOI
mcp22840	1268.26	13.59	1426.54
mcp22835	1276.59	16.65	1450.99
mcp22845	1069.10	4.32	1266.52
mcp22846	1211.77	3.52	1378.86
mcp22842	1243.76	4.98	1352.07
mcp22841	1286.84	4.04	1355.48
mcp22839	1330.66	10.84	1450.66
mcp22844	1250.75	4.45	1411.02
mcp22837	1282.13	6.23	1423.22
mcp22836	1230.88	3.54	1395.54
mcp22843	1288.80	1.13	1387.84
mcp22838	1313.71	4.36	1432.18
Amlitelimab	1128.82	3.34	1197.52
Human Isotype	1.00	0.36	1.00

[00203] Human OX40L antibodies demonstrated binding to human OX40L-conjugated beads with cross-reactivity to cynomolgus OX40L-conjugated beads. Test antibodies showed positive binding to human OX40L-conjugated beads with a range of observed FOIs between 1069.097-1330.664 at 25 nM. Test antibodies showed positive binding to cynomolgus OX40L-conjugated beads with a range of observed FOIs between 1266.524-1450.992 at 25 nM. Anti-OX40L positive control antibody, amlitelimab, showed positive binding to human OX40L-conjugated beads at 25 nM (FOI=1128.819) with cross-reactivity to cynomolgus OX40L-conjugated beads at 25 nM (FOI=1197.518).

[00204] Example 2

[00205] Cell-Based OX40L Antibody Binding Assay

[00206] Human recombinant OX40L-expressing CHO-K1 cells (Genscript) and CHO-K1 cells (Sigma Aldrich) were suspended in pre-warmed PBS + 2% FBS. CHO-K1 cells were washed with 1X PBS and centrifuged at 400xg for 5 minutes at room temperature. CHO-K1 were stained by incubating the cells with a final concentration of 5 μ M of prepared CELLTRACE™ Violet stock solution in 1X PBS for 10 minutes at 37°C. Stained cells were subsequently washed with PBS + 2% FBS, centrifuged at 400xg for 5 minutes at room temperature, and resuspended in PBS + 2% FBS.

[00207] CHO-K1 OX40L-expressing cells and stained CHO-K1 parental cells were then centrifuged at 400xg for 5 minutes at room temperature and resuspended in an equal ratio of CHO-K1 parental cells to CHO-K1 OX40L-expressing cells. Cell mixtures were seeded into a v-bottom 96-well plate, centrifuged at 400xg for 5 minutes at room temperature and resuspended with serial dilutions of anti-OX40L antibodies or isotype control antibodies in duplicate for 30 minutes at 37°C in the dark.

[00208] Following incubation, cells were washed twice with PBS + 2% FBS and centrifuged at 400xg for 5 minutes and resuspended in a solution containing 75 nM ALEXA FLUOR® F(ab')₂ 647 Goat anti-human IgG Fc Gamma in PBS + 2% FBS for 30 minutes at 37°C in the dark. Following incubation, cells were washed in PBS + 2% FBS and pelleted by centrifugation at 400xg for 5 minutes. Cells were resuspended, loaded into a CYTOFLEX® S Flow Cytometer, and measured using CYTEXPERT®. The average median fluorescence intensity (MFI) was calculated across all isotype replicates for each cell type, and the fold over isotype (FOI) by dividing the sample MFI by the average isotype MFI for each cell type. The results are shown in Table 2.

[00209] Table 2: Cell-Based OX40L Antibody Binding Assay

Antibody ID	CHO-K1 Parental		CHO-K1 OX40L	
	Mean FOI	SD	Mean FOI	SD
mcp22835	2.19	0.21	209.49	5.09
mcp22836	1.54	0.32	221.85	9.55
mcp22837	1.35	0.17	240.18	2.24
mcp22838	1.28	0.12	177.19	4.50
mcp22839	1.54	0.44	181.75	16.5F1
mcp22840	2.11	0.05	215.86	6.26
mcp22841	1.40	0.44	163.24	7.88
mcp22842	1.52	0.58	203.17	19.67
mcp22843	1.41	0.31	188.42	20.10
mcp22844	1.54	0.31	239.96	10.04
mcp22845	1.75	0.42	325.13	24.02

mcp22846	1.73	0.55	250.05	0.67
Amlitelimab	1.20	0.40	105.55	3.00
Human IgG ₄	1.00	0.16	1.00	0.15
Human IgG ₁	1.00	0.14	1.00	0.16

[00210] Human OX40L antibodies demonstrated binding to human OX40L expressing CHO-K1 cells with no binding to CHO-K1 parental cells. Test antibodies showed positive binding to CHO-K1 OX40L cells with a range of observed FOIs between 325.127-163.241 at top concentration of 25 nM. Test antibodies showed no marked binding to CHO-K1 parental cells at all concentrations tested. Range of observed FOIs between 1.282-2.185 on CHO-K1 parental cells at top concentration of 25 nM. Anti-OX40L positive control antibody, amlitelimab, showed positive binding to CHO-K1 OX40L cells at all concentrations tested (FOI=105.553 at top concentration of 25 nM; FOI=10.274 at 0.04 nM, lowest concentration tested) with no binding observed to CHO-K1 parental cells (FOI=1.203 at top concentration of 25 nM).

[00211] Example 3

[00212] Cell-Based OX40L Cross-Reactivity Antibody Binding Assay

[00213] ExpiCHO-S cells were transiently transfected via electroporation with human OX40L, mouse OX40L, rat OX40L or cyno OX40L plasmid DNA or buffer (control). Transfected cells were suspended in pre-warmed PBS + 2% FBS, washed with 1X PBS and centrifuged at 400xg for 5 minutes at room temperature. Human OX40L- and mouse OX40L-expressing transfected cells were stained by incubating the cells with a final concentration of 100 nM of prepared CELLTRACE™ CFSE stock solution in 1X PBS for 10 minutes at 37°C. Cyno OX40L- and rat OX40L-expressing transfected cells were stained by incubating the cells with a final concentration of 1 µM of prepared CELLTRACE™ Yellow stock solution in 1X PBS for 10 minutes at 37°C. Stained cells were subsequently washed with PBS + 2% FBS, centrifuged at 400xg for 5 minutes at room temperature, and resuspended in PBS + 2% FBS.

[00214] Unstained control cells, stained human OX40L-expressing cells, and stained cynomolgus OX40L-expressing cells were then combined in an equal ratio. Unstained control cells, stained mouse OX40L-expressing cells, and stained rat OX40L-expressing cells were then combined in an equal ratio. Cell mixtures were seeded into separate v-bottom 96-well plates. Serial dilutions of anti-OX40L antibodies or isotype control antibodies prepared at 2X were then added

in equal volume to seeded cell suspensions, in duplicate. Cells and antibodies were incubated for 30 minutes at 37°C in the dark.

[00215] Following incubation, cells were washed twice with PBS + 2% FBS and centrifuged at 400xg for 5 minutes and resuspended in a solution containing 200 nM ALEXA FLUOR® 647-F(ab')₂ goat anti-human IgG Gx gamma in PBS + 2% FBS for 30 minutes at 37°C in the dark. Following incubation, cells were washed in PBS + 2% FBS and pelleted by centrifugation at 400xg for 5 minutes. Cells were resuspended in PBS + 2% FBS and loaded into a CYTOFLEX® S Flow Cytometer, and measured using CYTEXPERT®. The average median fluorescence intensity (MFI) was calculated across all isotype replicates for each cell type, and the fold-over-isotype (FOI) was calculated by dividing the sample MFI by the average species-matched isotype MFI for each cell type. The results are shown in Table 3 and Figures 1A-1C for the control, human OX40L and cynomolgus OX40L binding assay, and in Table 4 and Figures 2A-2C for the control, mouse OX40L and rat OX40L binding assay.

[00216] Table 3: Cell-based OX40L Cross-Reactivity Antibody Binding Assay – Human and Cynomolgus OX40L

ExpiCHO-S Control	100nM		20 nM		4 nM		0.8 nM		0.16 nM		0 nM	
	Mean FOI	SD	Mean FOI	SD	Mean FOI	SD	Mean FOI	SD	Mean FOI	SD	Mean FOI	SD
mcp22841	1.15	0.08	1.07	0.02	1.19	0.02	1.55	0.20	1.03	0.03		
mcp32754	0.98	0.02	1.00	0.03	1.09	0.01	1.04	0.02	0.93	0.00		
mcp32757	1.13	0.00	1.21	0.14	1.09	0.06	1.10	0.06	1.00	0.02		
Amltelimab	1.05	0.10	1.08	0.07	1.09	0.00	1.05	0.05	0.97	0.06		
Hu 2°											0.84	0.10
ExpiCHO-S Human OX40L	100nM		20nM		4nM		0.8nM		0.16nM			
	Mean FOI	SD	Mean FOI	SD	Mean FOI	SD	Mean FOI	SD	Mean FOI	SD		
mcp22841	37.59	2.91	35.75	1.80	33.50	0.09	16.82	1.19	4.60	0.61		
mcp32754	39.04	1.98	40.08	2.40	31.54	0.87	16.16	2.19	5.11	0.67		
mcp32757	41.18	0.15	40.11	1.76	28.98	0.79	16.07	1.18	4.90	0.75		
Amltelimab	40.35	1.80	39.23	0.52	30.66	2.19	29.35	0.79	15.05	1.04		
Hu 2°											0.86	0.12
ExpiCHO-S	100nM		20nM		4nM		0.8nM		0.16nM			

Cyno OX40L	Mean FOI	SD	Mean FOI	SD	Mean FOI	SD	Mean FOI	SD	Mean FOI	SD		
mcp22841	9.20	0.05	7.64	0.08	6.08	0.09	3.89	0.52	1.79	0.12		
mcp32754	9.50	0.27	8.14	0.38	5.85	0.05	3.31	0.21	1.74	0.08		
mcp32757	9.39	0.08	8.51	0.02	5.46	0.02	3.39	0.13	1.78	0.07		
1c_IgG4	8.51	0.09	7.89	0.10	6.53	0.14	6.03	0.11	3.28	0.28		
Hu 2°											0.87	0.09

[00217] Table 4: Cell-based OX40L Cross-Reactivity Antibody Binding Assay – Mouse and Rat OX40L

ExpiCHO-S Control	100 nM		20 nM		4 nM		0.8 nM		0.16 nM			
	Mean FOI	SD	Mean FOI	SD	Mean FOI	SD	Mean FOI	SD	Mean FOI	SD	Mean FOI	SD
mcp22841	0.88	0.05	1.14	0.02	1.05	0.06	0.91	0.01	0.92	0.11		
mcp32754	1.00	0.01	1.15	0.01	1.08	0.01	1.18	0.01	1.01	0.06		
mcp32757	1.18	0.05	1.11	0.05	1.09	0.08	1.14	0.04	1.05	0.26		
Amlitelimab	0.98	0.07	1.01	0.15	1.05	0.02	1.14	0.08	0.98	0.18		
Ms anti-Rt OX40L	1.04	0.12	0.93	0.05	0.97	0.11	1.07	0.06	0.91	0.14		
Rt anti-Ms OX40L	1.10	0.24	1.06	0.21	1.17	0.11	1.12	0.01	0.92	0.01	0.93	0.07
Ms 2°											0.93	0.07
Rt 2°											0.90	0.04
Hu 2°											0.87	0.04
ExpiCHO-S Rat OX40L	100 nM		20 nM		4 nM		0.8 nM		0.16 nM			
	Mean FOI	SD	Mean FOI	SD	Mean FOI	SD	Mean FOI	SD	Mean FOI	SD		
mcp22841	0.84	0.05	1.12	0.00	1.05	0.04	0.89	0.01	0.90	0.12		
mcp32754	1.01	0.00	1.17	0.02	1.06	0.01	1.19	0.01	0.99	0.07		
mcp32757	1.18	0.06	1.11	0.05	1.10	0.07	1.17	0.06	1.05	0.26		
Amlitelimab	0.98	0.07	1.01	0.17	1.05	0.02	1.15	0.08	1.00	0.20		
Ms anti-Rt OX40L	506.1 4	7.64	471.2 7	11.35	218.5 0	17.74	87.16	11.41	15.49	0.70		
Rt anti-Ms OX40L	1.39	0.33	1.14	0.22	1.19	0.12	1.16	0.02	0.92	0.01		
Ms 2°											0.94	0.09

Rt 2°											0.94	0.04
Hu 2°											0.91	0.04
ExpiCHO-S Mouse OX40L	100 nM		20 nM		4 nM		0.8 nM		0.16 nM			
	Mean FOI	SD	Mean FOI	SD	Mean FOI	SD	Mean FOI	SD	Mean FOI	SD		
mcp22841	0.85	0.05	1.12	0.00	1.06	0.06	0.90	0.02	0.91	0.10		
mcp32754	1.01	0.02	1.15	0.02	1.08	0.00	1.18	0.00	1.00	0.05		
mcp32757	1.19	0.06	1.11	0.06	1.08	0.04	1.16	0.03	1.05	0.27		
1c_IgG4	0.98	0.07	1.00	0.15	1.06	0.03	1.16	0.08	0.98	0.19		
Ms anti-Rt OX40L	50.62	4.82	10.91	1.13	2.32	0.42	1.40	0.07	0.95	0.12		
Rt anti-Ms OX40L	407.78	3.95	460.52	9.04	305.74	13.38	99.37	4.63	19.15	1.47		
Ms 2°											0.92	0.09
Rt 2°											0.92	0.04
Hu 2°											0.89	0.05

[00218] Example 4**[00219]** Antibody Characterization Using High-Throughput Surface Plasmon Resonance**[00220]** Part A: mcp22835 through mcp22846

[00221] High-throughput surface plasmon resonance (SPR) coupled kinetic experiments were performed on a BIACORE® 8K+ instrument equipped with a Series S Protein A chip. Purified antibody samples were diluted in running buffer (1x HBS-EP+) to 10 nM. The human, cynomolgus, mouse, and rat OX40L analyte was centrifuged at top speed in the Eppendorf centrifuge 5425 R at 4°C. Human and cynomolgus OX40L was prepared as a 7 point, 3-fold dilution series in running buffer with a top concentration of 100 nM. Mouse and rat OX40L were prepared at a single concentration of 100 nM only in running buffer.

[00222] To measure binding kinetics, antibody samples were captured onto Flow cell 2 of all 8 channels on the Series S Protein A chip with a contact time of 30s and flow rate of 10 µL/min. Flow cell 1 of each channel was used as a reference. Human and cynomolgus OX40L at increasing concentrations (0 nM, 0.14 nM, 0.41 nM, 1.23 nM, 3.70 nM, 11.1 nM, 33.3 nM, 100 nM), mouse and rat OX40L at a single concentration (100 nM), as well as a blank buffer for baseline subtraction, were sequentially injected onto the chip surface with a contact time of 2 min

(association phase) followed by a running buffer injection for 5 min (dissociation phase) at a flow rate of 30 μ L/min. A 2 x 20 s regeneration step was performed between each cycle by injecting 10 mM glycine HCl, pH 2.0 onto the chip surface at a flow rate of 30 μ L/min: The chip surface was regenerated after each cycle i.e., multi-cycle kinetics. The method was performed at two different temperatures (25°C and 37°C).

[00223] The data were analyzed using the BIAcore® Insight Evaluation software. Briefly, the data were referenced with Flow cell 1 and double-referenced to a buffer cycle, then force-fit globally to a 1: 1 Langmuir binding model to determine the apparent association (k_a) and dissociation (k_d) kinetic rate constants, and binding affinity constants (KD). All antibodies demonstrated apparent affinities (KDapp) at 25°C for human OX40L and cynomolgus OX40L in the high pM to low nM range and did not show detectable binding to mouse or rat OX40L. See Tables 5-1 through 5-4.

[00224] Table 5-1: Binding Kinetics (Human OX40L at 25°C)

Antibody ID	k_a (M ⁻¹ .s ⁻¹)	k_d (s ⁻¹)	KD (nM)
mcp22835	2.3E+05	3.1E-04	1.4
mcp22836	2.8E+05	3.4E-04	1.2
mcp22837	8.2E+05	5.3E-04	0.65
mcp22838	2.7E+05	5.6E-04	2.1
mcp22839	8.3E+05	4.2E-04	0.51
mcp22840	5.7E+05	5.3E-04	0.93
mcp22841	9.4E+05	3.3E-04	0.35
mcp22842	1.1E+06	5.9E-04	0.54
mcp22843	9.7E+05	7.8E-04	0.80
mcp22844	2.7E+05	4.1E-04	1.5
mcp22845	1.2E+06	5.6E-04	0.47
mcp22846	2.8E+05	3.6E-04	1.3

[00225] Table 5-2: Binding Kinetics (Cynomolgus OX40L at 25°C)

Antibody ID	k_a (M ⁻¹ .s ⁻¹)	k_d (s ⁻¹)	KD (nM)
mcp22835	2.4E+05	4.4E-04	1.9
mcp22836	2.2E+05	5.1E-04	2.3
mcp22837	8.6E+05	8.0E-04	0.93
mcp22838	1.5E+05	1.2E-03	8.0
mcp22839	1.1E+06	5.8E-04	0.52

mcp22840	1.6E+06	5.7E-04	0.37
mcp22841	2.2E+06	3.2E-04	0.15
mcp22842	7.1E+05	1.0E-03	1.4
mcp22843	1.1E+06	1.3E-03	1.2
mcp22844	2.5E+05	8.8E-04	3.5
mcp22845	1.3E+06	6.0E-04	0.47
mcp22846	2.2E+05	6.4E-04	2.9

[00226] Table 5-3: Binding Kinetics (Human OX40L at 37°C)

Antibody ID	k_a (M ⁻¹ .s ⁻¹)	k_d (s ⁻¹)	KD (nM)
mcp22835	4.0E+05	9.5E-04	2.4
mcp22836	4.4E+05	1.1E-03	2.4
mcp22837	1.0E+06	1.0E-03	0.97
mcp22838	3.0E+05	1.0E-03	3.3
mcp22839	1.0E+06	1.2E-03	1.1
mcp22840	8.3E+05	1.2E-03	1.4
mcp22841	1.3E+06	9.8E-04	0.78
mcp22842	1.5E+06	1.0E-03	0.70
mcp22843	1.0E+06	1.1E-03	1.1
mcp22844	4.2E+05	9.1E-04	2.2
mcp22845	1.7E+06	1.1E-03	0.66
mcp22846	4.4E+05	1.1E-03	2.6

[00227] Table 5-4: Binding Kinetics (Cynomolgus OX40L at 37°C)

Antibody ID	k_a (M ⁻¹ .s ⁻¹)	k_d (s ⁻¹)	KD (nM)
mcp22835	3.7E+05	1.3E-03	3.5
mcp22836	3.2E+05	1.4E-03	4.5
mcp22837	7.9E+05	1.4E-03	1.7
mcp22838	1.4E+05	1.7E-03	12
mcp22839	1.2E+06	1.6E-03	1.3
mcp22840	1.9E+06	1.4E-03	0.76
mcp22841	2.2E+06	1.2E-03	0.53
mcp22842	5.3E+05	1.4E-03	2.6
mcp22843	6.5E+05	1.7E-03	2.5
mcp22844	3.6E+05	1.3E-03	3.7
mcp22845	1.4E+06	1.2E-03	0.86

mcp22846	4.1E+05	1.5E-03	3.7
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[00228] Part B: mcp32754 and mcp32757

[00229] Target binding (antibody capture) kinetics

[00230] Surface plasmon resonance (SPR) kinetic experiments were performed on a Biacore 8K instrument at 37°C. The antibody samples were captured on a Series S Protein A chip surface (flow cell 2) at a concentration of 1.5µg/mL, using a flow rate of 10ul/min. OX40L was prepared in a 1:3 dilution series starting at 100nM in HBS-EP+. For each concentration, the protein was injected for three minutes (association phase), followed by running buffer injection for five minutes (dissociation phase) at a flow rate of 30ul/min. One regeneration cycle of 30s was performed between each dilution series by injecting 10mM glycine pH 1.5 on the chip surface. A buffer only cycle was also performed between each dilution series for blank subtraction.

[00231] Data analysis

[00232] Data analysis was performed with the Biacore Insight Evaluation software. The data were double-referenced to flow cell one and a blank cycle. When possible, the data were globally fit to a 1:1 Langmuir binding model to determine their apparent association (k_a) and dissociation (k_d) kinetic rate constants and binding affinity constant (KD). The R_{max} was determined based on the maximum binding signal at the end of the association phase. The theoretical R_{max} were calculated as [Capture/Immobilization level (RU) * (Analyte MW (kDa) / Ligand MW (kDa) * Ligand binding sites] and the R_{max} ratio was calculated as [Observed R_{max} (RU) / Theoretical R_{max} (RU) * 100%]. χ^2 was calculated as [χ^2 / Observed R_{max} (RU) * 100%]. See Tables 5-5 and 5-6, Figures 11A through 11C, and Figure 12.

[00233] Table 5-5: Binding kinetics (Human OX40L at 37°C)

Antibody ID	Replicate #	k_a (M ⁻¹ .s ⁻¹)	k_d (s ⁻¹)	KD (M)
mcp32754	1	1.50E+06	1.63E-03	1.09E-09
mcp32754	2	1.52E+06	1.61E-03	1.06E-09
mcp32757	1	1.4E+06	1.12E-03	8.03E-10
mcp32757	2	1.38E+06	1.17E-03	8.48E-10
AmltelimabIgG4PE	1	1.25E+06	7.70E-04	6.18E-10

[00234] Table 5-6: Binding kinetics (Cynomolgus OX40L at 37°C)

Antibody ID	Replicate #	k_a	k_d (s ⁻¹)	KD (M)
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		(M-1.s-1)		
mcp32754	1	2.93E+05	2.10E-03	7.16E-09
mcp32754	2	3.08E+05	2.07E-03	6.74E-09
mcp32757	1	2.26E+05	1.48E-03	6.52E-09
mcp32757	2	2.38E+05	1.43E-03	6.00E-09
AmltelimabIgG4PE	1	3.52E+05	6.55E-04	1.86E-09

[00235] Example 5

[00236] OX40 Reporter Assay Dose-Response

[00237] A bioluminescent cell-based assay was performed to measure the antagonism of anti-OX40L antibodies in the presence of soluble human OX40L to prevent signaling through the OX40 receptor and to assess the OX40L-blocking activity of recombinant antibodies in a dose-dependent manner.

[00238] OX40L master mix (4X) was prepared 12-point 2-fold serial dilution of anti-OX40L antibodies and isotype control(s) starting at 100 nM (4X) and buffer-only controls and incubated for a minimum of 10 minutes. Note: buffer only wells were included with and without OX40L mastermix to determine maximum stimulation and background signal, respectively. Following the incubation step, pre-plated OX40 effector cells were removed from the incubator. An electronic multichannel pipette was used to add 10uL of the premixed anti-OX40L antibody titrations or controls and soluble OX40L (2x) to the assay plates according to the plate layout (1x). After the plates were prepared, the plates were covered with a lid and incubated in a 37°C, 5% CO₂ incubator for 5 hours.

[00239] After incubation, plates were allowed to cool to ambient temperature for 10-15 minutes and then 20 µL of ambient temperature reconstituted BIO-GLO™ reagent was added to each well. After incubating for 5-15 minutes, luminescence was measured using a BIOTEK® SYNERGY™ Neo2 plate reader in order to determine IC₅₀ values.

[00240] Briefly, relative light unit (RLU) values for each well were corrected by subtracting the average RLU value of the buffer only replicate wells on the same plate. The corrected RLU values for each well are normalized to percent inhibition values using the following equation:

$$\text{percent_inhibition} = (1 - (\text{corrected_rlu} / \text{ctrl_corrected_rlu})) * 100$$

where ctrl_corrected_rlu is the average corrected RLU value of the replicate wells with the maximum concentration of the isotype control and the same stimulation type on the same plate.

[00241] The sample concentrations and percent inhibition values are used to fit four parameter log Hill curves (using the `scipy.optimize.curve_fit` Python package) for each unique group of sample, plate and stimulation type. The EC_{50} parameter estimates are reported as asymptotic 95% confidence intervals, which are calculated using a critical value from the t-distribution. Samples were run in triplicate. See Table 6, below.

[00242] Table 6: IC_{50} values

Antibody ID	Soluble OX40L			OX40L CHO-K1 Cells		
	IC_{50} (nM)	IC_{50} (nM)	IC_{50} (nM)	IC_{50} (nM)	IC_{50} (nM)	IC_{50} (nM)
mcp22835	2.41	4.77	3.94	2.11	1.57	0.96
mcp22836	3.54	4.44	3.80	6.38	1.08	0.95
mcp22837	1.82	3.31	2.24	1.27	0.50	0.98
mcp22838	3.33	3.72	3.06	0.86	0.31	0.94
mcp22839	3.59	3.85	2.25	1.23	0.44	0.90
mcp22840	2.84	4.46	4.00	2.16	2.30	0.94
mcp22841	2.13	2.77	2.87	1.71	0.44	0.94
mcp22842	5.23	10.01	5.90	0.89	1.39	0.95
mcp22843	6.50	8.52	4.55	1.56	0.78	0.96
mcp22844	3.84	5.05	3.30	2.49	1.69	0.96
mcp22845	1.28	3.32	2.77	0.54	0.66	0.97
mcp22846	3.22	4.93	3.44	2.69	0.85	0.95
Amlitelimab	1.54	1.98	1.75	0.50	0.70	0.97

[00243] All anti-OX40L antibodies demonstrated dose-dependent inhibition of OX40L signaling through the OX40 receptor in the presence of CHO-K1 OX40L cells. The IC_{50} of anti-OX40L antibodies ranged from 0.5047 – 6.38 nM in biological replicate 1, 0.3132 – 2.296 nM in biological replicate 2 and 0.4373 – 2.609 nM in biological replicate 3 with a mean SD across replicates of 0.585 nM. Human isotype control showed no significant inhibition on membrane-bound OX40L activity, no OX40L stimulation was observed in the presence of CHO-K1 parental cells.

[00244] Additionally, the concentration of the antibody was plotted against percent inhibition to determine EC_{50} values for antibodies mcp32753 – mcp32758. See Table 7, below. See also Figure 3.

[00245] Table 7: EC_{50} values

Antibody ID	Soluble OX40L				
	Sample	EC_{50} (nM)	EC_{50} (nM) 95_pct_ci_min	EC_{50} (nM) 95_pct_ci_max	Fitted_max_pct_inhibition
mcp32753	abps68370	2.04	1.81	2.31	99.49

mcp32754	abps68374	1.97	1.77	2.20	99.94
mcp32755	abps68371	1.81	1.65	1.99	98.36
mcp32756	abps68375	1.98	1.85	2.11	99.13
mcp32757	abps68376	2.67	2.45	2.92	100.92
mcp32758	abps68372	2.31	2.01	2.64	99.77

Table 7 (continued)

Antibody ID	Soluble OX40L			
	Fitted_max_pct_ Inhibition_95_pct_ _ci_min	Fitted_max_pct_ Inhibition_95_pct_ _ci_min	Fitted_max_l sq_fit_sd	Hill_coefficient
mcp32753	94.69	104.28	2.36	3.04
mcp32754	96.23	103.65	1.82	4.00
mcp32755	93.86	102.87	2.21	3.96
mcp32756	96.55	101.71	1.27	4.04
mcp32757	97.44	104.40	1.71	2.92
mcp32758	93.78	105.76	2.94	2.65

Table 7 (continued)

Antibody ID	Soluble OX40L	
	Fitted_min_pct_ inhibition	r_squared
mcp32753	13.91	0.98
mcp32754	28.94	0.98
mcp32755	3.82	0.98
mcp32756	17.98	0.99
mcp32757	21.16	0.99
mcp32758	4.32	0.97

[00246] Example 6

[00247] Antibody Dependent Cell Cytotoxicity (ADCC)

[00248] Experiments were carried out to assess the ability of anti-OX40L antibodies to induce Fc receptor-mediated antibody-dependent cell-mediated cytotoxicity (ADCC) according to Promega Technical Manual ADCC Bioassay Effector Cells, Propagation Model, Instructions for Use of Product, G7102, with modifications.

[00249] Human recombinant OX-40L CHO-K1/OX-40L cells were cultured according to the GENSCRIPT® CHO-K1/OX40L manual and cells were prepared at a concentration of 0.3×10^6 cells/mL in RPMI media supplemented with 2% super low IgG FBS. 10 μ L of the target cell solution was added to each well of a microplate (for a total number of 3,000 cells per well). After

incubating the plates for 16-24 hours at 37°C 5% CO₂ to allow time for adherent cells to attach to plate, 5 µL of 10-point 4x serial dilution of anti-OX40L/isotype antibodies were added to each well (10-point 4x serial dilution, 0.0001 nM to 100 nM). Effector cells were prepared at a concentration of 3 x 10⁶ cells/mL in RPMI media supplemented with 2% super low IgG FBS and 5 µL of the effector cell solution were added to each well (for a total number of 15,000 cells per well). Plates were then incubated for 20 hours at 37°C 5% CO₂.

[00250] Plates were then removed from the incubator and equilibrated to room temperature for 15 minutes. 20 µL of BIOGLO® solution was added to each well, and after incubating for 5-30 minutes, luminescence was measured using a BIOTEK® SYNERGY™ Neo2 plate reader.

[00251] The negative control, isotype control, and all anti-OX40L antibodies but mAb mcp22843 showed an ADCC activity between 0.6 and 1.4 FOI at all concentrations. mAb mcp22843 showed higher than 1.4-fold over isotype ADCC activity at the three highest tested concentrations of 100 nM, 25 nM, and 6.25 M in both replicates, and a maximum FOI above 10. The positive control showed higher than 1.4-fold over isotype ADCC activity at nearly all concentrations, with a maximum FOI above 20. See, e.g., Figure 4A and 4B.

[00252] Example 7

[00253] Complement-Dependent Cytotoxicity

[00254] Experiments were carried out to assess the complement-dependent cytotoxicity activity of anti-OX40L antibodies on CHO-K1 expressing OX40L cells. 5 µL of serial diluted anti-OX40L antibodies and isotype control antibody were transferred into 384-well plates containing 10,000 CHO-K1 expressing OX40L cells per well. Cells were incubated at 37°C, 5% CO₂ for 30 minutes prior to adding complement. 5 µL of “no complement”, “complement”, and “heat-inactivated complement” samples were then added to each well and incubated at 37°C, 5% CO₂ for 3 hours. Cell supernatant was subsequently removed, and 10 µL of pre-warmed Ham’s F12 FBS-containing growth media was added to each well. Plates were equilibrated at RT for 30 minutes, and 10 µL CELLTITER-GLO® was added to each well. After shaking the plates for 2 minutes at 600xg, luminescence was measured using a BIOTEK® SYNERGY™ Neo2 plate reader 10 minutes later to allow for signal equilibration.

[00255] In absence of human pooled serum, live cell levels were equal or above 98% in cells treated with dose-responses of tested anti-OX40L antibodies, benchmark IgG1, benchmark IgG4, or isotype controls.

[00256] In the presence of human pooled sera, live cell levels of “no antibody” conditions were equal or greater than 94%. In the presence of human pooled sera, live cells levels in response to a dose response of isotype control were equal or above 90% with the exception of some cells treated with 0.16 nM of IgG1.

[00257] In the presence of human pooled sera, benchmark IgG1 induced a dose-dependent decrease in cell viability levels, reaching below 7% and 23% at a concentration of 4 nM. In the presence of human pooled sera, benchmark IgG4 induced a dose-dependent decrease in cell viability levels, reaching below 62% and 79% at a concentration of 20 nM. In the presence of human pooled sera, all tested anti-OX40L antibodies induced dose-dependent decreases in cell viability levels. IC₅₀ values were subsequently calculated from the observed results. Experiments were run in duplicate. See Table 8, below. See also Figures 5A-5F.

[00258] Table 8: IC₅₀ values

Antibody ID	IC ₅₀ (nM)	% of live cells	IC ₅₀ (nM)	% of live cells
mcp22835	4.08	38.44	n.d.	n.d.
mcp22836	3.48	36.15	3.78	47.76
mcp22837	5.09	33.67	6.21	45.89
mcp22838	2.34	30.30	3.49	42.59
mcp22839	1.46	43.60	3.84	41.8
mcp22840	3.67	26.79	1.85	32.81
mcp22841	9.62	62.09	5.51	70.40
mcp22842	3.42	16.64	3.84	35.72
mcp22843	3.22	19.22	3.18	40.30
mcp22844	3.22	29.07	3.92	33.18
mcp22845	3.75	41.78	5.44	51.39
mcp22846	3.77	30.62	5.56	34.54
BC1c IgG4	7.25	51.48	6.47	71.24
BC1c IgG1	0.86	4.3	1.12	15.82

[00259] All tested anti-OX40L antibodies exhibited CDC activities in presence of human pooled serum. CDC activities were measured with concentration equal or higher to 4 nM. IC₅₀ ranges from 1.5 nM to 9.6 nM and minimum live cell levels range from 19% to 70% were observed.

[00260] Example 8

[00261] T Cell Activation Assay

[00262] Experiments were carried out to assess the efficacy of anti-OX40L antibodies at inhibiting soluble OX40L-evoked primary T cell activation, measured as IL-2 secretion.

[00263] 25 μ L of anti-OX40L antibody (0-400 nM) and 25 μ L of 3.2 μ g/mL hOX40L were added to wells of a microplate and briefly mixed using a plate shaker. Plates were incubated at room temperature for 30-60 minutes.

[00264] After incubation, 25 μ L of primary T cells (4×10^6 cells/mL) and 25 μ L of 10 μ g/mL phytohemagglutinin-L (PHA-L) were added to wells containing anti-OX40L antibody and hOX40L, briefly mixed using a plate shaker, and incubated at 37°C and 5% CO₂ for 3 days. Non-specific T cell activation was also measured by replacing rhOX40L with 25 μ L of RPMI v/v 10% FBS and replacing PHA-L with 25 μ L of RPMI v/v 10% FBS for conditions without basal T cell activation (i.e., no OX40 expression). IL-2 release was measured using the LUMIT[™] IL-2 Immunoassay protocol (Promega).

[00265] The efficacy of anti-OX40L antibodies at inhibiting soluble OX40L-evoked primary T cell activation was tested in three different T cell donors (tcepbmc0074 (Fig. 6A and 6B), tcepbmc0075 (Fig. 6C and 6D), and tcepbmc0076 (Fig. 6E and 6F)). See Figures 6A, 6C, and 6E. All anti-OX40L antibodies displayed dose-dependent inhibition of IL-2 release from T cells in all T cell donors, with IC₅₀ range of 0.5-7 nM. None of the discovered anti-OX40L antibodies induced non-specific IL-2 release in T cells with or without OX40 expression. See Figures 6B, 6D, and 6F.

[00266] Example 9

[00267] Mixed Lymphocyte Reaction Assay

[00268] Experiments were carried out to assess the efficacy of anti-OX40L antibodies at inhibiting T cell proliferation and cytokine release in allogeneic mixed lymphocyte reaction. To this end, 25 μ L of an anti-OX40L antibody solution (0.06 nM to 200 nM) in RPMI v/v 10% FBS and 75 μ L of human mature Dendritic Cells (4,000 mDC cells) were added to wells of a microplate, briefly mixed using a plate shaker, and incubated at 37°C and 5% CO₂ for at least 60 minutes.

[00269] After incubation 100 μ L of CELLTRACE® Violet-labeled T cells (1×10^6 cells/mL in RPMI 1640 v/v 10% FBS) were added to each well containing anti-OX40L antibodies and mDCs, briefly mixed using a plate shaker, and incubated at 37°C and 5% CO₂ for 6 days. Cells and supernatants were harvested for T cell proliferation analysis via flow cytometry and cytokine release multiplexing via Meso Scale Discovery, respectively.

[00270] The efficacy of anti-OX40L antibodies at inhibiting T cell proliferation and cytokine release was evaluated in the three different donor pairs. A cut-off of 20% inhibition was used to indicate effective inhibitors. Most anti-OX40L antibodies showed dose-dependent inhibition of CD3 T cell proliferation in donor pair 1, but not in donor pairs 2 and 3, and all anti-OX40L antibodies demonstrated mean inhibition of CD3 T cell proliferation above 20%. The % inhibition of T cell proliferation strongly correlated between CD3 total T cells and CD4/CD8 subsets for all three donor pairs.

[00271] Most anti-OX40L antibodies showed dose-dependent inhibition of IFN- γ /IL-2/TNF- α release in all three donor pairs, and at 25 nM, all antibodies inhibited the release of two or more cytokines in all three donor pairs.

[00272] Example 10

[00273] c-Jun Signaling Assay

[00274] Experiments were carried out to assess levels of OX40L reverse signaling, measured as phosphorylated c-Jun (pc-Jun), in human umbilical vein endothelial cells (HUVECs) treated with anti-OX40L antibodies using the ALPHALISA® SUREFIRE® ULTRA™ detection kits.

[00275] While hOX40-Fc (10 μ g/mL (10X)) and hOX40-Avi (5.0 μ g/mL (10X)) were included in this assay as OX40L-specific assay controls, they showed no effect on levels of c-Jun phosphorylation. Accordingly, anisomycin (JNK activator, 250 μ g/mL (10X)) and SP600125 (JNK inhibitor, 50 μ M (1X)) were added as internal treatment controls for c-Jun phosphorylation. Anti-OX40L antibodies and IgG1 isotype antibodies were tested at three concentrations (1000 nM / 100 nM/ 10 nM (10X)).

[00276] Cells were washed using 100 μ L of pre-warmed serum-free F-12K media. 45 μ L of 50 μ M SP600125 was added to some wells. These represent wells that are pre-treated with SP600125 prior to treatment with anisomycin. 45 μ L of fresh serum-free F-12K media was added to the remainder of wells for serum starvation, and the plate was incubated for 40 mins at 37°C, 5% CO₂.

[00277] After incubation, 5 μ L of 10X anisomycin, hOX40-Fc, hOX40-Avi, and antibodies were added to wells and then incubated at 37°C, 5% CO₂ for 30 mins. Cells were lysed using 50 μ L of 1X lysis buffer and 10 μ L of lysates were added into two microplates for readouts of phosphorylated and total c-Jun, respectively, using a BIOTEK® SYNERGY™ Neo2 plate reader.

[00278] Control lysates (provided with the ALPHALISA® SUREFIRE® ULTRA™ kits as internal assay controls) showed high levels of measured phosphorylated c-Jun. Positive control

treatment anisomycin induced high levels of c-Jun phosphorylation (~20 to 25-fold), which was inhibited by pre-treatment with SP600125.

[00279] hOX40-Fc and hOX40-Avi were tested as OX40L-specific positive controls and showed no effect on levels of c-Jun phosphorylation, and anti-OX40L antibodies showed no evidence of inducing c-Jun phosphorylation at any test concentrations, except for antibody mcp22840. Test antibody mcp22840 induced a 1.8 to 2.7-fold increase in levels of phosphorylated c-Jun at 100 nM in one of two experiments. Accordingly, it was concluded that there were no consistent and significant changes in levels of phosphorylated c-Jun after treatment with anti-OX40L antibodies at tested concentrations.

[00280] Example 11

[00281] Antibody Dependent Cellular Phagocytosis (ADCP)

[00282] CHO-K1/OX40L cells were prepared at a concentration of 0.15×10^6 cells/mL in RPMI media supplemented with 4% super low IgG FBS. 10 μ L of cells were added to each well of a microplate for a total number of 1,500 cells per well. After centrifuging the plate to settle cells, samples were incubated for 16-24 hours at 37°C, 5% CO₂ to allow time for adherent cells to attach to the plate. Subsequently, an 11-point 4x serial dilution (0-100 nM) of anti-OX40L/Isotype antibodies in RPMI media supplemented with 4% super low IgG FBS in a v-bottom 96-well plate was prepared and 5 μ L of each dilution was transferred to the microplate.

[00283] Effector cells were washed with an equal volume of DPBS and prepared at a concentration of 3×10^6 cells/mL in RPMI media supplemented with 4% super low IgG FBS. 5 μ L of effector cells were aliquoted to each well of the microplate for a total number of 15,000 effector cells per well. After centrifuging the plate to settle cells, samples were incubated for 20 hours at 37°C, 5% CO₂.

[00284] After incubation was complete, 20 μ L of BIOGLO™ Luciferase Assay Reagent was added to each well of the microplate and incubated for 5-30 minutes at room temperature in the dark. Luminescence was subsequently measured using a BIOTEK® SYNERGY™ Neo2 plate reader in order to determine response curves. See Figures 7A and 7B.

[00285] Example 12

[00286] Th2 Inhibition Assay

[00287] Human PBMCs obtained from healthy donors were resuspended (5×10^6 cells/mL) in complete RPMI 1640 (1 mM sodium pyruvate, 25 mM HEPES, 2 mM L-glutamine, 1X non-essential amino acids) supplemented with 10% human serum, and seeded in duplicates in a 24-well plate (2.5×10^6 cells/well) coated with collagen I. Seeded cells were incubated with recombinant human thymic stromal lymphopoietin (TSLP; 120 ng/mL) and staphylococcal enterotoxin B (SEB; 2 ng/mL) in the presence or absence of increasing concentrations of tested anti-OX40L blocking antibodies (amlitelimab, mcp32754 and mcp32757; 0-100 nM) for 7 days. Every 2 days, complete RPMI was added to each well. On day 7, supernatants were harvested and stored at -80°C for subsequent analysis. Collected cell-free supernatants were then analyzed for cytokine concentration using the Meso Scale Discovery multiplex immunoassay as per manufacturer's instructions. Dose response curves demonstrating the effect of anti-OX40L blocking antibodies on normalized IL-9, IL-13 and IL-5 secretion in human PBMCs stimulated with TSLP and SEB are shown in Figures 8A-8I.

[00288] Example 13

[00289] IgG Recycling

[00290] MDCK-II (Madin-Darby Canine Kidney) cells expressing human FcRn or cynomolgus monkey FcRn were seeded and grown until confluent. Cells were washed and starved for 1 hour using HBSS (Hank's Balanced Salt Solution) (pH 7.4) + 1% BSA (Bovine Serum Albumin). The protein of interest was diluted in HBSS (pH 7.4) + 1% BSA and added to the cells, which were incubated for 2 hours. Media was removed, and the cells were extensively washed with ice-cold HBSS (pH 7.4) + 1% BSA before growth medium was added for overnight incubation. Supernatants were collected after the overnight incubation, and the collected samples were analyzed by MSD (Meso Scale Discovery) specific for human IgG. The data obtained are shown as mean $\pm$ standard deviation of one independent experiment performed in duplicate biological replicates and duplicate technical replicates. See Tables 9 and 10 below. See also Figure 9.

[00291] Table 9: Recycled mAb concentration in supernatant of Human FcRn-expressing Cells

mcp22841	mcp32754	mcp32757	Amlitelimab	Isotype control
ng/mL	ng/mL	ng/mL	ng/mL	ng/mL
253.8	214.0	256.8	8.7	4.2
291.2	197.8	273.0	8.9	4.1

[00292] Table 10: Recycled mAb concentration in supernatant of Cynomolgus Monkey FcRn-expressing Cells

mcp22841	mcp32754	mcp32757	Amltelimab	Isotype control
ng/mL	ng/mL	ng/mL	ng/mL	ng/mL
163.2	178.6	156.7	4.8	3.3
158.3	104.3	154.4	6.9	2.8

[00293] Example 14

[00294] Pharmacokinetic (PK) Study in hFcRn Tg32 Mice

[00295] Pharmacokinetic (PK) studies of monoclonal antibodies (mAbs) were conducted using male human FcRn homozygous transgenic mice (B6.Cg-Fcgrtm1Dcr Tg(FCGRT)32Dcr/DcrJ, Jackson Laboratories, Bar Harbor, ME, U.S.A). Each mAb (5 mg/kg) was administered intravenously to hFcRn Tg32 (n=4) via the tail vein. Blood samples were collected in MICROVETTE® CB 300 Z tubes from the tail nick at 5 min, 4 h, 1, 3, 7, 14, 21, 28, 35, 42, 49, 56 d after administration, and serum was obtained by centrifugation at 2500 ×g for 10 min at 4 °C. All animal experiments in this study were performed according to the Guidelines for the Care and Use of Laboratory Animals at Frontage Laboratories. Bioanalysis of serum concentrations of mAbs were determined by generic human IgG MSD (Meso Scale Discovery) assay. The serum samples were analyzed in technical duplicates. The pharmacokinetic profiles show mean ± standard deviation.

[00296] Noncompartmental PK analysis was performed using PhoenixWinNonlin (Version 8.5.1). The terminal elimination half-life ($t_{1/2}$) was determined using the equation $\ln(2)/|z|$, where $|z|$ is the slope of the terminal portion of the natural-log concentration–time curve, determined by linear regression of at least the last three timepoints. Systemic exposure was determined by calculating the area under the plasma concentration–time curve from time 0 to the last sampling time point (AUC_{0-t}), using the linear/log trapezoidal rule. Clearance was calculated using the Dose / AUC infinity. The noncompartmental PK parameters are reported as the average for individual animals, with the minimum and maximum values provided in brackets. See Table 11, below. See also Figures 10A and 10B.

[00297] Table 11: Pharmacokinetics

	Tg32 Clearance (mL/hr/kg)	Tg32 half-life (day)	Tg32 AUC (hour*µg/mL)
mcp22841	0.17 (0.15, 0.19)	17.1 (16.3, 18.5)	26,750 (23,400-29,200)
mcp32754	0.04 (0.03-0.05)	13 (12.5, 14.0)	121,331 (100,170, 136,509)
mcp32757	0.14 (0.13-0.16)	17.3 (14.8, 20.8)	30,865 (28,433, 32,481)

[00298] While this invention has been disclosed with reference to particular embodiments, it is apparent that other embodiments and variations of the inventions disclosed herein can be devised by others skilled in the art without departing from the true spirit and scope thereof. The appended claims include all such embodiments and equivalent variations.

CLAIMS

What is claimed is:

1. An antibody or antigen-binding fragment thereof that specifically binds to OX40L, wherein the antibody or antigen-binding fragment thereof comprises three CDRs of a heavy chain variable region set forth as SEQ ID NO: 13 and three CDRs of a light chain variable region set forth as SEQ ID NO: 14.

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

(a) CDR-H1 comprising residues 31-35 of the heavy chain variable region (VH), CDR-H2 comprising residues 50-66 of the VH, and CDR-H3 comprising residues 99-116 of the VH; and

(b) CDR-L1 comprising residues 24-34 of the light chain variable region (VL), CDR-L2 comprising residues 50-56 of the VL, and CDR-L3 comprising residues 89-97 of the VL; and wherein the CDR numbering is according to Kabat.

3. The antibody or antigen-binding fragment thereof as in claim 1 or claim 2, comprising a heavy chain variable region having an amino acid sequence that is at least 95% identical to the heavy chain variable region sequence set forth in SEQ ID NO: 13 and a light chain variable region having an amino acid sequence that is at least 95% identical to the light chain variable region sequence set forth in SEQ ID NO: 14.

4. The antibody or antigen-binding fragment thereof of any one of claims 1-3, comprising a heavy chain variable region set forth as SEQ ID NO: 13 and a light chain variable region set forth as SEQ ID NO: 14.

5. The antibody or antigen-binding fragment thereof of any one of claims 1-4, comprising:

(a) a heavy chain constant region set forth as SEQ ID NO: 61 and a light chain constant region set forth as SEQ ID NO: 63; or

(b) a heavy chain constant region set forth as SEQ ID NO: 62 and a light chain constant region set forth as SEQ ID NO: 63.

6. The antibody or antigen-binding fragment thereof of any one of claims 1-5, comprising:

(a) a heavy chain set forth as SEQ ID NO: 50 and a light chain set forth as SEQ ID NO: 38; or

(b) a heavy chain set forth as SEQ ID NO: 53 and a light chain set forth as SEQ ID NO: 38.

7. An anti-OX40L antibody or antigen-binding fragment thereof, comprising a heavy chain variable region and a light chain variable region, wherein the heavy chain variable region comprises:

(a) a CDR-H1 comprising the amino acid sequence of SEQ ID NO: 55;

(b) a CDR-H2 comprising the amino acid sequence of SEQ ID NO: 56; and

(c) a CDR-H3 comprising the amino acid sequence of SEQ ID NO: 57; and

wherein the light chain variable region comprises:

(a) a CDR-L1 comprising the amino acid sequence of SEQ ID NO: 58;

(b) a CDR-L2 comprising the amino acid sequence of SEQ ID NO: 59; and

(c) a CDR-L3 comprising the amino acid sequence of SEQ ID NO: 60.

8. The antibody or antigen-binding fragment thereof of claim 7, wherein the antibody or antigen-binding fragment thereof comprises a heavy chain variable region comprising an amino acid sequence at least 90% identical to SEQ ID NO: 13 and a light chain variable region comprising an amino acid sequence at least 90% identical to SEQ ID NO: 14.

9. The antibody or antigen-binding fragment thereof of claim 7, wherein the antibody or antigen-binding fragment thereof comprises a heavy chain variable region comprising an amino acid sequence at least 95% identical to SEQ ID NO: 13 and a light chain variable region comprising an amino acid sequence at least 95% identical to SEQ ID NO: 14.

10. The antibody or antigen-binding fragment thereof of claim 7, wherein the antibody or antigen-binding fragment thereof comprises a heavy chain variable region comprising an amino acid sequence at least 98% identical to SEQ ID NO: 13 and a light chain variable region comprising an amino acid sequence at least 98% identical to SEQ ID NO: 14.

11. The antibody or antigen-binding fragment thereof of claim 7, wherein the antibody or antigen-binding fragment thereof comprises a heavy chain variable region comprising an amino acid sequence at least 99% identical to SEQ ID NO: 13 and a light chain variable region comprising an amino acid sequence at least 99% identical to SEQ ID NO: 14.

12. The antibody or antigen-binding fragment thereof of claim 7, wherein the antibody or antigen-binding fragment thereof comprises a heavy chain variable region comprising the amino acid sequence of SEQ ID NO: 13 and a light chain variable region comprising the amino acid sequence of SEQ ID NO: 14.

13. The antibody or antigen-binding fragment thereof as in any one of claims 7-12, wherein the antibody or antigen-binding fragment thereof is selected from the group consisting of Fab, Fab', F(ab')₂, Fab'-SH, Fv, linear antibody, and single-chain antibody fragment.

14. The antibody or antigen-binding fragment thereof of any one of claims 7-13, wherein the antibody or antigen binding fragment thereof is a full-length antibody.

15. The antibody or antigen-binding fragment thereof of any one of claims 7-14, wherein the heavy chain variable region is fused to a heavy chain constant region and the light chain variable region is fused to a light chain constant region.

16. The antibody or antigen-binding fragment thereof of any one of claims 7-15, wherein the heavy chain constant region is selected from the group consisting of IgA1, IgA2, IgD, IgE, IgG1, IgG2, IgG3, IgG4, and IgM.

17. The antibody or antigen-binding fragment thereof of any one of claims 7-16, wherein the heavy chain constant region is of the IgG1 isotype.

18. The antibody or antigen-binding fragment thereof of any one of claims 7-17, wherein the antibody or antigen-binding fragment thereof comprises:

(a) a heavy chain constant region comprising an amino acid sequence at least 90% identical to SEQ ID NO: 61 and a light chain constant region comprising an amino acid sequence at least 90% identical to SEQ ID NO: 63; or

(b) a heavy chain constant region comprising an amino acid sequence at least 90% identical to SEQ ID NO: 62 and a light chain constant region comprising an amino acid sequence at least 90% identical to SEQ ID NO: 63.

19. The antibody or antigen-binding fragment thereof of any one of claims 7-18, wherein the antibody or antigen-binding fragment thereof comprises:

(a) a heavy chain constant region comprising an amino acid sequence at least 95% identical to SEQ ID NO: 61 and a light chain constant region comprising an amino acid sequence at least 95% identical to SEQ ID NO: 63; or

(b) a heavy chain constant region comprising an amino acid sequence at least 95% identical to SEQ ID NO: 62 and a light chain constant region comprising an amino acid sequence at least 95% identical to SEQ ID NO: 63.

20. The antibody or antigen-binding fragment thereof of any one of claims 7-19, wherein the antibody or antigen-binding fragment thereof comprises:

(a) a heavy chain constant region comprising an amino acid sequence at least 98% identical to SEQ ID NO: 61 and a light chain constant region comprising an amino acid sequence at least 98% identical to SEQ ID NO: 63; or

(b) a heavy chain constant region comprising an amino acid sequence at least 98% identical to SEQ ID NO: 62 and a light chain constant region comprising an amino acid sequence at least 98% identical to SEQ ID NO: 63.

21. The antibody or antigen-binding fragment thereof of any one of claims 7-20, wherein the antibody or antigen-binding fragment thereof comprises:

(a) a heavy chain constant region comprising an amino acid sequence at least 99% identical to SEQ ID NO: 61 and a light chain constant region comprising an amino acid sequence at least 99% identical to SEQ ID NO: 63; or

(b) a heavy chain constant region comprising an amino acid sequence at least 99% identical to SEQ ID NO: 62 and a light chain constant region comprising an amino acid sequence at least 99% identical to SEQ ID NO: 63.

22. The antibody or antigen-binding fragment thereof of any one of claims 7-21, wherein the antibody or antigen-binding fragment thereof comprises:

(a) a heavy chain constant region comprising the amino acid sequence of SEQ ID NO: 61 and a light chain constant region comprising the amino acid sequence of SEQ ID NO: 63; or

(b) a heavy chain constant region comprising the amino acid sequence of SEQ ID NO: 62 and a light chain constant region comprising the amino acid sequence of SEQ ID NO: 63.

23. The antibody or antigen-binding fragment thereof of any one of claims 7-22, comprising an Fc variant of a parent heavy chain constant region, the Fc variant comprising an amino acid substitution to block antibody binding to Fc γ RI, Fc γ RII, or Fc γ RIIIa.

24. The antibody or antigen-binding fragment thereof of claim 23, wherein the Fc variant comprises an amino acid substitution at a position selected from the group consisting of 234, 235, 252, 254, 256, 428, and 434, and wherein the Fc variant further comprises an amino acid deletion at position 447, and wherein amino acid numbering is according to the EU index.

25. The antibody or antigen-binding fragment thereof of claim 24, wherein the Fc variant is selected from one of:

(a) L234A, L235A, M428L, and N434S, and wherein K447 is deleted; or

(b) L234A, L235A, M252Y, S254T, and T256E, and wherein K447 is deleted, and wherein amino acid numbering is according to the EU index.

26. The antibody or antigen-binding fragment thereof of any one of claims 7-25, wherein the antibody or antigen-binding fragment thereof comprises:

(a) a heavy chain comprising an amino acid sequence at least 90% identical to SEQ ID NO: 50 and a light chain comprising an amino acid sequence at least 90% identical to SEQ ID NO: 38; or

(b) a heavy chain comprising an amino acid sequence at least 90% identical to SEQ ID NO: 53 and a light chain comprising an amino acid sequence at least 90% identical to SEQ ID NO: 38.

27. The antibody or antigen-binding fragment thereof of any one of claims 7-26, wherein the antibody or antigen-binding fragment thereof comprises:

(a) a heavy chain comprising an amino acid sequence at least 95% identical to SEQ ID NO: 50 and a light chain comprising an amino acid sequence at least 95% identical to SEQ ID NO: 38; or

(b) a heavy chain region comprising an amino acid sequence at least 95% identical to SEQ ID NO: 53 and a light chain region comprising an amino acid sequence at least 95% identical to SEQ ID NO: 38.

28. The antibody or antigen-binding fragment thereof of any one of claims 76-27, wherein the antibody or antigen-binding fragment thereof comprises:

(a) a heavy chain comprising an amino acid sequence at least 98% identical to SEQ ID NO: 50 and a light chain comprising an amino acid sequence at least 98% identical to SEQ ID NO: 38; or

(b) a heavy chain comprising an amino acid sequence at least 98% identical to SEQ ID NO: 53 and a light chain comprising an amino acid sequence at least 98% identical to SEQ ID NO: 38.

29. The antibody or antigen-binding fragment thereof of any one of claims 6-28, wherein the antibody or antigen-binding fragment thereof comprises:

(a) a heavy chain comprising an amino acid sequence at least 99% identical to SEQ ID NO: 50 and a light chain comprising an amino acid sequence at least 99% identical to SEQ ID NO: 38; or

(b) a heavy chain comprising an amino acid sequence at least 99% identical to SEQ ID NO: 53 and a light chain comprising an amino acid sequence at least 99% identical to SEQ ID NO: 38.

30. The antibody or antigen-binding fragment thereof of any one of claims 7-29, wherein the antibody or antigen-binding fragment thereof comprises:

(a) a heavy chain comprising the amino acid sequence of SEQ ID NO: 50 and a light chain comprising the amino acid sequence of SEQ ID NO: 38; or

(b) a heavy chain comprising the amino acid sequence of SEQ ID NO: 53 and a light chain comprising the amino acid sequence of SEQ ID NO: 38.

31. The antibody or antigen-binding fragment thereof of any one of claims 7-30, comprising a heavy chain comprising the amino acid sequence of SEQ ID NO: 50 and a light chain comprising the amino acid sequence of SEQ ID NO: 38.

32. The antibody or antigen-binding fragment thereof of any one of claims 7-30, comprising a heavy chain comprising the amino acid sequence of SEQ ID NO: 53 and a light chain comprising the amino acid sequence of SEQ ID NO: 38.

33. The antibody or antigen-binding fragment thereof of any one of claims 7-32, wherein the heavy chain constant region further comprises a C-terminal lysine residue.

34. A pharmaceutical composition comprising the antibody or antigen-binding fragment thereof as in any one of the preceding claims, and one or more pharmaceutically acceptable carriers, diluents, or excipients.

35. A nucleic acid encoding:

(a) an antibody heavy chain variable region or an antibody light chain variable region of an anti-OX40L antibody or antigen-binding fragment thereof; or

(b) an antibody heavy chain variable region and an antibody light chain variable region of an anti-OX40L antibody or antigen-binding fragment thereof,

wherein the heavy chain variable region comprises the amino acid sequence of SEQ ID NO: 13 and wherein the light chain variable region comprises the amino acid sequence of SEQ ID NO: 14.

36. An expression vector comprising the nucleic acid of claim 35.

37. A host cell comprising the nucleic acid of claim 35 or the expression vector of claim 36.

38. A method for producing an anti-OX40L antibody or antigen-binding fragment thereof comprising:

- (a) culturing the host cell of claim 37 under a condition suitable for production of the anti-OX40L antibody or antigen-binding fragment thereof; and
- (b) isolating the anti-OX40L antibody or antigen-binding fragment thereof produced by the host cell.

39. An article of manufacture for diagnosing or treating an autoimmune disorder comprising a receptacle comprising the antibody or antigen-binding fragment thereof of any one of claims 1-33, or the pharmaceutical composition of claim 34, and instructional materials for using the same to treat or diagnose the autoimmune disorder.

40. A method of preventing or treating an autoimmune disorder comprising administering a therapeutically effective amount of the antibody or antigen-binding fragment thereof of any one of claims 1-33 or the pharmaceutical composition of claim 34 to a subject.

41. The method as in claim 40, wherein the autoimmune disorder is caused by autoreactive T cells.

42. The method as in claim 40 or 41, wherein the autoimmune disorder is selected from the group consisting of Alopecia or Alopecia Areata (AA), Ankylosing spondylitis, Autoimmune Lymphoproliferative Syndrome (ALPS), Celiac disease, Crohn's Disease, Graft-versus-host disease, Graves' Disease, Guillain-Barre' syndrome, Hypersensitivity Vasculitis

(HV), Inflammatory bowel disease, Myasthenia Gravis Neutropenia, Rheumatoid arthritis (RA), Sarcoidosis, Systemic lupus erythematosus (SLE), Systemic sclerosis (scleroderma).

43. The method as in any one of claims 40-42, wherein:

- (a) the antibody or antigen-binding fragment thereof is administered via intravenous injection; or
- (b) the antibody or antigen-binding fragment thereof is administered via subcutaneous injection; or
- (c) the antibody or antigen-binding fragment thereof is administered via injection and the method comprises administering at least one injection at a dose of at least about 20 mg of the antibody or antigen-binding fragment thereof; or
- (d) the antibody or antigen-binding fragment thereof is a disease modifying drug; or
- (e) the antibody or antigen-binding fragment thereof is administered at least twice with at least one interval of 2 to 6 months; or
- (f) the antibody or antigen-binding fragment thereof is administered at least twice with at least one interval of around 3 months.

44. The method as in any one of claims 40-43, wherein:

- (a) each dose of the antibody or antigen-binding fragment thereof is between about 20 mg and about 1000 mg; or
- (b) each dose of the antibody or antigen-binding fragment thereof is about 75 mg, about 100 mg, about 150 mg, about 300 mg, or about 600 mg; or
- (c) the dose of the antibody or antigen-binding fragment thereof is about 75 mg; or
- (d) the dose of the antibody or antigen-binding fragment thereof is about 100 mg; or
- (e) the dose of the antibody or antigen-binding fragment thereof is about 150 mg; or
- (f) the dose of the antibody or antigen-binding fragment thereof is about 300 mg; or
- (g) the dose of the antibody or antigen-binding fragment thereof is about 600 mg; or
- (h) the dose of the antibody or antigen-binding fragment thereof is between about 0.45 mg/kg and about 12 mg/kg.

45. The method as in any one of claims 40-44, wherein:

- (a) the antibody or antigen-binding fragment thereof is administered via injection and the minimum blood serum concentration reached by the antibody or antigen-binding fragment thereof between any two injections (C_{min}) is at least 2.5 $\mu\text{g/mL}$; or
- (b) the maximum blood serum concentration reached by the antibody or antigen-binding fragment thereof after administration of an injection and prior to administration of a subsequent injection (C_{max}) is at least about 1.5 $\mu\text{g/mL}$; or
- (c) blood serum concentrations of the antibody or antigen-binding fragment thereof during treatment range between about 1.5 $\mu\text{g/mL}$ and about 550 $\mu\text{g/mL}$; or
- (d) the area under the serum concentration-time curve (AUC) following the first (AUC extrapolated to infinity [$AUC_{0-\infty}$]) injection may be at least around 100,000 $\text{ng}\cdot\text{day/mL}$ to at least around 15,500,000 $\text{ng}\cdot\text{day/mL}$.

46. The method as in any one of claims 40-45, wherein the method comprises an induction phase and a maintenance phase.

47. The method as in any one of claims 40-46, wherein:

- (a) the induction dose of the antibody or antigen-binding fragment thereof is between about 20 mg and about 1000 mg; or
- (b) the maintenance dose of the antibody or antigen-binding fragment thereof is between about 20 mg and about 600 mg; or
- (c) the antibody or antigen-binding fragment thereof is administered via injection and the interval between two or more induction phase injections is between about 2 weeks and about 24 weeks, and the interval between two or more maintenance phase injections is between about 2 weeks and about 52 weeks.

48. The method as in any one of claims 40-47, wherein:

- (a) the first injection of the antibody or antigen-binding fragment thereof can be a loading dose, wherein:
 - (i) the loading dose of the antibody or antigen-binding fragment thereof is up to three times the mass of the treatment dose, or
 - (ii) the loading dose of the antibody or antigen-binding fragment thereof is between about 100 mg and about 600 mg; and

(b) the second and additional injections of the antibody or antigen-binding fragment thereof can be treatment doses of at least 5 treatment injections, wherein:

(i) the treatment dose of the antibody or antigen-binding fragment is between about 50 mg and about 300 mg.

49. A method of treating Atopic Dermatitis in a subject in need thereof, the method comprising administering a therapeutically effective amount of the antibody or antigen-binding fragment thereof of any one of claims 1-33 or the pharmaceutically composition of claim 34 to the subject.

50. The method of claim 49, wherein:

(a) the antibody or antigen-binding fragment thereof is administered via injection; or

(b) the antibody or antigen-binding fragment thereof is administered via subcutaneous injection; or

(c) the antibody or antigen-binding fragment thereof is administered via injection and the method comprises administering at least one injection at a dose of at least about 20 mg of the antibody or antigen-binding fragment thereof; or

(d) the antibody or antigen-binding fragment thereof is a disease modifying drug; or

(e) the antibody or antigen-binding fragment thereof is a disease modifying drug, the subject achieves an IGA-AD score of 0 or 1 for at least six months; or

(f) the antibody or antigen-binding fragment thereof is administered at least twice with at least one interval of 2 to 6 months; or

(g) the antibody or antigen-binding fragment thereof is administered at least twice with at least one interval of around 3 months.

51. The method of claim 49 or 50, wherein:

(a) each dose of the antibody or antigen-binding fragment thereof is between about 20 mg and about 1000 mg; or

(b) each dose of the antibody or antigen-binding fragment thereof is about 75 mg, about 100 mg, about 150 mg, about 300 mg, or about 600 mg; or

(c) the dose of the antibody or antigen-binding fragment thereof is about 75 mg; or

(d) the dose of the antibody or antigen-binding fragment thereof is about 100 mg; or

(e) the dose of the antibody or antigen-binding fragment thereof is about 150 mg; or

- (f) the dose of the antibody or antigen-binding fragment thereof is about 300 mg; or
- (g) the dose of the antibody or antigen-binding fragment thereof is about 600 mg; or
- (h) the dose of the antibody or antigen-binding fragment thereof is between about 0.45 mg/kg and about 12 mg/kg.

52. The method as in any one of claims 49-51, wherein:

- (a) the antibody or antigen-binding fragment thereof is administered via injection and the minimum blood serum concentration reached by the antibody or antigen-binding fragment thereof between any two injections (C_{min}) is at least 2.5 $\mu\text{g/mL}$; or
- (b) the maximum blood serum concentration reached by the antibody or antigen-binding fragment thereof after administration of an injection and prior to administration of a subsequent injection (C_{max}) is at least about 1.5 $\mu\text{g/mL}$; or
- (c) blood serum concentrations of the antibody or antigen-binding fragment thereof during treatment range between about 1.5 $\mu\text{g/mL}$ and about 550 $\mu\text{g/mL}$; or
- (d) the area under the serum concentration-time curve (AUC) following the first (AUC extrapolated to infinity [$AUC_{0-\infty}$]) injection may be at least around 100,000 $\text{ng}\cdot\text{day/mL}$ to at least around 15,500,000 $\text{ng}\cdot\text{day/mL}$.

53. The method as in any one of claims 49-52, wherein the method comprises an induction phase and a maintenance phase.

54. The method as in any one of claims 49-53, wherein:

- (a) the induction dose of the antibody or antigen-binding fragment thereof is between about 20 mg and about 1000 mg; or
- (b) the maintenance dose of the antibody or antigen-binding fragment thereof is between about 20 mg and about 600 mg; or
- (c) the antibody or antigen-binding fragment thereof is administered via injection and the interval between two or more induction phase injections is between about 2 weeks and about 24 weeks, and the interval between two or more maintenance phase injections is between about 2 weeks and about 52 weeks.

55. The method as in any one of claims 49-54, wherein:

(a) the first injection of the antibody or antigen-binding fragment thereof can be a loading dose, wherein:

(i) the loading dose of the antibody or antigen-binding fragment thereof is up to three times the mass of the treatment dose, or

(ii) the loading dose of the antibody or antigen-binding fragment thereof is between about 100 mg and about 600 mg; and

(b) the second and additional injections of the antibody or antigen-binding fragment thereof can be treatment doses of at least 5 treatment injections of the antibody or antigen-binding fragment, wherein:

(i) the treatment dose of the antibody or antigen-binding fragment thereof is between about 50 mg and about 300 mg.

56. The method as in any one of claims 49-55, wherein:

(a) the subject is at least 6 years of age and/or less than 100 years of age; or

(b) the atopic dermatitis is moderate-to-severe atopic dermatitis; or

(c) the atopic dermatitis is moderate-to-severe atopic dermatitis and wherein the subject is a patient candidate for systemic therapy; or

(d) the atopic dermatitis is moderate-to-severe atopic dermatitis and wherein the subject is a patient whose disease is not adequately controlled with topical prescription therapies or a patient for whom topical prescription therapies are not advisable; or

(e) the atopic dermatitis is resistant, non-responsive, or inadequately responsive to treatment by either topical corticosteroids and/or systemic therapies; or

(f) either topical corticosteroids and/or systemic therapies are not advisable for the subject; or

(g) the subject has had an inadequate response to, was intolerant to, or is refractory to one or more topical corticosteroids.

57. The method as in any one of claims 49-56, wherein the method further comprises administering a therapeutically effective amount of one or more topical corticosteroids.

58. The method of claim 57, wherein the topical corticosteroid is selected from the group consisting of betamethasone dipropionate, clobetasol propionate, dexamethasone,

methylprednisolone, methylprednisolone aceponate, mometasone furoate, diflorasone diacetate, halobetasol propionate, amcinonide, augmented betamethasone dipropionate, fluocinonide, halcinonide, triamcinolone acetonide, betamethasone valerate, clocortolone pivalate, desoximetasone, fluocinolone acetonide, flurandrenolide, fluticasone propionate, hydrocortisone butyrate, hydrocortisone probutate, hydrocortisone valerate, prednicarbate, alclometasone dipropionate, desonide, gentamicin sulphate, hydrocortisone, and hydrocortisone acetate.

59. The method as in any one of claims 49-58, wherein administering the antibody or antigen-binding fragment thereof results in at least one improvement selected from:

- (a) a decrease from baseline in validated investigator global assessment atopic dermatitis (vIGA-AD) score of at least 2 points, or
- (b) achieving clear or almost clear skin (vIGA-AD 0/1) from baseline, or
- (c) a decrease from baseline in eczema area and severity index (EASI) score of at least 50%, or
- (d) a decrease from baseline in EASI score of at least 75%, or
- (e) achieving EASI-75, or
- (f) achieving EASI-90, or
- (g) achieving an improvement of at least 3 points in pruritus numerical rating scale (NRS) score, or
- (h) achieving an improvement of at least 4 points in pruritus NRS score, or
- (i) a decrease from baseline in affected BSA (Body Surface Area) score of at least 60%, or
- (j) a decrease from baseline in affected BSA score of at least 70%.

60. The method as in any one of claims 49-59, wherein:

- (a) the administration of the anti-OX40L antibody or antigen-binding fragment thereof results in the decrease in serum levels of at least one biomarker selected from the group consisting of IL-4, IL-5, IL-9, IL-13, IL-22, IL-17A, IL-31, IgE, TARC, MDC, PARC, CTACK, eosinophils, and OX40L; or
- (b) the administration of the anti-OX40L antibody or antigen-binding fragment thereof results in the decrease in serum levels of at least one biomarker selected from the group consisting of: IL-4, IL-5, IL-9, IL-13, IL-22, IL-17A, IL-31, IgE, TARC, MDC, PARC, CTACK,

Eosinophils, and OX40L, and wherein the decrease is maintained for at least 12 weeks or at least 24 weeks following the final dose; or

(c) the atopic dermatitis has been assessed by determining a baseline EASI score; or

(d) the atopic dermatitis has been assessed by determining a baseline EASI score and wherein the post-administration EASI score is maintained, without additional administration of an anti-OX40L antibody, or antigen-binding fragment thereof, for:

(i) at least 2 months, at least 3 months, at least 4 months, at least 5 months, or at least 6 months after administration of the last injection; or

(ii) at least about 4, 8, 15, 22, 25, 29, 36, 43, 50, 57, 64, 71, 85, 113, 169, or 253 days; or

(e) the atopic dermatitis has been assessed by determining a baseline EASI score and wherein the post-administration EASI and/or further post-administration EASI is determined at least around 113 days after administering a first injection of the antibody or antigen-binding fragment thereof and wherein the post-administration EASI and/or further post-administration EASI is EASI50, EASI75, EASI90 or EASI100; or

(f) the atopic dermatitis has been assessed by determining a baseline EASI score and wherein the post-administration EASI and/or further post-administration EASI is determined at least around 169 days after administering a first injection of the antibody or antigen-binding fragment thereof and wherein the post-administration EASI and/or further post-administration EASI is EASI50, EASI75, EASI90 or EASI100; or

(g) the atopic dermatitis has been assessed by determining a baseline EASI score and wherein the post-administration EASI and/or further post-administration EASI is determined at least around 253 days after administering a first injection of the antibody or antigen-binding fragment thereof and wherein the post-administration EASI and/or further post-administration EASI is EASI50, EASI75, EASI90 or EASI100; or

(h) the atopic dermatitis has been assessed by determining a baseline EASI score and wherein the atopic dermatitis is treated as evidenced by a reduction in the EASI score by at least 40% after the third injection as a treatment dose and wherein the reduction in EASI score is persistent for at least 2 months, at least 3 months, at least 4 months, at least 5 months, or at least 6 months after administration of the last injection as a treatment dose.

61. The method as in any one of claims 49-60, wherein the atopic dermatitis has been assessed by determining a baseline vIGA-AD score, and wherein:

- (a) the post-administration vIGA-AD score is 0 or 1; or
- (b) the post-administration vIGA-AD score is maintained, without additional administration of an anti-OX40L antibody, or antigen-binding fragment thereof, for:
 - (i) at least 2 months, at least 3 months, at least 4 months, at least 5 months, or at least 6 months after administration of the last injection; or
 - (ii) at least about 4, 8, 15, 22, 25, 29, 36, 43, 50, 57, 64, 71, 85, 113, 169 or 253 days; or
- (c) the post-administration vIGA-AD and/or further post-administration vIGA-AD is determined at least around 113 days after administering a first injection of the antibody or antigen-binding fragment thereof and wherein the post-administration vIGA-AD and/or further post-administration vIGA-AD is:
 - (i) a vIGA-AD score of 0 or 1, and/or
 - (ii) reduced at least 2 points relative to the baseline vIGA-AD score; or
- (d) the post-administration vIGA-AD and/or further post-administration vIGA-AD is determined at least around 169 days after administering a first injection of the antibody or antigen-binding fragment thereof and wherein the post-administration vIGA-AD and/or further post-administration vIGA-AD is:
 - (i) a vIGA-AD score of 0 or 1, and/or
 - (ii) reduced at least 2 points relative to the baseline vIGA-AD score; or
- (e) the post-administration vIGA-AD and/or further post-administration vIGA-AD is determined at least around 253 days after administering a first injection of the antibody or antigen-binding fragment thereof and wherein the post-administration vIGA-AD and/or further post-administration vIGA-AD is:
 - (i) a vIGA-AD score of 0 or 1, and/or
 - (ii) reduced at least 2 points relative to the baseline vIGA-AD score; or
- (f) the atopic dermatitis is treated as evidenced by a reduction in the vIGA-AD score by at least 2 points after the third injection as a treatment dose and wherein the reduction in vIGA-AD score is persistent for at least 2 months, at least 3 months, at least 4 months, at least 5 months, or at least 6 months after administration of the last injection as a treatment dose.

62. The antibody or antigen-binding fragment thereof of any one of claims 1-33 or the pharmaceutical composition of claim 34 for use in preventing or treating an autoimmune disorder or Atopic Dermatitis in a subject.

63. The antibody or antigen-binding fragment thereof of any one of claims 1-33 or the pharmaceutical composition of claim 34, for use in the manufacture of a medicament for the prevention or treatment of an autoimmune disorder or Atopic Dermatitis.